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«Молекулярные основы эпидемиологии, диагностики, профилактики и лечения актуальных инфекций»

Санкт-Петербург, 4–6 декабря 2018 года

110-летие со дня основания Института (1908)

95-летие со дня присвоения Институту имени Пастера (1923)

Молекулярные основы эпидемиологии, диагностики, профилактики и лечения актуальных инфекций

Международная конференция, посвященная 110-летию со дня основания Санкт-Петербургского института эпидемиологии и микробиологии имени Пастера и 95-летию со дня присвоения Институту имени Пастера

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САНКТ-ПЕТЕРБУРГСКОЕ РЕГИОНАЛЬНОЕ ОТДЕЛЕНИЕ РОССИЙСКОЙ АССОЦИАЦИИ АЛЛЕРГОЛОГОВ И КЛИНИЧЕСКИХ ИММУНОЛОГОВ (СПб РО РААКИ)

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МОЛЕКУЛЯРНЫЕ ОСНОВЫ ЭПИДЕМИОЛОГИИ, ДИАГНОСТИКИ, ПРОФИЛАКТИКИ И ЛЕЧЕНИЯ АКТУАЛЬНЫХ ИНФЕКЦИЙ

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Russian Journal of Infection and Immunity = Infektsiya i immunitet
2018, vol. 8, no. 4, pp. 405

Инфекция и иммунитет
2018, Т. 8, № 4, с. 405

ПРЕДИСЛОВИЕ

Уважаемые коллеги!

В этом году журналу «Инфекция и иммунитет» исполнилось 8 лет. За это время журнал прошел очень интенсивный путь развития, в результате чего был включен в две основные международные научные базы данных: Web of Science и Scopus. В итоге среди отечественных журналов эпидемиологического, микробиологического и иммунологического профиля «Инфекция и иммунитет», несмотря на «молодость», занимает одно из ведущих мест по данным Российского индекса научного цитирования.

Данный номер журнала содержит материалы Международной научной конференции «Молекулярные основы эпидемиологии, диагностики, профилактики и лечения актуальных инфекций», посвященной 110-летию со дня основания Санкт-Петербургского института эпидемиологии и микробиологии имени Пастера и 95-летию со дня присвоения институту имени Пастера. Учитывая широкую географию участников конференции, которые представляют 33 зарубежных страны с 4 континентов, мы сочли возможным публикацию большей части материалов на английском языке.

Первый раздел материалов конференции состоит из статей, представляющих избранные устные доклады. Статья, посвященная новым страницам истории Санкт-Петербургского Института имени Пастера, подводит итоги двухлетних активных поисков документов, показывающих, что Институт в действительности на 15 лет старше, чем считалось до сих пор и что в этом году ему исполняется не 95, а 110 лет. Это во многом объясняет почему в 1923 году, буквально через месяц после приказа об организации Института, ему присваивают имя Пастера. Только 100-летием со дня рождения Луи Пастера объяснить данное решение вряд ли было возможно.

Частная лаборатория, трансформированная в дальнейшем в частный бактериологический институт, была основана двумя микробиологами (Яков Юльевич Либерман и Петр Петрович Маслаковец) и иммунологом (Георгий Дмитриевич Белоновский) в 1908 году. Именно этот институт после революции 1917 года был преобразован во Вторую городскую бактериологическую лабораторию и, наконец, в 1923 году — в Петроградский бактериологический и диагностический институт. К сожалению найти доказательства этому удалось в архивных документах, оставшихся от трагических страниц истории страны и связанных с годами сталинских репрессий. Георгий Дмитриевич Белоновский, которого также не обошли годы репрессий, но который прошел Школу Парижского Пастеровского института в 1905–1906 годах у И.И. Мечникова, явился одним из основных инициаторов присвоения Институту имени великого Пастера.

Кроме исторических статей в этом разделе также содержатся обзорные и оригинальные работы по кори, энтеровирусным инфекциям, микобактериям, гриппу и т.д.

Второй раздел юбилейной конференции состоит из тезисов устных и стендовых докладов, представленных в 10 главах: «Итоги и направления деятельности по обеспечению эпидемиологической безопасности населения в современных условиях», «Современные методы молекулярной диагностики инфекционных заболеваний», «Вирусные инфекции, управляемые средствами вакцинопрофилактики на этапе ликвидации и элиминации», «Зоонозные и паразитарные инфекции: клинико-эпидемиологические и лабораторно-инструментальные аспекты», «Иерсиниозы: таксономия, филогеография, полиморфизм факторов патогенности и избирательная вирулентность», «Туберкулез и микобактерии: молекулярный подход», «ВИЧ, гепатиты и другие социально значимые инфекции», «Инфекционная иммунология на современном этапе», «Антибиотикорезистентность микроорганизмов: актуальные вопросы диагностики и пути преодоления» и «Новые химиопрепараты для терапии инфекционных заболеваний». Из большинства этих тезисов была сформирована не только программа секционных заседаний, но и программы Рабочего совещания «Реализация Программы элиминации острого вирусного гепатита В на территории Северо-Западного федерального округа Российской Федерации», Совещания полиомиелитных лабораторий ВОЗ с участием институтов Международной Сети Институтов Пастера «Энтеровирусные инфекции после реализации программы ликвидации полиомиелита», Симпозиума «ВИЧ-инфекция и иммуносупрессии» (10-е Виноградовские чтения), а также «2-го Санкт-Петербургского Симпозиума по туберкулезу и микобактериям: молекулярный подход», который проводится при поддержке Российского Фонда Фундаментальных Исследований (грант № 18-04-20102).

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Инфекция и иммунитет
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FOREWORD

Dear Colleagues!

This year journal "Infektsiya i immunitet" ("Russian Journal of Infection and Immunity") turned 8 years old.

Over the years, the journal has developed intensively and as a result was included in two main international databases: Web of Science and Scopus. Meanwhile despite its youth it occupies one of the leading positions among Russian journals in the field of epidemiology, microbiology, immunology according to the Russian Science Citation Index (RSCI).

This issue contains materials of the International Scientific Conference "Molecular Bases of Epidemiology, Diagnostics, Prevention and Treatment of Infectious Diseases" dedicated to the 110 years since the establishment of St. Petersburg Pasteur Institute and 95 years since its naming after Louis Pasteur. Considering the wide geography of the conference participants, who represent 33 foreign countries from 4 continents, we found it possible to publish most of the materials in English.

The first section of the Conference materials consists of selected oral presentations. The article devoted to the new pages of St. Petersburg Pasteur Institute's history summarizes the two-year search for the documents showing that the Institute is in fact 15 years older than it was previously considered and proving that the Institute is not 95, but 110 years old this year. This explains a lot why in 1923, just a month after the order for organizing the Institute, it was named after Pasteur. It would not have been possible to explain this decision only by the 100th anniversary of Louis Pasteur.

The private laboratory, later transformed into a private Bacteriological Institute, was founded in 1908 by two microbiologists (Yakov Lieberman and Petr Maslakovets) and an immunologist (Georgy Belonovsky). It was this Institute that after the Revolution of 1917 was transformed into the Second Bacteriological Laboratory and later in 1923 into the Petrograd Bacteriological and Diagnostic Institute. Sadly, this data were found only in the archive documents left over from the tragic pages of the country's history related to the years of Stalinist repression. Georgy Belonovsky, who also has not been able to escape the years of repression, but studied at School of the Institut Pasteur (Paris) in 1905–1906 under Ilya Mechnikov, was one of the main initiators of the naming the Institute after Lois Pasteur.

In addition to the historical articles, this section also contains reviews and original articles on measles, enterovirus infections, Mycobacteria, influenza, etc.

The second section of the Jubilee Conference consists of oral and poster presentations presented in 10 chapters: Viral infections managed by means of vaccination at the stage of destruction and elimination; Modern methods of molecular diagnostics of infectious diseases; Results and directions of activities to ensure the epidemiological safety of the population in modern conditions; Zoonotic and parasitic infections: clinical, epidemiological and laboratory aspects; Yersiniosis: taxonomy, phylogeography, polymorphism of pathogenicity factors and selective virulence; Tuberculosis and Mycobacteria: Molecular Approach; Diagnosis and treatment of socially significant infections; Infectious immunology at the present stage; Antibiotic resistance of microorganisms: current issues of diagnosis and ways to overcome and New chemotherapy for the treatment of infectious diseases.

Most of these abstracts have formed not only the Programme of the Conference Sessions but also the Programme of the Workshop "Implementation of the Programme for Hepatitis B Elimination in North-Western Federal District of Russia: History, Results of the 2nd Stage, Prospects", Satellite event "Enterovirus diseases following poliomyelitis eradication", X Anniversary Scientific and Practical Conference with International Participation "HIV Infection of Immunosuppression and 2nd St. Petersburg Symposium on Tuberculosis and Mycobacteria: Molecular Approach, which is supported by the Russian Foundation for Basic Research (Grant No 18-04-20102).

Editor in Chief of the "Russian Journal of Infection and Immunity",
Director of St. Petersburg Pasteur Institute,
Co-Chair of the Organizing Committee of the International
Scientific Conference "Molecular Bases of Epidemiology,
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Areg A. Totolian

Молекулярные основы эпидемиологии, диагностики, профилактики и лечения актуальных инфекций

Международная конференция, посвященная 110-летию со дня основания Санкт-Петербургского института эпидемиологии и микробиологии имени Пастера и 95-летию со дня присвоения Институту имени Пастера

Санкт-Петербург, 4–6 декабря 2018 года

Избранные устные доклады

Molecular bases of epidemiology, diagnostics, prevention and treatment of infectious diseases

International conference, dedicated to the 110th anniversary of St. Petersburg Pasteur Institute and to the 95th anniversary of naming the Institute after Pasteur

December 4–6, 2018, St. Petersburg

Selected oral presentations

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Светлой памяти основателей Института, Пастеровцам и нашим семьям посвящается

ЮБИЛЕЙНЫЙ 2018: 110-ЛЕТИЕ СО ДНЯ ОСНОВАНИЯ САНКТ-ПЕТЕРБУРГСКОГО НИИ ЭПИДЕМИОЛОГИИ И МИКРОБИОЛОГИИ ИМЕНИ ПАСТЕРА И 95-ЛЕТИЕ СО ДНЯ ПРИСВОЕНИЯ ИНСТИТУТУ ИМЕНИ ПАСТЕРА

Н.Г. Алексеева, Арег А. Тотолян

ФБУН НИИ эпидемиологии и микробиологии имени Пастера, Санкт-Петербург, Россия

Резюме. В статье приведены ранее не публиковавшиеся архивные данные и копии документов о первом директоре Петроградского Бактериологического Института имени Пастера (ныне — Санкт-Петербургский НИИ эпидемиологии и микробиологии имени Пастера) Якове Юльевиче Либермане. На основании личных показаний Я.Ю. Либермана, Петра Петровича Маслаковца и Георгия Дмитриевича Белоновского (учредителей и организаторов Института), содержащихся в фондах архива УФСБ РФ по г. Санкт-Петербургу и Ленинградской области, а также с учетом Акта комиссии по обследованию Института от декабря 1930 г., представленного в статье, прослеживается путь преобразований, которые претерпел Институт со дня своего основания. Эти данные проиллюстрированы копиями объявлений об Институте, содержащихся в справочниках «Весь Петербург» за 1910, 1911, 1912, 1914, 1923, 1924, 1925 гг. (из фондов Российской национальной библиотеки). В статье также приведены копии служебной документации, датированные 1913 и 1914 гг. и хранящиеся в фондах Центрального государственного исторического архива Санкт-Петербурга. Представленные документы фактически устанавливают, что датой создания Института является 1908 г.

Ключевые слова: *Диагностический и Бактериологический Институт, Первая серодиагностическая и бактериологическая лаборатория, Санкт-Петербургский Частный Диагностический и Бактериологический Институт, Либерман, Маслаковец, Белоновский, Санкт-Петербургский НИИЭМ имени Пастера.*

JUBILEE 2018: 110th ANNIVERSARY OF THE FOUNDATION OF THE ST. PETERSBURG PASTEUR RESEARCH INSTITUTE OF EPIDEMIOLOGY AND MICROBIOLOGY AND THE 95th ANNIVERSARY OF THE NAMING OF THE INSTITUTE AFTER PASTEUR

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Abstract. The article presents not previously published archive data and copies of documents about Lieberman Yakov Yulievich, the first director of the Petrograd Bacteriological Institute named after Pasteur (now called Saint Petersburg Pasteur Institute). Based on the personal testimony of Ya.Yu. Lieberman, Pyotr Petrovich Maslakovets and Georgii Dmi-

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trievich Belonovsky (the founders and leaders of the Institute) preserved in the archive funds of the Directorate of the Federal Security Service (FSB) of Russian Federation for St. Petersburg and the Leningrad Region, and taking into account the Act of the Commission for the Institute Inspection from December 1930, provided in the article, the authors consistently trace the path of transformations that the Institute has undergone since its foundation. These data are illustrated by copies of publication advertisements contained in the annual city reference book "All Petersburg" for years 1910, 1911, 1912, 1914, 1923, 1924, and 1925 (from the funds of the National Library of Russia). The article also contains copies of official documents dated 1913 and 1914 and stored in the funds of the Central State Historical Archive of St. Petersburg. All documents provided establish the fact that the date of creation of the Institute is 1908.

Key words: *Diagnostics and Bacteriological Institute, The First Serodiagnostic and Bacteriological Laboratory, The St. Petersburg Private Diagnostics and Bacteriological Institute, Lieberman, Maslakovets, Belonovsky, St. Petersburg Pasteur Institute.*

Работа над сохранением исторического, научного и культурного наследия Санкт-Петербургского НИИ эпидемиологии и микробиологии имени Пастера в части сбора информации об истории и руководителях учреждения непосредственным образом оказалась связана со взаимодействием с государственными архивами. Поиск сведений о биографиях директоров Института привел нас в архивы Управления ФСБ России по Санкт-Петербургу и Ленинградской области. На официальный запрос с просьбой оказать содействие в поиске указанной информации был получен ответ, что в архивных фондах ФСБ имеются сведения в отношении Якова Юльевича Либермана — первого директора нашего Института. Нам был открыт доступ к изучению документов архивных уголовных дел, а также оказано содействие в подготовке ксерокопий, а кроме того, в исключительном порядке, — проведению фотосъемки отдельных страниц документов. В результате, в наших руках оказались материалы, основанные на личных показаниях Я.Ю. Либермана, а впоследствии и на показаниях других фигурантов, проходящих по ряду следственных уголовных дел, а также на заключениях комиссий, согласно которым датой образования Института, следует считать не 1923, а 1908 год. Другими словами, в 2018 г. Институт отмечает не 95-летие со дня основания, а свое 110-летие.

К сожалению, так сложилось в истории нашей страны, что в определенные периоды происходили события кардинально меняющие основополагающие принципы существования и устройства российского государства. Так было и на рубеже XIX и XX вв., когда Россия была ввергнута в пучину революционных событий 1905 г., стала участницей Первой мировой войны, затем — февральской и октябрьской революций 1917 г.

В это непростое для страны время ученые продолжали свою работу — каждый в своем направлении. Так было и с тремя микробиологами: Яковом Юльевичем Либерманом, Петром Петровичем Маслаковцом и Георгием Дмитриевичем Белоновским, которые под руководством академика Даниила Кирилловича

Work on the preservation of the historical, scientific and cultural heritage of the St. Petersburg Pasteur Research Institute of Epidemiology and Microbiology in the collection of information about the history and the heads of the institution was directly linked to interaction with state archives. The search for information about the biographies of the Institute's directors led us to the archives of the Directorate of the Federal Security Service of Russia for St. Petersburg and the Leningrad Region. An official request for assistance in the search for this information was sent and we received an answer stating that the archive funds of the FSB have information regarding Yakov Yulievich Lieberman, the first director of our Institute. We were given access to the study of documents of archival criminal cases, as well as we have been assisted in the preparation of photocopies, and also, by exception, — in the photography of non-sequential pages of the documents. As a result, we received the materials based on the personal testimony of Y.Y. Lieberman and later — based on the testimonies of other persons involved in a number of investigative criminal cases, as well as on the conclusions of the commissions according to which the date of the Institute's formation was year 1908, not 1923. In other words, in 2018 the Institute marks not its 95th anniversary, but its 110th anniversary.

Unfortunately, there were certain periods events took place in the history of our country that radically changed the fundamental principles of the existence and structure of the Russian state. So it was at the turn of the 19th and 20th centuries, when Russia was plunged into the abyss of the revolutionary events of 1905, became a participant in the First World War, then the February and October Revolutions of 1917.

In this difficult time for the country, scientists continued their work — each in its own direction. So it was with three microbiologists — Yakov Yulievich Lieberman, Pyotr Petrovich Maslakovets and Georgii Dmitrievich Belonovsky, who, under the leadership of Academician Daniil Kirillovich Zabolotniy, were working at the laboratory of syphilology at the Clinic of Skin and Sexually Transmitted Diseases named after V.K. Sinyagin and A.K. Chekaleva at the Imperial Institute of Experimental Medicine. Serious scientific work in this direction required the creation of

Заболотного работали в это время в лаборатории сифилидологии при Клинике кожных и венерических болезней им. В.К. Синягина и А.К. Чекалевой Императорского Института Экспериментальной Медицины. Для серьезной научной работы в этом направлении требовалось наличие соответствующей лабораторной базы. И в 1908 г. ими была создана Первая серодиагностическая и бактериологическая лаборатория, которая разместилась на Большом проспекте Петроградской стороны в доме № 35-в. Инициаторы создания лаборатории стали ее заведующими. Вот как они писали об этом (по материалам уголовных дел № 19112 и № 39514 из фондов архива УФСБ РФ по г. Санкт-Петербургу и Ленинградской области):

Я.Ю. Либерман: *«...У меня совместно с Маслаковец и Белоновским имелась серодиагностическая лаборатория, переименованная потом в Диагностический Институт. С Маслаковец и Белоновским я работал в Институте Э[кспериментальной] Медицины по вопросам сифилиса. Вместе с ними я практически применял реакцию Вассермана. Сначала эта работа, в ее научной части проводилась в стенах Института Эксп. Медицины, но с ростом ее, стал вопрос о ее организации вне его стен, а так как разработку этих вопросов вели мы — я, Маслаковец и Белоновский, то это и явилось одной из причин появления этой лаборатории...»* (рис. 1).

П.П. Маслаковец: *«В 1908 г. я, Либерман и Белоновский открыли Серо-диагностическую Лабораторию, которая была в годы революции национализирована и из которой ныне вырос Бакт. Ин-т имени Пастера...»* (рис. 2).

Г.Д. Белоновский: *«До революции являлся частным владельцем лаборатории «Частный бактериологический ин-т» совместно с докторами Либерманом и Маслаковцом и работал в нем до 1919 года... С 1920 г. по 1930 г. работал в ин-те «Пастера» в Ленинграде заведующим вакцинным отделением...»* (рис. 3).

Крайне важным историческим свидетельством, не оставляющим сомнений в определении настоящей даты основания Института является Акт комиссии по материалам уголовного дела от 1931 г. № 19112, лист 410, орфография и пунктуация сохранены) (рис. 4):

«Мы нижеподписавшиеся Пом.Нач.2-го Отдела ВСО ЛВО тов. Найда В.А., эксперт Пружанская Е.М. и Михайловский Н.Г., по полномочию Реввоенсовета ЛВО от 19/XII-30 г. за № 220/с, с 21 по 23/XII-30 г. в присутствии Зам. Директора тов. Лебединского, а при обследовании отделов — в присутствии и Заведывающих ими — Вержиковского, Ципа, Белоновского, Кадлеца, Либермана, Иванова, Гартоха и Гаха, — произвели обследование Бахтериологического an appropriate laboratory base. In 1908 they created the first serodiagnostic and bacteriological laboratory, which was located on Bolshoy Prospect of the Petrograd side in the house number 35-v. The initiators of the creation of the laboratory became its managers. Here is how they wrote about it (based on the materials of criminal cases No. 19112 and No. 39514 from the archives of the Federal Security Service of the Russian Federation for St. Petersburg and the Leningrad region):

Ya.Yu. Liberman: *"...I had a serodiagnostic laboratory with Maslakovets and Belonovsky, renamed the Diagnostic Institute later. I worked at the Institute of Epidemiological Medicine for syphilis with Maslakovets and Belonovsky and together we have been practically applying the Wasserman's reaction. At the beginning, this work, in its scientific part, was conducted within the walls of the Institute of Medicine, but with its growth, the question of creating some outside organization, and since the development of these issues was led by me, Maslakovets and Belonovsky, this was one of the reasons of the creating this lab..."* (fig. 1).

P.P. Maslakovets: *"In 1908 Lieberman, Belonovsky and I opened the Sero-Diagnostic Laboratory, which was nationalized during the revolution and from which Bacteriological Institute of Pasteur has now grown..."* (fig. 2).

G.D. Belonovsky: *"Prior to the Revolution, I was the private owner of the laboratory "The Private Bacteriological Institute" in conjunction with doctors Lieberman and Maslakovets and worked there until 1919... From 1920 to 1930 I worked at the Pasteur Institute in Leningrad as the head of the vaccine department..."* (fig. 3).

From the funds of the Archive of the Federal Security Service of the Russian Federation for St. Petersburg and the Leningrad Region (the act of the commission based on the materials of the criminal case No. 19112, sheet 410):

"The Institute was created in 1908 as "The Institute of Diagnostics and Bacteriology". After the revolution in 1918–1919 it continued working as "The Private Bacteriological and Diagnostics Institute". It belonged to Ya.Yu. Lieberman, P.P. Maslakovets and G.D. Belonovsky. Then it was nationalized and reorganized into the Second City Bacteriological Laboratory and it was led by Prof. Rosenberg, Prof. Nedrigailov and Belonovsky one after another..." (fig. 4).

The following information is an extract of the book "The Petrograd Side: Bolshoy Prospect " by its authors G.Y. Nikitenko and V.D. Privalov (fig. 5):

"The private bacteriological and diagnostic institute was located here (note — at Bolshoy pr., 35-v), whose head and owner was G.D. Belonovsky M.D. — Associate Professor of the Imperial Clinical Institute, Privat-docent of the Military Medical Academy and the Women's Medical Institute, the senior resident of the Maritime Hospital. He had two assistants: Ya.Yu. Lieberman — the doctor at the Clinical Institute,

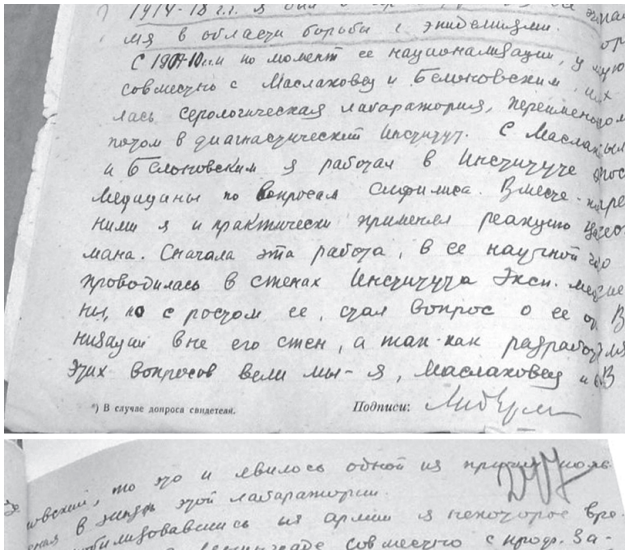


Рисунок 1. Фотокопия показаний Я.Ю. Либермана (по материалам уголовного дела № 19112, листы 246, 247, из фондов архива УФСБ РФ по Санкт-Петербургу и Ленинградской области)

Figure 1. A photocopy of the testimony of Ya.Yu. Lieberman (based on the materials of the criminal case No. 19112, sheets 246, 247, from the funds of the Archive of the Federal Security Service of the Russian Federation for St. Petersburg and the Leningrad Region)

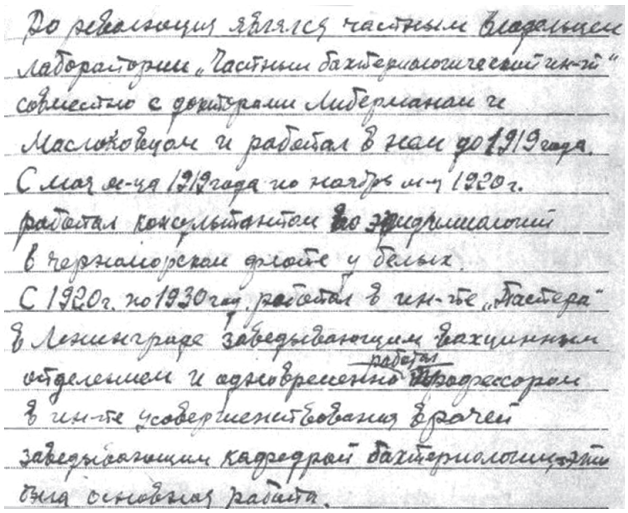


Рисунок 3. Из показаний Г.Д. Белоновского (по материалам уголовного дела № 39514, лист 206, из фондов архива УФСБ РФ по Санкт-Петербургу и Ленинградской области)

Figure 3. Testimony of G.D. Belonovsky (based on the materials of the criminal case No. 39514, sheet 206, No. 39514, from the funds of the Archive of the Federal Security Service of the Russian Federation for St. Petersburg and the Leningrad Region)

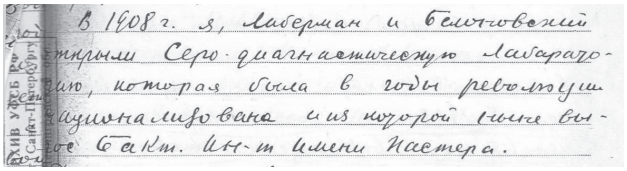
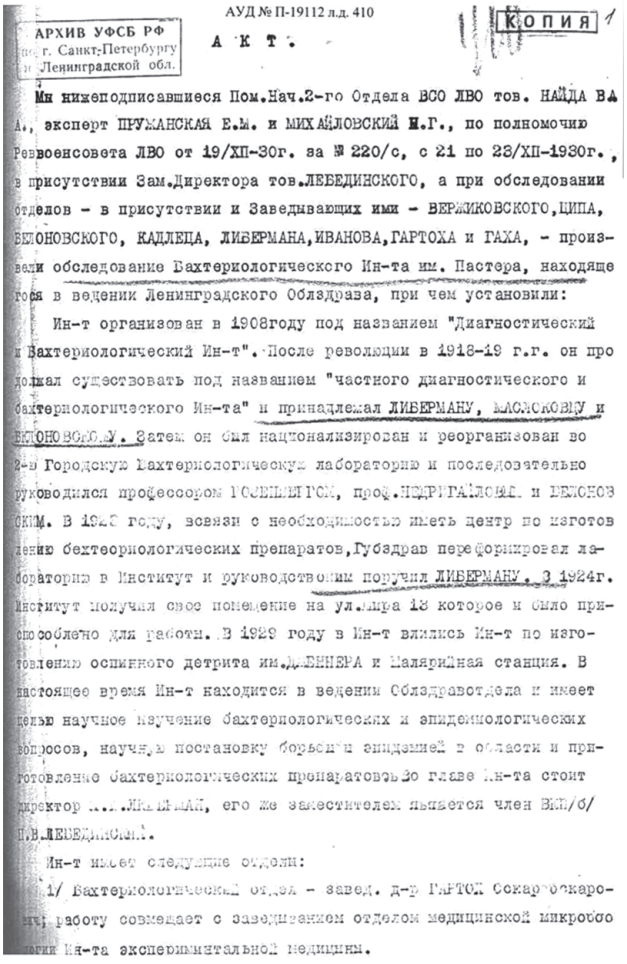


Рисунок 2. Из показаний П.П. Маслаковца (по материалам уголовного дела № 19112, т. 2, лист 8, из фондов архива УФСБ РФ по Санкт-Петербургу и Ленинградской области)

Figure 2. Testimony of P.P. Maslakovets (based on the materials of the criminal case No. 19112, vol. 2, sheet 8, from the funds of the Archive of the Federal Security Service of the Russian Federation for St. Petersburg and the Leningrad Region)



АУД № П-19112 л.д. 410

АРХИВ УФСБ РФ по г. Санкт-Петербургу и Ленинградской обл.

КОПИЯ 1

А К Т.

Мы нижеподписавшиеся Пом.Нач.2-го Отдела ВСО ЛВО тов. НАЙДА В.А., эксперт ПРУЖАНСКАЯ Е.М. и МИХАЙЛОВСКИЙ Н.Г., по полномочию Реввоенсовета ЛВО от 19/XII-30г. за № 220/с, с 21 по 28/XII-1930г., в присутствии Зам.Директора тов.ЛЕБЕДИНСКОГО, а при обследовании отделов - в присутствии и Заведывающих ими - ВЕРЖИКОВСКОГО, ЦИПА, БЕЛОНОВСКОГО, КАДЛЕЦА, ЛИБЕРМАНА, ИВАНОВА, ГАРТОХА и ГАХА, - произвели обследование Бактериологического Ин-та им. Пастера, находящегося в ведении Ленинградского Облздрава, при чем установили:

Ин-т организован в 1908году под названием "Диагностический и Бактериологический Ин-т". После революции в 1918-19 г.г. он продолжал существовать под названием "частного диагностического и бактериологического Ин-та" и принадлежал ЛИБЕРМАНУ, МАСЛАКОВЦУ и БЕЛОНОВСКОМУ. Затем он был национализирован и реорганизован во 2-ю Городскую Бактериологическую лабораторию и последовательно руководился профессором ГОЛЬШМИТОМ, проф.НЕДРИГАЙЛОВЫМ и БЕЛОНОВСКИМ. В 19..0 году, вовзи с необходимостью иметь центр по изготовлению бактериологических препаратов, Губздрав переформировал лабораторию в Институт и руководством поручил ЛИБЕРМАНУ. В 1924г. Институт получил свое помещение на ул.Мира 18 которое и было приспособлено для работы. В 1929 году в Ин-т влились Ин-т по изготовлению оспинного детрита им.ДЕЛЬНЕРА и Малярийная станция. В настоящее время Ин-т находится в ведении Облздравотдела и имеет целью научное изучение бактериологических и эпидемиологических вопросов, научную постановку борьбы с эпидемией в области и приготовление бактериологических препаратов. Во главе Ин-та стоит директор ....ЖИЛЬЯНИ, его же заместителем является член ВКП/б/ Н.В.ЛЕБЕДИНСКИЙ.

Ин-т имеет следующие отделы:

1/ Бактериологический отдел - завед. д-р ГАРТОХ Оскар Оскарович; работу совмещает с заведыванием отделом медицинской микробиологии Ин-та экспериментальной медицины.

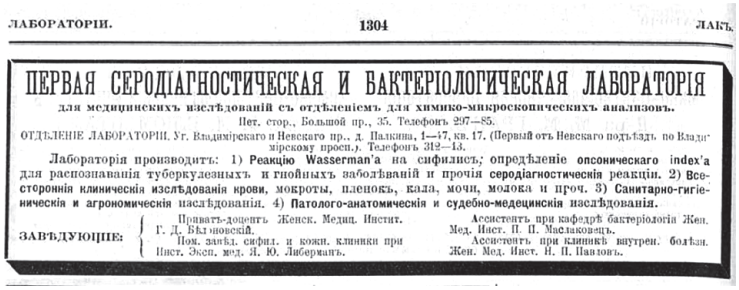
Рисунок 4. Копия акта комиссии по материалам уголовного № 19112, лист 410 (из фондов архива УФСБ РФ по Санкт-Петербургу и Ленинградской области)

Figure 4. The copy of the act of the commission based on the materials of the criminal case No. 19112, sheet 410 (from the funds of the Archive of the Federal Security Service of the Russian Federation for St. Petersburg and the Leningrad Region)

Здесь же находился частный бактериологический и диагностический институт. Его руководитель и владелец доктор медицины Г.Д. Белоновский – доцент Императорского клинического института, приват-доцент Военно-медицинской академии и Женского медицинского института, старший ординатор Морского госпиталя. У него было два помощника: Я.Ю. Либерман – врач Клинического института, и П.П. Маслаковец, служивший в лаборатории Женского медицинского института. Институт в 1920-х гг. был преобразован во 2-ю Городскую лабораторию и продолжал производить вакцины, сыворотки.

Рисунок 5. Фрагмент книги Г.Ю. Никитенко и В.Д. Привалова «Петроградская сторона. Большой проспект» [8, с. 158]

Figure 5. G.Y. Nikitenko and V.D. Privalov. “The Petrograd Side: Bolshoy Prospect”. Fragment of the book [8, p. 158]



ЛАБОРАТОРІИ. 1304 ЛАБ.

ПЕРВАЯ СЕРОДІАГНОСТИЧЕСКАЯ И БАКТЕРІОЛОГИЧЕСКАЯ ЛАБОРАТОРІЯ

для медицинскихъ изслѣдованій съ отдѣленіемъ для химико-микроскопическихъ анализовъ.

Пет. стор., Большой пр., 35. Телефонъ 297—85.

ОТДѢЛЕНІЕ ЛАБОРАТОРІИ. Уг. Владимірскаго и Невскаго пр., д. Палкина, 1—17, кв. 17. (Первый отъ Невскаго подъѣздъ по Владимірскому просп.). Телефонъ 312—13.

Лабораторія производитъ: 1) **Реакцію Wasserman'a** на сифилисъ; **опредѣленіе опсоническаго index'a** для распознаванія туберкулезныхъ и гнойныхъ заболѣваній и прочія **серодіагностическія** реакціи. 2) **Всесторонніе клиническія изслѣдованія крови**, мокроты, пленокъ, кала, мочи, молока и проч. 3) **Санитарно-гигіеническія и агрономическія** изслѣдованія. 4) **Патолого-анатомическія и судебно-медецинскія** изслѣдованія.

ЗАВѢДУЮЩІЕ: Приватъ-доцентъ Женск. Медиц. Институт. Г. Д. Бѣлоновскій. Ассистентъ при кафедрѣ бактеріологіи Жен. Мед. Инст. П. П. Маслаковецъ. Пом. завѣд. сифил. и кожн. клиники при Инст. Эксп. мед. Я. Ю. Либерманъ. Ассистентъ при клиникѣ внутрен. болѣзн. Жен. Мед. Инст. П. П. Павловъ.

Рисунок 6. Копия объявления в справочнике «Весь Петербург», 1910 г. [3, с. 1304]

Figure 6. Copy of the announcement in the guide “All Petersburg”, 1910 [3, p. 1304]

ЛАБОРАТОРІИ. 1365 ЛАБОРАТОРІИ.

Бактеріологическій и Діагностическій Институтъ

БЫВШ. ПЕРВАЯ СЕРОДІАГНОСТИЧЕСКАЯ И БАКТЕРІОЛОГИЧЕСКАЯ ЛАБОРАТОРІЯ

Пет. стор., Большой пр., 35. Телефонъ 497—85.

ОТДѢЛЕНІЕ: Уг. Владимірскаго и Невскаго пр., д. Палкина, 1—17, кв. 17. Телефонъ 512—13.

Институтъ изготовляетъ: 1) **Вакцины**; гонококковую, стафилококковую, скарлатинозную и др. 2) **Туберкулины** по проф. Denys и Koch'y. 3) **Антигены** и гемолитическую сыворотку для реакціи Wasserman'a. 4) **Питательныя среды** и разводки бактерій.

Институтъ производитъ: 1) **Реакцію Wasserman'a** на сифилисъ; опредѣленіе **опсоническаго index'a** и **прочія серодіагностическія** реакціи. 2) **Всесторонніе клиническія изслѣдованія крови**, мокроты, пленокъ, кала, мочи, молока и проч. 3) **Санитарно-гигіеническія и агрономическія** изслѣдованія. 4) **Патолого-анотомическія и судебно-медицинскія** изслѣдованія.

ЗАВѢДУЮЩІЕ: Приватъ-доцентъ Женск. Медиц. Институт. Г. Д. Бѣлоновскій. Ассистентъ при кафедрѣ бактеріологіи Жен. Мед. Инст. П. П. Маслаковецъ. Пом. завѣд. сифил. и кожн. клиники при Инст. Эксп. мед. Я. Ю. Либерманъ. Ассистентъ при клиникѣ внутрен. болѣзн. Жен. Мед. Инст. П. П. Павловъ.

Рисунок 7. Копия объявления в справочнике «Весь Петербург», 1911 г. [4, с. 1365]

Figure 7. Copy of the announcement in the guide “All Petersburg”, 1911 [4, p. 1365]

ЛАБОРАТОРІИ. 1408 ЛАБОРАТОРІИ.

С.-Петербургскій Частный

Бактеріологическій и Діагностическій Институтъ

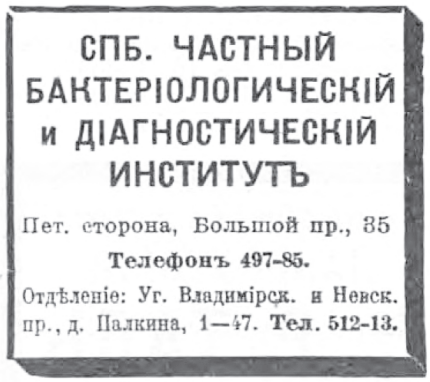
Петерб. стор. Большой пр., 35. Тел. 497—85.

ОТДѢЛЕНІЯ: 1) Уг. Невскаго и Владимірск., д. Палкина. Тел. 512—13.
2) Лѣсной, 2-ой Муринскій, д. 18. Тел. 82.
3) Пятигорскъ, Царская, д. Баумгольца.

I. Производство всевозможныхъ клиническихъ, бактеріологическихъ и серодіагностическихъ изслѣдованій. Реакція Wassermann'a, р. опсониновъ, мейостагминовая р., р. Calmetta-Pirquet.
II. Производство: 1) вакцинъ по методу Wright'a (гонококковой, стафилококковой, пневмококковой и др.); 2) Туберкулиновъ Denys и Koch'a; 3) Культуръ бактерій и питательныхъ средъ; 4) Культуръ крысоубивающихъ бактерій—«Эликтана»; 5) Оспеннаго детрита.
III. Дезинфекція помѣщеній (съ бактеріологическимъ контролемъ) и испытаніе дезинфекціонныхъ средствъ.
IV. Курсы для врачей и фармацевтовъ по общей бактеріологіи, серодіагностикѣ и инфекціоннымъ заболѣваніямъ.

Рисунок 8. Копия объявления в справочнике «Весь Петербург», 1912 г. [5, с. 1408]

Figure 8. Copy of the announcement in the guide “All Petersburg”, 1912 [5, p. 1408]


СПБ. ЧАСТНЫЙ
БАКТЕРІОЛОГИЧЕСКІЙ
и ДІАГНОСТИЧЕСКІЙ
ИНСТИТУТЪ

Пет. сторона, Большой пр., 35
Телефонъ 497-85.
Отдѣленіе: Уг. Владимірск. и Невск. пр., д. Палкина, 1—47. Тел. 512-13.

Рисунок 9. Копия объявления в справочнике «Весь Петербург», 1914 г. [6, с. 1303]
Figure 9. Copy of the announcement in the guide “All Petersburg”, 1914 [6, p. 1303]

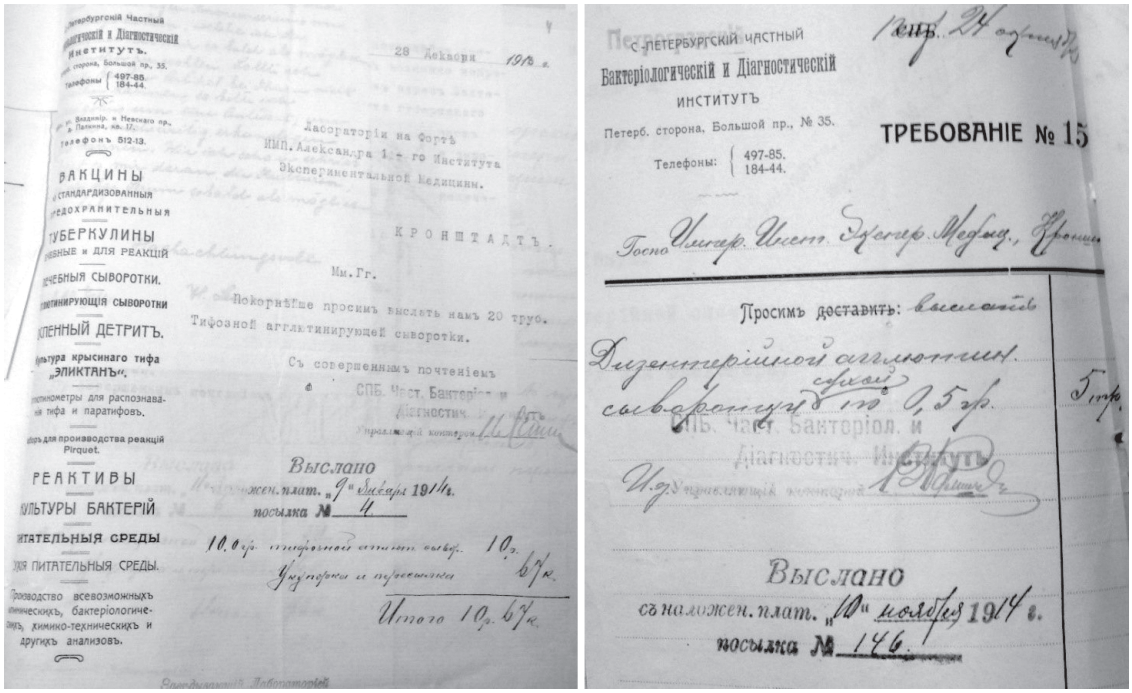

ВАКЦИНЫ
СТАНДАРДИЗОВАННЫЯ
ПРЕДОХРАНИТЕЛЬНЫЯ
ТУБЕРКУЛИНЫ
ЛЕЧЕБНЫЕ и ДЛЯ РЕАКЦІЙ
ЛЕЧЕБНЫЯ СЫВОРОТКИ.
АГГЛЮТИНИРУЮЩІЯ СЫВОРОТКИ
ЛЕННЫЙ ДЕТРИТЪ.
Культура крысинаго тифа „ЭЛИКТАНЪ“.
РЕАКТИВЫ
КУЛЬТУРЫ БАКТЕРІЙ
ИТАТЕЛЬНЫЯ СРЕДЫ
ЯЯ ПИТАТЕЛЬНЫЯ СРЕДЫ.
Производство всевозможныхъ клиническихъ, бактеріологическихъ, химико-техническихъ и другихъ анализовъ.
Лаборатоpіи на фортъ
ИМП. Александра 1-го Института
Экспериментальной Медицины.
КРОНШТАДТЪ.
Мм. Гг.
Покорнѣйше просимъ выслать намъ 20 трубо.
Тифозной агглютинирующей сыворотки.
Выслано
Итого 10 р. 6 к.
С.-ПЕТЕРБУРГСКІЙ ЧАСТНЫЙ
Бактеріологическій и Діагностическій
ИНСТИТУТЪ
Петерб. сторона, Большой пр., № 35.
Телефоны: 497-85. 184-44.
ТРЕБОВАНІЕ № 15
Просимъ доставить:
Выслано
посылка № 146.

Рисунок 10. Копии запросов (требований) Института на высылку вакцин, 1913 и 1914 гг. (из фондов архива ЦГИА СПб)
Figure 10. Copies of the Institute’s requests (demands) for dispatch of vaccines, 1913 and 1914 (from the funds of the Central Governmental Historical Archive)

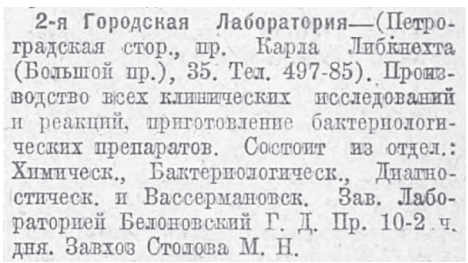
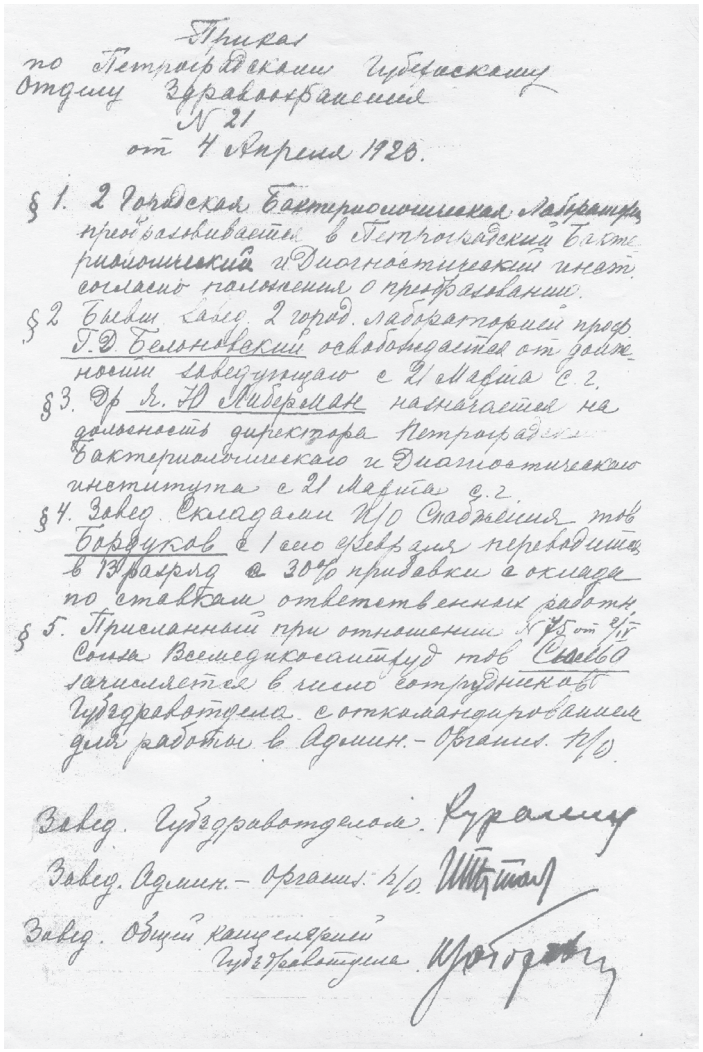

2-я Городская Лаборатория—(Петроградская стор., пр. Карла Либкнехта (Большой пр.), 35. Тел. 497-85). Производство всех клинических исследований и реакций, приготовление бактериологических препаратов. Состоит из отдел.: Химическ., Бактериологическ., Диагностическ. и Вассермановск. Зав. Лабораторией Белоновский Г. Д. Пр. 10-2 ч. дня. Завхоз Столова М. Н.

Рисунок 11. Копия объявления в справочнике «Весь Петроград», 1923 г. [7, с. 61]
Figure 11. Copy of the announcement in the guide “All Petrograd”, 1923 [7, p. 61]



Приказ
по Петроградскому Губернскому
Отделу Здравоохранения
№ 21
от 4 Апреля 1923.

§ 1. 2 Городская Бактериологическая Лаборатория преобразовывается в Петроградский Бактериологический и Диагностический институт согласно положения о преобразовании.

§ 2. Бывш. Зав. 2 город. Лабораторией проф. Г.Д. Белоновский освобождается от должности заведующаго с 21 Марта с.г.

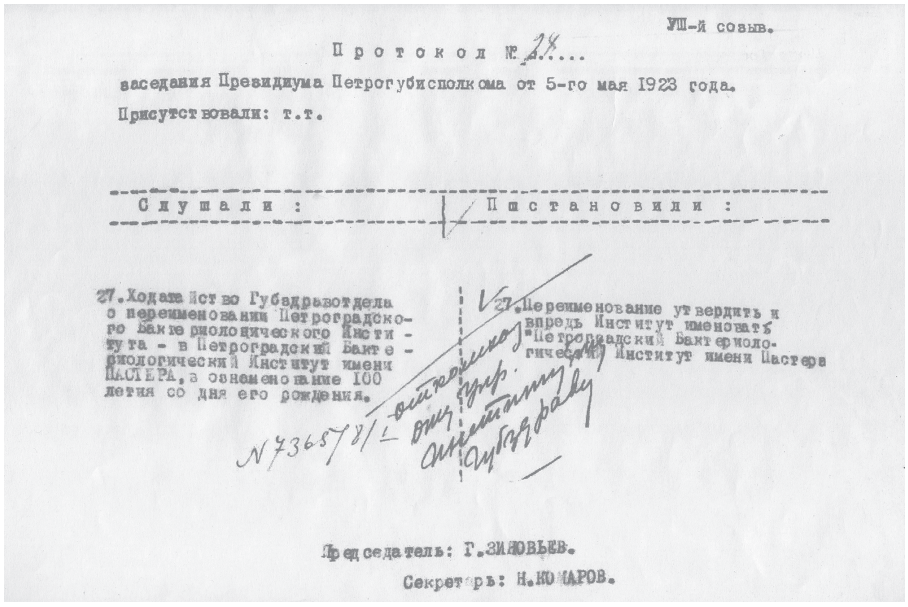
§ 3. Др. Я.Ю. Либерман назначается на должность директора Петроградскаго Бактериологическаго и Диагностическаго института с 21 Марта с.г.

§ 4. Завед. Складами Н/О Снабжения тов. Бордуков с 1-го Апреля переводится в 13 разряд с 30% прибавки с оклада по ставкам ответственных работн.

§ 5. Присланный при отношении № 75 от 2/IV Союза Всемедсантруд тов. Сидоров зачисляется в число сотрудников Губздравотдела с откомандированием для работы в Админ.-Органах. Н/О.

Завед. Губздравотдела. Куралим
Завед. Админ.-Органах. Н/О. Штейн
Завед. Общей канцелярией Губздравотдела. Кротович

Рисунок 12. Копия приказа от 4 апреля 1923 г. (из архива Института)
Figure 12. Copy of the order of April 4, 1923 (from the archive of the Institute)



VIII-й созыв.

Протокол № 28....

заседания Президиума Петрогубисполкома от 5-го мая 1923 года.

Присутствовали: т.т.

Слушали :	Постановили :
27. Ходатайство Губздравотдела о переименовании Петроградского Бактериологического Института - в Петроградский Бактериологический Институт имени ПАСТЕРА, в ознаменование 100 летия со дня его рождения.	27. Переименование утвердить и впредь Институт именовать "Петроградский Бактериологический Институт имени Пастера

№ 7365/8/1 — отправлено

Председатель: Г. ЗИНОВЬЕВ.
Секретарь: Н. КОМАРОВ.

Рисунок 13. Копия протокола от 5 мая 1923 г. (из архива Института)
Figure 13. Copy of protocol of May 5, 1923 (from the Institute's archive)

Ин-та им. Пастера, находящегося в ведении Ленинградского Облздрава, при чем установили:

Ин-т организован в 1908 г. под названием «Диагностический и Бахтериологический Ин-т». После революции в 1918–1919 гг. он продолжал существовать под названием «частного диагностического и бактериологического Ин-та» и принадлежал Либерману, Маслаковцу и Белоновскому. Затем он был национализирован и реорганизован во 2-ю Городскую Бахтериологическую лабораторию и последовательно руководился проф. Розенбергом, проф. Недригайловым и Белоновским. В 1923 г., всвязи с необходимостью иметь центр по изготовлению бехтеориологических препаратов, Губздрав преобразовал лабораторию в Институт и руководство им поручил Либерману. В 1924 г. Институт получил свое помещение на ул. Мира 13 которое и было приспособлено для работы. В 1929 году в Ин-т влились Ин-т по изготовлению оспинного детрита им. Дженнера и Малярийная станция. В настоящее время Ин-т находится в ведении Облздравотдела и имеет целью научное изучение бахтериологических и эпидемиологических вопросов, научную постановку борьбы с эпидемией в области и приготовление бахтериологических препаратов. Во главе Ин-та стоит директор Я.Ю. Либерман...» (рис. 4).

А вот какие сведения приводят в книге «Петроградская сторона. Большой проспект» ее авторы Г.Ю. Никитенко и В.Д. Привалов (рис. 5):

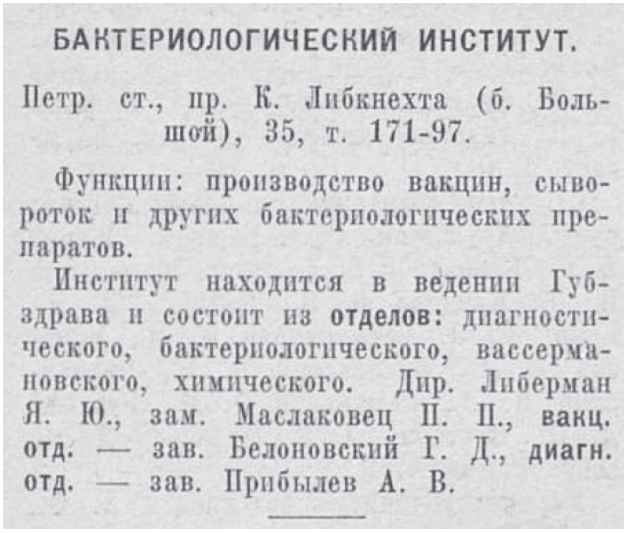
«Здесь же [Большой пр., д. 35-в] находился частный бактериологический и диагностический институт. Его руководитель и владелец доктор медицины Г.Д. Белоновский — доцент Императорского клинического института, приват-доцент Военно-медицинской академии и Женского медицинского института, старший ординатор Морского госпиталя. У него было два помощника: Я.Ю. Либерман — врач Клинического института, и П.П. Маслаковец, служивший в лаборатории Женского медицинского института. Институт в 1920-х гг. был преобразован во 2-ю Городскую лабораторию и продолжал производить вакцины, сыворотки» [8].

Первое упоминание о лаборатории содержится в справочнике «Весь Петербург» за 1910 г. (уже тогда лаборатория имела дополнительное отделение, располагавшееся на углу Владимирского и Невского проспектов в д. 1-47). Лаборатория проводила реакции Вассермана на сифилис, серодиагностические реакции, клинические исследования крови и пр.; выполнялись санитарно-гигиенические и агрономические, а также патолого-анатомические и судебно-медицинские исследования (рис. 6) [3].

В 1910 г. лаборатория была преобразована в Бактериологический и Диагностический Институт. В это время в Институте налажено

and P.P. Maslakovets, who was working at the laboratory of the Women's Medical Institute. In the 1920's the Institute was transformed into the 2nd City laboratory and continued to produce vaccines and serums" [8]:

The first mention of the laboratory is contained in the reference book "All Petersburg" for 1910 (even then the laboratory had an additional department located at the corner of Vladimirsky and Nevsky Prospects, at 1-47. The laboratory conducted Wasserman's reactions to syphilis, serodiagnostic reactions, clinical blood tests, etc., conducted sanitary



БАКТЕРИОЛОГИЧЕСКИЙ ИНСТИТУТ.

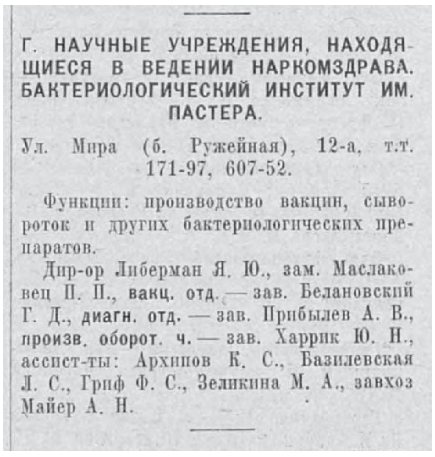
Петр. ст., пр. К. Либкнехта (б. Большой), 35, т. 171-97.

Функции: производство вакцин, сывороток и других бактериологических препаратов.

Институт находится в ведении Губздрава и состоит из **отделов:** диагностического, бактериологического, вассермановского, химического. Дир. Либерман Я. Ю., зам. Маслаковец П. П., **вакц. отд.** — зав. Белоновский Г. Д., **диагн. отд.** — зав. Прибылев А. В.

Рисунок 14. Копия объявления в справочнике «Весь Ленинград», 1924 г. [1, с. 115]

Figure 14. Copy of the announcement in the guide "All Leningrad", 1924 [1, p. 115]



Г. НАУЧНЫЕ УЧРЕЖДЕНИЯ, НАХОДЯЩИЕСЯ В ВЕДЕНИИ НАРКОМЗДРАВА. БАКТЕРИОЛОГИЧЕСКИЙ ИНСТИТУТ ИМ. ПАСТЕРА.

Ул. Мира (б. Ружейная), 12-а, т.т. 171-97, 607-52.

Функции: производство вакцин, сывороток и других бактериологических препаратов.

Дир-ор Либерман Я. Ю., зам. Маслаковец П. П., **вакц. отд.** — зав. Бедановский Г. Д., **диагн. отд.** — зав. Прибылев А. В., **произв. оборот. ч.** — зав. Харрик Ю. Н., ассист-ты: Архипов К. С., Базилевская Л. С., Гриф Ф. С., Зеликина М. А., завхоз Майер А. Н.

Рисунок 15. Копия объявления в справочнике «Весь Ленинград», 1925 г. [2, с. 88]

Figure 15. Copy of the announcement in the guide "All Leningrad", 1925 [2, p. 88]

производство вакцин (гонококковой, стафилоккоковой, скарлатиновой и др.), туберкулинов, антигенов и гемолитических сывороток для реакций Вассермана, питательных сред и др. (рис. 7) [4].

С 1911 г. учреждение имеет статус Санкт-Петербургского Частного Бактериологического и Диагностического Института. Помимо отделения на углу Владимирского и Невского пр., в его составе появляются новые отделения в Санкт-Петербурге (2-й Муринский пр., д. 18) и в г. Пятигорске (ул. Царская, дом Баумгольца). Увеличивается производство вакцин, организуются курсы для врачей и фармацевтов по общей бактериологии, серодиагностике и инфекционным заболеваниям, проводится дезинфекция помещений и испытание дезинфекционных средств (рис. 8, 9) [5].

Институт активно сотрудничает с противочумной лабораторией Императорского Института Экспериментальной Медицины, располагавшейся на форте «Император Александр I» в Кронштадте (рис. 10).

Как уже отмечалось ранее, после революции 1917 г. Институт продолжал какое-то время существовать под названием «Частного диагностического и бактериологического Института». Затем он был национализирован и реорганизован во Вторую Городскую бактериологическую лабораторию (рис. 11) [7].

Из фондов архива УФСБ РФ по СПб и ЛО: *«В 1923 г. в связи с необходимостью иметь Центр по изготовлению бактериологических препаратов, Губздрав переформировал лабораторию в Институт и руководство им поручил Либерману Я.Ю. В 1924 г. Институт получил свое помещение на ул. Мира»* (рис. 4).

4 апреля 1923 г. приказом Петроградского Губернского отдела здравоохранения Вторая Городская бактериологическая лаборатория была преобразована в Петроградский Бактериологический и Диагностический Институт; доктор Яков Юльевич Либерман был назначен директором Института; бывший владелец 2-й Городской бактериологической лаборатории проф. Г.Д. Белоновский освобожден от должности заведующего преобразованной лаборатории (рис. 12).

На основании ходатайства Губздравотдела Президиумом Петрогубисполкома 5 мая 1923 г. учреждение переименовано в Петроградский Бактериологический Институт имени Пастера (в ознаменование 100-летия со дня рождения Луи Пастера, рис. 13).

Следует отметить, что какое-то время учреждение продолжало располагаться и работать по-прежнему адресу (рис. 14) [1]. И лишь только в 1924 г. Институт переезжает на ул. Мира, д. 12-а (ныне: ул. Мира, д. 14, рис. 15) [2].

and hygienic and agronomic, as well as pathological anatomical and forensic studies (fig. 6) [3].

The laboratory was transformed into the Bacteriological and Diagnostic Institute in 1910. At this time the Institute established the production of vaccines (gonococcal, staphylococcal, scarlet fever, etc.), tuberculin, antigens and haemolytic serums for Wassermann's reactions, nutrient media, etc. (fig. 7) [4].

Since 1911, the institution has the status of the St. Petersburg Private Bacteriological and Diagnostic Institute. In addition to the department at the corner of Vladimirsky and Nevsky Prospect, new sections opened in its structure in St. Petersburg (2nd Murinsky Ave, 18) and in Pyatigorsk (Tsarskaya Street, the house of Baumgolts). The production of the vaccines was increasing, courses for doctors and pharmacists on general bacteriology, serodiagnostic and infectious diseases were being organized, disinfection of premises and testing of disinfectants were carried out (fig. 8, 9) [5].

The Institute actively cooperated with the anti-plague laboratory of the Imperial Institute of Experimental Medicine, located at the fortress "Emperor Alexander I" in Kronstadt (fig. 10).

As it was mentioned earlier, after the revolution of 1917 the Institute continued to exist for some time under the name of the "Private Diagnostic and Bacteriological Institute". Then it was nationalized and reorganized into the Second City Bacteriological Laboratory (fig. 11) [7].

From the funds of the archive of the Federal Security Service of the Russian Federation for St. Petersburg and the Leningrad Region: *"In 1923, in connection with the need to have a Centre for the production of bacteriological agents, Gubzdrav (the health department of the region) reorganized the laboratory into the Institute and appointed Ya.Yu. Liberman as its head. In 1924 the Institute moved to Mira Street"* [2] (fig. 4).

On April 4, 1923, by order of the Petrograd Provincial Health Department, the Second City Bacteriological Laboratory was transformed into the Petrograd Bacteriological and Diagnostic Institute; Dr. Yakov Yulievich Lieberman was appointed as the Director of the Institute; the former owner of the 2nd City bacteriological laboratory prof. Belonovsky G.D. was dismissed from the position of the head of the transformed laboratory (fig. 12).

Based on the petition of Gubzdravotdel (the health department of the region) by the Presidium of the Petrogubispolkom (the Executive Committee of the region) on May 5, 1923, the institution was renamed the Petrograd Bacteriological Institute named after Pasteur (in commemoration of the 100th anniversary of the birth of Louis Pasteur, fig. 13).

It should be noted that for some time the Institute was located at the former address (fig. 14) [1]. Only in 1924 the Institute moved to Mira Street, 12-a (now: Mira Street, 14, fig. 15) [2].

Заместителем директора Якова Юльевича Либермана становится Петр Петрович Маслаковец, заведующим вакцинным отделением — Георгий Дмитриевич Белоновский.

Таким образом, в 1923 г. был начат новый отсчет истории Института. И у его истоков, попрежнему, стояли все те же ученые, которые начали его в 1908 г.

Восстанавливая историческую справедливость и отдавая дань памяти основателям Серодиагностической лаборатории, на базе которой был создан наш Институт, следует считать годом образования Института — 1908 год, а 1923 — годом присвоения Институту имени Пастера.

Pyotr Petrovich Maslakovets became the deputy director, Yakov Yulievich Lieberman became the chief and Georgii Dmitrievich Belonovsky became the senior physician of the vaccine department.

Thus, a new countdown of the history of the Institute was launched in 1923. And at its sources, as before, stood all the same scientists who had founded it in 1908.

Restoring historical justice and paying tribute to the founders of the Sero-diagnostic laboratory, on the basis of which our Institute was established, it should be considered that 1908 was the year of the Institute's formation, and 1923 was the year when the Institute was named after Pasteur.

Список литературы/References

1. Весь Ленинград на 1924 год: адресная и справочная книга г. Ленинграда. Л.: Орг. отд. Ленингр. губисполкома, 1924. 920 с. [Ves' Leningrad na 1924 god: adresnaya i spravochnaya kniga g. Leningrad [All Leningrad for 1924: address and reference book of the city of Leningrad]. *Leningrad: Org. otd. Leningr. gubispolkoma, 1924. 920 p.*]
2. Весь Ленинград на 1925 год: адресная и справочная книга г. Ленинграда. Л.: Орг. отд. Ленингр. губисполкома, 1925. 1080 с. [All Leningrad for 1925: address and reference book of the city of Leningrad]. *Leningrad: Org. otd. Leningr. gubispolkoma, 1925. 1080 p.*]
3. Весь Петербург на 1910 год: адресная и справочная книга г. С.-Петербурга. СПб.: Издание А.С. Суворина, 1910. 2312 с. [Ves' Peterburg na 1910 god: adresnaya i spravochnaya kniga g. S.-Peterburga [All Petersburg for 1910: address and reference book of the city of St. Petersburg]. *St. Petersburg: Izdanie A.S. Suvorina, 1910. 2312 p.*]
4. Весь Петербург на 1911 год: адресная и справочная книга г. С.-Петербурга. [СПб.: Издание А.С. Суворина, 1911. 2410 с. [Ves' Peterburg na 1911 god: adresnaya i spravochnaya kniga g. S.-Peterburga [All Petersburg for 1911: address and reference book of the city of St. Petersburg]. *St. Petersburg: Izdanie A.S. Suvorina, 1911. 2410 p.*]
5. Весь Петербург на 1912 год: адресная и справочная книга г. С.-Петербурга. СПб.: Издание А.С. Суворина, 1912. 2492 с. [Ves' Peterburg na 1912 god: adresnaya i spravochnaya kniga g. S.-Peterburga [All Petersburg for 1912: address and reference book of the city of St. Petersburg]. *St. Petersburg: Izdanie A.S. Suvorina, 1912. 2492 p.*]
6. Весь Петербург на 1914 год: адресная и справочная книга г. С.-Петербурга. Под ред. А.П. Шашковского. СПб.: Издание т-ва А.С. Суворина – «Новое время», 1914. 1999 с. [Ves' Peterburg na 1914 god: adresnaya i spravochnaya kniga g. S.-Peterburga. Pod red. A.P. Shashkovskogo [All Petersburg for 1914: address and reference book of the city of St. Petersburg. Ed. A.P. Shashkovskyi]. *St. Petersburg: Izdanie A.S. Suvorina* — "*Novoe vremya*". *1999 p.*]
7. Весь Петроград на 1923 год: адресная и справочная книга г. Петрограда. Пг.: Петропечать, 1923. 1231 с. [Ves' Petrograd na 1923 god: adresnaya i spravochnaya kniga g. Petrograda [All Petrograd for 1914: address and reference book of the city of Petrograd]. *Petrograd: Petropechat', 1923. 1231 p.*]
8. Никитенко Г.Ю., Привалов В.Д. Петроградская сторона. Большой проспект. М.: Центрполиграф, 2009. 368 с. [Nikitenko G.Yu., Privalov V.D. Petrogradskaya storona. Bol'shoi prospekt [Petrograd side. Bolshoi prospect]. *Moscow: Tsentrpoligraf, 2009. 368 p.*]

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УЧЕНИКИ ПАСТЕРА ИЗ РОССИИ

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Резюме. Выдающийся ученый, микробиолог, химик Луи Пастер оставил будущим поколениям великое наследство: множество открытий в соответствующих сферах наук, большое количество учеников, последователей и Парижский институт, носящий его имя. Среди учеников Пастера было более ста российских ученых, судьба которых сложилась по-разному. Некоторые из них вернулись на Родину и смогли внести существенный вклад в развитие микробиологии и иммунологии в России. Не все фамилии учеников Пастера известны широкому кругу микробиологов. Поэтому в статье представлены краткие материалы о некоторых более или менее известных ученых — последователях Луи Пастера. Основоположником микробиологии в России вполне может считаться Л.С. Ценковский, который в 1882 г. выпустил книгу «Микроорганизмы», а позже смог самостоятельно создать вакцину против сибирской язвы. Неоднократные командировки в Институт Пастера в Париж имели свое положительное значение — Лев Семенович смог оценить, как должны быть оснащены лаборатории, которые занимаются решением серьезных задач в области микробиологии. Ученики Пастера Л.Л. Гейденрейх, А.Д. Павловский, М.Ф. Попов, А.И. Судаков, А.А. Раевский смогли не только развивать научные направления в микробиологии, но и передавать свои знания студентам медицинских и ветеринарных факультетов университетов в различных городах России, слушателям Петербургской Военно-медицинской академии и военным врачам. Большими друзьями и коллегами Пастера долгие годы были И.И. Мечников и Н.Ф. Гамалея. Вместе с Пастером они внесли большой вклад в процветание Парижского института и поддержание славы его основоположника. Роль этих ученых в общемировом наследии в области иммунологии и микробиологии трудно переоценить. Сподвижником И.И. Мечникова был также Д.К. Заболотный, которому удалось организовать кафедры микробиологии и эпидемиологии в нескольких высших учебных заведениях России и Украины. И, конечно, непревзойденный вклад в организацию института Пастера в России по образцу Парижского института принадлежит Г.Д. Белоновскому. Благодаря его стараниям Серо-диагностическая лаборатория постепенно переросла в Санкт-Петербургский институт Пастера. Многие ученики Пастера заложили фундамент целого ряда научных направлений в России: микробиологии, иммунологии, токсикологии, гигиены. Благодаря усилиям энтузиастов среди последователей Луи Пастера удалось основать в России (Санкт-Петербурге) институт, подобный Парижскому, также названный его именем. Каждый из учеников Пастера благодаря самоотверженному труду смог доказать свое высокое звание и внести весомый вклад в развитие науки на благо здоровья населения Родины.

Ключевые слова: *Луи Пастер, микробиология, иммунология, ученые из России.*

DISCIPLES OF PASTEUR FROM RUSSIA

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Abstract. Outstanding scientist, microbiologist, chemist Louis Pasteur left a great legacy to future generations: many discoveries in the relevant fields of science, a large number of disciples, followers and the Paris Institute, bearing his name. Among Pasteur's disciples were more than a hundred Russian scientists, whose fate was different. Some of them returned Home

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and were able to make a significant contribution to the development of microbiology and immunology in Russia. Not all the names of Pasteur's disciples are known to a wide range of microbiologists. Therefore, the article presents brief materials about some more or less well known scientists-followers of Louis Pasteur. L.S. Tsenkovsky may be considered the founder of microbiology in Russia, who in 1882 published the book "Microorganisms", and later was able to independently create a vaccine against anthrax. Numerous trips to the Pasteur Institute in Paris had a positive value — Lev Semenovich was able to assess how to be equipped laboratories that deal with serious problems in the field of microbiology. Students of Pasteur L.L. Heydenreich, A.D. Pavlovsky, M.F. Popov, A.I. Sudakov, A.A. Raevsky was able not only to develop scientific areas in microbiology, but also to transfer their knowledge to students of medical and veterinary faculties of universities in various cities of Russia, students of the St. Petersburg Military medical Academy and military doctors. I.I. Mechnikov and N.F. Gamalea were great friends and colleagues of Pasteur for many years. Together with Pasteur, they made a great contribution to the prosperity of the Paris Institute and the maintenance of the glory of its founder. The role of these scientists in the world heritage in the field of immunology and microbiology is difficult to overestimate. Associate I.I. Mechnikova was also D.K. Zabolotny, who managed to organize the departments of microbiology and epidemiology in several higher educational institutions of Russia and Ukraine. And, of course, the unsurpassed contribution to the organization of the Pasteur Institute in Russia on the model of the Paris Institute belongs to G.D. Belonovsky. Thanks to his efforts, the Sero-diagnostic laboratory gradually developed into the St. Petersburg Pasteur Institute. Many disciples of Pasteur laid the foundation of a number of scientific areas in Russia: microbiology, immunology, toxicology, hygiene. Thanks to the efforts of enthusiasts among the followers of Louis Pasteur managed to establish in Russia (St. Petersburg) Institute, similar to Paris, also named after him. Each of the disciples of Pasteur thanks to selfless work was able to prove his high rank and make a significant contribution to the development of science for the health of the population of the Homeland.

Key words: *Louis Pasteur, microbiology, immunology, scientists from Russia.*

В период «золотого века» микробиологии (вторая половина XIX в.) в Европе было по меньшей мере два места, куда стремились попасть люди для того, чтобы совершенствовать свои знания в науке микробиологии: Париж (Л. Пастер) и Берлин (Р. Кох).

Институт Пастера в Париже носит имя своего создателя, великого французского ученого и организатора науки Луи Пастера (1822–1895). Институт был организован на деньги, собранные по международной подписке; он начал работу 4 июня 1887 г., а его торжественное открытие состоялось 14 ноября 1888 г.

С начала своего существования Институт Пастера стал международным научным центром. Его филиалы были созданы во Вьетнаме, Китае, Камбодже, Тунисе, Эфиопии и других странах. В 1889 г. по инициативе ученика и сотрудника Л. Пастера, Эмиля Ру, в Пастеровском институте были организованы Микробиологические курсы; на них стажировались ученые из многих государств, в том числе из России.

Научную деятельность Л. Пастера нельзя рассматривать в отрыве от его педагогической деятельности. В 1857 г. он стал деканом факультета естественных наук Высшей школы, а с 1867 г. — профессором химии Парижского университета. С 1887 г. Л. Пастер возглавил организованный на частные пожертвования, в том числе с российской стороны, институт, названный впоследствии его именем. В Институте Пастера, наряду с другими иностранными учеными, плодотворно работали и русские ученые — И.И. Мечников, С.Н. Виноградский, Н.Ф. Гамалея, В.А. Хавкин, А.М. Безредка и другие.

Так, И.И. Мечников создал первую русскую школу микробиологов, иммунологов и патологов [9, 13].

Именно благодаря такому великому научному вкладу Ильи Ильича в 1915 г. во время празднования 70-летия И.И. Мечникова Эмиль Ру в своей речи сказал: «В Париже, как в Петрограде и в Одессе, Вы стали главой школы и зажгли в этом институте научный очаг, далеко разливающий свой свет. Ваша лаборатория — самая жизненная в нашем доме, и желающие работать толпой стекаются в нее. Здесь исследователь ищет мысль, которая вывела бы его из затруднения... Ваш огонь делает горячим равнодушного и скептику внушает веру. Вы — несравненный товарищ в работе; я могу это сказать, ибо не раз мне выпадало счастье участвовать в Ваших изысканиях. В сущности все делали Вы. Институт Пастера многим обязан Вам. Вы принесли ему престиж Вашего имени, и работами своими и Ваших учеников Вы в широкой мере способствовали его славе. В нем Вы показали пример бескорыстия, отказываясь от всякого жалованья в годы, когда с трудом сводились концы с концами... Оставаясь русским по национальности, Вы заключили с Институтом франко-русский союз задолго до того, как мысль о нем возникла у дипломатов» («Природа», июль-август 1916).

По подсчетам Л.А. Тарасевича число русских, прошедших в разное время стажировку в Париже, достигало 100 человек.

Среди них были люди различных национальностей и профессий – врачи, судебные медики, патологоанатомы, фармакологи, хирурги, ветеринары, ботаники и даже химики. Некоторые

из них, вернувшись на Родину, внесли большой вклад в развитие микробиологии и иммунологии в России, других ждала судьба эмигрантов. Кому-то из учеников Пастера больше повезло в том смысле, что об их жизни и работах свидетельствуют многочисленные биографические и научные труды. В данной работе мы хотим также вспомнить некоторых русских учеников Пастера, ставших первыми проводниками учения выдающегося основоположника микробиологии.

Лев Семенович Ценковский (1822–1887)

В 1844 г. Лев Семенович окончил курс Санкт-Петербургского университета со степенью кандидата естественных наук и был оставлен при университете, а через два года после защиты диссертации «Несколько фактов из истории развития хвойных растений» получил степень магистра. В 1855 г. Ценковский занял кафедру ботаники Санкт-Петербургского университета, а в следующем году блестяще защитил диссертацию на степень доктора ботаники. Именно Л.С. Ценковский, сверстник Пастера, выпустил в 1882 г. в России книгу «Микроорганизмы» и, по справедливости, должен считаться основоположником микробиологии в России [7, 16].

Лев Семенович много занимался со своими учениками изучением сибирской язвы, самостоятельно разработал (1883) метод изготовления сибиреязвенной вакцины, так как пастеровская вакцина была монополизирована «Обществом пастеровских вакцин» и метод ее изготовления не был опубликован. Вакцину Л.С. Ценковского применяли в нашей стране до введения новой сибиреязвенной вакцины СТИ (1942).

Для ознакомления с технологией приготовления сибиреязвенной вакцины Л. Пастера профессор Л.С. Ценковский был командирован «Вольным экономическим обществом» в Париж [1, 2].

Прибыв в Париж, профессор обратился к Пастеру с просьбой разрешить ему ознакомиться с технологией приготовления вакцин, но получил отказ, поскольку, как объяснил Пастер, право на производство вакцин было продано недавно созданному акционерному обществу. Таким образом, лаборатория ученого оказалась предназначенной только для научно-исследовательских, а не учебных целей. Однако присутствовать при вакцинации овец гость Пастера все-таки смог.

Правительство России отнеслось к проблеме вакцинации против сибирской язвы безучастно, не соизволив даже заслушать отчет о командировке ученого в Париж. Из письма Л.С. Ценковского работающему в Институте Пастера И.И. Мечникову от 22 февраля 1886 г.: «...Три года я работал в поте лица над вакцинацией сибирской язвы без надлежащей поддержки. В настоящее время начинает что-то шевелиться, вроде желания выделить мне, наконец, средства, обставить занятия как следует. Пока же я бьюсь, чтобы выработать более удобный способ получения вакцин, остаться же при пастеровском неудобно потому, что вакцины крепнут со временем, и приходится каждый раз путем бесконечных проб на животных добывать новые. Не можете ли Вы, Илья Ильич, помочь мне в этом? Очевидно, что Пастер знает, как сохранять вакцины, чтобы они не портились...». Мечников пообещал помочь. Ученые договорились о встрече в Париже. Однако и на этот раз в Париже не пожелали или не смогли помочь Льву Семеновичу. И Ценковскому ничего не оставалось, как, возвратившись домой, самому искать ответы на возникающие в ходе исследований вопросы.

Было от чего опустить руки. И отказ Пастера, и авторитетное мнение коллеги — одесского бактериолога Н.Ф. Гамалеи, длительное время работавшего у Пастера и считавшего, что без знания секретов вакцинации, тонкостей процесса приготовления и хранения вакцин, все попытки отечественных бактериологов обречены на неудачу. Не добавляла оптимизма и огромная разница в условиях исследований: просторные, отлично оснащенные лаборатории, опытные, высококвалифицированные сотрудники в Париже и университетская комнатушка Ценковского, именуемая лабораторией, без современного оборудования и средств. Тем ценнее для отечественной науки был достигнутый результат — создание вакцины против сибирской язвы, выдержавшей проверку временем.

Людвиг Людвигович Гейденрейх (1846–1920)

В 1869 г. Людвиг Людвигович окончил Императорскую медико-хирургическую академию. После ее окончания он служил уездным врачом на Валдае, затем в Санкт-Петербургском воспитательном доме, работая одновременно в медико-хирургической академии.

В 1876 г. Гейденрейх защитил диссертацию по этиологии и клинике «возвратной горячки» («возвратного тифа») и уехал за границу, где работал в лабораториях Р. Коха, Л. Пастера, К. Негели и П. Эрлиха.

В 1884 г. он вернулся в Россию и в 1887 г. был избран приват-доцентом Петербургской

Военно-медицинской академии, где организовал при клинике бактериологическую лабораторию. С 1889 г. Людвиг Людвигович — главный врач Виленского, а в 1903–1911 гг. — Одесского окружных военных госпиталей. Л.Л. Гейденрейх — автор около 50 научных работ. В 1876 г. он ввел анилиновые краски в бактериологическую технику; в 1883–1885 гг. применил автоклав для стерилизации плотных питательных сред; в 1885 г. предложил двойные стеклянные чашки (на два года раньше немецкого микробиолога J.R. Petri, ассистента P. Koxa), которые вытеснили из микробиологической практики пластинки Koxa; в 1888–1889 гг. изучал этиологию и клинику возвратного тифа и кожного лейшманиоза в Туркестане [6].

Л.Л. Гейденрейх усовершенствовал ряд микробиологических методик, опубликовал одно из первых полных руководств по микробиологической методике: «Методы исследований низших организмов».

Александр Дмитриевич Павловский (1857–1946)

Александр Дмитриевич Павловский оставил о себе след в науке как русский и советский бактериолог, патологоанатом, фармаколог и хирург.

В 1885 г. в Императорской Военно-медицинской академии Александр Дмитриевич служил ординатором хирургической клиники, а затем преподавателем. В том же году он успешно защитил докторскую диссертацию и получил научную степень доктора медицинских наук. В 1886 г. Павловский отправился в двухгодичную командировку в Берлин, где с 1886 по 1889 гг. специализировался в лабораториях Р. Вирхова и Р. Koxa, а в 1889 г. переехал в Париж, где некоторое время специализировался в лаборатории Луи Пастера. В 1889 г. Александр Дмитриевич переехал в Киев, где до 1918 г. работал профессором Киевского университета. В 1894 г. Киевский государственный университет направил Павловского в командировку в Париж, и тот устроился на работу в Пастеровский институт. В 1895 г. ученый основал Киевский бактериологический институт, где до 1918 г. был научным руководителем, заведовал сывороточным отделением. Во время Русско-Японской войны он работал врачом в Маньчжурии, а в годы Первой мировой войны работал хирургом в военных госпиталях Киева.

В 1918 г. Александр Павловский принял решение покинуть Россию навсегда и переехал жить в Бессарабию. В Бессарабии Александр Дмитриевич продолжил научную и врачебную деятельность, работая хирургом в Кишиневе и Сороках.

Основные научные работы А.Д. Павловского посвящены изучению этиологии, патогенеза и клиники дифтерии, риносклеромы, туберкулеза, холеры и хирургическим исследованиями. В 1885 г. он исследовал рожистое воспаление; в 1887 г. предложил лечение сибирской язвы бактериями-антагонистами; в 1889 г. изучал различные формы туберкулеза суставов; в 1892 г. одним из первых доказал, что воспалительный очаг в организме имеет защитный характер; в 1893 г. организовал изготовление противохолерной сыворотки, которая нашла широкое применение в Германии и Японии во время эпидемии холеры; в 1895 г. организовал изготовление противодифтерийной сыворотки; в 1897 г. создал лекарственный препарат Риносклерин для лечения риносклеромы; в 1929 г. исследовал природу бактериофага и его роли в иммунитете и терапии.

А.Д. Павловский глубоко изучал антагонизм бактерий, исследовал взаимодействия микробов и организма хозяина, разработал питательную среду для туберкулезных бактерий.

Михаил Федорович Попов (1854–1919)

Михаил Федорович Попов — русский ученый-медик, профессор, декан медицинского факультета и ректор (1913–1916) Императорского Томского университета. Михаил Федорович окончил в 1880 г. с отличием медицинский факультет Императорского Харьковского университета. Там же в 1888 г. получил степень доктора медицины и звание приват-доцента. В 1889 г. был командирован на 2 года в Европу, где занимался в Берлине у профессоров Koxa и Гертера, в Мюнхене — у профессоров Петтенкофера и Фойта, в Париже — у Пастера, Готье и Бруарделя [11].

В 1891 г. М.Ф. Попов назначен экстраординарным профессором Императорского Томского университета по кафедре судебной медицины, с 1895 г. он — ординарный профессор; заведовал кафедрой до 1916 г. С 1898 по 1913 гг. Михаил Федорович — декан медицинского факультета, в 1913–1916 гг. — ректор. Заслуженный профессор Томского университета (1913) [12].

Александр Иванович Судаков (1851–1914)

А.И. Судаков — русский врач, ординарный профессор по кафедре гигиены, ректор Императорского Томского университета.

В 1884 г. Главное военно-медицинское управление послало Судакова за границу с целью подготовить из него специалиста для заведования

гигиенической лабораторией при Николаевском военном госпитале, что и было ему поручено после заграничной командировки. В Европе Судаков занимался у Петтенкофера, Пастера и Коха [14].

В 1887 г. Александр Иванович был избран приват-доцентом по кафедре гигиены Военно-медицинской академии. Он был командирован в ряд городов Московского, Виленского, Киевского и Варшавского военных округов для обучения военных врачей методам исследования холеры по способу Коха. С 1887 по 1890 гг. Александр Иванович являлся редактором газеты «Военно-Санитарное Дело». В 1890 г. был назначен профессором гигиены в Императорский Томский университет, ректором которого был дважды: в 1892–1894 и 1895–1903 гг.; в 1898–1899 гг. одновременно управлял Западно-Сибирским учебным округом [15].

Аркадий Александрович Раевский (1848–1916)

По окончании курса в ветеринарном отделении при медико-хирургической академии, Аркадий Александрович Раевский был оставлен при академии. После защиты диссертации «О росте и строении копыт у домашних животных» на степень магистра был командирован за границу. Первые научные работы Раевского: «Трансплантация хрящей» (1878), «К сибирской язве», «К чуме рогатого скота», «К вопросу о гнилостном заражении» (1873), «О способности человеческой диафрагмы к всасыванию при ее нормальном и патологическом состоянии» (1875), «О внутреннем развитии раковых опухолей в диафрагме» (1875). По возвращении из-за границы Раевский назначен в 1875 г. адъюнкт-профессором по кафедре общей патологии и патологической анатомии при ветеринарном отделении академии, причем ему же было поручено чтение лекций по эпизоотологии студентам-ветеринарам и медикам [5].

Получив кафедру, Аркадий Александрович устроил при ней специальную лабораторию. По результатам проведенных в ней исследовательских работ его учеников был написан ряд магистерских и докторских диссертаций. В конце 70-х гг. и в начале 80-х гг. Раевским напечатаны два руководства: «Патологическая анатомия и гистология домашних животных» и «Руководство к изучению инфекционных болезней домашних животных». В 1884 г. он был назначен директором и профессором Харьковского ветеринарного института. Раевский, как ученый-эпизоотолог, с успехом популяризировал пастеровские предохранительные прививки против сибирской язвы.

Николай Федорович Гамалея (1859–1949)

Николай Федорович Гамалея — известнейший российский микробиолог и эпидемиолог, почетный член АН СССР (1940), академик АМН (1945), большой друг и коллега И.И. Мечникова. В 1886 г. он работал в Париже у Л. Пастера. Изучая прививки против бешенства, усовершенствовал пастеровский метод предохранительных прививок. Гамалея говорил, что, подобно Пастеру, этиология инфекций его интересует только с точки зрения их профилактики и лечения, чему он и посвятил свою жизнь. Сам Николай Федорович считал себя микробиологом.

В 1886 г. при содействии Луи Пастера, Н.Ф. Гамалея учредил совместно с И.И. Мечниковым и Я.Ю. Бардахом первую в России (и вторую в мире) бактериологическую станцию и впервые в России осуществил вакцинацию людей против бешенства. За первые три года своей деятельности Одесская станция привила приблизительно 1500 человек.

В течение года (с лета 1886 по осень 1887 гг.) Гамалея изучает, усовершенствует и анализирует метод Пастера, находясь с ним в постоянной переписке. В результате этой работы параллельно с Пастером (часто по инициативе Гамалеи) были изменены метод приготовления вакцины и схема иммунизации. Безвредность более активных препаратов (более «ядовитых мозгов») Гамалея проверял на себе [8].

Через год успешной работы Гамалея был вызван Пастером в Париж, чтобы оградить его от необоснованных нападок, причиной которых стали неудачи внедрения прививок, выполненных преимущественно другими лицами, нежели Пастером и его помощниками. Основной причиной неудач, как выявил Гамалея, было несоблюдение правил асептики. Помимо доказательства безвредности метода и его высокой эффективности, Гамалея разобрался в «паралитическом» бешенстве и показал с помощью ретроспективного анализа случаев, что эта форма встречалась во Франции еще до применения прививок, а вовсе не являлась их результатом. Пришлось съездить и в Лондон, чтобы на месте ознакомиться с неудачными случаями и восстановить добрую славу прививок и их создателя.

В 1899 г. под руководством Н.Ф. Гамалеи был создан бактериологический институт в Одессе. Н.Ф. Гамалея открыл вещества, вызывающие разрушение бактерий — бактериолизины (1898). Внес много нового в учение о ядах микробов. В 1901–1902 гг. руководил противоэпидемическими мероприятиями во время чумы в Одессе. В 1912–1928 гг. — научный руководи-

тель Института оспопрививания в Ленинграде, в 1930–1938 гг. — Центрального института эпидемиологии и бактериологии в Москве. С 1938 г. до конца жизни Николай Федорович — профессор кафедры микробиологии 2-го Московского медицинского института и с 1939 г. — заведующий лабораторией Института эпидемиологии и микробиологии АМН СССР.

Даниил Кириллович Заболотный (1866–1929)

Авторы не могут не упомянуть Даниила Кирилловича Заболотного, который являлся учеником и другом верного последователя дела Л. Пастера — И.И. Мечникова и основателем кафедры микробиологии Военно-медицинской академии, где работают авторы данной статьи.

Перечисление всех его титулов заняло бы много места, поэтому здесь приведем лишь некоторые: советский микробиолог и эпидемиолог, один из основоположников отечественной эпидемиологии, академик (1922) и президент (1928–1929) АН Украинской ССР, академик АН СССР (1929) [3, 4].

С 1897 г. Даниил Кириллович участвовал в командировках для изучения тропических болезней (чумы и холеры) в Индии, Аравии, Китае, Персии. По возвращении Заболотный год работал в Институте Пастера в Париже вместе с И.И. Мечниковым (интересная подробность — последний был у него свидетелем на свадьбе).

В последующем много и плодотворно ученый работал в Институте экспериментальной медицины, занимался в том числе и исследованием патогенеза сифилиса (докторская диссертация). Лишь случайность помешала ему стать первооткрывателем возбудителя сифилиса — бледной трепонемы (Шаудин и Гофман описали ее в 1905 г.).

В 1898 г. Д.К. Заболотный организовал в Петербургском женском медицинском институте первую в России кафедру бактериологии и заведовал ею до 1928 г., в 1920 г. в Одессе основал первую в мире кафедру эпидемиологии. В 1921 г. основал и был первым ректором Одесского медицинского института. В 1923 г. в Военно-медицинской академии основал кафедру микробиологии и эпидемиологии с курсом дезинфекции [10].

На базе Института имени Пастера была создана первая в истории российской медицины вакцинно-сывороточная комиссия, положившая начало экспертизе, контролю и стандартизации национальных средств специфической диагностики, лечению и профилактике инфекционных заболеваний. Ее возглавлял академик Д.К. Заболотный.

Георгий Дмитриевич Белоновский (1875–1950)

Закончив в 1899 г. Петербургскую Военно-медицинскую академию, уже в 1902 Георгий Дмитриевич Белоновский защитил докторскую диссертацию «О влиянии специфической гемолитической сыворотки на искусственное и естественное малокровие».

В 1905–1907 гг. Георгий Дмитриевич работал за границей, в том числе в лаборатории И.И. Мечникова в Институте Пастера (Париж).

В 1908 г. при самом активном участии Г.Д. Белоновского, а также Я.Ю. Либермана и П.П. Маслаковца в Петербурге была создана Серо-диагностическая лаборатория, преобразованная в Диагностический и Бактериологический институт, который после революции, в 1918–1919 гг., назывался Частным Диагностическим и Бактериологическим институтом, а позднее был национализирован и реорганизован во Вторую Городскую Бактериологическую лабораторию под руководством Г.П. Белоновского. На ее базе 4 апреля 1923 г. был образован Петроградский Бактериологический и Диагностический Институт, которому в ознаменование 100-летия со дня рождения Луи Пастера 5 мая 1923 г. присвоено имя этого великого ученого. Тем самым была осуществлена идея принца А.П. Ольденбургского о создании в России Института, «подобного Пастеровскому».

Все российские ученые, ученики Пастера, были продолжателями дела, начатого их великим учителем. Они внесли огромный вклад в развитие микробиологии не только в нашей стране, но и за рубежом. Благодарные потомки увековечивают имена великих соотечественников, называя в их честь университеты, больницы, научно-исследовательские институты, улицы и различные научные общества не только в России, но и за рубежом.

Список литературы/References

1. Бучинский П. Лев Семенович Ценковский. // Записки Новороссийского общества естествоиспытателей. 1888. Т. XIII, Вып. I. [Buchinsky P. Lev Semenovich Tsenkovsky. *Zapiski Novorossiiskogo obshchestva estestvoispytatelei = Notes of the Novorossiysk society of naturalists, 1888. vol. XIII, iss. I. (In Russ.)*]
2. Гайдуков Н.М. Ценковский, Лев Семенович // Энциклопедический словарь Брокгауза и Ефрона: в 86 т. (82 т. и 4 доп.). СПб., 1890–1907. [Gaidukov N.M. Tsenkovsky Lev Semenovich. In: Encyclopedic dictionary Brockhaus and Efron. *St. Petersburg, 1890–1907.*]

3. Гиммельфарб Я.К., Гродский К.М. Д.К. Заболотный (1866–1929). М.: Медгиз, 1958. 224 с. (Серия: Выдающиеся деятели отечественной медицины). [Himmelfarb J.C., Grodsky K.M. D.K. Zabolotnyi (1866–1929). Series: Outstanding figures of national medicine. *Moscow: Medgiz, 1958. 224 p.*]
4. Голубев Г.Н. Житие Даниила Заболотного. М.: Молодая гвардия, 1962. 256 с. (Жизнь замечательных людей: сер. биогр.; вып. 18 (351)). [Golubev G.N. The Life of Daniel Zabolotny. Series: Life of remarkable people; iss. 18 (351). *Moscow: Molodaya gvardiya, 1962. 256 p.*]
5. Калугин В.И. Профессор А.А. Раевский. К 35-летию со дня смерти // Ветеринария, 1951, № 10. С. 60–62. [Kalugin V.I. Professor A. Raevsky. *Veterinariya = Veterinary, 1951, no. 10, pp. 60–62. (In Russ.)*]
6. Метелкин А.И. Гейденрейх Людвиг Людвигович // Большая медицинская энциклопедия. Под ред. Б.В. Петровского. 3-е изд. М.: Советская энцикопедия, 1977. Т. 5. С. 73. [Metelkin A.I. Heydenreich Ludwig Ludwig. In: Great Medical Encyclopedia; Ed. B.V. Petrovskii; 3rd ed. *Moscow: Sovetskaya entsikopediya, 1977. Vol. 5. P. 73.*]
7. Метелкин А.И. Л.С. Ценковский. Основопоожник отечественной школы микробиологов. М.: Медгиз, 1950. 264 с. (Серия: Выдающиеся деятели отечественной медицины). [Metelkin A.I. L.S. Cenkowski. The founder of the national school of microbiologists. Series: Outstanding figures of national medicine. *Moscow: Medgiz, 1950. 264 p.*]
8. Миленушкин Ю.И. Николай Федорович Гамалея: очерк жизни и научной деятельности. М.: Изд-во АН СССР, 1954. 160 с. [Milanoski Yu.I. Nikolai Fedorovich Gamaleya: sketch of life and scientific activities. *Moscow: Publishing house of USSR Academy of Sciences, 1954. 160 p.*]
9. Могилевский Б.Л. Жизнь Мечникова. Повесть о великом русском биологе. Харьков: Обл. изд., 1955. 296 с. [Mogilevskii B.L. Mechnikov's life. The story of the great russian biologist. *Kharkov: Regional Publishing house, 1955. 296 p.*]
10. Пицык Н.Е. Даниил Кириллович Заболотный, 1866–1929. Отв. ред. Л.Б. Борисов. М.: Наука, 1988. 304 с. [Pitsyk N.E. Daniil Kirillovich Zabolotny, 1866–1929. *Moscow: Nauka, 1988. 304 p.*]
11. Попов Михаил Федорович // Энциклопедический словарь Брокгауза и Ефрона: в 86 т. (82 т. и 4 доп.). СПб., 1890–1907. [Popov Mikhail Fedorovich. In: Encyclopedic dictionary Brockhaus and Efron. *St. Petersburg, 1890–1907.*]
12. Попов, Михаил Федорович // Томск от А до Я: Краткая энциклопедия города / Под ред. д-ра ист. наук Н.М. Дмитренко. 1-е изд. Томск: Изд-во НТЛ, 2004. С. 266. [Popov, Mikhail Fedorovich. In: Tomsk from A to Z: a concise encyclopedia of the Tomsk city. Ed. N.M. Dmitrenko. 1st ed. *Tomsk: Publishing house "NTL", 2004. P. 266.*]
13. Резник С.Е. Мечников. М.: Молодая гвардия, 1973. 366 с. (Серия: Жизнь замечательных людей). [Reznik S.E. Mechnikov. Series: Life of remarkable people. Moscow: Molodaya gvardiya, 1973. 366 p.]
14. Судаков Александр Иванович // Энциклопедический словарь Брокгауза и Ефрона: в 86 т. (82 т. и 4 доп.). СПб., 1890–1907. [Sudakov Alexander Ivanovich. In: Encyclopedic dictionary Brockhaus and Efron. *St. Petersburg, 1890–1907.*]
15. Судаков Александр Иванович // Томск от А до Я: Краткая энциклопедия города / Под ред. д-ра ист. наук Н.М. Дмитренко. 1-е изд. Томск: Изд-во НТЛ, 2004. С. 342–343. [Sudakov Alexander Ivanovich. In: Tomsk from A to Z: a concise encyclopedia of the Tomsk city. Ed. N.M. Dmitrenko. 1st ed. *Tomsk: Publishing house "NTL", 2004. P. 342–343.*]
16. Теренин Н.В. Ценковский Лев Семенович // Русский биографический словарь: в 25 т. СПб.—М., 1896–1918. [Terenin N.V. Tsenkovsky Lev Semenovich. Russian biographical dictionary. *St. Petersburg – Moscow, 1896–1918.*]

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PYRAZINAMIDE/PYRAZINOIC ACID RESISTANCE IN *MYCOBACTERIUM TUBERCULOSIS*: RECENT FINDINGS AND IMPLICATIONS FOR IMPROVING THE TREATMENT OF TUBERCULOSIS

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Abstract. Pyrazinamide (PZA) is unique in that it is a component of the first line therapy for drug sensitive tuberculosis and in most current and experimental treatments also for multi drug resistant tuberculosis. Furthermore, PZA has been shown to help to ensure lasting cure and prevent relapse in shorter multi drug regimens. PZA is a prodrug. *Mycobacterial tuberculosis* (MTB) PncA enzyme activates the anti-mycobacterial prodrug PZA by transforming it into pyrazinoic acid (POA). The majority of clinical PZA resistant isolates contain mutations within the *pncA* gene and therefore remain sensitive to POA as they no longer activate PZA. Resistance to the active compound POA requires an alternative resistance mechanism and *in vitro* selected spontaneous MTB POA resistant mutants typically acquire a range of mutations in *panD* or mutations in one of a series of genes most of which are associated with the regulation of the bacterial stringent response. Clinically isolated PZA resistant MTB strains resistant to PZA and POA with mutations in any of these genes are unusual. Thus, it is likely the stringent response is critical for MTB *in vivo* and a damaged stringent response results in at least a reduction in fitness. Various lead compounds that disrupt the MTB stringent response have been identified that might form the basis for drugs with activity against latent mycobacteria with the potential to shorten tuberculosis treatment. Here we discuss the role of latency in the lifecycle of MTB and possible links to the activity PZA with a focus on potential new targets and drugs.

Key words: *Mycobacterium tuberculosis, drug resistance, pyrazinamide, pyrazinoic acid, latent tuberculosis.*

УСТОЙЧИВОСТЬ *MYCOBACTERIUM TUBERCULOSIS* К ПИРАЗИНАМИДУ/ПИРАЗИНОЕВОЙ КИСЛОТЕ: НОВЫЕ СВЕДЕНИЯ И ИХ ЗНАЧЕНИЕ ДЛЯ ПОВЫШЕНИЯ ЭФФЕКТИВНОСТИ ЛЕЧЕНИЯ ТУБЕРКУЛЕЗА

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Резюме. Пиразинамид (PZA) уникален тем, что является противотуберкулезным препаратом первого ряда как при лечении лекарственно-чувствительного туберкулеза, так и компонентом современных курсов лечения мультирезистентного туберкулеза. Также было показано, что PZA помогает обеспечить длительное лече-

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ние и предотвратить рецидив в более коротких схемах приема нескольких лекарств. Пиразинамид является неактивным пролекарством и фермент PncA *Mycobacterium tuberculosis* превращает его в активную форму — пиразиноевую кислоту (POA). Большинство клинических PZA-резистентных штаммов содержат мутации внутри гена *pncA* и поэтому остаются восприимчивыми к POA, поскольку не активируют PZA. Устойчивость к активному соединению POA требует альтернативного механизма резистентности, и полученные *in vitro* POA-резистентные спонтанные мутанты MTB имеют ряд мутаций в гене *panD* или в серии генов, большинство из которых связаны с регуляцией строгого ответа бактерий. Клинические штаммы MTB, устойчивые к PZA и POA с мутациями в любом из этих генов, являются нетипичными. Таким образом, вероятно, строгий ответ имеет важное значение для MTB в условиях *in vivo*, а нарушенный ответ приводит к снижению жизнеспособности микроорганизма. Были идентифицированы различные лекарственные соединения-прототипы, нарушающие строгий ответ MTB, которые могут стать основой для препаратов с активностью против латентных форм микобактерий с целью сокращения сроков противотуберкулезного лечения. В данном обзоре мы обсуждаем роль латентного периода в жизненном цикле MTB и возможные связи с активностью PZA с особым вниманием к потенциально новым мишенями и препаратам.

Ключевые слова: *Mycobacterium tuberculosis, лекарственная устойчивость, пиразинамид, пиразиноевая кислота, латентный туберкулез.*

Latency and the activity of (PZA) POA

The critical importance of bacterial latency on the epidemiology and treatment of tuberculosis is widely accepted [11]. Lethal infectious diseases as well as transient infections that result in protective immunity require a continuous supply of naive hosts to be maintained in a population. *Mycobacterium tuberculosis* (MTB) adopts a distinct strategy establishing active infections in only a small proportion of individuals and latent infection in the majority of the infected population. Latent TB infections may spontaneously clear, reactivate or die with the host. The continuing long-term success of *M. tuberculosis* is thus largely due to its ability to (undetected) spread in a population by establishing large numbers of slowly progressing incipient or dormant infections [14]. Subclinical MTB infections have the potential to transform into new transmittable active infections, predominantly in vulnerable populations, maintaining the epidemic in a human population over a long period [25, 37]. To establish a long-term latent infection requires the infecting mycobacteria to respond effectively to stress and to have the capacity to enter a dormant/latent phenotype (variously termed; latent, fat lazy, viable non-culturable, persister). The transition to these phenotypes thus appears to be critical for the long term success of MTB and we will argue here is closely linked to transmission dynamics, treatment outcomes, and probably also the emergence of drug resistant clones.

PZA is a pro drug which can be modified by the mycobacterial enzyme PncA to form the active compound POA. Recent reports demonstrate that resistance to pyrazinoic acid, a drug primarily active against stressed/dormant MTB *in vitro* can be caused by disrupting the stress responses resulting in the a failure to express the sensitive phenotype. As regulation of bacterial stress responses in this pathogen resulting in a dormant/latent phenotype is essential for the spread and survival of the MTB species, this form of resistance comes with a cost. And it is thus logical to investigate the disrupted stress responses seen in PZA and POA resistant strains, and use these data to identify potential targets for new drugs.

A further complication is neither PZA nor POA show any activity in routine culture, an effect is only seen when cultured bacteria are subjected to environmental stress. Typically an acidic growth medium is used for sensitivity testing, but a wide range of other stresses, that result in a switch to a latent/dormant phenotype, have a similar effect [30, 46]. In order to identify the target of POA multiple groups have generated POA resistant mutants *in vitro* and identified mutations in a range of genes, for example: *panD, clpC1*, and *gpsI* [26, 45, 55, 66, 67]. Recent evidence suggests *panD* is the primary target of POA [5, 26] therefore, other inhibitors of the pantothenate synthetase pathway [13] would be expected to have similar activity to PZA/POA against MTB under stressed conditions.

Apart from mutations in the likely primary target of POA *panD*, many of the mutations observed in spontaneous *in vitro* POA resistant mutants are the result of a damaged ability to enter the stressed state in which the activity of POA is inhibitory for bacterial growth [5, 26].

Disrupting the stringent response

Based on the range of genes identified in *in vitro* POA resistant spontaneous mutants the stringent response appears to play a key role in susceptibility to POA. The bacterial stringent response is a specific and very rapid cascade response to a change in environment. In *E. coli* the stringent response has been shown to be induced within seconds and is initiated by the accumulation of the so called stringent response alarmone (p)ppGpp [6]. This

response was detected 20 minutes after *M. tuberculosis* log-phase cultures were transferred into nutrient free buffer and (p)ppGpp declined to a new steady state by 90 to 120 min [57]. Interestingly, an enzyme (Gps1) involved in the metabolism of (p)ppGpp was recently suggested as a new target for POA after it was observed in 4 clinical PZA resistant isolates with wildtype *pncA*, *panD* and *clpC1* [45]. When this gene *(gps1)* was mutated in a sensitive strain the PZA MIC was increased. An altered enzymatic activity of mutated Gps1 in the presence and absence of POA was also demonstrated. Based on the activity of the mutant gene in the presence of POA the authors suggested Gps1 as yet another target of POA [45]. This may be correct but the role of *gps1* in the initiation of the stringent response suggests that absence of the wild type *gps1* may disrupt the regulation of entry into a fully POA susceptible phenotype [5].

Because of its importance for the regulation of latency and virulence in multiple species, the bacterial stringent response has already received attention as a potential drug target. A compound (relacin) structurally similar to the alarmone (p)ppGpp has been shown to disrupt the bacterial stringent response [64]. Relacin and related compounds are of interest also against MTB [7] but to our knowledge have not been investigated in detail.

In most bacteria the Clp protease complex is a non-essential ATP-dependent protease that regulates the response to various stresses. The Clp protease complex is composed of two heptameric sections ClpP1 and ClpP2 which are involved in substrate unfolding and breakdown into short peptides. In *M. tuberculosis* the ClpP1 ClpP2 complex is active when bound to either hexameric ClpX or ClpC1. In *M. tuberculosis* ClpX and ClpC1 are both essential and involved in substrate recognition and specificity [36, 49]. Along with *panD* point mutations in *clpC1* are among the most frequently reported mutations in *in vitro* selected *M. tuberculosis* POA resistant mutants [26, 66, 67].

It has been proposed that *clpC1* is involved in the stringent response by regulating CarD levels, a probable substrate of ClpP1P2 [48]. In *E. coli* DksA is a key regulator of the stringent response. Despite having little structural similarity mycobacterial CarD can functionally complement an *E. coli* DksA deficient mutant. Thus both DksA/CarD can work as general transcription factors when combined with

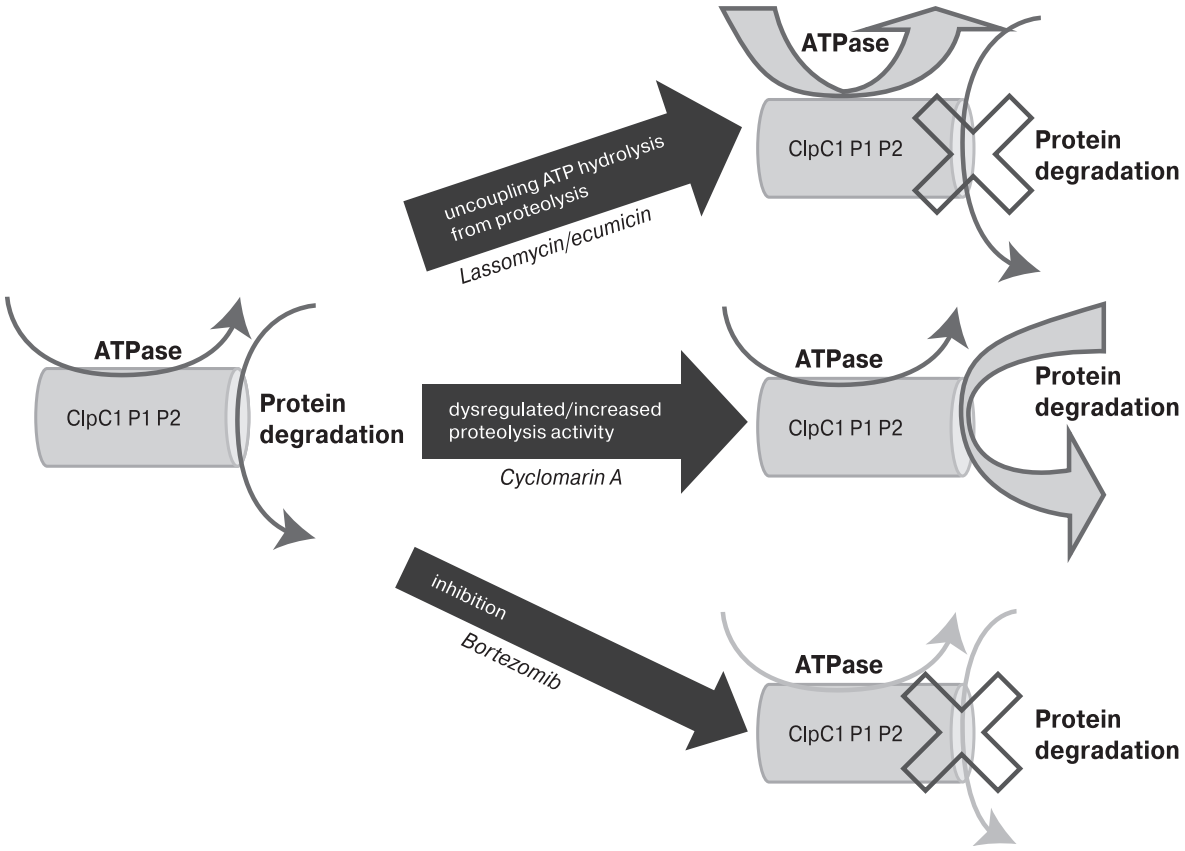

Figure. Overview of the different mechanisms of compounds known to disrupt the activity of the ClpP1P2 complex in *M. tuberculosis*

Proposed mechanisms are indicated in the straight arrows and compounds identified with this mechanism in italic below. ATPase and Protein degradation activity level is illustrated by the thickness of the curved arrows (left hand side normal activity), an X indicates inhibition

the stringent response alarmone (p)ppGpp to activate the stringent response [57]. It is therefore possible that the POA resistance associated mutations seen in *clpC1* are the result of a disrupted stringent response [5] due to dysregulation of the control of *carD* levels in the mycobacterial cell.

The absence of the Clp protease complex in eukaryotic cells and its requirement for normal growth of *M. tuberculosis* make this complex an interesting drug target [49]. The potential of this target is further supported by the fact that although *clpC1* mutants are resistant to both PZA and POA *in vitro*, *clpC1* mutants have, to our knowledge, never been observed in PZA resistant clinical isolates. Multiple lead compounds with activity against this protease complex with different mechanisms of action have been described [36] illustrated in Fig. and briefly described here: Cyclomarin A is a naturally occurring cyclic peptide isolated from a marine *Streptomyces* spp. which is active against mycobacteria with reportedly good specificity [53]. Cyclomarin A appears to act by binding to ClpC1 and dysregulating the proteolysis activity pf the MTB ClpC1P1P2 complex resulting in uncontrolled protein degradation [60]. Two other cyclic and looped peptides, lassomycin and ecumicin found after screening libraries of extracts obtained from actinomycetes, have also been reported to disrupt the activity of ClpC1P1P2 activity but by a different mechanism, uncoupling the ATPase activity from the proteolysis activity [20, 22]. These are far from being fully developed drugs but lassomycin demonstrated good specificity with activity against mycobacterial ClpC1 but none of the other bacterial ClpC homologs or eukaryotic proteases screened [22]. Finally, bortezomib is a compound which disrupts the MTB ClpC1P1P2 proteolytic catalytic sites [42]. Unfortunately, as this compound is used as a proteasome inhibitor approved by the U.S. FDA for the treatment of human multiple myeloma [1] it lacks (myco)bacterial specificity. However, recent work on derivatives of bortezomib, by the group who identified the potential of this compound, demonstrates scope for improving its specificity [43].

Trans-translation and PZA?

RpsA is a component of trans-translation, a rescue mechanism for stalled ribosomes. Although the role of *rpsA* mutations in PZA resistance is disputed, association studies of larger collections of clinical isolates does suggest some involvement [63]. RspA does not appear to be a target of PZA/POA, as recent work using laboratory mutants did not show any effect of PZA (POA) on trans-translation or the expression of RpsA [17], but RspA does seem to play a role in the susceptibility to PZA/POA. It has also been shown that overexpression of RpsA increases the PZA MIC [54]. Trans-translation is also closely linked to the stringent response and the available data supports that it is probable that disturbances in the balance of these interacting pathways, may disrupt entry into a latent, pyrazinamide-susceptible phenotype [5].

Inhibitors of trans-translation have been identified and at least one (KKL-35) was shown to be active against MTB under both aerobic and anoxic conditions [3], although others have questioned KKL-35's mode of action [10].

Based on this idea above we have suggested RpsA and trans-translation do not have a direct role in the mode of action of pyrazinamide but mutations in *rpsA* reduce the efficiency of a switch to a pyrazinamide-susceptible phenotype [5]. Interestingly, in 2018 a mutation in another gene lprG was associated with PZA resistance in 4 POA-resistant laboratory mutants [55]. The authors speculated this mutation probably results in a state of higher metabolism during *in vitro* culture that antagonizes PZA/POA activity *in vitro*, a suggestion that appears to be analogous with our speculation on the role of *rpsA* mutations [5].

Novel strategies to target PZA resistant *pncA* mutants?

As a clear majority of clinically PZA resistant isolates are resistant due to mutations in *pncA* and do not transform PZA into its active form POA [40] might it be possible to reverse this resistance? Although it has been frequently argued that the loss of the *pncA* gene has little or no cost this may not be the case [32]. NAD^+ is produced in *M. tuberculosis* by either the de novo or the salvage pathway. PncA is part of the NAD^+ salvage pathway. *M. bovis* PncA has dramatically reduced activity and as a result *M. bovis* is resistant to PZA and has a negative result in the niacin test [56]. The *pncA* gene in *M. bovis* has a single mutation (His57Asp) and does appear to retain some activity as *M. bovis* strains in which the de novo NAD^+ pathway is also deleted remain viable but are killed if starved of nicotinamide [62]. This indicates that either a (partially) functional de novo or active salvage pathway for NAD^+ is essential for the viability of the MTB complex. In support of this interpretation detailed work on these pathways suggests when the do novo pathway cannot function due to prolonged starvation recycling of NAD^+ by the salvage pathway prevents cell death [62]. This implies that the total loss of PncA activity, probable in a large proportion of PZA resistant *pncA* mutants as a result of AA substitutions frame shifts or even *pncA* gene deletion, will have a lethal cost under extended starvation.

Based on the argument above, either exposing the cost of a total loss of the salvage pathway or conversion of PZA to POA by another (host) pathway would be expected to restore sensitivity to PZA

in MTB PZA resistant mutants lacking any PncA activity. Therefore, inhibition of the *de novo* NAD^+ pathway should be lethal for PZA resistant strains with no PncA activity making this pathway a potential target for a large proportion of M(X)DR-TB isolates as suggested by Vilcheze et al. in 2010 [62].

Secondly, PZA is not only converted into the active form (POA) by bacterial PncA but also in the infected patient's liver by microsomal deamidase. POA is then further metabolized by human xanthine oxidase. It has been shown that compounds inhibiting human xanthine oxidase activity, such as allopurinol, result in increased the levels of circulating pyrazinoic acid [44, 61] but it does not appear to be known if this increase in circulating host-derived pyrazinoic acid would restore pyrazinamide activity against infecting bacteria with *pncA* mutations.

Preventing the accumulation of PZA resistance

The lack of PZA activity in routine culture and an incomplete understanding of the mechanism of action have added to the complexity of optimising the use of this compound in patients. The diversity of resistance mutations in *pncA* in PZA resistant clinical isolates suggests ongoing selection of PZA resistance in most settings [4]. This may be a particular problem for MDR-TB patients who are not detected as infected with resistant TB at diagnosis and receive first line therapy which is in fact likely to be monotherapy with PZA supplemented with ineffective drugs [65]. PZA is usually given only for the first two months of standard TB therapy because clinical trials conducted by the British Medical Research Council in the 1960s and 1970s did not detect any benefit of PZA beyond 2 months [19]. However, an effect of PZA beyond 2 months was seen in second line regimens in a murine model [2] and treatment of MDR-TB frequently includes longer periods of PZA exposure [59]. RIF and INH are very effective at clearing replicating bacilli in an infection whereas PZA is known to be most active against difficult to eradicate non-replicating mycobacteria therefore, PZA given at the end of therapy to eradicate any remaining bacteria instead of exposing the large numbers of bacilli at the start of therapy appears logical. Furthermore delaying the use of PZA in this way should reduce the chance of inadvertent monotherapy with PZA for yet to be identified M(X)DR-TB patients starting TB therapy [4]. To our knowledge, the utility of PZA at the end of therapy vs at the beginning of therapy has not been tested.

Recent insights into the pharmacological mechanism underlying PZA's unique clinical efficacy and modelling suggest a potential benefit of PZA beyond the first 2 months in some patients [9]. This combined with knowledge on the mechanism of action of PZA, which has become much clearer by the efforts of different groups in the past few years, should provide a basis for trials to explore the more optimal use of this drug in multidrug regimens as well as how to time and dose new drugs with related mechanisms of action.

The regulation of latency and the success of MTB strains

Latency is critical for the success of MTB in the population and in an individual patient. It is often stated that the infectious dose of TB is low possibly as low a single viable cell [52]. This may be true but caution is warranted as routine culture often misses > 90% of viable cells [18]. Also, the observation that infections with double MIRU-VNTR bands are mixed infections that can be transmitted between patients [33] provides circumstantial evidence that new infections are often the result of larger numbers of mycobacterial cells that preserve some of the genetic diversity present in the source case. This preserved diversity within the infecting bacterial population may ultimately be useful to identify more details of transmission dynamics [8, 33, 39]. Even more interestingly, it was recently observed that patients with a higher proportion of latent bacteria in their sputum appeared more likely to transmit the disease to close contacts [16]. At first sight this may appear to be a paradox but establishing a new infection is a critical step in the life cycle of *M. tuberculosis* and expressing a phenotype able to remain viable in the environment and initiate a new infection without provoking a lethal immune response from the host could well be an essential ability for continued success. It has long been recognised that microscopically the cells in patient's sputum appear different to those in culture, being slightly more elongated and with more apparent internal structure when stained with ZN or auramine O. These elongated cells with structure were studied in detail by Garton et al. (2008) [21] and termed "fat lazy bacteria". Presumably these bacteria are the subpopulation of cells that are transmitting in the study of Datta et al. (2017) [16]. Downregulation of growth in a large proportion of the mycobacteria in an infection thus appears to be normal and is probably important for efficient transmission.

The critical importance of persistence on the natural life cycle of MTB raises the possibility that different linages may have adapted their propensity to enter or exit latency as a survival strategy. The presence of bacilli simultaneously in different states in an active infection is assumed to be one of the primary reasons why the curative treatment of tuberculosis is so ineffective and requires at least 6 months to eliminate all the latent cells, even

Table. Potential drug targets and compounds of interest likely to be active against stressed mycobacteria based on our current understanding of PZA activity

Target/mechanism of action	Compounds of interest	Notes	Literature
Alarmone (p)ppGpp analogue	Relacin	Disrupt the stringent response shown to be active against MTB	[64] [7] [58]
Disruption of clpCP1P2 complex activity	Cyclomarin A Bortezomib Lassomycin Ecumicin	Disrupt the regulation of clpC targeted protein degradation to disrupt the stress response *(see Fig.)*	[53] [42] [22] [36]
Reversion of PZA resistance in *pncA* mutants	Alopurinol + PZA	Inhibit host degradation of POA to increase levels of host derived POA, restoring sensitivity in *pncA* mutants	[61] [44]
	unidentified inhibitor of the *de novo* NAD^+ pathway + PZA	Inhibition of the NAD^+ *de novo* pathway. Loss of PncA activity is predicted to make the *de novo* pathway essential	[62]
Avoiding the selection of *de novo* PZA resistance	PZA dosed differently/ given at the end of therapy	The diversity of *pncA* mutations suggests ongoing selection of resistance in some settings	[4] [9]
Disrution of trans-translation	KKL-35	Trans-translation has been shown to be essential for MTB and the activity of of KKL-35 agains MTB demonstrated but the mechanism of action of KKL-35 is disputed	[3] [10]
Alternative inhibitors of pantothenate synthesis	Unknown (sulfamoyl analogues)	Based on the ideas presented here would be expected to have similar activity as PZA	[13]

though an active tuberculosis infection is probably effectively "cured" within the first few weeks [27]. The majority of drugs used to treat tuberculosis appear to be at best only partially effective at eliminating latent bacteria. Two notable exceptions may be pyrazinamide (PZA) and high dose rifampicin but even these compounds probably do not rapidly eliminate all persistent MTB [28, 29]. Here the discussion focusses on the bacteria but this is certainly also in part due to the location of many of these less active mycobacterial cells in tissue or granulomas where the concentration of antimicrobials is suboptimal as a result of limited penetration [23], a situation that may also help amplify resistance. Furthermore, as an active MTB infection does not usually result in protective immunity [51] the immune system of even a "virtually cured" patient may be unable to eliminate even a few reactivating MTB cells that escaped the treatment.

Differences in the propensity of strains to enter a latent phenotype may result in some strains being more likely to rapidly breakdown into active disease with others being more likely to establish latent infections. An association with specific clades with treatment failure and drug resistance is established [24, 41] but the explanation for this association remains controversial [12, 35]. Although representatives of virtually all genotypes of *M. tuberculosis* have independently acquired multi-drug resistance by similar mechanisms it does appear that in many settings similar genotypes are more often associated with an MDR genotype than other genotypes. This may be chance, but as these differences between lineages are consistent between different geographical areas and because first line treatment of tuberculosis is highly standardised throughout the world, it is probable that certain genotypes are more able to develop resistance or are more likely to maintain their ability to spread and cause disease after having acquired resistance. There is tantalising evidence that variation in the initial bactericidal effect of antibiotic exposure plays a role in the development of resistance in *M. tuberculosis*: it has recently been observed that when different genotypes of *M. tuberculosis* are initially exposed to rifampicin the rate of killing and initial response differs [31, 34]. The increased ability to resist exposure to an antibiotic in the absence of a specific resistance mechanism (mutation) by a proportion of the bacterial cells in a population is termed persistence. It is likely these effects are linked to differing proportions of metabolically active cells in a nutrient rich environment for different genotypes [50], termed Class 1 persistence [38], possibly a result of differences in the "magic spot" setting of the stringent response [15].

Conclusions

In this paper we discuss the insights that mutations seen in in vitro PZA/POA resistant strains have provided regarding the formation of a latent/dormant phenotype of TB. The increasing amount of data on POA resistance mechanisms many of which appear to disrupt the formation of these phenotypes, provides an opportunity to determine the clinical relevance of blocking this phenotype switching and research compounds capable of specifically blocking the activity of the relevant enzymes.

In the discussion above we present a series of arguments that suggests the precise regulation of latency in MTB is of critical importance for the disease process, the development of resistance, as well as the epidemiology of tuberculosis. If the argument that PZA attacks latent cells is accepted this explains the value of PZA in (shortening) tuberculosis treatment regimens. We further suggest that it is likely the regulation of latency is disrupted in many unsuccessful POA resistant mutants, seen in culture but rarely in clinical isolates, and compounds that disrupt related targets (Table) would be expected to also help shorten treatment duration and prevent relapse if developed into drugs.

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Список литературы/References

1. Adams J., Kauffman M. Development of the proteasome inhibitor Velcade™ (Bortezomib). *Cancer Invest., 2004, vol. 22, no. 2, pp. 304–311. doi: 10.1081/CNV-120030218*
2. Ahmad Z., Tyagi S., Minkowski A., Peloquin C.A., Grosset J.H., Nuermberger E.L. Contribution of moxifloxacin or levofloxacin in second-line regimens with or without continuation of pyrazinamide in murine tuberculosis. *Am. J. Respir. Crit. Care Med., 2013, vol. 188, no. 1, pp. 97–102. doi: 10.1164/rccm.201212-2328OC*
3. Alumasa J.N., Manzanillo P.S., Peterson N.D., Lundrigan T., Baughn A.D., Cox J.S., Keiler K.C. Ribosome rescue inhibitors kill actively growing and nonreplicating persister Mycobacterium tuberculosis cells. *ACS Inf. Dis., 2017, vol. 3, no. 9, pp. 634–644. doi: 10.1021/acsinfecdis.7b00028*
4. Anthony R.M., Cynamon M., Hoffner S., Werngren J., den Hertog A.L., van Soolingen D. Protecting pyrazinamide, a priority for improving outcomes in multidrug-resistant tuberculosis treatment. *Antimicrob. Agents. Chemother., 2017, vol. 61, no. 6: e00258-17. doi: 10.1128/AAC.00258-17*
5. Anthony R.M., den Hertog A.L., van Soolingen D. 'Happy the man, who, studying nature's laws, Thro'known effects can trace the secret cause.' Do we have enough pieces to solve the pyrazinamide puzzle? *J. Antimicrob. Chemother., 2018, vol. 73, no. 7, pp. 1750–1754. doi: 10.1093/jac/dky060*
6. Arenz S., Abdelshahid M., Sohmen D., Payoe R., Starosta A.L., Berninghausen O., Vasili Hauryliuk V., Beckmann R., Wilson D.N. The stringent factor RelA adopts an open conformation on the ribosome to stimulate ppGpp synthesis. *Nucleic Acids Res., 2016, vol. 44, no. 13, pp. 6471–6481. doi: 10.1093/nar/gkw470*
7. Bag S., Das B., Dasgupta S., Bhadra R.K. Mutational analysis of the (p) ppGpp synthetase activity of the Rel enzyme of Mycobacterium tuberculosis. *Arch. Microbiol., 2014, vol. 196, no. 8, pp. 575–588. doi: 10.1007/s00203-014-0996-9*
8. Black P.A., De Vos M., Louw G.E., Van der Merwe R.G., Dippenaar A., Streicher E.M., Abdallah A.M., Sampson S.L., Victor T.C., Dolby T., Simpson, J.A., van Helden, P.D., Warren, R.M., Pain A. Whole genome sequencing reveals genomic heterogeneity and antibiotic purification in Mycobacterium tuberculosis isolates. *BMC genomics, 2015, vol. 16, no. 1: 857. doi: 10.1186/s12864-015-2067-2*
9. Blanc L., Sarathy J.P., Cabrera N.A., O'Brien P., Dias-Freedman I., Mina M., Sacchettini J., Savic R.M., Gengenbacher M., Podell B.K., Prideaux B., Ioerger T., Dick T., Dartois V. Impact of immunopathology on the antituberculous activity of pyrazinamide. *J. Exp. Med., 2018, vol. 215, no. 8: 1975. doi: 10.1084/jem.20180518*
10. Brunel R., Descours G., Durieux I., Doublet P., Jarraud S., Charpentier X. KKL-35 exhibits potent antibiotic activity against Legionella species independently of trans-translation inhibition. *Antimicrob. Agents. Chemother., 2017, AAC-01459. doi: 10.1128/AAC.01459-17*
11. Caño-Muñiz S., Anthony R., Niemann S., Alffenaar J.W.C. New approaches and therapeutic options for Mycobacterium tuberculosis in a dormant state. *Clin. Microbiol. Rev., 2018, vol. 31, no. 1: e00060-17. doi: 10.1128/CMR.00060-17*
12. Carey A.F., Rock J.M., Krieger I.V., Chase M.R., Fernandez-Suarez M., Gagneux S., Sacchettini, J.C., Ioerger, T.R., Fortune S.M. TnSeq of Mycobacterium tuberculosis clinical isolates reveals strain-specific antibiotic liabilities. *PLoS Pathog., 2018, vol. 14, no. 3, e1006939. doi: 10.1371/journal.ppat.1006939*
13. Ciulli A., Scott D.E., Ando M., Reyes F., Saldanha S.A., Tuck K.L., Chirgadze, D.Y., Blundell, T.L., Abell C. Inhibition of Mycobacterium tuberculosis pantothenate synthetase by analogues of the reaction intermediate. *ChemBioChem, 2008, vol. 9, no. 16, pp. 2606–2611. doi: 10.1002/cbic.200800437*
14. Cobelens F., Kik S., Esmail H., Cirillo D.M., Lienhardt C., Matteelli A. From latent to patent: rethinking prediction of tuberculosis. *The Lancet Respiratory Medicine, 2017, vol. 5, no. 4, pp. 243–244. doi: 10.1016/S2213-2600(16)30419-2*

15. Connolly L.E., Cox J.S. CarD tricks and magic spots: mechanisms of stringent control in mycobacteria. *Cell Host Microbe, 2009, vol. 6, no. 1, pp. 1–2. doi: 10.1016/j.chom.2009.07.001*
16. Datta S., Sherman J.M., Tovar M.A., Bravard M.A., Valencia T., Montoya R., Quino W., D'Arcy N., Ramos E.S., Gilman R.H., Evans C.A. Sputum microscopy with fluorescein diacetate predicts tuberculosis infectiousness. *J. Infect. Dis., 2017, vol. 216, no. 5, pp. 514–524. doi: 10.1093/infdis/jix229*
17. Dillon N.A., Peterson N.D., Feaga H.A., Keiler K.C., Baughn A.D. Anti-tubercular activity of pyrazinamide is independent of trans-translation and RpsA. *Scientific Reports, 2017, vol. 7, no. 1: 6135. doi: 10.1038/s41598-017-06415-5*
18. Dillon N.A., Peterson N.D., Rosen B.C., Baughn A.D. Pantothenate and pantetheine antagonize the antitubercular activity of pyrazinamide. *Antimicrob. Agents Chemother., 2014, vol. 58, pp. 7258–7263. doi: 10.1128/AAC.04028-14*
19. East African/British Medical Research Councils. Controlled clinical trial of four short-course (6-month) regimens of chemotherapy for treatment of pulmonary tuberculosis: second report. *Lancet, 1973, vol. 1, pp. 1331–1339. doi: 10.1016/S0140-6736(74)91411-1*
20. Gao W., Kim J.Y., Anderson J.R., Akopian T., Hong S., Jin Y.Y., Kandror O., Kim J.-W., Lee I.-A., Lee S.-W., McAlpine J.B., Mulugeta S., Sunoqrot S., Wang Y., Yang S.H., Yoon T-M., Goldberg A.L., Pauli G.F., Syh J.-W., Franzblau S.G., Cho S. The cyclic peptide ecumicin targeting ClpC1 is active against Mycobacterium tuberculosis *in vivo. Antimicrob. Agents. Chemother., 2014, vol. 59, no. 2, pp. 880–889. doi: 10.1128/AAC.04054-14*
21. Garton N.J., Waddell S.J., Sherratt A.L., Lee S.M., Smith R.J., Senner C., Hinds J., Rajakumar K., Adegbola R.A., Besra G.S., Butcher P.D. Cytological and transcript analyses reveal fat and lazy persister-like bacilli in tuberculous sputum. *PLoS Med., 2008, vol. 5: e75. doi: 10.1371/journal.pmed.0050075*
22. Gavrish E., Sit C.S., Cao S., Kandror O., Spoering A., Peoples A., Ling L., Fetterman A., Hughes D., Bissell A., Torrey H., Akopian T., Mueller A., Epstein S., Goldberg A., Clardy J., Lewis K. Lassomycin, a ribosomally synthesized cyclic peptide, kills Mycobacterium tuberculosis by targeting the ATP-dependent protease ClpC1P1P2. *Chem. Biol., 2014, vol. 21, no. 4, pp. 509–518. doi: 10.1016/j.chembiol.2014.01.014*
23. Gillespie S.H. Evolution of drug resistance in Mycobacterium tuberculosis: clinical and molecular perspective. *Antimicrob. Agents. Chemother., 2002, vol. 46, no. 2, pp. 267–274. doi: 10.1128/AAC.46.2.267-274.2002*
24. Glynn J.R., Whiteley J., Bifani P.J., Kremer K., van Soolingen D. Worldwide occurrence of Beijing/W strains of Mycobacterium tuberculosis: a systematic review. *Emerg. Infect. Dis., 2002, vol. 8, no. 8: 843. doi: 10.3201/eid0808.020002*
25. Gomez J.E., McKinney J.D. M. tuberculosis persistence, latency, and drug tolerance. *Tuberculosis, 2004, vol. 84, no. 1, pp. 29–44. doi: 10.1016/j.tube.2003.08.003*
26. Gopal P., Tasneen R., Yee M., Lanoix J.-P., Sarathy J., Rasic G., Li L., Dartois V., Nuermberger E., Dick T. In vivo-selected pyrazinoic acid-resistant Mycobacterium tuberculosis strains harbor missense mutations in the aspartate decarboxylase PanD and the unfoldase ClpC1. *ACS Infect. Dis., 2017, vol. 3, pp. 492–501. doi: 10.1021/acsinfecdis.7b00017*
27. Honeyborne I., McHugh T.D., Phillips P.P., Bannoo S., Bateson A., Carroll N., Perrin F.M., Ronacher K., Wright L., Van Helden P.D., Walzl G. Molecular bacterial load assay, a culture-free biomarker for rapid and accurate quantification of sputum Mycobacterium tuberculosis bacillary load during treatment. *J. Clin. Microbiol., 2011, vol. 49, no. 11, pp. 3905–3911. doi: 10.1128/JCM.00547-11*
28. Hu Y., Coates A.R., Mitchison D.A. Sterilising action of pyrazinamide in models of dormant and rifampicin-tolerant Mycobacterium tuberculosis. *Int. J. Tuberc. Lung Dis., 2006, vol. 10, no. 3, pp. 317–322.*
29. Hu Y., Mangan J.A., Dhillon J., Sole K.M., Mitchison D.A., Butcher P.D., Coates A.R. Detection of mRNA transcripts and active transcription in persistent Mycobacterium tuberculosis induced by exposure to rifampin or pyrazinamide. *J. Bacteriol., 2000, vol. 182, no. 22, pp. 6358–6365. doi: 10.1128/JB.182.22.6358-6365.2000*
30. Hertog A.L. den, Menting S., Pfeltz R., Warns M., Siddiqi S.H., Anthony R.M. PZA is active against Mycobacterium tuberculosis cultures at neutral pH with reduced temperature. *Antimicrob. Agents. Chemother., 2016, vol. 60, no. 8, pp. 4956–4960. doi: 10.1128/AAC.00654-16.*
31. Hertog A.L. den, Menting S., van Soolingen D., Anthony R.M. Mycobacterium tuberculosis Beijing genotype resistance to transient rifampin exposure. *Emerg. Infect. Dis., 2014, 20(11), 1932. doi: 10.3201/eid2011.130560*
32. Hertog A.L. den, Sengstake S., Anthony R.M. Pyrazinamide resistance in Mycobacterium tuberculosis fails to bite? *Pathog. Dis., 2015, vol. 73, no. 6, doi: 10.1093/femspd/ftv037*
33. Jajou R., Kamst M., van Hunen R., de Zwaan C.C., Mulder A., Supply P., Anthony R., van der Hoek W., van Soolingen D. The occurrence and nature of double alleles in VNTR patterns of more than 8,000 Mycobacterium tuberculosis complex isolates in The Netherlands. *J. Clin. Microbiol., 2017, vol. 56, iss. 2: e00761-17. doi: 10.1128/JCM.00761-17*
34. Keijzer J. de, Mulder A., de Ru A.H., van Soolingen D., van Veelen P.A. Parallel reaction monitoring of clinical Mycobacterium tuberculosis lineages reveals pre-existent markers of rifampicin tolerance in the emerging Beijing lineage. *J. Proteomics, 2017, vol. 150, pp. 9–17. doi: 10.1016/j.jprot.2016.08.022*
35. Lan N.T. N., Lien H.T. K., Tung L.B., Borgdorff M.W., Kremer K., Van Soolingen D. Mycobacterium tuberculosis Beijing genotype and risk for treatment failure and relapse, Vietnam. *Emerg. Infect. Dis., 2003, vol. 9, no. 12: 1633. doi: 10.3201/eid0912.030169*
36. Lee H., Suh J.W. Anti-tuberculosis lead molecules from natural products targeting Mycobacterium tuberculosis ClpC1. *J. Ind. Microbiol. Biotechnol., 2016, vol. 43, no. 2–3, pp. 205–212. doi: 10.1007/s10295-015-1709-3*
37. Lillebaek T., Dirksen A., Baess I., Strunge B., Thomsen V.Ø., Andersen Å.B. Molecular evidence of endogenous reactivation of Mycobacterium tuberculosis after 33 years of latent infection. *J. Infect. Dis., 2002, vol. 185, pp. 401–404. doi: 10.1086/338342*
38. Lupoli T.J., Vaubourgeix J., Burns-Huang K., Gold B. Targeting the proteostasis network for mycobacterial drug discovery. *ACS Inf. Dis., 2018, vol. 4, no. 4, pp. 478–498. doi: 10.1021/acsinfecdis.7b00231*
39. Meehan C.J., Moris P., Kohl T.A., Pecerska J., Akter S., Merker M., Utpatel C., Beckert P., Gehre F., Lempens P., Stadler T. The relationship between transmission time and clustering methods in Mycobacterium tuberculosis epidemiology. *bioRxiv, 2018, 302232. doi: 10.1101/302232*

40. Miotto P., Cabibbe A.M., Feuerriegel S., Casali N., Drobniewski F., Rodionova Y., Bakonyte D., Stakenas P., Pimkina E., Augustynowicz-Kopeć. E., Degano M. Mycobacterium tuberculosis pyrazinamide resistance determinants: a multicenter study. *MBio, 2014, vol. 5, no. 5: e01819-14. doi: 10.1128/mBio.01819-14*
41. Mokrousov I., Jiao W.W., Sun G.Z., Liu J.W., Valcheva V., Li M., Li M., Narvskaya O., Shen A.D. Evolution of drug resistance in different sublineages of Mycobacterium tuberculosis Beijing genotype. *Antimicrob. Agents. Chemother., 2006, vol. 50, no. 8, pp. 2820–2823. doi: 10.1128/AAC.00324-06*
42. Moreira W., Ngan G.J.Y., Low J. L, Poulsen A., Chia B.C.S., Ang M.J.Y., Yap A., Fulwood J., Lakshmanan U., Lim J., Khoo A.Y.T., Flotow H., Hill J., Raju R.M., Rubin E.J., Dick T. Target mechanism-based whole-cell screening identifies bortezomib as an inhibitor of caseinolytic protease in mycobacteria. *mBio, 2015, vol. 6, no. 3: e00253-15. doi: 10.1128/mBio.00253-15*
43. Moreira W., Santhanakrishnan S., Dymock B.W., Dick T. Bortezomib warhead-switch confers dual activity against mycobacterial caseinolytic protease and proteasome and selectivity against human proteasome. *Front. Microbiol., 2017, vol. 8: 746. doi: 10.3389/fmicb.2017.00746*
44. Naftalin C.M., Verma R., Gurumurthy M., Lu Q., Zimmerman M., Yeo B.C.M., Tan K.H., Lin W., Yu B., Dartois V., Paton N.I. Co-administration of allopurinol to increase anti-mycobacterial efficacy of pyrazinamide: evaluation in a whole-blood bactericidal activity model. *Antimicrob. Agents. Chemother., 2017, vol. 61, no. 10: e00482-17. doi: 10.1128/AAC.00482-17*
45. Njire M., Wang N., Wang B., Tan Y., Cai X., Liu Y., Mugweru J., Guo J., Hameed H.A., Tan S., Liu J. Pyrazinoic acid inhibits a bifunctional enzyme in Mycobacterium tuberculosis. *Antimicrob. Agents Chemother., 2017, vol. 61, no. 7: e00070-17. doi: 10.1128/AAC.00070-17*
46. Peterson N.D., Rosen B.C., Dillon N.A. Baughn A.D. Uncoupling environmental pH and intrabacterial acidification from pyrazinamide susceptibility in Mycobacterium tuberculosis. *Antimicrob. Agents Chemother., 2015, vol. 59, pp. 7320–7326. doi: 10.1128/AAC.00967-15*
47. Primm T.P., Andersen S.J., Mizrahi V., Avarbock D., Rubin H., Barry C.E. The stringent response of Mycobacterium tuberculosis is required for long-term survival. *J. Bacteriol., 2000, vol. 182, no. 17, pp. 4889–4898. doi: 10.1128/JB.182.17.4889-4898.2000*
48. Raju R.M., Jedrychowski M.P., Wei J.R., Pinkham J.T., Park A.S., O'Brien K., Rehren G., Schnappinger D., Gygi, S.P., Rubin E.J. Post-translational regulation via Clp protease is critical for survival of Mycobacterium tuberculosis. *PLoS Pathog., 2014, vol. 10, no. 3: e1003994. doi: 10.1371/journal.ppat.1003994*
49. Raju R.M., Unnikrishnan M., Rubin D.H., Krishnamoorthy V., Kandror O., Akopian T.N., Rubin E.J. Mycobacterium tuberculosis ClpP1 and ClpP2 function together in protein degradation and are required for viability *in vitro* and during infection. *PLoS Pathog., 2012, vol. 8, no. 2: e1002511. doi: 10.1371/journal.ppat.1002511*
50. Reed M.B., Gagneux S., DeRiemer K., Small P.M., Barry C.E. The W-Beijing lineage of Mycobacterium tuberculosis overproduces triglycerides and has the DosR dormancy regulon constitutively upregulated. *J. Bacteriol., 2007, vol. 189, no. 7, pp. 2583–2589. doi: 10.1128/JB.01670-06*
51. Rie A. van, Warren R., Richardson M., Victor T.C., Gie R.P., Enarson D.A., Beyers N., van Helden P.D. Exogenous reinfection as a cause of recurrent tuberculosis after curative treatment. *N. Eng. J. Med., 1999, vol. 341, no. 16, pp. 1174–1179. doi: 10.1056/NEJM199910143411602*
52. Russell D.G., Barry C.E., Flynn J.L. Tuberculosis: what we don't know can, and does, hurt us. *Science, 2010, vol. 328, no. 5980, pp. 852–856. doi: 10.1126/science.1184784*
53. Schmitt E.K., Riwanto M., Sambandamurthy V., Roggo S., Miault C., Zwingelstein C., Krastel P., Noble C., Beer D., Rao S.P., Au M. The natural product cyclomarin kills Mycobacterium tuberculosis by targeting the ClpC1 subunit of the caseinolytic protease. *Angewandte Chemie, 2011, vol. 123, no. 26, pp. 6011–6013. doi: 10.1002/ange.201101740*
54. Shi W., Chen J., Zhang S., Zhang W., Zhang Y. Identification of novel mutations in LprG (rv1411c), rv0521, rv3630, rv0010c, ppsC, cyp128 associated with pyrazinoic acid/pyrazinamide resistance in Mycobacterium tuberculosis. *Antimicrob. Agents. Chemother., 2018, vol. 62, no. 7: e00430-18. doi: 10.1128/AAC.00430-18*
55. Shi W., Zhang X., Jiang X., Yuan H., Lee, J.S., Barry C.E., Wang H., Zhang W., Zhang, Y.Pyrazinamide inhibits trans-translation in Mycobacterium tuberculosis. *Science, 2011, vol. 333, pp. 1630–1632. doi: 10.1126/science.1208813*
56. Sreevatsan S., Pan X., Zhang Y., Kreiswirth B.N., Musser J.M. Mutations associated with pyrazinamide resistance in *pncA* of Mycobacterium tuberculosis complex organisms. *Antimicrob. Agents. Chemother., 1997, vol. 41, no. 3, pp. 636–640.*
57. Stallings C.L., Stephanou N.C., Chu L., Hochschild A., Nickels B.E., Glickman M.S. CarD is an essential regulator of rRNA transcription required for Mycobacterium tuberculosis persistence. *Cell, 2009, vol. 138, no. 1, pp. 146–159. doi: 10.1016/j.cell.2009.04.041*
58. Syal K., Flentie K., Bhardwaj N., Maiti K., Jayaraman N., Stallings C.L., Chatterji D. Synthetic (p) ppGpp analogue: inhibitor of stringent response in mycobacteria. *Antimicrob. Agents. Chemother., 2017, vol. 61, iss. 6: e00443-17. doi: 10.1128/AAC.00443-17*
59. Van Deun A., Salim H., Kumar Das A.P., Bastian I., Portaels F. Results of a standardised regimen for multidrug-resistant tuberculosis in Bangladesh. *Int. J. Tuberc. Lung Dis., 2004, vol. 8, no. 5, pp. 560–567.*
60. Vasudevan D., Rao S.P., Noble C.G. Structural basis of mycobacterial inhibition by cyclomarin A. *J. Biol. Chem., 2013, vol. 288, no. 43: 30883-91. doi: 10.1074/jbc.M113.493767*
61. Via L.E., Savic R., Weiner D.M., Zimmerman M.D., Prideaux B., Irwin S.M., Lyon E., O'Brien P., Gopal P., Eum S., Lee M. Host-mediated bioactivation of pyrazinamide: implications for efficacy, resistance, and therapeutic alternatives. *ACS Inf. Dis., 2015, vol. 1, no. 5, pp. 203–214. doi: 10.1021/id500028m*
62. Vilchèze C., Weinrick B., Wong K.W., Chen B., Jacobs, Jr W.R. NAD^+ auxotrophy is bacteriocidal for the tubercle bacilli. *Mol. Microbiol., 2010, vol. 76, no. 2, pp. 365–377. doi: 10.1111/j.1365-2958.2010.07099.x*
63. Werngren J., Alm E., Mansjö M. Non-*pncA* Gene-mutated but pyrazinamide-resistant Mycobacterium tuberculosis: why is that? *J. Clin. Microbiol., 2017, vol. 55, pp. 1920-1927. doi: 10.1128/JCM.02532-16*
64. Wexselblatt E., Oppenheimer-Shaanan Y., Kaspy I., London N., Schueler-Furman O., Yavin E., Glaser G., Katzhendler J., Ben-Yehuda S. Relacin, a novel antibacterial agent targeting the stringent response. *PLoS Pathog., 2012, vol. 8, no. 9: e1002925. doi: 10.1371/journal.ppat.1002925*

65. Wollenberg K.R., Desjardins C.A., Zalutskaya A., Slodovnikova V., Oler A.J., Quiñones Abeel T., Chapman S.B., Tartakovsky M., Gabrielian A., Hoffner S., Skrahin A., Birren B.W., Rosenthal A., Skrahina A., Earl A.M. Whole-genome sequencing of Mycobacterium tuberculosis provides insight into the evolution and genetic composition of drug-resistant tuberculosis in Belarus. *J. Clin. Microbiol., 2017, vol. 55, pp. 457–469. doi: 10.1128/JCM.02116-16*
66. Yee M., Gopal P., Dick T. Missense mutations in the unfoldase ClpC1 of the caseinolytic protease complex are associated with pyrazinamide resistance in Mycobacterium tuberculosis. *Antimicrob. Agents Chemother., 2017, vol. 61: e02342-16. doi: 10.1128/AAC.02342-16*
67. Zhang S., Chen J., Shi W., Cui P., Zhang J., Cho S., Zhang W., Zhang Y. Mutation in clpC1 encoding an ATP-dependent ATPase involved in protein degradation is associated with pyrazinamide resistance in Mycobacterium tuberculosis. *Emerg. Microbes Infect., 2017, vol. 6: e8. doi: 10.1038/emi.2017.1*

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ПРОДУКЦИЯ БИОФИЛЬМОВ КЛИНИЧЕСКИМИ ШТАММАМИ ВОЗБУДИТЕЛЯ ТУБЕРКУЛЕЗА

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Резюме. Приведены доказательства малой вероятности обнаружения феномена образования биофильмов (БФ) при росте современных клинических штаммов *M. tuberculosis* на жидкой питательной среде. Высказана гипотеза о роли МЛУ/ШЛУ как препятствия для продукции БФ. Обнаружено, что штаммы, способные к продукции БФ, на среде Левенштейна–Йенсена растут специфическими колониями в R-форме в виде диска с выпуклым центром — «НЛО-колонии». Исследована способность к продукции БФ, устойчивость к антибиотикам и их принадлежность к основным эпидемическим кластерам генотипа Beijing (CC1 и CC2-W148) у 67 НЛО-штаммов. Показано что, МЛУ/ШЛУ штаммы так же способны к продукции БФ, однако значимо чаще это наблюдается у штаммов CC1 и CC2-W148 генотипов. Выдвигается гипотеза о потенциальной роли БФ в исходах хронических форм туберкулеза.

Ключевые слова: *продукция биофильмов, Mycobacterium tuberculosis, генотипы, МЛУ/ШЛУ.*

BIOFILM FORMATION INDUCED BY CLINICAL ISOLATES OF *MYCOBACTERIUM TUBERCULOSIS*

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Abstract. The data proving low probability of observing Biofilm Formation (BF) by contemporary clinical strains of *M. tuberculosis* growing on liquid medium in vitro are discussed. A hypothesis about the role of MDR/XDR development hindering BF production was proposed. It was found that strains capable of producing BF grow on Lewenstein–Jensen medium generated R-form specific colonies shaped as a disk with a convex center, "UFO-colonies". Sixty seven "UFO"-strains were investigated to BF production, resistance to antibiotics and their belonging to the main epidemics clusters of the Beijing genotype (CC1 and CC2-W148). It was shown that MDR/XDR strains were also capable of BF production that, however, was remarkably more frequent in strains of CC1 and CC2-W148 genotypes. Thus, it was hypothesized that BF production might potentially influence an outcome of chronic forms of TB-infection.

Key words: *biofilm production, Mycobacterium tuberculosis, genotypes, MDR/XDR.*

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Введение

Лечение туберкулеза легких предполагает терапию в течение 6–9 месяцев для исключения рецидива. Причина подобной продолжительности лечения не вполне ясна, поскольку эффективная гибель *Mycobacterium tuberculosis* (МБТ) должна произойти в течение первых 14 дней [9]. Предполагается, что существует субпопуляция возбудителя, которая или недоступна терапевтическому воздействию или не реагирует на него [14]. Современные модели устойчивости МБТ обычно объясняют этот феномен присутствием покоящихся (нерастущих) клеток-персисторов возбудителя, способных переживать в условиях гипоксии длительное время [15]. В то же время МТБ может в равной степени рассматриваться как внутриклеточный, так и внеклеточный патоген, который значительную часть своего жизненного цикла *in vivo* проводит внутри биофильмоподобной структуры [12]. Способность к продукции биофильмов (БФ) широко распространена у патогенных микроорганизмов, когда происходит формирование многоклеточной структуры возбудителя, окруженной внеклеточным матриксом, состоящим из различных полимеров [16]. Одним из наиболее значимых свойств возбудителя внутри БФ является резкое снижение его чувствительности к воздействию антибиотиков. МБТ не являются исключением: возбудитель внутри БФ в 20–1000 раз более устойчив к противотуберкулезным препаратам (ПТП) [6]. Однако продукция БФ клиническими штаммами туберкулеза, остается весьма загадочным феноменом. Анализ более трех сотен клинических штаммов из двух областных противотуберкулезных учреждений Иркутска и Улан-Уде [3] показал, что в подавляющем большинстве случаев клинические изоляты МБТ не имеют способности к продукции БФ. Анализ литературы свидетельствует, что ни в одном из исследований за последние десятилетия нет описания масштабной продукции БФ клиническим штаммами, и все описанные эксперименты проведены на лабораторных штаммах, зачастую подвергнутых генной модификации [6, 7, 11, 16]. В то же время в русскоязычной литературе 80-х гг. прошлого века [2] продукция БФ описывается как рутинное явление при росте на жидкой питательной среде. Нами было сделано предположение, что основной причиной изменения культуральных свойств клинических изолятов возбудителя (отсутствие роста в виде БФ на жидкой среде) за последние десятилетия могло стать массовое распространение множественной и широкой лекарственной устойчивости (МЛУ и ШЛУ). Мы предполагаем, что мутации, вызывающие МЛУ и ШЛУ, в первую очередь однонуклеотидные замены (SNP), влияющие на процессы синтеза клеточной стенки возбудителя, являются причиной потери МБТ способности формировать БФ в модели *in vitro*. Эта гипотеза была прията как основная на первоначальных этапах настоящего исследования.

Материалы и методы

Соблюдение этических норм. Настоящее исследование одобрено Этическим комитетом ФГБНУ НЦ ПЗСРЧ.

Штаммы микобактерий. Штаммы были получены с твердой питательной среды Левенштейна–Йенсена. Среда Школьниковой готовилась согласно действующим рекомендациям [1], для получения биопленок штаммы МБТ выращивали в 5 мл среды в стеклянных пробирках без добавления сыворотки крови человека [2]. Оценку устойчивости к ПТП проводили на базе бактериологической лаборатории Иркутской областной клинической больницы согласно утвержденным стандартным операционным процедурам (СОП) на твердых питательных средах. Для уточнения минимальной ингибирубющей концентрации (МИК) в ряде случаев проведена оценка устойчивости штаммов к 12-ти ПТП 1-го и 2-го рядов в жидкой среде Middlebrook 7H9 с тест-системой Sensititre Myco TB (Thermo Scientific, США).

Модельный эксперимент по исследованию продукции БФ клиническими штаммами МБТ проводили в стеклянных пробирках с 5 мл среды Школьниковой. Посевная доза составляла 100 мкл культуры, приготовленной по стандарту мутности 0,5 ед. и разведенному в 100 раз. В первые 10 дней рост происходил в аэробных условиях (пробирки закрывались ватно-марлевыми пробками), в последующие дни эксперимента во избежание высыхания среды пробки заменялись пластиковыми. Длительность экспериментов во всех случаях составляла 45 дней. Классификация интенсивности образования БФ проводилась по следующим критериям: 1) исключительным (++++) считался обильный рост БФ, заходящий на стенки пробирки; 2) хороший рост (+++) предполагал наличие толстой пленки, покрывающей всю поверхность среды, не заходящей вверх на стенки пробирки; 3) слабый рост (++) отмечался при наличии тонкой пленки и/или отдельных островков на поверхности жидкой среды; 4) отрицательный результат рассматривался при отсутствии поверхностного, но при наличии обильного придонного роста.

Выделение геномной ДНК из биофильмов и штаммов. Экстракцию ДНК штаммов МБТ проводили из образцов, убитых прогреванием при 100°С в течение 30 мин. Для удаления ингибиторов ПЦР проводили предваритель-

ную обработку биофильмов протеиназой К (AppliChem) и хлороформом. Фермент добавляли в количестве 2 ед. на образец в равном образцу объеме 0,5-кратного лизирующего буфера [8] от набора ДНК-сорб-В (Интерлабсервис, Россия), интенсивно встряхивали и прогревали при 55°С 30 мин. В дальнейшем к образцу добавляли равный объем хлороформа, перемешивали и центрифугировали на максимальных оборотах. Полученный супернатант отбирали в чистый виал и выделяли ДНК набором «ДНК-сорб-В» (Интерлабсервис), согласно протоколу производителя.

ПЦР проводили в варианте с детекцией в реальном времени (ПЦР-РВ) на амплификаторе LightCycler Nano (Roche). Олигонуклеотидные праймеры и зонды собственного дизайна синтезированы НПФ «Синтол», реагенты для ПЦР приобретались в компаниях «Интерлабсервис» и «Силекс».

Статистическую обработку результатов проводили в таблицах Excel и программой Statistica v6.0.

Результаты

Первоначально был проведен целенаправленный скрининг чувствительных штаммов на способность образовывать БФ при росте на синтетической среде Школьниковой. Результатом этого поиска стало обнаружение трех культур, способных с разной эффективностью образовывать БФ (рис., А, III обложка). Было так же обнаружено, что все 3 культуры имеют специфическую R-форму колоний при росте на среде Левенштейна–Йенсена. В отличие от стандартной R-формы МБТ эти штаммы на твердой питательной среде образовывали сухую колонию, окруженную кольцом вторичного роста.

Данная R-форма колоний была условно названа нами «НЛО-колонии» (рис. Б, III обложка). Для получения более представительной выборки в течение года был предпринят развернутый поиск штаммов, продуцирующих такого года НЛО-колонии. Скрининг более полутора тысяч посевов на средах Левенштейна–Йенсена и Финн II позволил выявить 67 пробирок с искомыми колониями. В 12-ти случаях штаммы были парными, то есть принадлежали одному больному. НЛО-штаммы значимо чаще продуцировали БФ при росте на среде Школьниковой ($p < 0,01$). Однако около трети штаммов (25 из 67) с НЛО-колониями оказались неспособны продуцировать биопленку при росте на жидкой среде в течение 45 дней, или продуцировали ее слабо (++). Ретроспективный анализ профилей устойчивости к ПТП выбранных культур обнаружил, что вопреки исходному предположе-

Таблица 1. Список праймеров и зондов, использованных для идентификации штаммов, принадлежащих к эпидемическим кластерам

Table 1. List of primers and probes used for identify strains belonging to epidemics clusters

Название гена и (позиция SNP в геноме) The name of the gene and (SNP position in the genome)	**Структура олигонуклеотида 5' → 3'** The structure of the oligonucleotide 5' → 3'	**Размер ампликона (п.н.)** The size of the amplicon (b.p.)	**Tm, °C**	**Ссылка на источник** Source reference	**Примечание** Note
Pks17 (1887060)	CC1 FAM-ATGAGCTCAC(G-LNA)CGGC(A-LNA)CCTG-RTQ1	71	65	[13]	**Зонды и праймеры для выявления ДНК CC1/ non-CC1 кластеров (дизайн выполнен в рамках данного исследования)** Probes and primers for DNA detection of CC1/ non-CC1 clusters (designed as part of this study)
	nonCC1 R6G-ATGAGCTCAC(C-LNA)CGGC(A-LNA)CCTG-BHQ2				
	CC1F AGGTCGATGGGGCCTGGAATT				
	CC1R GAAAACAACACAAACGCTGACAC				
Инсерция IS6110 Rv2664-Rv2665 Insertion IS6110 Rv2664-Rv2665	W148 (R6G)-AGACTCTCTGATCT(G-LNA)AGAC (C-LNA)TCA-(BHQ2)	198	65	[5]	**Зонды и праймеры для выявления ДНК W148/ nonW148 кластеров** Probes and primers for DNA detection of W148/ nonW148 clusters
	nonW148 (FAM)-TT(C-LNA)CTCTGACAGCAACA(C-LNA)CAGTT-(RTQ1)				
	GCGACCCGCCGTCTCCTGA				
	TCGGCCGTACGGACGACGAT				

нию подавляющее большинство несет ЛУ, в том числе МЛУ/ШЛУ. Все 67 исследуемых штаммов были разделены на 2 группы: 1) хорошие продуценты БФ (++++ и +++) и плохие продуценты БФ (++, отсутствие поверхностного роста).

В процессе анализа был отмечена особенность штаммов кластера W148 продуцировать БФ и в то же время нести МЛУ/ШЛУ. После исключения из выборки высокотрансмиссивных кластеров CC2-W148 и CC1 [10], среди оставшихся штаммов отмечено появление значимой разницы в способности к продукции БФ, относительно распределения МЛУ/ШЛУ. Выборка МЛУ/ШЛУ штаммов без CC1 и CC2-W148 значимо чаще теряла способность к продукции БФ (табл. 2). Проведен углубленный анализ 24 парных штаммов (по 2 изолята от больного), с целью оценки изменения способности к продукции БФ в процессе лечения ПТП. Для всех штаммов определены минимальные ингибирующие концентрации (МИК) с помощью теста Sensititre Myco TB. В трех случаях пару составляли культуры, выделенные из мокроты и из операционного материала, а в 9 случаях, из мокрот, выделенных с интервалом не менее 1 месяца. Ни в одном из 12 парных случаев не обнаружено значимых изменений в МИК большинства антибиотиков. Был выявлен 1 случай значимых различий в способности к продукции БФ. Парадоксально, но штамм, способный продуцировать полноценный БФ (++++), был выделен от больной в более поздние сроки лечения, чем штамм сравнения БФ (++) из мокроты. Углубленное количественное исследование устойчивости к противотуберкулезным препаратам (ПТП) этих штаммов не выявило больших различий в профилях чувствительности к антибиотикам, за исключением уменьшения минимальной ингибирующей концентрации к этионамиду с 8 мг/мл у штамма БФ (++), до 2 мкг/мл у штамм БФ (++++). Интересно, что штамма БФ (++++) в отличие от остальных парных штаммов, был выделен из натечника (холодного абсцесса).

Обсуждение

Вопреки основной гипотезе настоящего исследования нам не удалось выявить единственный ПТП или комбинацию антибиотиков, которые однозначно нарушают процесс образования поверхностного БФ на голодной среде Школьниковой (без добавления сыворотки крови). Проведенные ранее эксперименты с имеющимися продуцентами БФ показали, что добавление 10% инактивированной сыворотки крови человека не оказывает значительного влияния на возможность продукции БФ штаммом (данные не приводятся). В то же время после исключения из анализа штаммов высотрансмиссивных кластеров CC1 и CC2-W148 генотипа Beijing среди оставшихся МЛУ/ШЛУ штаммов наблюдались значимые различия в продукции БФ. Как и ожидалось, активных продуцентов БФ среди МЛУ/ШЛУ штаммов встречалось значимо реже. По всей видимости штаммы высокотрансмиссивных кластеров CC2-W148 и CC1 [10], называемых также «Europe-Russia B0/W148 outbreak и Central Asia outbreak» [13], обладают наибольшим фитнес-потенциалом по отношению SNP, вызывающих устойчивость к ПТП. Достаточно сказать, что более 99% штаммов генотипа CC2-W148 имеют первичную устойчивость к рифампицину [13] и к изониазиду [5]. С этой точки зрения, исключение генетически гомогенных эпидемических штаммов, распространившихся в последние десятилетия [4], позволило увидеть картину более близкую к описываемой в нормативных документах прошлого века [2].

Таблица 2. Свойства исследуемых штаммов

Table 2. Properties of the studied strains

Характеристика штамма Properties of the strain	**БФ** ++++/+++ BF ++++/+++	**БФ** ++/**отр.** БФ ++/neg.	χ^2; ρ
МЛУ/ШЛУ MDR/XDR	17/34 (50%)	23/33 (69,7%)	NS
CC1 CC1	1/34 (2,9%)	2/33 (6,1%)	NS*
CC2-W148 CC1-W148	12/34 (35,3%)	7/33 (21,2%)	NS*
«Иные генотипы» «Other genotypes»	21/34 (61,8%)	24/33 (72,7%)	NS
МЛУ/ШЛУ среди «иных генотипов» MDR/XDR among «other genotypes»	4/21 (19,1%)	14/24 (58,4%)	5,7; < 0,5*

Примечания. NS — отсутствие значимых различий. * Значение χ^2 с коррекцией по Йетсу.

Notes. NS — no significant differences; * χ^2 with Yates correction.

Пожалуй, наибольшее клиническое значение имеет результат, показывающий возможность восстановления активной продукции БФ штаммами в процессе лечения. Ретроспективный анализ этого случая не выявил какого-либо изменения в процессе химиотерапии за анализируемый период, однако больная отличалась низкой приверженностью к лечению, результатом чего, по-видимому, стало развитие натечного абсцесса. Объективно у штаммов из мокроты (более раннее выделение) и из натечного абсцесса (выделен через месяц) профили ПТП практически одинаковы (МЛУ, пред-ШЛУ штаммы, сохранившие устойчивость к канамицину), разница наблюдалась только в МИК к этионамиду 8 и 2 мкг/мл. Следует отметить, что оба эти значения остаются в пограничных зонах чувствительности к препарату. По всей видимости, наиболее важным фактором, спровоцировавшим активную БФ-продукцию на жидкой среде Школьниковой, был объект выделения (натечный абсцесс).

Исходя из полученных результатов можно предположить, что лечение ПТП препаратами может оказывать косвенное влияние на способность МБТ штаммов образовывать БФ при росте на жидкой питательной среде. Однако в условиях роста *in vivo*, гораздо более сильное влияние на способность к формированию БФ оказывает окружающая патоген среда макроорганизма и (возможно) микробиота легкого. Можно думать, что образование БФ — процесс требующий больших затрат энергии, и для такого медленно растущего микроорганизма как МБТ это далеко не всегда целесообразная стратегия размножения. В пользу этого говорят наши наблюдения (данные не приводятся), о том, что пассирование активных продуцентов БФ на голодной среде Школьниковой в подавляющем большинстве случаев приводит штаммы к утрате способности к поверхностному росту при сохранении обильного придонного осадка.

Без сомнения, феномен образования БФ *in vitro* и, особенно, *in vivo* возбудителем туберкулеза требует дальнейшего изучения, поскольку вполне очевидно, что данный процесс многократно осложняет процессы лечения хронических форм туберкулеза.

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Список литературы/References

1. О совершенствовании противотуберкулезных мероприятий в Российской Федерации: приказ Министерство Здравоохранения Российской Федерации № 109 от 21 марта 2003 г. [On the improvement of tuberculosis measures in the Russian Federation: Order of the Russia Ministry of Health, March 21, 2003 No. 109]. *URL: http://www.consultant.ru/document/cons_doc_LAW_100829 (10.09.2018)*
2. Об унификации микробиологических методов исследования при туберкулезе: приказ Минздрава СССР № 558 от 8 июня 1978 г. [On the unification of microbiological research methods for tuberculosis: order of the USSR Ministry of Health No. 558, June 8, 1978]
3. Огарков О.Б., Бадлеева М.В., Белькова Н.Л., Адельшин Р.В., Цыренова Т.А., Хромова П.А., Синьков В.В., Костюнин К.Ю., Дашацыренова С.Б., Кощеев М.Е., Зарбуев А.Н., Жданова С.Н. Феномен образования биопленок штаммами Brevibacillus spp. и Bacillus spp. в присутствии клинических штаммов Mycobacterium tuberculosis // Молекулярная генетика, микробиология и вирусология. 2017. Т. 35, № 3. С. 98–103. [Ogarkov O.B., Badleeva M.V., Belkova N.L., Adelshin R.V., Tsyrenova T.A., Khromova P.A., Sinkov V.V., Kostjunin K.Yu., Dashatsyrenova S.B., Koshcheyev M.E., Zarbuev A.N., Zhdanova S.N. The phenomenon of the formation of biofilms by Brevibacillus spp. and Bacillus spp. in the presence of clinical strains of Mycobacterium tuberculosis. *Molekulyarnaya genetika, mikrobiologiya i virusologiya = Molecular Genetics, Microbiology and Virology, 2017, vol. 35, no. 3, pp. 98–103. doi: 10.3103/S0891416817030065 (In Russ.)*]
4. Синьков В.В., Савилов Е.Д., Огарков О.Б. Реконструкция эпидемической истории «пекинского» генотипа Mycobacterium tuberculosis в России и странах бывшего СССР по результатам сполиготипирования // Молекулярная генетика, микробиология и вирусология. 2011. № 3. С. 25–29. [Sinkov V.V., Savilov E.D., Ogarkov O.B. Reconstruction of the epidemic history of the Beijing genotype of Mycobacterium tuberculosis in Russia and former soviet countries using spoligotyping. *Molekulyarnaya genetika, mikrobiologiya i virusologiya = Molecular Genetics, Microbiology and Virology, 2011, no. 3, pp. 25–29. doi: 10.3103/S0891416811030050 (In Russ.)*]
5. Хромова П.А., Огарков О.Б., Жданова С.Н., Синьков В.В., Моисеева Е.Я., Цыренова Т.А., Кощеев М.Е., Зоркальцева Е.Ю., Савилов Е.Д. Выявление высокотрансмиссивных генотипов возбудителя в клиническом материале для прогноза неблагоприятного течения туберкулеза // Клиническая лабораторная диагностика. 2017. Т. 62, № 10. С. 622–627. [Khromova P.A., Ogarkov O.B., Zhdanova S.N., Sinkov V.V., Moiseeva E.Ya., Tzyrenova T.A., Koscheev M.E., Zorkaltseva E. Yu., Savilov E.D. The detection of highly-transmissible genotypes of agent in clinical samples for prognosis of unfavorable course of tuberculosis. *Klinicheskaya laboratornaya diagnostika = Clinical Laboratory Diagnostics, 2017, vol. 62, no. 10, pp. 622–627. doi: 10.18821/0869-2084-2017-62-10-622-627 (In Russ.)*]
6. Dalton J.P., Uy B., Phummarin N., Copp B.R., Denny W.A., Swift S., Wiles S. Effect of common and experimental anti-tuberculosis treatments on Mycobacterium tuberculosis growing as biofilms. *Peer J., 2016, vol. 22, no. 4: e2717. doi: 10.7717/peerj.2717*

7. Esteban J., García-Coca M. Mycobacterium biofilms. *Front Microbiol., 2018, vol. 18, no. 8, p. 2651. doi: 10.3389/fmicb.2017.02651*
8. Hilz H., Wiegers U., Adamietz P. Stimulation of proteinase K action by denaturing agents: application to the isolation of nucleic acids and the degradation of "masked" proteins. *Eur. J. Biochem., 1975, vol. 56, no. 1, pp. 103–108. doi: 10.1111/j.1432-1033.1975.tb02211.x*
9. Jindani A., Dore C.J., Mitchison D.A. Bactericidal and sterilizing activities of antituberculosis drugs during the first 14 days. *Am. J. Respir. Crit. Care Med., 2003, vol. 167, pp. 1348–1354. doi: 10.1164/rccm.200210-1125OC*
10. Merker M., Blin C., Mona S., Duforet-Frebourg N., Lecher S., Willery E., Blum M.G., Rüsch-Gerdes S., Mokrousov I., Aleksic E., Allix-Béguec C., Antierens A., Augustynowicz-Kopeć E., Ballif M., Barletta F., Beck H.P., Barry C.E. 3rd, Bonnet M., Borroni E., Campos-Herrero I., Cirillo D., Cox H., Crowe S., Crudu V., Diel R., Drobniewski F., Fauville-Dufaux M., Gagneux S., Ghebremichael S., Hanekom M., Hoffner S., Jiao W.W., Kalon S., Kohl T.A., Kontsevaya I., Lillebæk T., Maeda S., Nikolayevskyy V., Rasmussen M., Rastogi N., Samper S., Sanchez-Padilla E., Savic B., Shamputa I.C., Shen A., Sng L.H., Stakenas P., Toit K., Varaine F., Vukovic D., Wahl C., Warren R., Supply P., Niemann S., Wirth T. Evolutionary history and global spread of the Mycobacterium tuberculosis Beijing lineage. *Nat. Genet., 2015, vol. 47, no. 3, pp. 242–249. doi: 10.1038/ng.3195*
11. Ojha A.K., Baughn A.D., Sambandan D., Hsu T., Trivelli X., Guerardel Y., Alahari A., Kremer L., Jacobs W.R. Jr, Hatfull G.F. Growth of Mycobacterium tuberculosis biofilms containing free mycolic acids and harbouring drug-tolerant bacteria. *Mol. Microbiol., 2008, vol. 69, pp. 164–174. doi: 10.1111/j.1365-2958.2008.06274.x*
12. Orme I.M. A new unifying theory of the pathogenesis of tuberculosis. *Tuberculosis (Edinb), 2014, vol. 94, no. 1, pp. 8–14. doi: 10.1016/j.tube.2013.07.004*
13. Shitikov E., Kolchenko S., Mokrousov I., Bespyatykh J., Ischenko D., Ilina E., Govorun V. Evolutionary pathway analysis and unified classification of East Asian lineage of Mycobacterium tuberculosis. *Sci. Rep., 2017, vol. 7, pp. 9227. doi: 10.1038/s41598-017-10018-5*
14. Wallis R.S., Patil S., Cheon S.H., Edmonds K., Phillips M., Perkins M.D., Joloba M., Namale A., Johnson J.L., Teixeira L., Dietze R., Siddiqi S., Mugerwa R.D., Eisenach K., Ellner J.J. Drug tolerance in Mycobacterium tuberculosis. *Antimicrob. Agents Chemother., 1999, vol. 43, no. 11, pp. 2600–2606*.
15. Wayne L.G., Sohaskey C.D. Nonreplicating persistence of Mycobacterium tuberculosis. *Annu. Rev. Microbiol., 2001, vol. 55, pp. 139–163.*
16. Xiang X., Deng W., Liu M., Xie J. Mycobacterium biofilms: factors involved in development, dispersal, and therapeutic strategies against biofilm-relevant pathogens. *Crit. Rev. Eukaryot. Gene Expr., 2014, vol. 24, no. 3, pp. 269–279. doi: 10.1615/CritRevEukaryotGeneExpr.2014010545*

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GENETIC DIVERSITY OF *MYCOBACTERIUM AVIUM* subsp. *HOMINISSUIS* STRAINS ISOLATED IN ITALY BASED ON VNTR LOCI ANALYSIS

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Abstract. *Background. Mycobacterium avium* subsp. *hominissuis* (MAH) is an important pathogen responsible for most of the human-associated nontuberculous mycobacteria infections. Over the past few decades the incidence of MAH infections is increasing in Italy, as in many countries worldwide. The present study is aimed to elucidate the genetic characteristics of MAH strains isolated from human patients using VNTR typing and to show the genetic relatedness among them. *Methods.* The genetic diversity of 108 human isolates of MAH was determined by VNTR analysis targeting 8 loci, coded 32, 292, X3, 25, 3, 7, 10 and 47. *Results.* The VNTR analysis revealed 25 distinct VNTR patterns; of these, 13 patterns were unique, while 12 patterns were shared by 2 or more isolates, thus yielding 12 clusters including a total of 95 isolates. The discriminatory power of our VNTR analysis yielded an HGDI of 0.990, indicating that VNTR typing has an excellent discriminatory power. No association of a particular VNTR pattern with a particular clinical feature, such as the disseminated, pulmonary or extrapulmonary type of infection, was observed. Minimum spanning tree analysis showed that 21 VNTR patterns, occurring either as clustered or unique isolates, differed from the nearest one for one allelic variation. *Conclusions.* The results obtained through the VNTR analysis showed that most MAH strains displayed a close genetic relationship. This high phylogenetic proximity of the VNTR loci over a long time period supports the concept that the MAH genotype is highly homogeneous in our geographical area, suggesting the hypothesis of the presence of possible sources of infection and transmission pathways at the local level.

Key words: *Mycobacterium avium, population structure, Italy, VNTR loci, mycobacteriosis.*

ГЕНЕТИЧЕСКОЕ РАЗНООБРАЗИЕ ШТАММОВ *MYCOBACTERIUM AVIUM* subsp. *HOMINISSUIS*, ВЫДЕЛЕННЫХ В ИТАЛИИ, НА ОСНОВЕ АНАЛИЗА ЛОКУСОВ VNTR

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Резюме. *Mycobacterium avium* subsp. *hominissuis* является наиболее актуальным возбудителем микобактериоза человека. За последние несколько десятилетий в Италии заболеваемость микобактериозом *M. avium* subsp. *hominissuis* растет, как и во многих странах мира. Целью исследования была молекулярно-генетическая характеристика и оценка генетического родства штаммов *M. avium* subsp. *hominissuis*, выделенных от больных микобактериозом в Италии, с использованием VNTR (variable number of tandem repeats)-типирования. Аллельный полиморфизм 108 штаммов *M. avium* subsp. *hominissuis* оценивали методом VNTR-типирования по 8 локусам — 32, 292, X3, 25, 3, 7, 10 и 47. С помощью VNTR-типирования было выявлено 25 вариантов VNTR-типов; из них 13 профилей были уникальными, а 12 профилей представлены кластерами (включающими

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2 и более изолятов), в состав которых входило 95 изолятов. Дискриминирующая способность VNTR-типирования (индекс Хантера–Гастона, Hunter Gaston discriminatory index) составила 0.990, что указывает на высокую дискриминирующую способность использованной схемы VNTR. Связи между профилем VNTR и клинической формой микобактериоза (генерализованная, легочная или внелегочная) не обнаружено. Анализ минимального связывающего дерева профилей VNTR показал, что 21 VNTR-тип (как уникальные изоляты, так и кластеры двух и более изолятов) входили в единый клональный комплекс в котором соседние узлы различались по одному локусу. Полученные результаты VNTR-типирования выявили близкое родство изученных штаммов *M. avium* subsp. *hominissuis*. Высокий уровень филогенетического родства по локусам VNTR для штаммов, выделенных в течение длительного периода, подтверждает концепцию о том, что *M. avium* subsp. *hominissuis* очень гомогенен в нашей географической области в Италии, что, в свою очередь, подкрепляет гипотезу о наличии возможных источников инфекции и путей ее передачи на местном уровне.

Ключевые слова: *Mycobacterium avium, структура популяции, Италия, локусы VNTR, микобактериоз.*

Introduction

In many countries worldwide the incidence of nontuberculous mycobacteria (NTM) infections is increasing over the past few decades [11]. *Mycobacterium avium* complex is responsible for most of the human-associated nontuberculous mycobacteria infections [1]. *Mycobacterium avium*, one of the members of the *M. avium* complex, includes 4 subspecies, each endowed with specific pathogenetic and host range characteristics: *M. avium* subsp. *paratuberculosis*, that causes the Johne's disease in ruminants; *M. avium* subsp. *avium*, that infects birds; *M. avium* subsp. *silvaticum*, that infects wood pigeons; and *M. avium* subsp. *hominissuis* (MAH), that is usually isolated from human and swine sources [14, 20]. MAH is an important pathogen that causes not only disseminated diseases in patients with human immunodeficiency virus infection but also pulmonary disease, even in immunocompetent patients [19], and the incidence of pulmonary MAH infection is increasing in Italy [14].

Control of MAH infections in humans requires knowledge of its epidemiology and biodiversity of the strains. The variable numbers of tandem repeats (VNTR) analysis is a genotyping method that has been proven to be a rapid and reliable method with a high discriminatory power for MAH isolates [5, 17]. The present study is aimed to elucidate the genetic characteristics of MAH strains isolated from human patients using VNTR typing and to show the genetic relatedness among them.

Materials and Methods

Clinical isolates. A set of 108 MAH strains, identified by InnoLipa probes and by a multiplex PCR designed to discriminate MAC organisms [16], isolated from 1990 to 2016 in the Laboratory of Clinical Mycobacteriology of the University Hospital of Pisa, Italy, from the same number of patients, were studied. Fifty isolates were from respiratory specimens, 19 from blood, 15 from lymph nodes, 7 from specimens other than respiratory specimens, blood and lymph nodes, and 17 from an unknown source.

VNTR analysis. Genomic DNA was extracted by the cetyltrimethyl-ammonium bromide (CTAB) method. VNTR typing was performed by PCR using specific primers for the eight loci identified as polymorphic for *M. avium* subsp. *paratuberculosis* K10 and coded 32, 292, X3, 25, 3, 7, 10 and 47, as described previously [17]. The PCR fragments were analyzed by gel electrophoresis using 2% NuSieve agarose (Cambrex Bio Science Rockland). For each locus, sizes of amplicons were estimated by comparison with 20 bp and 100 bp markers (Superladder-low; GenSura, CA, USA) and the numbers of repetitive units were determined according with a previously described allele-calling table [17]. VNTR profile is expressed as a string of 8 numbers, each representing the number of tandem repeats (TR) at a given VNTR position, in the order given above. The allelic diversity (h) of the VNTR loci was calculated using the equation $h = 1 - \Sigma\, x_i^2\, \{n/(n-1)\}$ where n is the number of isolates and x_i the frequency of the i^{th} allele at the locus (Selander et al., 1986). The global discriminatory power of complete VNTR scheme (HGDI) was determined using the Hunter and Gaston discriminatory index (HGDI) [2]. The HGDI was calculated using the following formula:

$$D = 1 - \frac{1}{N(N-1)} \sum_{J=1}^{s} xj\,(xj-1),$$

where N is the total number of isolates in the typing scheme, s is the total number of distinct subtypes discriminated by the typing method, and xj is the number of isolates belonging to the x^{th} subtype.

Genetic relationships analysis. VNTR data were analyzed by the MIRU-VNTRplus web application available at www.miru-vntrplus.org; VNTR profile similarities were visualized by generating a dendrogram using the unweighted pair group method with arithmetic averages (UPGMA); the genetic relationships among the isolates were analyzed by constructing a minimum spanning tree (MST), an undirected network in which all the VNTR profiles are linked together with the smallest possible linkages between nearest neighbours, by the UPGMA method.

Table 1. VNTR allelic distribution in 108 MAH clinical isolates

No. of tandem repeat copies	No. of isolates at the VNTR locus							
	32	**292**	**X3**	**25**	**3**	**7**	**10**	**47**
0		19						
1		1		1	108	108	1	
2		85	48	82			104	96
3		1	4	23				12
4			25	1				
5	2		30				3	
6								
7	2							
8	62							
9	39							
10	2							
nd*	1	2	1	1				
*h***	0.57	0.32	0.66	0.36	0	0	0.06	0.19

* not determined (no PCR product was obtained). ** allelic diversity (h) was calculated as described by Selander et al. (1986).

Results

The genetic diversity of 108 MAH human strains, isolated over a two 25 year-period in the Laboratory of Clinical Mycobacteriology of the University Hospital of Pisa, Italy, was investigated by determining the polymorphism of a set of eight MIRU-VNTR loci as previously described by Thibault et al. [17]. We first quantified the resolution provided by each VNTR locus by calculating its allelic diversity, which depends upon both the number and the distribution of the alleles, according to Selander et al. [15]. As shown in Table 1, the allelic diversity *(h)* of the VNTR loci of our collection varied widely, from 0 to 0.66. The VNTR loci 32 and X3 had a high diversity index ($h \geq 0.5$); three loci (292, 25, 47) showed medium diversity index ($0.1 \leq h \leq 0.5$); the locus 10 achieved a low diversity index ($h \leq 0.1$); the last two loci (3, 7) did not show any allelic diversity.

The VNTR analysis revealed 25 distinct VNTR patterns; of these, 13 patterns were unique, while 12 patterns were shared by 2 or more isolates, thus yielding 12 clusters including a total of 95 isolates. In particular, 1 cluster consisting of 24 strains, 1 cluster of 15 strains, 2 clusters of 11 strains, 1 cluster of 9 strains, 1 cluster of 7 strains, 1 cluster of 5 strains, 1 cluster of 4 strains, 1 cluster of 3 strains and finally 3 clusters of 2 strains were identified. The discriminatory power of our VNTR analysis yielded an HGDI of 0.990, indicating that VNTR typing has an excellent discriminatory power. Table 2 shows VNTR profiles and localization of infection of clustered and unique MAH strains; no association of a particular VNTR pattern with a particular clinical feature, such as the disseminated, pulmonary or extrapulmonary type of infection, was observed.

The genetic relationships between the study isolates were then visualized by constructing a minimum spanning tree (MST) based on the VNTR profiles. The MST reflects the variations from one

Table 2. Characteristics of MAH strains

VNTR pattern[a]	No. of Isolates	No. of isolates with specific localization[b]			
		Respiratory tract	Blood	Lymphnode	Other
82221122	24	12	5	5	1
92221122	15	10	–	3	–
82421122	11	2	6	1	1
92421122	11	4	–	–	–
82521122	9	3	1	3	–
80531122	7	2	1	–	4
80531123	5	3	2	–	–
92521122	4	1	1	1	–
90531122	3	–	–	1	–
82231122	2	2	–	–	–
52421122	2	–	2	–	–
90221122	2	2	–	–	–
82321122	1	–	–	–	1
10 2221122	1	1	–	–	–
90421122	1	1	–	–	–
82231123	1	1	–	–	–
82241123	1	1	–	–	–
82221123	1	1	–	–	–
92221113	1	1	–	–	–
82531122	1	–	–	1	–
10 0531122	1	1	–	–	–
72511123	1	–	1	–	–
71331152	1	–	–	–	–
93331153	1	1	–	–	–
90331153	1	1	–	–	–

[a] VNTR patterns are expressed as strings of 8 numbers, each representing the number of tandem repeats at a given VNTR position, in the following order: locus 32, 292, X3, 25, 3, 7, 10, 47.
[b] Localization was unknown for 17 patients.

allele to another due to the loss or gain of one tandem repeat sequence at a single VNTR locus. The MST, illustrated in Figure, shows that most (21 out of 25) VNTR patterns, occurring either as clustered or unique isolates, differed from the nearest one for one allelic variation; one VNTR pattern differed for 2 allelic variations; three VNTR patterns differed for 3 allelic variations. By this analysis, the 25 VNTR profiles described above yielded two clonal complexes, termed CC1 and CC2, including 21 and 2 unique profiles, respectively. CC1 (white in Fig.) included a total of 104 isolates, 95 of which clustered in the 12 clusters. CC2 (grey in Fig.), that differed from CC1 for three allelic variations, included 2 isolates with unique VNTR profile.

Discussion

The aim of the present study was to determine the genetic diversity of MAH strains isolated in a region of Italy by analyzing a set of eight VNTR loci. The VNTR typing assay employed in the present study showed that 5 VNTR loci of our MAH isolates (i.e., loci 32, 292, X3, 25 and 47) were enough polymorphic to yield an acceptable allelic diversity. Indeed, in agreement with previous reports [4, 10, 12, 17, 18], locus VNTR X3 turn out to be the most polymorphic, while loci VNTR 3, VNTR 7 and VNTR 10 were the least suitable for VNTR typing of MAH isolates. Our VNTR analysis, that yielded 25 unique VNTR patterns and identified 12 clusters including a total 95 isolates, showed an excellent discriminatory power (HGDI = 0.990), similar to that obtained with VNTR schemes used by other authors [4, 5]. The results obtained through the VNTR analysis showed that most MAH strains displayed a close genetic relationship, as indicated by the minimum spanning tree analysis; in fact, 21 out of 25 VNTR patterns of the MAH isolates, occurring either as clustered or unique isolates, differed from the nearest one only for one allelic variation. This high phylogenetic proximity of the VNTR loci over a long time period supports the concept that the MAH genotype is highly homogeneous in our geographical area. Other studies demonstrated geographical differences in genetic diversity of MAH, suggesting the hypo-

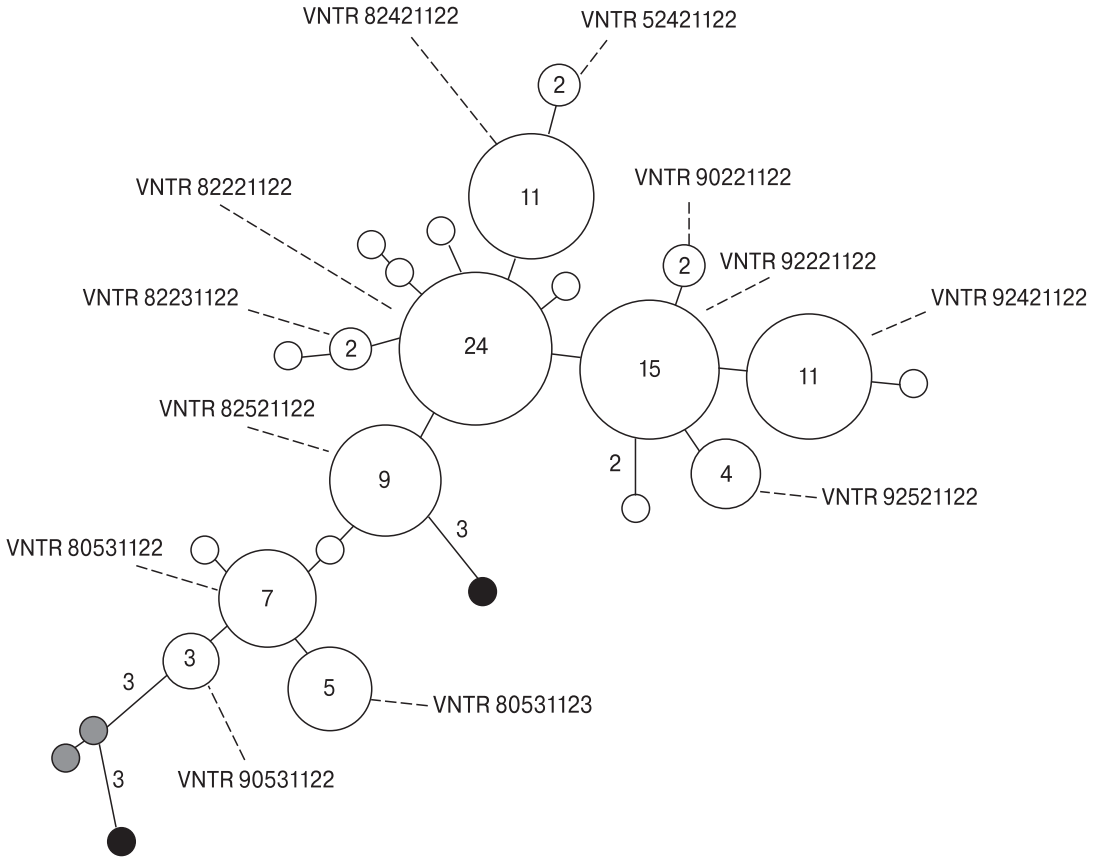

Figure. Minimum spanning tree based on VNTR profiles of a set of 8 loci of 108 MAH clinical isolates
Each small-size circle represents a single isolate; larger circles represent clusters of 2–24 isolates, depending on the circle size, with identical VNTR profiles. For each cluster the number of the isolates is given in the circle and the VNTR profile in the callouts. Numbers next to the branches indicate the level of changes more than 1 induced by loss or gain of VNTR copies at a given locus, yielding a change from one allele to another. White and grey circles indicate VNTR profiles belonging to clonal complexes CC1 and CC2, respectively, detected by the analysis at single locus variance. The tree was generated using the UPGMA method by the MIRU-VNTRplus web application available at www.miru-vntrplus.org.

thesis of the presence of possible sources of infection and transmission pathways at the local level [3, 6, 7, 8, 9]. Interestingly on this subject, a recent population structure study postulated the emergence of human-adapted MAH lineages on local scale, and suggested that recombination facilitates local adaptation of MAH [21].

In order to achieve a better control of MAH infection, further investigations on larger collections of MAH strains of human, animal and environmental origin are needed to clarify the sources of infection, the specific transmission pathway and the local adaptation mechanisms of MAH.

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Список литературы/References

1. Griffith D.E., Aksamit T., Brown-Elliott B.A., Catanzaro A., Daley C., Gordin F., Holland S.M., Horsburgh R., Huitt G., Iademarco M.F., Iseman M., Olivier K., Ruoss S., von Reyn C.F., Wallace R.J. Jr, Winthrop K. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am. J. Respir. Crit. Care Med., 2007, vol. 175, pp. 367–416. doi: 10.1164/rccm.200604-571ST*
2. Hunter P.R., Gaston M.A. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. *J. Clin. Microbiol., 1988, vol. 26, pp. 2465–2466.*
3. Ichikawa K., van Ingen J., Koh W.J., Wagner D., Salfinger M., Inagaki T., Uchiya K.I., Nakagawa T., Ogawa K., Yamada K., Yagi T. Genetic diversity of clinical Mycobacterium avium subsp. hominissuis and Mycobacterium intracellulare isolates causing pulmonary diseases recovered from different geographical regions. *Infect. Genet. Evol., 2015, vol. 36, pp. 250–255. doi: 10.1016/j.meegid.2015.09.029*
4. Imperiale B.R., Moyano R.D., Di Giulio A.B., Romero M.A., Alvarado Pinedo M.F., Santangelo M.P., Traveria G.E., Morbillo N.S., Romano M.I. Genetic diversity of Mycobacterium avium complex strains isolated in Argentina by MIRU-VNTR. *Epidemiol. Infect., 2017, vol. 145, pp. 1382–1391. doi: 10.1017/S0950268817000139*
5. Inagaki T., Nishimori K., Yagi T., Ichikawa K., Moriyama M., Nakagawa T., Shibayama T., Uchiya K., Nikai T., Ogawa K. Comparison of a variable-number tandem-repeat (VNTR) method for typing Mycobacterium avium with mycobacterial interspersed repetitive-unit-VNTR and IS1245 restriction fragment length polymorphism typing. *J. Clin. Microbiol., 2009, vol. 47, pp. 2156–2164. doi: 10.1128/JCM.02373-08*
6. Iwamoto T., Nakajima C., Nishiuchi Y., Kato T., Yoshida S., Nakanishi N., Tamaru A., Tamura Y., Suzuki Y., Nasu M. Genetic diversity of Mycobacterium avium subsp. hominissuis strains isolated from humans, pigs, and human living environment. *Infect. Genet. Evol., 2012, vol. 12, pp. 846–852. doi: 10.1016/j.meegid.2011.06.018*
7. Kalvisa A., Tsirogiannis C., Silamikelis I., Skenders G., Broka L., Zirnitis A., Jansone I., Ranka R. MIRU-VNTR genotype diversity and indications of homoplasy in M. avium strains isolated from humans and slaughter pigs in Latvia. *Infect. Genet. Evol., 2016, vol. 43, pp. 15–21. doi: 10.1016/j.meegid.2016.05.013*
8. Muwonge A., Oloya J., Kankya C., Nielsen S., Godfroid J., Skjerve E., Djønne B., Johansen T.B. Molecular characterization of Mycobacterium avium subspecies hominissuis isolated from humans, cattle and pigs in the Uganda cattle corridor using VNTR analysis. *Infect. Genet. Evol., 2014, vol. 21, pp. 184–191. doi: 10.1016/j.meegid.2013.11.012*
9. Nishiuchi Y., Iwamoto T., Maruyama F. Infection sources of a common non-tuberculous mycobacterial pathogen, Mycobacterium avium complex. *Front. Med., 2017, vol. 4: 27. doi: 10.3389/fmed.2017.00027*
10. Pate M., Kušar D., Zolnir-Dovč M., Ocepek M. MIRU-VNTR typing of Mycobacterium avium in animals and humans: heterogeneity of Mycobacterium avium subsp. hominissuis versus homogeneity of Mycobacterium avium subsp. avium strains. *Res. Vet. Sci., 2011, vol. 91, pp. 376–381. doi: 10.1016/j.rvsc.2010.10.001*
11. Prevots D.R., Marras T.K. Epidemiology of human pulmonary infection with nontuberculous mycobacteria: a review. *Clin. Chest Med., 2015, vol. 36, pp. 13–34. doi: 10.1016/j.ccm.2014.10.002*
12. Radomski N., Thibault V.C., Karoui C., de Cruz K., Cochard T., Gutiérrez C., Supply P., Biet F., Boschiroli M.L. Determination of genotypic diversity of Mycobacterium avium subspecies from human and animal origins by mycobacterial interspersed repetitive-unit-variable-number tandem-repeat and IS1311 restriction fragment length polymorphism typing methods. *J. Clin. Microbiol., 2010, vol. 48, pp. 1026–1034. doi: 10.1128/JCM.01869-09*
13. Rindi L., Garzelli C. Genetic diversity and phylogeny of Mycobacterium avium. *Infect. Genet. Evol., 2014, vol. 21, pp. 375–383. doi: 10.1016/j.meegid.2013.12.007*
14. Rindi L., Garzelli C. Increase in non-tubercolous mycobacteria isolated from humans in Tuscany, Italy, from 2004 to 2014. *BMC Infect. Dis., 2016, vol. 16: 44. doi: 10.1186/s12879-016-1380-y*
15. Selander R.K., Caugant D.A., Ochman H., Musser J.M., Gilmour M.N., Whittam T.S. Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. *Appl. Environ. Microbiol., 1986, vol. 51, pp. 873–884.*
16. Shin S.J., Lee B.S., Koh W.J., Manning E.J., Anklam K., Sreevatsan S., Lambrecht R.S., Collins M.T. Efficient differentiation of Mycobacterium avium complex species and subspecies by use of five-target multiplex PCR. *J. Clin. Microbiol., 2010, vol. 48, pp. 4057–4062. doi: 10.1128/JCM.00904-10*
17. Thibault V.C., Grayon M., Boschiroli M.L., Hubbans C., Overduin P., Stevenson K., Gutierrez M.C., Supply P., Biet F. New variable number tandem-repeat markers for typing Mycobacterium avium subsp. paratuberculosis and M. avium strains: comparison with IS900 and IS1245 restriction fragment length polymorphism typing. *J. Clin. Microbiol., 2007, vol. 45, pp. 2404–2410. doi: 10.1128/JCM.00476-07*

18. Tirkkonen T., Pakarinen J., Rintala E., Ali-Vehmas T., Marttila H., Peltoniemi O.A., Mäkinen J. Comparison of variable-number tandem-repeat markers typing and IS1245 restriction fragment length polymorphism fingerprinting of Mycobacterium avium subsp. hominissuis from human and porcine origins. *Acta Vet. Scand., 2010, vol. 52: 21. doi: 10.1186/1751-0147-52-21*
19. Tortoli E. Clinical manifestation of nontuberculous mycobacteria infections. *Clin. Microbiol. Infect., 2009, vol. 15, pp. 906–910. doi: 10.1111/j.1469-0691.2009.03014.x*
20. Turenne C.Y., Wallace R. Jr, Behr M.A. Mycobacterium avium in the postgenomic era. *Clin. Microbiol. Rev., 2007, vol. 20, pp. 205–229. doi: 10.1128/CMR.00036-06*
21. Yano H., Iwamoto T., Nishiuchi Y., Nakajima C., Starkova D.A., Mokrousov I., Narvskaya O., Yoshida S., Arikawa K., Nakanishi N., Osaki K., Nakagawa I., Ato M., Suzuki Y., Maruyama F. Population structure and local adaptation of MAC lung disease agent Mycobacterium avium subsp. hominissuis. *Genome Biol. Evol., 2017, vol. 9, pp. 2403–2417. doi: 10.1093/gbe/evx183*

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RETROSPECTIVE ANALYSIS OF SLOVENIAN *MYCOBACTERIUM AVIUM* COMPLEX AND *MYCOBACTERIUM ABSCESSUS* COMPLEX ISOLATES AND MOLECULAR RESISTANCE PROFILE

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Abstract. Mycobacteria belonging to *Mycobacterium (M.) avium* complex (MAC) and *M. abscessus* complex (MABSC) are the most frequent causes of mycobacteriosis in the world. In the last few years MAC and MABSC taxonomy was rapidly changing due to new molecular methods conveying the possibility to differentiate between species. New techniques are able to identify *M. chimaera* that was previously recognized as *M. intracellulare* and also differentiate subspecies of MABSC. Due to their natural habitat, non-tuberculous mycobacteria (NTM) are constantly exposed to various concentrations of antimicrobial drugs and other chemicals and consequently they had developed different mechanisms of resistance. Macrolides and aminoglycosides are frequently used drugs to treat MAC and MABSC infections. The aim of our nation-wide survey was to obtain information about MABSC subspecies prevalence in Slovenia and to assess the percentage of misidentifications of *M. chimaera* isolates as *M. intracellulare* in the past. Moreover, the purpose of our study was to reveal, which of the two species *M. intracellulare* or *M. chimaera* is clinically more relevant in Slovenia. Further, the aim of the study was to detect mutations in *erm(41), rrl* and *rrs* genes, which are known to convey macrolide resistance (*erm(41)* and *rrl*) and aminoglycoside resistance *(rrs).* One hundred and thirty-two Slovenian mycobacterial isolates obtained from the National Mycobacterial Collection that belong to MAC and MABSC were analysed. GenoType NTM-DR was used to differentiate *M. intracellulare* from *M. chimaera* and subspecies of MABSC. Our results showed that 48% of previously identified *M. intracellulare* isolates were actually *M. chimaera* isolates and that *M. abscessus* subsp. *abscessus* was the most frequent subspecies of MABSC. Most of the MABSC isolates carried the inducible macrolide resistance genes (*erm(41)* and *rrl*), however none of the isolates of MAC and MABSC had mutations in *rrs* genes for aminoglycoside resistance.

Key words: *nontuberculous mycobacteria, Mycobacterium abscessus, Mycobacterium avium, macrolide resistance, aminoglycoside resistance, nation-wide study.*

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РЕТРОСПЕКТИВНЫЙ АНАЛИЗ СЛОВЕНСКИХ ИЗОЛЯТОВ *MYCOBACTERIUM AVIUM* COMPLEX И *MYCOBACTERIUM ABSCESSUS* COMPLEX И МОЛЕКУЛЯРНЫЙ ПРОФИЛЬ УСТОЙЧИВОСТИ

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Резюме. Микобактерии, принадлежащие к *Mycobacterium avium* complex (MAC) и *Mycobacterium abscessus* complex (MABSC), являются наиболее частыми причинами микобактериоза в мире. В последние несколько лет таксономия MAC и MABSC быстро менялась в результате появления новых молекулярно-генетических методов, позволяющих выявлять различия в пределах вида. Это позволило идентифицировать вид *M. chimaera*, который ранее относили к *M. intracellulare*, а также дифференцировать подвиды MABSC. Нетуберкулезные микобактерии являются типичными обитателями окружающей среды и в значительной мере подвержены воздействию различных концентраций противомикробных препаратов и других химических веществ, что привело к развитию различных механизмов природной резистентности. Макролиды и аминогликозиды наиболее часто используются для лечения инфекций, вызванных MAC и MABSC. Целью общенационального исследования являлась оценка распространенности подвидов MABSC в Словении, а также выявление случаев ошибочной идентификации изолятов *M. chimaera* как *M. intracellulare* ранее. Вместе с тем целью работы было выявить, какой из двух видов *M. intracellulare* или *M. chimaera* являлся клинически значимым на территории Словении, а также обнаружение мутаций в генах *erm(41), rrl* и *rrs*, которые, как известно, ассоциированы с развитием устойчивости к макролидам (*erm(41)* и *rrl*) и аминогликозиду *(rrs).* Нами были проанализированы 132 изолята MAC и MABSC, полученных из Национальной коллекции микобактерий Словении. GenoType NTM-DR использовался для дифференциации видов *M. intracellulare* и *M. chimaera*, а также подвидов MABSC. Результаты исследования показали, что 48% изолятов, ранее идентифицированных как *M. intracellulare*, относились к виду *M. chimaera*; наиболее распространенным подвидом MABSC являлся *M. abscessus* subsp. *abscessus.* Большинство изолятов MABSC обладали генами устойчивости к макролидам (*erm (41)* и *rrl*), однако ни один из изолятов MAC и MABSC не выявлено мутаций устойчивости к аминогликозиду в гене *rrs.*

Ключевые слова: *нетуберкулезные микобактерии, Mycobacterium abscessus, Mycobacterium avium, резистентность, макролиды, аминогликозиды, общенациональное исследование.*

Introduction

Non-tuberculous mycobacteria (NTM) are environmental microorganisms that colonise different surfaces and can be isolated from soil, natural waters, air, household plumbing systems, animals and human specimens too. They are resistant to many disinfectants and antibiotics and therefore many infections caused by NTM cannot be cured with commonly used antibiotics [1]. They are causing diseases especially among immunocompromised patients.

Among *Mycobacterium (M.) avium* complex (MAC) there are two well known species causing disease in humans, *M. avium* and *M. intracellulare.* In 2004, development of more specific molecular methods revealed a new species among MAC, *M. chimaera.* Previously *M. chimaera* was, due to similar phenotypic and genotypic characteristics, misidentified as *M. intracellulare.* When *M. chimaera* was first described in 2004 by Tortoli et al. [11] it was estimated that it is a highly virulent species. Afterwards results showed that *M. intracelullare* was more virulent than *M. chimaera* [9, 11]. In MAC, genes connected with macrolide and aminoglycoside resistance are *rrl* and *rrs*, respectively [6]. In 2012 *M. chimaera* caused two invasive infections after cardiac surgery [8]. Afterwards more than 100 cases of *M. chimaera* infections were revealed in European countries and around the world. This opportunistic pathogen became linked with heater-cooler units (HCUs) used during cardiac surgeries. *M. chimaera* has preferences to colonise warm, humid surfaces where it forms biofilms and has high potential to aerosolize. During surgeries, HCUs produce aerosols and *M. chimaera* is dispersed into the air and can colonize the patient. Due to *M. chimaera*'s slow-growth, it can take even several years after surgery to develop disease [4, 8].

Mycobacterium abscessus, belonging to *M. abscessus* complex (MABSC), is one of the most resistant pathogens as it possesses acquired and innate drug resistance. In the last years, MABSC was divided into three subspecies: *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *bolletii* in *M. abscessus* subsp. massiliense. It is known that the three subspecies have different resistance profiles, hence correct species identification is clinically important. Three genes are important for MABSC resistance: *erm(41), rrl* and *rrs.* Gene *erm(41)* encodes the inducible 23S rRNA methylase and contributes to inducible macrolide resistance. Two *erm(41)* sequevars depending on the T/C polymorphism at nucleotide 28, are present in the MABSC population. *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *bolletii* harbour gene *erm(41)* with a T at the nucleotide position 28 that leads to inducible

macrolide resistance. *M. abscessus* subsp. *massiliense* however, has due to a deletion in this region a non-functional gene and is therefore macrolide susceptible. Further, high level of macrolide resistance is caused by point mutations in the peptidyl-transferase-binding region of *rrl* gene, which can be present in all three subspecies. The macrolide antibiotic clarithromycin was the drug of choice in last decade, and still is for cystic fibrosis (CF) patients. Aminoglycoside resistance is caused by single point mutations in the *rrs* gene encoding 16S rRNA and is also present in all three MABSC subspecies [2, 5].

The aim of our study was to perform a retrospective analysis of all Slovenian MAC and MABSC isolates with new molecular test GenoType NTM-DR, which is known to successfully identify *M. chimaera* isolates and also enables mutation identification in *rrl, rrs* and *erm(41)* genes [2, 4]. Our purpose was therefore to identify how many *M. chimaera* isolates were misidentified as *M. intracellulare* and to estimate how many isolates are resistant to macrolides and aminoglycosides and which mutations are prevalent in Slovenia. Moreover, information about clinical relevance of isolates was obtained.

Materials and Methods

In total 133 clinical isolates (obtained from 126 patients in the period from January 2007 to September 2016) from the Slovenian National Mycobacterial Collection at the Clinic Golnik were included in our county-wide survey. Clinical isolates were retrieved from 70 male and 56 female patients. Fisher's exact test was used to statistically evaluate the data related to clinical relevance. The threshold for statistical significance was set at a P value of < 0.05. All isolates were previously identified with the diagnostic test GenoType CM/AS (Hain Lifescience, Nehren, Germany) as MABSC (n = 31) or *M. intracelullare* (n = 102). The previously used test cannot differentiate subspecies in MABSC and M. intracelullare from the closely related species *M. chimaera*. All investigated isolates were stored at –20°C on glass beads and subcultured on Löwenstein–Jensen medium or Middlebrook 7H10 agar plates. Total DNA was extracted from two loops of mycobacterial culture resuspended in 0,3 mL of sterile water. Cell lysis in the mycobacterial culture was done with incubation at 95°C for 20 minutes followed by sonication for 15 minutes. Samples were centrifuged at maximum speed 14 000 RPM for 5 minutes. Supernatant with the extracted DNA was used for GenoType NTM-DR. PCR protocol and DNA hybridisation, was done according to manufacturer instructions as previously described [6].

Results and Discussion

In Slovenia in the last decade, the number of NTM isolates is increasing [12]. In the period 2000–2016 MAC and MABSC isolates were second and seventh most frequently isolated NTM in Slovenia, respectively. A similar trend — increasing number of NTM's — was noticed in other countries around the world too [10].

Our nation-wide analysis of 102 MAC isolates showed that 53/102 (52%) isolates belonged to *M. intracellulare* and 49/102 (48%) isolates belonged to *M. chimaera*. We can therefore conclude that *M. chimaera* is nearly as common in our country as *M. intracellulare*. Schweickert et al. [9] reported that in Germany almost 86% of previously identified species as *M. intracelullare* are actually *M. chimaera*. Mok et al. [4] reported data from Ireland where 55% of *M. intracelullare* isolates were misidentified and are actually *M. chimaera*. Our study showed that *M. intracelullare* was more often clinically relevant than *M. chimaera* (29% vs. 6% of clinical isolates, respectively). Our obtained results are concordant with results of Schweickert et al. [9] and in contrary with Tortoli et al. [11] who proposed *M. chimaera* strains as more clinically relevant than other MAC species.

Retrospective analysis of Slovenian MABSC isolates from January 2007 to September 2016 showed that predominant species in our country was *M. abscessus* subsp. *abscessus* 24/31 (77.4%), followed by *M. abscessus* subsp. *bolletii* 4/31 (12.9%) and *M. abscessus* subsp. massiliense 3/31 (9.7%). Our results are comparable with other countries in Europe

Table. Isolates of *M. abscessus* complex, *M. intracellulare* and included in the study presented by patients status and clinical relevance

Mycobacterial species	All patients		Cystic fibrosis patients	
	No. of all isolates	No. (%) of CR isolates	No. (%) of all isolates	No. (%) of CR isolates
M. abscessus* subsp. *abscessus	24	7 (29.1)	4 (16.7)	4 (16.7)
M. abscessus* subsp. *massiliense	4	0	0	0
M. abscessus* subsp. *bolletii	3	1 (33.3)	0	0
M. intracellulare	53	15 (28.3)*	0	0
M. chimaera	49	3 (6.1)*	3 (6.1)	0
Total	133	26 (19.5)	7 (5.2)	4 (57.1)

P values obtained following Fisher exact test are indicated by asterisks as follows: * $P < 0.05$; CR: clinically relevant

and in US, where *M. abscessus* subsp. *abscessus* represents around 45–65% of all MABSC isolates [7, 13]. Meanwhile in East Asia, the percentage of MABSC isolates is much higher among all NTM isolates. Furthermore, Asian countries also report *M. abscessus* subsp. *abscessus* as frequently isolated as *M. abscessus* subsp. *massiliense* [3] Our hypothesis was also that *M. abscessus* subsp. *bolletii* is rarest subspecies among MABSC in Slovenia, which would be concordant with results yielded in other studies [3, 7, 13]. Our hypothesis failed, but the number of samples was relatively small so in future more isolates will be need to be tested to confirm it.

Molecular analysis of resistance genes in MABSC showed that all 4/4 (100%) *M. abscessus* subsp. *bolletii*i and 22/24 (92%) *M. abscessus* subsp. *abscessus* had the T polymorphism at position 28 in *erm(41)* gene, which leads to inducible resistance to macrolides. All 3/3 (100%) *M. abscessus* subsp. massiliense isolates also had the T polymorphism in *erm(41)* gene but due to deletion in this gene, isolates did not show inducible resistance to macrolides. No isolate of MABSC had a point mutation in *rrl* gene or in *rrs* gene. Thus, it can be concluded that high percentage of MABSC isolates can develop inducible macrolide resistance but high-level macrolide resistance is not present at the moment. None of Slovenian MABSC isolates from the study had aminoglycoside resistance.

Also in MAC isolates no mutation in *rrl* nor *rrs* gene was detected. Based on this observation it can be concluded that all analysed MAC isolates were sensitive to both, macrolides and aminoglycosides with molecular methods.

Slovenian *M. intracellulare* isolates were found to be statistically significantly more clinical relevant than *M. chimaera*. None of *M. intracellulare* isolates was obtained from CF patient specimens. On the other hand, three of *M. chimaera* isolates were isolated from CF patient specimens, but were not clinically relevant. Higher percentage of *M. abscessus* subsp. *abscessus* isolates were found as clinical relevant, but with no statistical significance ($P > 0,05$). Furthermore, *M. abscessus* subsp. *abscessus* was isolated from CF patients too (see Table).

To sum up, in Slovenia the number of MABSC isolates is slowly increasing, with *M. abscessus* subsp. *abscessus* being predominant subspecies. *M. abscessus* subsp. *abscessus* subspecies is the only subspecies isolated from specimens from patients with CF. Our MABSC isolates have high proportion of inducible resistance to macrolides. This fact needs to be considered when treating patients with MABSC infections, especially CF patients. *M. intracellulare* was a slightly more frequently isolated from human specimens than *M. chimaera*, and was more often clinically relevant in the last 10 years.

Список литературы/References

1. Falkinham J.O. Surrounded by mycobacteria: nontuberculous mycobacteria in the human environment. *J. App. Microbiol., 2009, vol. 107, no. 2, pp. 356–367. doi: 10.1111/j.1365-2672.2009.04161.x*
2. Kehrmann J., Kurt N., Rueger K., Bange F.C., Buer J. GenoType NTM-DR for identifying Mycobacterium abscessus subspecies and determining molecular resistance. *J. Clin. Microbiol., 2016, vol. 54, no. 6, pp. 1653–1655. doi: 10.1128/JCM.00147-16*
3. Kim H.Y., Kook Y., Yun Y.J., Park C.G., Lee N.Y., Shim T.S., Kim B.J., Kook Y.H. Proportions of Mycobacterium massiliense and Mycobacterium bolletii strains among Korean Mycobacterium chelonae-Mycobacterium abscessus group isolates. *J. Clin. Microbiol., 2008, vol. 46, pp. 3384–3390. doi: 10.1128/JCM.00319-08*
4. Mok S., Rogers T.R., Fitzgibbon M. Evaluation of GenoType NTM-DR assay for identification of Mycobacterium chimaera. *J. Clin. Microbiol., 2017, vol. 55, no. 6, pp. 1821–1826. doi: 10.1128/JCM.00009-17*
5. Mougari F., Bouziane F., Crockett F., Nessar R., Chau F., Veziris N., Sapriel G., Raskine L., Cambau E. Selection of resistance to clarithromycin in Mycobacterium abscessus subspecies. *Antimicrob. Agents Chemother., 2016, vol. 61, no. 1, pp. 9. doi: 10.1128/AAC.00943-16*
6. Mougari F., Loiseau J., Veziris N., Bernard C., Bercot B., Sougakoff W., Jarlier V., Raskine L., Cambau E. Evaluation of the new GenoType NTM-DR kit for the molecular detection of antimicrobial resistance in non-tuberculous mycobacteria. *J. Antimicrob. Chemother., 2017, vol. 72, no. 6, pp. 1669–1677. doi: 10.1093/jac/dkx021*
7. Roux A.-L., Catherinot E., Ripoll F., Soismier N., Macheras E., Ravilly S., Bellis G., Vibet M.-A., Le Roux E., Lemonnier L., Gutierrez C., Vincent V., Fauroux B., Rottman M., Guillemot D., Gaillard J.L. Multicenter study of prevalence of nontuberculous mycobacteria in patients with cystic fibrosis in France. *J. Clin. Microbiol., 2009, vol. 47, pp. 4124–4128. doi: 10.1128/JCM.01257-09*
8. Sax H., Bloemberg G., Hasse B., Sommerstein R., Kohler P., Achermann Y., Rössle M., Falk V., Kuster P.S., Böttger C.E., Weber R. Prolonged outbreak of Mycobacterium chimaera infection after open- chest heart surgery. *Clin. Infect. Dis., 2015, vol. 61, no. 1, pp. 67–75. doi: 10.1093/cid/civ198*
9. Schweickert B., Goldenberg O., Richter E., Göbel U. B., Petrich A., Buchholz P., Moter A. Occurrence and clinical relevance of Mycobacterium chimaera sp. nov., Germany. *Emerg. Infect. Dis., 2008, vol. 14, no. 9, pp. 1443–1446. doi: 10.3201/eid1409.071032: 1443-1446*
10. Stout J.E., Koh W.J., Yew W.W. Update on pulmonary disease due to non-tuberculous mycobacteria. *Int. J. Infect. Dis., 2016, vol. 45, pp. 123–134. doi: 10.1016/j.ijid.2016.03.006*
11. Tortoli E., Rindi L., Garcia M.J., Chiaradonna P., Dei R., Garzelli C., Kroppenstedt R.M., Lari N., Mattei R., Mariottini A., Mazzarelli G., Murcia M.I., Nanetti A., Piccoli P., Scarparo C. Proposal to elevate the genetic variant MAC-A, included in the Mycobacterium avium complex, to species rank as Mycobacterium chimaera sp. nov.. *Int. J. Syst. Evol. Microbiol., 2004, vol. 54, no. 4, pp. 1277–1285. doi: 10.1099/ijs.0.02777-0*

12. Van der Werf M.J., Ködmön C., Katalinić-Janković V., Kummik T., Soini H., Richter E., Papaventsis D., Tortoli E., Perrin M., van Soolingen D., Žolnir-Dovč M., Thomsen V.Ø. Inventory study of non-tuberculous mycobacteria in the European Union. *BMC Infect. Dis., 2014, vol. 14, no. 62. doi: 10.1186/1471-2334-14-62*
13. Zelazny A.M., Root J.M., Shea Y.R., Colombo R.E., Shamputa I.C., Stock F., Conlan S., McNulty S., Brown-Elliott B.A., Wallace R.J. Jr., Olivier K.N., Holland S.M., Sampaio E.P. Cohort study of molecular identification and typing of Mycobacterium abscessus, Mycobacterium massiliense, and Mycobacterium bolletii. *J. Clin. Microbiol., 2009, vol. 47, no. 7, pp. 1985–1995. doi: 10.1128/JCM.01688-08*

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СОВРЕМЕННЫЕ ВОЗМОЖНОСТИ И НАПРАВЛЕНИЯ РАЗВИТИЯ МОЛЕКУЛЯРНО-ЭПИДЕМИОЛОГИЧЕСКОГО МОНИТОРИНГА В НАДЗОРЕ ЗА ЭНТЕРОВИРУСНЫМИ ИНФЕКЦИЯМИ. ОПЫТ РОССИЙСКОЙ ФЕДЕРАЦИИ

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Резюме. Энтеровирусы — мелкие РНК-содержащие вирусы, которые распространены повсеместно и регулярно становятся причиной вспышек заболеваемости с различной симптоматикой. В Российской Федерации с 2006 г. действует надзор за энтеровирусами. За эти годы в России и в мире были отработаны молекулярно-биологические и биоинформатические инструменты для изучения эпидемиологии энтеровирусов. В настоящее время идентификация энтеровирусов в мире осуществляется практически исключительно на основании анализа нуклеотидной последовательности области генома VP1 (около 900 нуклеотидов). При этом в рутинной работе определяется только фрагмент этой области генома (около 300 нуклеотидов). В большинстве случаев этого достаточно, чтобы достоверно типировать вирус, однако точность анализа короткого участка генома ниже, и достоверным критерием типа для короткого фрагмента следует считать 80% сходства нуклеотидной последовательности, а не 75%, как в случае полной области генома VP1. Для даль-

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нейшего анализа полученных нуклеотидных последовательностей в настоящее время широко применяются Байесовы филогенетические методики, которые позволяют использовать метод молекулярных часов в расследовании вспышек заболеваемости. Энтеровирусы накапливают порядка 0,5–1% нуклеотидных замен в год, поэтому даже короткий фрагмент генома позволяет анализировать филодинамику вирусов на уровне передачи между странами или смены циркулирующего варианта вируса. На более короткой временной шкале короткий фрагмент ограниченно пригоден для изучения молекулярной эпидемиологии, поскольку позволяет достоверно различать не более 1–2 случаев передачи вируса в год. Таким образом, для расследования вспышек желательно определение нуклеотидной последовательности полной области генома VP1 или полногеномной последовательности. В результате анализа имеющихся в базах данных нуклеотидных последовательностей энтеровирусов все более очевидными становятся ограничения, связанные с неравномерной эффективностью надзора в разных странах мира и короткой длиной фрагмента генома, определяемой при рутинном надзоре. Как следствие, полноценный анализ молекулярной эпидемиологии энтеровирусов в глобальном масштабе остается проблематичным. Количество известных нуклеотидных последовательностей энтеровирусов за последние 20 лет выросло в сотни раз, однако понимание закономерностей возникновения вспышек энтеровирусной инфекции практически отсутствует. Эффективное развитие надзора за энтеровирусами потребует внедрения новых методов исследования сточных вод, экономически эффективного высокопроизводительного секвенирования, гармонизации систем надзора в разных странах мира.

Ключевые слова: *энтеровирус, менингит, эпидемиология, надзор, филогенетика, эволюция.*

CURRENT POSSIBILITIES AND POTENTIAL DEVELOPMENT OF MOLECULAR ENTEROVIRUS SURVEILLANCE. EXPERIENCE OF RUSSIAN FEDERATION

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Abstract. Enteroviruses are small RNA viruses, which are ubiquitous and commonly cause outbreaks with various clinical manifestations. In 2006, the Program on enterovirus surveillance was approved in the Russian Federation. Over the last years, molecular-biological and bioinformatics methods for enterovirus epidemiology studies have been developed both in Russia and worldwide. Currently, identification of enteroviruses is carried out by analyzing nucleotide sequence of the full-length VP1 genome region (ca. 900 nt). Routinely, it is sufficient to obtain a partial VP1 genome region sequence (ca. 300 bp) for enteroviruse verification in most cases; however, a more stringent type criterion of 80% sequence identity should be used compared to the 75% sequence identity cut-off for the complete VP1 genome region. Further sequence analysis may be performed by using Bayesian phylogenetic methods, which allow using molecular clock to trace outbreak emergence. Enteroviruses accumulate about 0.5–1% nucleotide substitutions per year. Therefore, a short genome fragment may be used to analyze virus phylodynamics at the level of international transfers and circulating virus variants. On a shorter timescale, a full-length VP1 genome region or a complete genome sequence are preferred for investigating molecular epidemiology, because a short sequence allows to reliably distinguish not more than 1–2 transmission events per year. Thus, determining enterovirus sequences for full-length VP1 genome region or full-genome sequence is preferred for examining viral outbreaks. It is increasingly apparent that analyzing available enterovirus nucleotide sequences reveals limitations related to uneven surveillance efficacy in various countries and short length of genome fragment measured in routine control. As a result, a proper global-scale analysis of enterovirus molecular epidemiology remains problematic. Over the last 20 years, the number of available enterovirus nucleotide sequences increased by hundred times, but understanding emergence of enterovirus infection outbreaks remains limited. Further development of enterovirus surveillance would require new methods for sewage monitoring, affordable high-throughput sequencing and harmonization of global surveillance systems.

Key words: *enterovirus, meningitis, epidemiology, surveillance, phylogenetics, evolution.*

Энтеровирусы — мелкие безоболочечные РНК-содержащие вирусы, входящие в семейство *Picornaviridae*, род *Enterovirus*. В соответствии с недавними решениями Международного комитета по таксономии вирусов (ICTV, International Committee on Taxonomy of Viruses) энтеровирусы подразделяют на 13 видов: Enterovirus A-J и Rhinovirus A-C. У человека выделяют виды Enterovirus A-D и Rhinovirus A-C. Данный обзор посвящен только собственно энтеровирусам человека, то есть видам Enterovirus A-D. Используемое ранее название Human enterovirus более не применяется в связи с тем, что представители видов A-D регулярно обнаруживаются у приматов. Поскольку в последние годы практически все энтеровирусы идентифицируют и относят к известным или новым типам на основании анализа нуклеотидной последовательности, вместо применяемого ранее термина «серотип» рекомендовано использовать термин «тип». На сегодняшний день известно более 120 типов энтеровирусов видов A-D. По современным правилам типы энтеровирусов обозначают с указанием вида, например, «энтеровирус A-71». Исторически некоторые типы называют вирусами Коксаки A и B (например, КВ-А2/CV-A2 и КВ-Б3/CV-B3) и вирусами ECHO (например, E11).

Энтеровирусы распространены повсеместно. Энтеровирусная инфекция (ЭВИ) может протекать бессимптомно, о чем свидетельствует регулярное выделение энтеровирусов от здоровых лиц. В зависимости от страны и времени года частота выявления энтеровирусов в фекалиях здоровых детей может достигать 20% [18, 35, 39]. В случае развития клинической ЭВИ чаще всего наблюдается легкое фебрильное (простудоподобное) заболевание без ярко выраженной специфической симптоматики, в связи с чем большинство таких случаев лечится амбулаторно, не диагностируется и не регистрируется как ЭВИ. Обычно госпитализируют больных с экзантемными формами ЭВИ (чаще всего везикулярный дерматит), герпетической ангиной (везикулярный тонзиллит или фарингит), серозным менингитом. В странах умеренного климата энтеровирусы являются основной причиной вирусного менингита. В редких случаях энтеровирусы могут вызывать менингоэнцефалит, тяжелые поражения сердца и легких, мультисистемную инфекцию новорожденных, клинически сходную с сепсисом. ЭВИ может проявляться в виде спорадических случаев и вспышек, но периодически на отдельных территориях формируются эпидемические подъемы заболеваемости.

Энтеровирусы являются важным источником возникающих заболеваний. В конце XIX в. началась пандемия паралитического полиомиелита. В 1970 г. имела место пандемия геморрагического конъюнктивита, вызванного энтеровирусом 70 типа [32, 40]. В 1980-е гг. в РФ произошли крупные вспышки энтеровирусного увеита, вызванного E11 и E19 [2]. Начиная с конца XX в. в Восточной и Юго-Восточной Азии продолжается пандемия вызванного EV-A71 везикулярного дерматита, который у некоторых больных протекает с неврологическими осложнениями (менингоэнцефалит) [23]. В настоящее время в России регулярно происходят вспышки серозного менингита, чаще всего вызываемого E30. Начиная с 2010 г. в мире распространяются атипичные формы инфекции CV-A6, протекающие с необычными кожными проявлениями [33].

С 2008 г. в Российской Федерации реализуется программа «Эпидемиологический надзор и профилактика энтеровирусной (неполио) инфекции». После сертификации в 2002 г. Российской Федерации как страны, свободной от полиомиелита [9], надзор за циркуляцией энтеровирусов рассматривается как важная дополнительная составляющая надзора за полиомиелитом и острыми вялыми параличами. Рост заболеваемости ЭВИ в отдельных субъектах и в целом по РФ, наличие сезонных подъемов заболеваемости, регистрация эпидемических очагов с групповыми случаями заболеваний, высокий риск завоза высоковирулентных штаммов определяют самостоятельную актуальность надзора за энтеровирусами для Российской Федерации.

В настоящее время надзор за энтеровирусной инфекцией представляет собой непрерывное наблюдение за эпидемическим процессом с целью оценки ситуации, своевременного принятия управленческих решений, разработки и реализации санитарно-противоэпидемических (профилактических) мероприятий, обеспечивающих снижение рисков распространения ЭВИ, предупреждения тяжелых форм ЭВИ и формирования очагов с множественными случаями заболеваний. Неотъемлемыми составляющими эффективного надзора являются точная идентификация энтеровирусов и информативные молекулярно-эпидемиологические исследования.

Первичная диагностика энтеровирусов у больных и в объектах внешней среды проводится на местах на базе лабораторий медицинских учреждений и ФБУЗ «Центров гигиены и эпидемиологии в субъектах РФ», преимущественно методом ПЦР.

Классическим и пока еще достаточно широко применяемым методом идентификации энтеровирусов в вирусологических лабораториях РФ является определение серотипа вируса в реакции нейтрализации с помощью типоспецифических диагностических сывороток. Главные недостатки этого метода — низкая цитопатогенность некоторых типов и недостаточная специфичность, приводящая к ошибочной идентификации типа вируса. Проблема специфичности практически не актуальна для сывороток, изготовленных в США и в Голландии, однако на сегодняшний день их производство прекращено. Молекулярная идентификация (определение нуклеотидной последовательнос-

ти фрагмента генома, кодирующего структурные белки) позволяет определить тип и подтип (генотип) как при исследовании клинических образцов, так и вирусов, выделенных в культуре клеток. Молекулярная идентификация применяется все чаще, однако в полной мере доступна только нескольким специализированным центрам, на базе которых ежегодно в целом по РФ исследуется около 2000–2500 вируссодержащих образцов. На первом этапе формирования системы молекулярно-генетического мониторинга энтеровирусов происходила отработка методик и накопление первичных данных. В данном обзоре рассмотрены основные методические подходы, применяемые при анализе генетической информации энтеровирусов, их возможности, ограничения и перспективы развития.

По итогам десятилетнего молекулярного мониторинга только в референс-центре по мониторингу энтеровирусных инфекций (Нижегородский НИИ эпидемиологии и микробиологии имени академика И.Н. Блохиной) было типировано около 3500 штаммов энтеровирусов. Идентифицировано 52 типа неполиомиелитных ЭВ: вирусы вида Энтеровирус А: Коксаки А2–6, 8, 10, 14, 16, EV-A71, EV-A76, EV-A120; вирусы вида Энтеровирус В: Коксаки А9, Коксаки В1–5, ECHO 1–7, 9, 11, 13–19, 21, 25, 29, 30, 31, 33, ЭВВ75; вирусы вида Энтеровирус С: Коксаки А1, 13, 17, 19, 20, 21, 22, 24, EV-C99, EV-C113, ЭВС116. Ежегодно выявлялось около 30-ти типов неполиомиелитных ЭВ, при этом многие типы энтеровирусов характеризовались наличием нескольких одновременно циркулирующих геновариантов. Установлены этиологические агенты 161 группового заболевания ЭВИ [1].

На основе данных, опубликованных в 1999 г. [30], Международный комитет по таксономии вирусов определяет тип энтеровируса на основании сходства с другими вирусами типа более чем на 75% нуклеотидной и 85% аминокислотной последовательности в полной области генома VP1 (около 900 нуклеотидов, от 840 до 930 у разных типов). Благодаря тому, что рекомбинация в области генома, кодирующей структурные белки VP1, VP2 и VP3 (но не VP4) встречается редко [4, 20, 21], любой из этих участков генома может быть использован для типирования энтеровирусов [5, 27], однако точные критерии типа будут несколько отличаться из-за разной вариабельности этих участков генома. На практике молекулярное типирование энтеровирусов в большинстве лабораторий мира и в РФ проводят на основании фрагмента области генома VP1 длиной около 370 нуклеотидов (так называемый «типирующий» фрагмент), который может быть амплифицирован с использованием универсальных праймеров [29]. Для этого фрагмента генома в базе данных GenBank доступно более 30 000 нуклеотидных последовательностей. Использование других структурных белков, например, VP2, затрудняет последующий анализ из-за небольшого количества последовательностей этих участков генома в базе данных GenBank (около 4000) и с неравномерным распределением по типам. Определение типа удобно производить на основании сравнения выявленной нуклеотидной последовательности с базой данных GenBank при помощи поискового алгоритма BLAST. При использовании такого подхода необходимо, чтобы перекрытие типируемой последовательности с последовательностями из GenBank (Query coverage) было не менее 90%. Только в таком случае возможно интерпретировать результат. При использовании для типирования программ для филогенетического анализа необходимо использовать нескорректированные дистанции (p-distance). Использование любой коррекции или модели замен, которые применяются, например, для филогенетического анализа (модели Jukes-Cantor, Kimura, GTR и т.п.) делает невозможным применение указанных критериев.

На сегодняшний день нет четкого критерия типа для этого короткого фрагмента VP1, поскольку вариабельность в полной и частичной области VP1 может несколько различаться. Для определения такого критерия мы построили распределение попарных нуклеотидных дистанций в полной области генома VP1 и в области типирующего фрагмента для 34 наиболее распространенных типов энтеровирусов (рис. 1А, Б; рис. 2А, Б). В полной области генома VP1 внутритиповые сходства нуклеотидных последовательностей находятся в пределах 72–100% (рис. 1В), а аминокислотных — в пределах 84–100% (рис. 2В). Более 99% попарных сравнений находятся в пределах предложенных 20 лет назад критериев типа — более 85% сходства аминокислотной и более 75% нуклеотидной последовательности [30]. Таким образом, за последние 20 лет данные значения практически не изменились, несмотря на приблизительно 100-кратное увеличение числа доступных последовательностей энтеровирусов и постоянное накопление мутаций [22]. В типирующем фрагменте наблюдается сходная картина, однако граница между дистанциями внутри и между типами не такая выраженная (рис. 1Б, Г; рис. 2Б, Г). Из почти что 7 млн проанализированных попарных различий энтеровирусов в типирующем фрагменте значения сходств при внутритиповых сравнениях были не ниже 72% для нуклеотидных и 84% аминокислотных последовательностей вирусов одного типа (рис. 1Г, рис. 2Г). Однако межтиповые сходства между короткими фрагментами VP1 разных типов достигали 78 и 89% для нуклеотидных и аминокислотных последовательностей, соответственно, то есть между 72–78% сходства нуклеотидной и 84–89% аминокислотной последовательности существует «серая зона» значений, в которой достоверное определение типа невозможно. Таким образом, при использовании короткого типирующего фрагмента сходство более 80% нуклеотидной и более 90% аминокислот-

ной последовательности с известными вирусами позволяет уверенно определить тип образца. В спорных случаях, например, для некоторых типов вида Энтеровирус C [8], в случае верификации открытия ЭВ нового типа или обнаружения нового генотипа известного типа, рекомендовано определение полной нуклеотидной последовательности области генома VP1, причем желательно рассматривать сходство как нуклеотидной, так и аминокислотной последовательности. В случае попадания попарных сходств между полными последовательностями VP1 предположительно нового типа вируса в «серую зону» (72–75% для полной нуклеотидной последовательности VP1 и 84–87% для аминокислотной последовательности) желательно экспериментальное подтверждение принадлежности вируса к новому типу с помощью серологических методов.

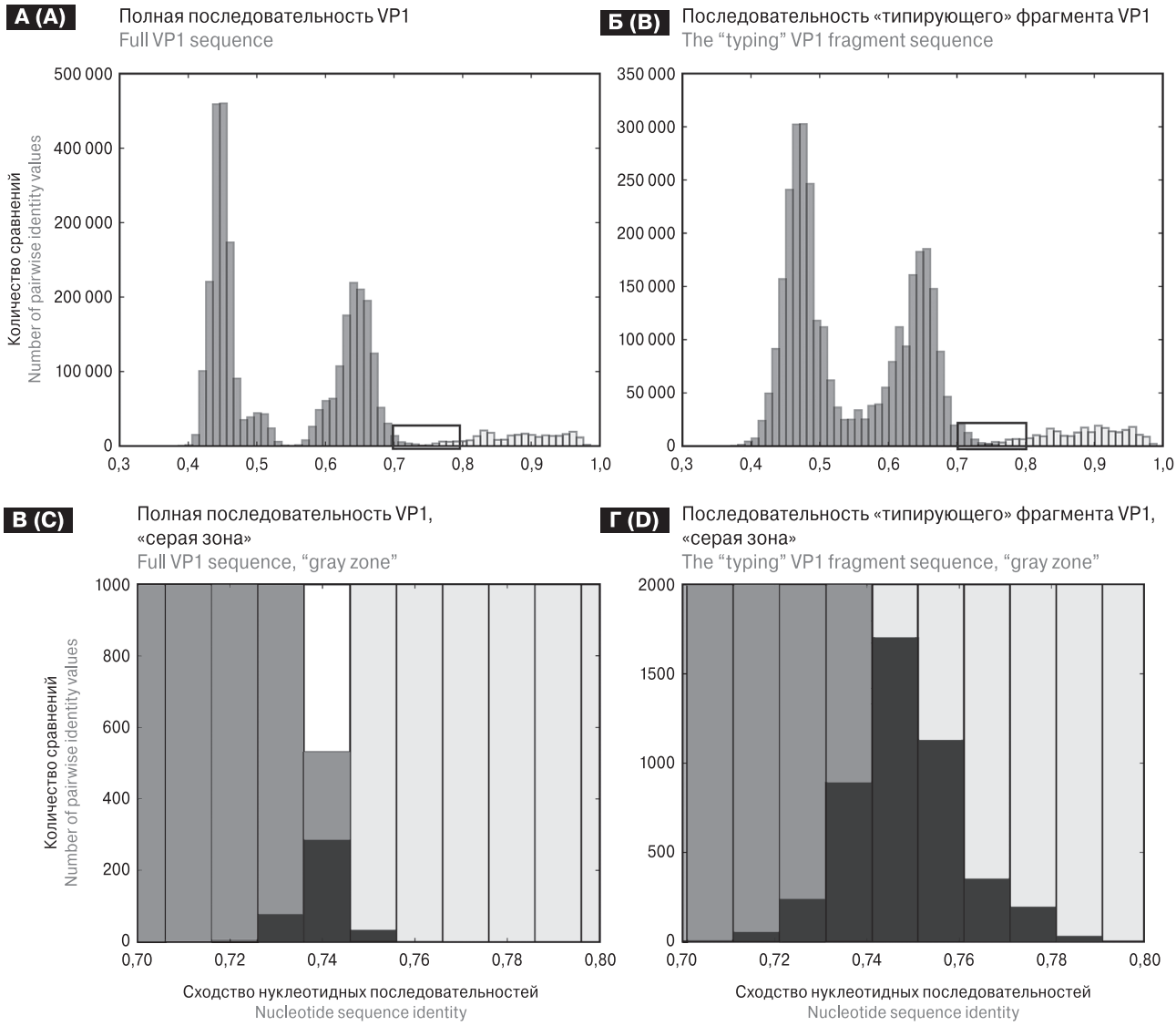

Рисунок 1. Гистограммы распределений значений попарных сходств между нуклеотидными последовательностями полных (А, В) и коротких (Б, Г) участков VP1 для 34 наиболее распространенных типов энтеровирусов

Figure 1. Distribution of pairwise identity values between nucleotide sequences of full-length (A, C) and short (B, D) VP1 fragments derived from most common 34 types of enteroviruses

Примечание. Рисунки В и Г являются фрагментами рисунков А и Б соответственно в области границы межтиповых и внутритиповых сравнений. Темно-серым и светло-серым цветом показаны значения межтипового и внутритипового попарного сходства соответственно. На рисунках В и Г черным цветом показаны перекрывающиеся значения сходств, которые находятся в «серой зоне». Матрицы попарного сходства между 2615 последовательностями энтеровирусов были рассчитаны в программе MEGA6 [37].

Note. Panels C and D represent magnified fragments of panels A and B, respectively, within the range of Intertypic and intratypic comparisons. Intertypic and intratypic similarity values are shown in dark-gray and light-gray, respectively. Panels C and D highlight in black the overlapping values presented in "gray zone". Matrices of pairwise similarity values among 2615 sequences were calculated by using MEGA6 software [37].

Важным преимуществом молекулярного типирования является возможность проведения филогенетического анализа полученных вирусов. В случае энтеровирусов одного типа для анализа используется нуклеотидная, а не аминокислотная последовательность, поскольку нуклеотидные замены накапливаются приблизительно в 10 раз быстрее. Филогенетический анализ помогает выяснить источники заноса вируса и пути распространения, проследить развитие вспышки или эпидемии. Простота получения и анализа нуклеотидных последовательностей позволяют проводить такой анализ пользователям с минимальной квалификацией, причем методы не содержат механизмов контроля качества полученных последовательностей, а критерии достоверности оценивают значимость филогенетических взаимоотношений, но не их последующей интерпретации. Ниже мы рассмотрим основные ограничения для филогенетического анализа энтеровирусов.

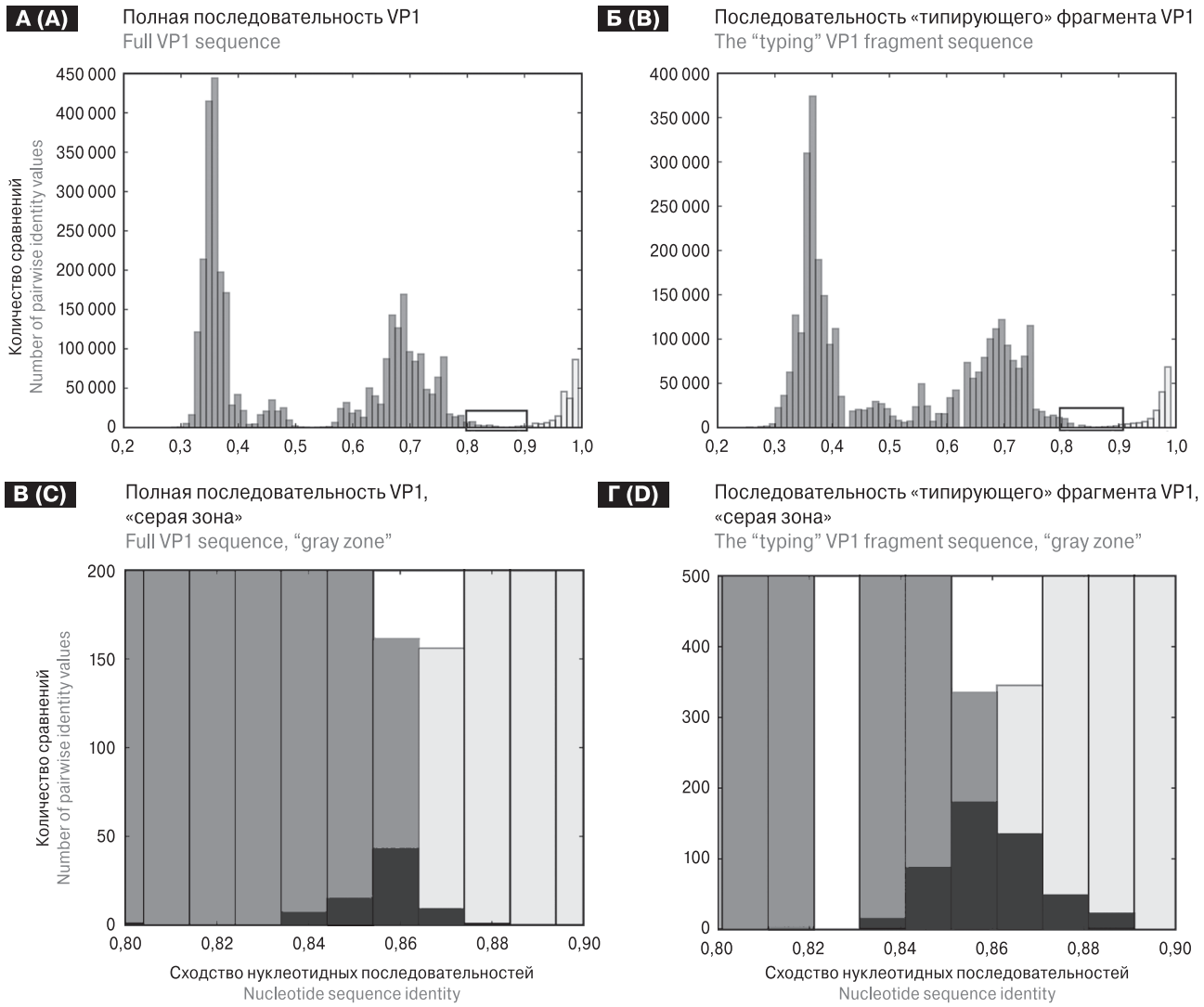

Рисунок 2. Гистограммы распределений значений попарных сходств между аминокислотными последовательностями полных (А, В) и коротких (Б, Г) участков VP1 для 34 наиболее распространенных типов энтеровирусов

Figure 2. Distribution of pairwise identity values between amino acid sequences of full-length (A, C) and short (B, D) VP1 fragments derived from most common 34 types of enteroviruses

Примечание. Рисунки В и Г являются фрагментами рисунков А и Б соответственно в области границы межтиповых и внутритиповых сравнений. Темно-серым и светло-серым цветом показаны значения межтипового и внутритипового попарного сходства соответственно. На рисунках В и Г черным цветом показаны перекрывающиеся значения сходств, которые находятся в «серой зоне». Матрицы попарного сходства между 2615 последовательностями энтеровирусов были рассчитаны в программе MEGA6 [37].

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На сегодняшний день (май 2018 г.) в базе данных GenBank представлено около 53 500 нуклеотидных последовательностей энтеровирусов A-D. Около 39 000 из них относятся к области генома VP1. Распределение известных нуклеотидных последовательностей по времени и месту выделения крайне неравномерное. Большая часть вирусов была выделена после 2000 г. (рис. 3) в Китае, Франции, Японии, США, Индии, Нидерландах, РФ — странах, которые проводят активный надзор за энтеровирусами (табл. 1).

При этом даже в пределах указанных стран существуют значительные различия между представленными типами и годами выделения. Для многих типов филогенетический анализ неинформативен из-за крайне неоднородной выборки доступных в базе данных GenBank нуклеотидных последовательностей. Например, 33 из 35 известных последовательностей EV-B85 были выделены в Китае в 2011 г.

На сегодняшний день в большинстве стран депонирование последовательностей вирусов при рутинном надзоре не практикуется, и доступны в первую очередь массивы данных для отдельных типов, полученные в результате научных исследований. Более того, количество последовательностей не в полной мере отражает их информативность, так как возможно депонирование большого количества практически идентичных последовательностей, полученных, например, во время расследования крупных вспышек заболеваемости. Таким образом, при сравнении полученной последовательности можно определить ее сходство только с известными вирусами. Это объясняет, почему в литературе часто обсуждаются заносы энтеровирусов из Европы и Китая, но не из других стран, в которых не ведется систематического секвенирования энтеровирусов и депонирования данных в базу данных GenBank. Точно так же выводы

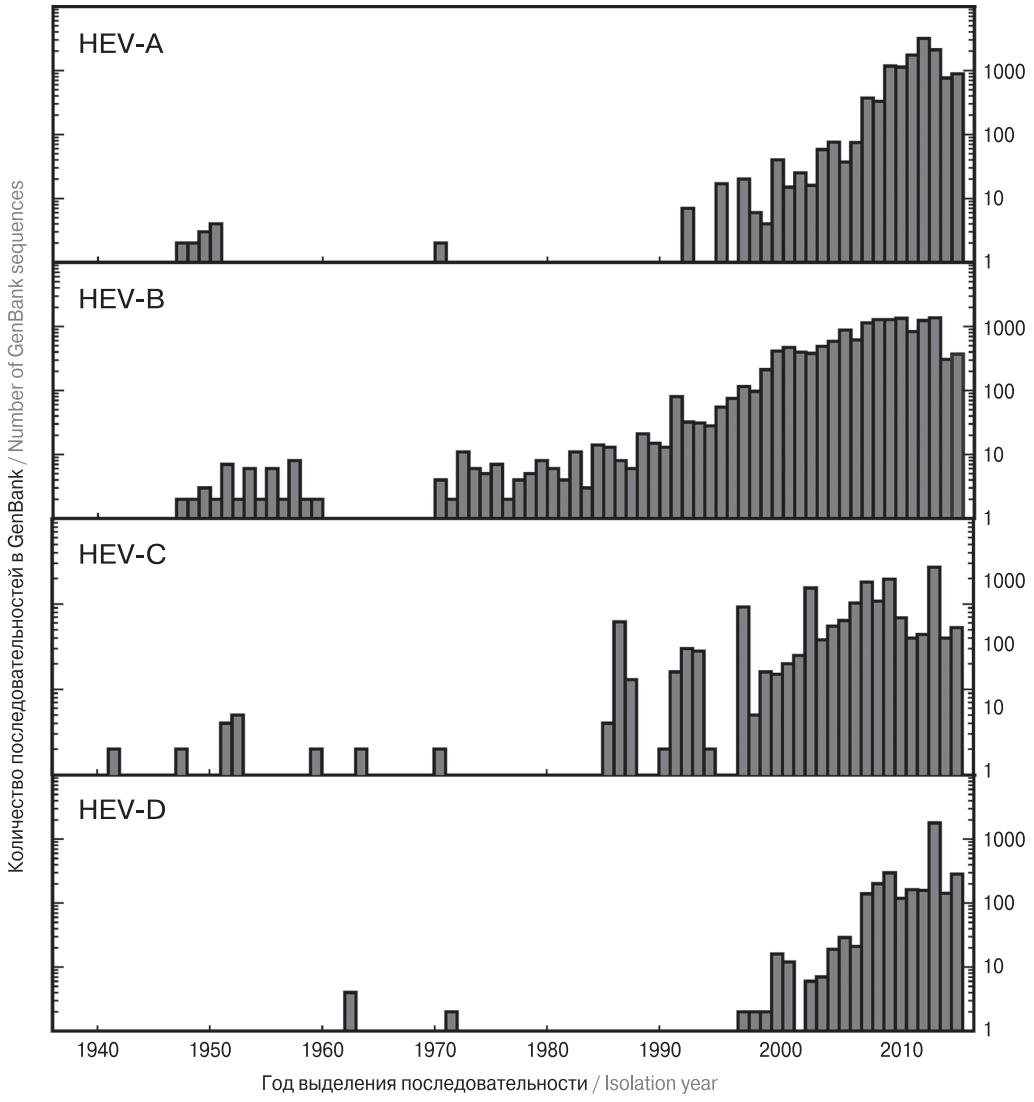

Рисунок 3. Количество депонированных в базе данных GenBank последовательностей энтеровирусов, выделенных в разные годы, с 1940 по 2017 гг. [22]

Figure 3. The number of enterovirus sequences isolated within 1940–2017 deposited in the GenBank [22]

о формировании в странах с высокой плотностью надзора эндемичных штаммов, которые не выявлялись в других странах, могут быть связаны с неравномерностью надзора в мире, а не с истинной эпидемиологией вирусов.

Для наиболее распространенных типов энтеровирусов количество известных нуклеотидных последовательностей исчисляется сотнями или тысячами. Филогенетический анализ сразу всех известных сиквенсов в таком случае будет и затруднен технически и ограниченно информативен. Выбор референсных сиквенсов для филогенетического анализа имеет ключевое значение для получения максимально достоверных результатов. При ручном выборе референсных последовательностей существует риск серьезного искажения результатов. Оптимальным подходом может быть исключение из анализа слишком сходных референсных последовательностей, причем критерии для исключения могут быть разными для различных групп вирусов. Например, при расследовании вспышки целесообразно включать все последовательности, близкие к выделенному во время вспышки вирусу, а для более отличных вирусов (например, отличающихся от исследуемого изолята более чем на 5%) исключить «повторные» последовательности, которые отличаются друг от друга менее чем на 2–5%. Каких-либо универсальных стандартов и алгоритмов подбора референсных нуклеотидных последовательностей для анализа не существует, хотя именно этот этап анализа является определяющим для его достоверности. На сегодняшний день подготовка репрезентативного набора сиквенсов для сравнения является более сложной задачей (как технически, так и с точки зрения эпидемиологии и эволюции), нежели собственно филогенетический анализ.

Качество депонированных в GenBank данных в целом высокое, однако встречаются последовательности (менее 1%) с очевидными ошибками секвенирования или аннотации (даты, места выделения, типа). Исключение таких последовательностей является важным этапом для дальнейшего анализа, особенно с использованием Байесовых филогенетических методов, поскольку даже несколько ошибочных последовательностей могут значительно исказить все результаты расчетов. Признаками ошибочных последовательностей являются несоответствие соотношения процента синонимических и несинонимических замен наблюдаемому в среднем в VP1, группы последовательных аминокислотных замен в относительно консервативных участках генома, непропорционально длинные ветви на филогенетическом дереве, скорость накопления замен (rate) на отдельных ветвях дерева, значительно отличающаяся от средней для данного филогенетического дерева и для VP1 в среднем (6×10^{-3} — 12×10^{-3} замен на сайт в год, табл. 2).

Таблица 1. Количество доступных в базе данных GenBank последовательностей неполиомиелитных энтеровирусов, выделенных в разных странах

Table 1. Number of non-polio enterovirus sequences isolated in various countries available in the GenBank database

Страна Country	**Число последовательностей в базе данных GenBank** The number of GenBank entries
Китай China	16 525 (+2506 — Тайвань/Taiwan)
Франция France	2795
Япония Japan	2777
Индия India	2069
Нидерланды Netherlands	1987
Таиланд Thailand	1758
Россия Russia	1679
США USA	1468
Прочие страны либо страна не указана Other countries	20 606

Благодаря достаточно высокой и предсказуемой скорости накопления нуклеотидных замен у энтеровирусов даже на основании процента сходства нуклеотидной последовательности можно выполнить грубую оценку источника и времени происхождения вируса. Следует принимать во внимание, что скорость накопления замен может различаться в 1,5–2 раза между разными генотипами одного типа [25]. Кроме того, после того как два вируса имели общего предка, накапливали замены оба, то есть процент замен между вирусами отражает суммарное время от общего предка до каждого из вирусов. Например, если два вируса, выделенные в 2010 г., различаются на 4% нуклеотидной последовательности в области генома VP1, то их общий предок скорее всего существовал в 2007–2008 гг. (при скорости накопления замен каждым вирусом 0,6 или 1% в год соответственно).

Удобным подходом для быстрой оценки молекулярной эпидемиологии энтеровирусов является определение генотипов внутри типа. Для наиболее распространенных типов, таких как E11 [31], E30 [7], EV-A71 [23], существуют устоявшиеся системы генотипов. Использование генотипов значительно облегчает описание результатов и взаимодействие между исследователями. У некоторых типов, например, E11, EV-A71, генотипы четко отделены друг от друга на филогенетическом дереве, хотя определять генотип

Таблица 2. Скорости замен для наиболее распространенных типов [22]

Table 2. Nucleotide substitution rates for the most common enterovirus strains [22]

Тип Serotype	**Скорость замен, $\times 10^{-3}$ замен/сайт/год** Substitution rate, $\times 10^{-3}$ substitutions/site/year	**95% доверительный интервал, $\times 10^{-3}$ замен/сайт/год** 95% confidence interval, $\times 10^{-3}$ substitutions/site/year
CV-A6	9,0	7,2–11,2
CV-A10	8,3	6,3–10,3
CV-A16	8,2	6,6–9,9
CV-A20	4,1	2,2 -6,1
CV-B1	9,1	7,0–11,3
CV-B3	6,8	5,5–8,0
CV-B4	6,7	5,5–8,0
CV-B5	7,7	6,6–8.9
E-11	6,5	5,4–7,7
E-1	2,2	8,4–16,3
E-30	7,5	6,4–8,6
E-6	9,6	7,9–11,1
E-9 [26]	5,8	3,7–8,1
EV-A71	6,0	5,5–6,5

только на основании степени отличия нуклеотидной последовательности не всегда возможно. У E30, напротив, определение генотипов в ряде случаев, особенно для архивных штаммов, носит субъективный характер [7]. Универсальных критериев генотипа не существует. У приблизительно половины типов существуют четко ограниченные филогенетические подгруппы. Для таких типов характерно мультимодальное распределение внутритиповых значений попарных сходств (различий) со множеством пиков, что позволяет определить критерий генотипа, однако у различных типов эти критерии будут разными, в пределах 12–20% различия нуклеотидной последовательности области генома VP1 [22]. У другой части типов не наблюдается мультимодального распределения попарных дистанций внутри типа. В этих случаях невозможно определить количественные критерии генотипов в виде фиксированного процента сходства/различия нуклеотидной последовательности, однако возможно определение генотипов на основе филогенетического группирования для облегчения обсуждения результатов.

Для более точной оценки филодинамики энтеровирусов в настоящее время широко используют Байесовы филогенетические методы [11], которые стали доступны только в 2007 г. В случае достаточно большого объема исходных данных метод является вполне воспроизводимым и позволяет получить ответ о времени существования последнего общего предка вирусов с учетом 95% доверительного интервала. Например, расследование вспышки менингоэнцефалита, вызванного ЭВ71 в Ростове-на-Дону в 2013 г., выявило, что вирус, скорее всего, был занесен на территорию РФ из Китая за 3 года до вспышки и затем циркулировал в РФ, не вызывая значимых вспышек заболеваемости [6]. За эти три года нет свидетельств передачи этой линии вируса из Китая, однако, поскольку количество сиквенсов из большинства стран Азии остается недостаточным, не исключено, что этот вирус циркулировал не только в РФ, но и «где угодно, кроме Китая и Европы».

Байесовы филогенетические методы также позволяют исследовать филогеографию вирусов [17]. В этом случае метод Монте-Карло с Марковскими цепями используется для оптимизации расчета филогенетических взаимоотношений геномных последовательностей во времени и в пространстве. Расчет филогеографии хорошо работает для зоонозов, таких как бешенство [17]. В случае энтеровирусов его применение в значительной степени ограничено лимитом филогенетического разрешения.

Теоретическая возможность различить 2 штамма и достоверно оценить их филогенетические взаимоотношения определяется скоростью накопления замен и длиной известной нуклеотидной последовательности. При скорости накопления замен 0,6–1,2% в год в участке генома длиной 300 нуклеотидов можно ожидать появление 2–4 замен за год. Таким образом, анализ «типирующего» фрагмента VP1 теоретически позволяет выявить не более 2–4 эволюционных событий в год. Этого достаточно для оценки циркуляции вирусов в масштабах страны, но недостаточно для расследования филодинамики вирусов, например, внутри вспышки. Даже полная последовательность области генома VP1 (около 900 нт) теоретически позволяет определять не более 5–15 событий в год и в целом подходит только для расследования крупных вспышек, например, вспышки полиомиелита в Таджикистане в 2010 г. [41]. Учитывая то, что длительность одного цикла энтеровирусной инфекции составляет порядка 3–10 дней, для достоверного расследования распространения вируса во время вспышек может быть рекомендовано определение полной нуклеотидной последовательности. На сегодняшний день в системе надзора за энтеровирусами в РФ таких работ на регулярной основе не ведется.

Проблема разрешения филогенетических методов неотъемлемо связана с вопросом необходимой плотности молекулярно-генетического надзора за энтеровирусами. Анализ филогенетических последовательностей позволяет оценить адекватность существующей системы надзора. Целью молекулярно-эпидемиологического надзора можно считать полноценное представление обо всех вариантах энтеровирусов, циркулирующих на территории, и путях их распространения. Частота обнаружения разных типов различается в сотни раз и в РФ (http://www.nniiem.

ru/development/informanalit/evi.html), и в других странах [15, 38]. Более того, внутри типов возможно существование редких вариантов вирусов, которые в течение десятилетий не выявлялись даже при достаточно интенсивном надзоре [42]. С точки зрения выявления новых вариантов вирусов, плотность действующего надзора все еще нельзя признать адекватной. Этот показатель можно будет считать достигнутым, когда любой редкий генотип, присутствующий на территории РФ, будет представлен как минимум десятью образцами. На сегодняшний день определение большого количества нуклеотидных последовательностей практически идентичных вирусов нецелесообразно экономически, поскольку минимальную стоимость типирования одного вируса, без учета трудозатрат и накладных расходов, можно оценить в 3000 рублей.

Целью профилактических мероприятий, разработанных на основе данных эпидемиологического надзора за ЭВИ, является снижение рисков формирования и распространения очагов с множественными случаями заболеваний. Эта цель является, вероятно, наиболее труднодостижимой. Прогнозирование в настоящее время опирается на анализ ретроспективной заболеваемости. Очевидно, что прогнозирование должно базироваться на анализе множественных факторов, таких как заболеваемость в предшествующие периоды, данные молекулярно-эпидемиологического мониторинга, показатели коллективного иммунитета.

На сегодняшний день в мире опубликовано достаточно большое количество работ, посвященных возможным причинам и механизмам возникновения вспышек и эпидемий энтеровирусной инфекции, однако целостного понимания этого процесса на сегодняшний день нет. К числу факторов, которые могут быть ассоциированы с возникновением вспышек энтеровирусной инфекции, относят колебание уровня коллективного иммунитета, климатические и социальные факторы. Изменение активности циркуляции вируса, связанное с сезонностью энтеровирусной инфекции (в РФ подъем заболеваемости с пиком в июле–сентябре) может способствовать возникновению и исчезновению из циркуляции новых вариантов энтеровирусов. Показано, что этот процесс происходит даже в пределах одного типа. Например, рекомбинантные формы E30 регулярно появлялись, получали широкое распространение в течение одного или нескольких сезонов и самопроизвольно исчезали из циркуляции в Европе [24]. В целом можно сказать, что случайные факторы могут иметь не меньшее значение для эпидемиологии вируса, чем коллективный иммунитет.

Роль генетических характеристик вируса в возникновении вспышек заболеваемости остается неясной. Во время эпидемии везикулярной экзантемы в Китае в 2008–2011 гг. EV-A71 был основным циркулирующим вирусом, но более чем в половине случаев от больных с экзантемой выделяли CV-A16 и другие типы [19]. В других странах Юго-Восточной Азии вспышки EV-A71 могли быть вызваны в разные годы различными генотипами вируса (B и C), при этом принципиальных различий клинической картины выявлено не было [23]. Во время вспышки серозного менингита в Хабаровске в 2006 г. от больных чаще всего выделяли E6, однако у части лиц предполагаемым возбудителем менингита был E30, при этом явных различий в течении заболевания, вызванного вирусами разных типов не наблюдалось. Более того, филогенетический анализ показал, что выделенные от больных варианты E6 в ряде случаев имели общего предка за много лет до вспышки, то есть не были связаны между собой эпидемиологически [4]. Кроме того, в ряде работ описана циркуляция варианта вируса, вызвавшего вспышку, до и после нее [6, 13, 34], что также ставит под сомнение роль конкретных мутаций в развитии вспышек.

С другой стороны, есть много примеров указывающих на то, что во время вспышек вирус может получать способность намного чаще вызывать симптоматическую инфекцию или атипичное заболевание. Так, во время вспышки менингоэнцефалита, вызванного EV-A71 в Ростове-на-Дону в 2013 г., 32% (25 из 78) детей с симптомами энтеровирусной инфекции имели неврологические симптомы [6]. Такая частота нейроинфекции резко контрастирует с частотой нейроинфекции в 0,3% от 129 000 детей с везикулярной экзантемой во время вспышки, вызванной EV-A71 на Тайване в 1998 г. [14]. В другой ситуации возникновение новой клинической разновидности экзантемы, вызванной CV-A6, было связано с новым вариантом вируса, который появился незадолго до выявления первых случаев [12]. В подобных ситуациях роль генетических детерминант вирулентности представляется весьма вероятной. В то же время до сегодняшнего дня не опубликовано работ, достоверно доказывающих роль конкретных мутаций в возникновении вспышек заболеваемости. В случае вируса полиомиелита (входящего в вид EV-C) вирулентность, как правило, определяется несколькими мутациями [16, 28], поэтому поиск генетических детерминант вирулентности неполиомиелитных энтеровирусов может быть очень сложной задачей, решаемой только при масштабном применении высокопроизводительного секвенирования. Не исключено также, что для вирулентности энтеровирусов имеет значение роль минорных мутантных вариантов вирусов, которые присутствуют в популяции, но не определяются при обычном секвенировании по Сэнгеру. Например, безопасность аттенуированной вакцины от полиовируса 3 типа определяется долей нейровирулентной мутации 472 U > C в популяции вируса. Добиться полного отсутствия этой мутации в вакцине технически невозможно,

но при доле мутантов менее 0,8% вакцина практически безопасна, а при доле более 1,2% риск вакцинно-ассоциированного полиомиелита резко возрастает [10]. При этом стандартное секвенирование может выявить только мутации, составляющие более 20% вирусной популяции [36], то есть в принципе не позволяет анализировать такие события. Проверка гипотезы о роли минорных генетически вариантов в возникновении вспышек заболеваемости будет возможна также только при масштабном применении высоко производительного секвенирования.

В ряде работ рассматривается возможность предсказывать появление новых энтеровирусных инфекций на основе детекции бессимптомно циркулирующих вирусов. Проблема интерпретации такого результата заключается в том, что расследуются только положительные события (вспышки), и неизвестно, часто ли появление в циркуляции нового варианта вируса не сопровождается подъемом заболеваемости. Более того, такие исследования в идеале должны быть основаны на систематическом мониторинге сточных вод. На сегодняшний день исследование сточных вод имеет определенные ограничения: недостаточная эффективность методов концентрирования, ингибирующее влияние состава сточных вод, затрудняющее применение молекулярных методов, и ряд других. Отсутствие массива данных по надзору за сточными водами не позволяет оценить статистическую значимость эпизодических наблюдений. Проведение скрининговых исследований материалов от здоровых людей для определения «эпидемического потенциала циркулирующих штаммов» [5] вряд ли может быть широко практически внедрено, а скорее может быть использовано для решения научных задач.

Созданная в последние десять лет в РФ система надзора за энтеровирусами в целом соответствует уровню стран — лидеров в этой области и работает на современном методическом и научном уровне. К сожалению, на сегодняшний день не вполне понятно, как использовать большой объем данных о заболеваемости и молекулярном составе циркулирующих энтеровирусов для эффективного воздействия на эпидемический процесс и принятия оптимальных управленческих решений. Для конвертации достижений надзора в снижение заболеваемости необходимо более глубокое понимание молекулярно-генетических механизмов формирования вариантов энтеровирусов, способных вызывать очаговый подъем заболеваемости. Другой стратегической целью развития надзора за энтеровирусами можно считать внедрение экономически эффективных (то есть высоко мультиплексированных) методов определения нуклеотидной последовательности с использованием высокопроизводительного секвенирования.

Список литературы/References

1. Голицына Л.Н., Зверев В.В., Парфенова О.В., Новикова Н.А. Эпидемические варианты неполиомиелитных энтеровирусов в России // Медицинский альманах. 2015. Т. 40, № 5. С. 136–140. [Golitsyna L.N., Zverev V.V., Parfenova O.V., Novikova N.A. Epidemic variants of non-poliomyelitic enteroviruses in Russia. *Meditsinskii al'manakh = Medical Almanac, 2015, vol. 40, no. 5, pp. 136–140. (In Russ.)*]
2. Лашкевич В.А., Королева Г.А., Лукашев А.Н., Денисова Е.В., Каргина Л.А., Хорошилова-Маслова И.П. Острый энтеровирусный увеит у детей младшего возраста // Вопросы вирусологии. 2005. Т. 50, № 3. С. 36–45. [Lashkevich V.A., Koroleva G.A., Lukashev A.N., Denisova E.V., Katargina L.A., Khoroshilova-Maslova I.P. Acute enterovirus uveitis in young children. *Voprosy virusologii = Problems of Virology, 2005, vol. 50, no. 3, pp. 36–45. (In Russ.)*]
3. Лукашев А.Н. Роль рекомбинации в эволюции энтеровирусов // Вопросы вирусологии. 2005. Т. 50, № 3. С. 46–52. [Lukashev A.N. Role of recombination in evolution of enteroviruses. *Voprosy virusologii = Problems of Virology, 2005, vol. 50, no. 3, pp. 46–52. (In Russ.)*]
4. Лукашев А.Н., Резник В.И., Иванова О.Е., Еремеева Т.П., Каравянская Т.Н., Перескокова М.А., Лебедева Л.А., Лашкевич В.А., Михайлов М.И. Молекулярная эпидемиология вируса ECHO 6 — возбудителя вспышки серозного менингита в Хабаровске в 2006 г. // Вопросы вирусологии. 2008. Т. 53, № 1. С. 16–21. [Lukashev A.N., Reznik V.I., Ivanova O.E., Eremeyeva T.P., Karavyanskaya T.N., Pereskokova M.A., Lebedeva L.A., Lashkevich V.A., Mikhallov M.I. Molecular epidemiology of ECHO 6 virus, the causative agent of the 2006 outbreak of serous meningitis in Khabarovsk. *Voprosy virusologii = Problems of Virology, 2008, vol. 53, no. 1, pp. 16–21. (In Russ.)*]
5. Устюжанин А.В., Резайкин А.В., Сергеев А.Г., Снитковская Т.Э. Значение молекулярно-генетического мониторинга в оценке степени вирулентности и эпидемической значимости штаммов неполиомиелитных энтеровирусов, циркулирующих среди населения // Вестник Уральской медицинской академической науки. 2015. Т. 52, № 1. С. 72–76. [Ustyuzhanin A.V., Rezaykin A.V., Sergeev A.G., Snitkovskaya T.E. Molecular genetic surveillance of circulating human non-poliomyelitis viruses strains: implications for the estimates of epidemic significance and virulence. *Vestnik Ural'skoi meditsinskoi akademicheskoi nauki = Journal of Ural Medical Academic Science, 2015, vol. 52, no. 1, pp. 72–76. (In Russ.)*]
6. Akhmadishina L.V., Govorukhina M.V., Kovalev E.V., Nenadskaya S.A., Ivanova O.E., Lukashev A.N. Enterovirus A71 meningoencephalitis outbreak, Rostov-on-Don, Russia, 2013. *Emerg. Infect. Dis., 2015, vol. 21, no. 8, pp. 1440–1443. doi: 10.3201/eid2108.141084*
7. Bailly J.L., Mirand A., Henquell C., Archimbaud C., Chambon M., Charbonne F., Traore O., Peigue-Lafeuille H. Phylogeography of circulating populations of human echovirus 30 over 50 years: nucleotide polymorphism and signature of purifying selection in the VP1 capsid protein gene. *Infect. Genet. Evol., 2009, vol. 9, no. 4, pp. 699–708. doi: 10.1016/j.meegid.2008.04.009*
8. Brown B.A., Maher K., Flemister M.R., Naraghi-Arani P., Uddin M., Oberste M.S., Pallansch M.A. Resolving ambiguities in genetic typing of human enterovirus species C clinical isolates and identification of enterovirus 96, 99 and 102. *J. Gen. Virol., 2009, vol. 90, no. 7, pp. 1713–1723. doi: 10.1099/vir.0.008540-0*

9. Centers for Disease Control and Prevention. Certification of poliomyelitis eradication — European Region, June 2002. *MMWR Morb. Mortal. Wkly Rep., 2002, vol. 51, no. 26, pp. 572–574.*
10. Chumakov K.M., Powers L.B., Noonan K.E., Roninson I.B., Levenbook I.S. Correlation between amount of virus with altered nucleotide sequence and the monkey test for acceptability of oral poliovirus vaccine. *Proc. Natl. Acad. Sci. USA, 1991, vol. 88, no. 1, pp. 199–203.*
11. Drummond A.J., Rambaut A. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol., 2007, vol. 7, pp. 214. doi: 10.1186/1471-2148-7-214*
12. Gaunt E., Harvala H., Osterback R., Sreenu V.B., Thomson E., Waris M., Simmonds P. Genetic characterization of human coxsackievirus A6 variants associated with atypical hand, foot and mouth disease: a potential role of recombination in emergence and pathogenicity. *J. Gen. Virol., 2015, vol. 96, no. 5, pp. 1067–1079. doi: 10.1099/vir.0.000062*
13. Hassel C., Mirand A., Lukashev A., Terletskaialadwig E., Farkas A., Schuffenecker I., Diedrich S., Huemer H.P., Archimbaud C., Peigue-Lafeuille H., Henquell C., Bailly J.L. Transmission patterns of human enterovirus 71 to, from and among European countries, 2003 to 2013. *Euro Surveill., 2015, vol. 20, no. 34. doi: 10.2807/1560-7917.ES.2015.20.34.30005*
14. Ho M., Chen E.R., Hsu K.H., Twu S.J., Chen K.T., Tsai S.F., Wang J.R., Shih S.R. An epidemic of enterovirus 71 infection in Taiwan. Taiwan Enterovirus Epidemic Working Group. *N. Engl. J. Med., 1999, vol. 341, no. 13, pp. 929–935. doi: 10.1056/NEJM199909233411301*
15. Khetsuriani N., Lamonte-Fowlkes A., Oberste S., Pallansch M.A. Enterovirus surveillance — United States, 1970–2005. *MMWR Surveill. Summ., 2006, vol. 55, no. 8, pp. 1–20.*
16. Lancaster K.Z., Pfeiffer J.K. Mechanisms controlling virulence thresholds of mixed viral populations. *J. Virol., 2011, vol. 85, no. 19, pp. 9778–9788. doi: 10.1128/JVI.00355-11*
17. Lemey P., Rambaut A., Welch J.J., Suchard M.A. Phylogeography takes a relaxed random walk in continuous space and time. *Mol. Biol. Evol., 2010, vol. 27, no. 8, pp. 1877–1885. doi: 10.1093/molbev/msq067*
18. Liste M.B., Natera I., Suarez J.A., Pujol F.H., Liprandi F., Ludert J.E. Enteric virus infections and diarrhea in healthy and human immunodeficiency virus-infected children. *J. Clin. Microbiol., 2000, vol. 38, no. 8, pp. 2873–2877.*
19. Liu W., Wu S., Xiong Y., Li T., Wen Z., Yan M., Qin K., Liu Y., Wu J. Co-circulation and genomic recombination of coxsackievirus A16 and enterovirus 71 during a large outbreak of hand, foot, and mouth disease in Central China. *PLoS One, 2014, vol. 9, no. 4: e96051. doi: 10.1371/journal.pone.0096051*
20. Lukashev A.N., Lashkevich V.A., Ivanova O.E., Koroleva G.A., Hinkkanen A.E., Ilonen J. Recombination in circulating enteroviruses. *J. Virol., 2003, vol. 77, no. 19, pp. 10423–10431. doi: 10.1128/JVI.77.19.10423-10431.2003*
21. Lukashev A.N., Lashkevich V.A., Ivanova O.E., Koroleva G.A., Hinkkanen A.E., Ilonen J. Recombination in circulating enterovirus B: independent evolution of structural and non-structural genome regions. *J. Gen. Virol., 2005, vol. 86, no. 12, pp. 3281–3290. doi: 10.1099/vir.0.81264-0*
22. Lukashev A.N., Vakulenko Y.A. Molecular evolution of types in non-polio enteroviruses. *J. Gen. Virol., 2017, vol. 98, no. 12, pp. 2968–2981. doi: 10.1099/jgv.0.000966*
23. McMinn P.C. Recent advances in the molecular epidemiology and control of human enterovirus 71 infection. *Curr. Opin. Virol., 2012, vol. 2, no. 2, pp. 199–205. doi: 10.1016/j.coviro.2012.02.009*
24. McWilliam Leitch E.C., Bendig J., Cabrerizo M., Cardosa J., Hyypia T., Ivanova O.E., Kelly A., Kroes A.C., Lukashev A., MacAdam A., McMinn P., Roivainen M., Trallero G., Evans D.J., Simmonds P. Transmission networks and population turnover of echovirus 30. *J. Virol., 2009, vol. 83, no. 5, pp. 2109–2118. doi: 10.1128/JVI.02109-08*
25. McWilliam Leitch E.C., Cabrerizo M., Cardosa J., Harvala H., Ivanova O.E., Koike S., Kroes A.C., Lukashev A., Perera D., Roivainen M., Susi P., Trallero G., Evans D.J., Simmonds P. The association of recombination events in the founding and emergence of subgenogroup evolutionary lineages of human enterovirus 71. *J. Virol., 2012, vol. 86, no. 5, pp. 2676–2685. doi: 10.1128/JVI.06065-11*
26. McWilliam Leitch E.C., Cabrerizo M., Cardosa J., Harvala H., Ivanova O.E., Kroes A.C., Lukashev A., Muir P., Odoom J., Roivainen M., Susi P., Trallero G., Evans D.J., Simmonds P. Evolutionary dynamics and temporal/geographical correlates of recombination in the human enterovirus echovirus types 9, 11, and 30. *J. Virol., 2010, vol. 84, no. 18, pp. 9292–9300. doi: 10.1128/JVI.00783-10*
27. Nasri D., Bouslama L., Omar S., Saoudin H., Bourlet T., Aouni M., Pozzetto B., Pillet S. Typing of human enterovirus by partial sequencing of VP2. *J. Clin. Microbiol., 2007, vol. 45, no. 8, pp. 2370–2379. doi: 10.1128/JCM.00093-07*
28. Nomoto A. Molecular aspects of poliovirus pathogenesis. *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci., 2007, vol. 83, no. 8, pp. 266–275. doi: 10.2183/pjab/83.266*
29. Oberste M.S., Maher K., Kilpatrick D. R., Flemister M.R., Brown B.A., Pallansch M.A. Typing of human enteroviruses by partial sequencing of VP1. *J. Clin. Microbiol., 1999, vol. 37, pp. 1288–1293.*
30. Oberste M.S., Maher K., Kilpatrick D.R., Pallansch M.A. Molecular evolution of the Human enteroviruses: correlation of serotype with VP1 sequence and application to picornavirus classification. *J. Virol., 1999, vol. 73, pp. 1941–1948.*
31. Oberste M.S., Nix W.A., Kilpatrick D.R., Flemister M.R., Pallansch M.A. Molecular epidemiology and type-specific detection of echovirus 11 isolates from the Americas, Europe, Africa, Australia, southern Asia and the Middle East. *Virus Res., 2003, vol. 91, pp. 241–248. doi: 10.1016/S0168-1702(02)00291-5*
32. Palacios G., Oberste M.S. Enteroviruses as agents of emerging infectious diseases. *J. Neurovirol., 2005, vol. 11, no. 5, pp. 424–433. doi: 10.1080/13550280591002531*
33. Puenpa J., Vongpunsawad S., Osterback R., Waris M., Eriksson E., Albert J., Midgley S., Fischer T.K., Eis-Hubinger A.M., Cabrerizo M., Gaunt E., Simmonds P., Poovorawan Y. Molecular epidemiology and the evolution of human coxsackievirus A6. *J. Gen. Virol., 2016, vol. 97, no. 12, pp. 3225–3231. doi: 10.1099/jgv.0.000619*
34. Savolainen-Kopra C., Paananen A., Blomqvist S., Klemola P., Simonen M.L., Lappalainen M., Vuorinen T., Kuusi M., Lemey P., Roivainen M. A large Finnish echovirus 30 outbreak was preceded by silent circulation of the same genotype. *Virus Genes, 2011, vol. 42, no. 1, pp. 28–36. doi: 10.1007/s11262-010-0536-x*
35. Simonen-Tikka M.L., Hiekka A.K., Klemola P., Poussa T., Ludvigsson J., Korpela R., Vaarala O., Roivainen M. Early human enterovirus infections in healthy Swedish children participating in the PRODIA pilot study. *J. Med. Virol., 2012, vol. 84, no. 6, pp. 923–930. doi: 10.1002/jmv.23284*

36. Solmone M., Vincenti D., Prosperi M.C., Bruselles A., Ippolito G., Capobianchi M.R. Use of massively parallel ultradeep pyrosequencing to characterize the genetic diversity of hepatitis B virus in drug-resistant and drug-naive patients and to detect minor variants in reverse transcriptase and hepatitis B S antigen. *J. Virol., 2009, vol. 83, no. 4, pp. 1718–1726. doi: 10.1128/JVI.02011-08*
37. Tamura K., Stecher G., Peterson D., Filipski A., Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol., 2013, vol. 30, no. 12, pp. 2725–2729. doi: 10.1093/molbev/mst197*
38. Van der Sanden S.M., Koopmans M.P., van der Avoort H.G. Detection of human enteroviruses and parechoviruses as part of the national enterovirus surveillance in the Netherlands, 1996-2011. *Eur. J. Clin. Microbiol. Infect. Dis., 2013, vol. 32, no. 12, pp. 1525–1531. doi: 10.1007/s10096-013-1906-9*
39. Witso E., Palacios G., Cinek O., Stene L.C., Grinde B., Janowitz D., Lipkin W.I., Ronningen K.S. High prevalence of human enterovirus a infections in natural circulation of human enteroviruses. *J. Clin. Microbiol., 2006, vol. 44, no. 11, pp. 4095–4100. doi: 10.1128/JCM.00653-06*
40. Wright P.W., Strauss G.H., Langford M.P. Acute hemorrhagic conjunctivitis. *Am. Fam. Physician, 1992, vol. 45, no. 1, pp. 173–178.*
41. Yakovenko M.L., Gmyl A.P., Ivanova O.E., Eremeeva T.P., Ivanov A.P., Prostova M.A., Baykova O.Y., Isaeva O.V., Lipskaya G.Y., Shakaryan A.K., Kew O.M., Deshpande J.M., Agol V.I. The 2010 outbreak of poliomyelitis in Tajikistan: epidemiology and lessons learnt. *Euro Surveill., 2014, vol. 19, no. 7, pp. 20706.*
42. Yarmolskaya M.S., Shumilina E.Y., Ivanova O.E., Drexler J.F., Lukashev A.N. Molecular epidemiology of echoviruses 11 and 30 in Russia: different properties of genotypes within an enterovirus serotype. *Infect. Genet. Evol., 2015, vol. 30, pp. 244–248. doi: 10.1016/j.meegid.2014.12.033*

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IMPLEMENTATION OF THE WORLD HEALTH ORGANIZATION WESTERN PACIFIC REGIONAL PLAN OF ACTION FOR MEASLES ELIMINATION

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Abstract. The Western Pacific Region (WPR) is comprised of 37 countries such as China, Japan, Mongolia, Republic of Korea, The Socialist Republic of Vietnam, Papua-New Guinea, Australia, including Pacific Island Countries and Territories (21 countries of PICTs, approx. 3 million people) etc., with a population of 1.85 billion people. Among them, China is the largest and most populous (1.3 billion people) country of the Region. Large measles outbreaks were documented to occur in the Region. In 2003, the Regional Committee announced officially about the WPR action plan on measles elimination 2005, which, however, failed. Since 2012, WPR countries joined the WHO 2012–2020 Global Measles and Rubella Strategic Plan performing a routine measles vaccination (national immunization schedule) or within Expanded Programme on Immunization (EPI). Basically, a two-dose immunization strategy is followed in the WPR countries. Since 2002, measles supplementary immunization activities (SIAs) in children were conducted in the following countries: Japan, Laos, Vietnam, Philippines, Mongolia, Cambodia, Papua New Guinea, and China. Starting from 2005, measles management was considerably improved, demonstrating by 2012 decreased measles incidence rate down to 5.9 cases per million population. In last years, a decreased measles immunization coverage in decreed population groups was noted in the WPR countries that resulted in 2013–2015 measles epidemic involving almost all regional countries. In particular, in China measles incidence rate was 19.6 cases per million population, whereas in the Vietnam Papua New Guinea and Philippines it progressively increased reaching 182.8, 345.9 and 548.0 cases per million population, respectively. Early children not vaccinated according to schedule, adolescents and young adults dominated among measles patients. It was found that measles outbreaks were due to missed vaccination and increased level of vulnerability to measles. Children under one, adolescents and young adults who did not receive a two-dose measles vaccination were in risk group. Analyzing WPR measles epidemiology demonstrated that refusal of parents to vaccinate children, poor knowledge of advantages related to vaccination, insufficient immunization coverage in immigrants, travelers, subjects changing place of residence, workers of healthcare and educational facilities require special attention. In 2017–2018 season, the following measles genotypes were found in the WPR: D8 — Australia, New Zealand, Republic of Korea, Singapore, Japan; H1 — China; B3 — Philippines, Australia and Japan; D9 — Singapore, Australia, Macau (China), Malaysia and Japan, H2 strains endemic in Vietnam. According to the WHO, measles endemic transmission has been successfully interrupted; Australia, Macau, Mongolia and Republic of Korea are being verified to eliminate measles; Hong Kong (China) and Singapore (based on available information) are ready to verify measles elimination. Thus, in the Western Pacific Region measles elimination is achievable after solving current issues such as increasing and maintaining high-level routine vaccination and conducting measles supplementary immunization campaigns in epidemically important contact clusters.

Key words: *measles, Western Pacific region, disease incidence, elimination program, vaccination schedule, genotype.*

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К ВОПРОСУ О РЕАЛИЗАЦИИ ПРОГРАММЫ ЭЛИМИНАЦИИ КОРИ В СТРАНАХ ЗАПАДНО-ТИХООКЕАНСКОГО РЕГИОНА ВОЗ

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Резюме. Западно-Тихоокеанский регион (ЗТР) объединяет 37 государств, в том числе Китай, Японию, Монголию, Республику Корея, Вьетнам, Папуа-Новую Гвинею, Австралию и др. Из них 21 страна известна как Тихоокеанские Островные государства и территории (ТОГТ). Население Западно-Тихоокеанского региона составляет 1,85 млрд человек. Самой большой страной региона по площади и по численности населения (1,3 млрд человек) является Китай. В странах ТОГТ проживает около 3 млн человек. На островах развивались особенно крупные вспышки кори. В 2003 г. Региональный Комитет формально объявил целью элиминацию кори в ЗТР к 2005 г., однако достичь ее не удалось. С 2012 г. страны ЗТР присоединились к программе элиминации кори ВОЗ и Глобальному плану элиминации кори к 2020 г. Все страны ЗТР проводят вакцинацию против кори в рамках рутинной иммунизации (национальные календари прививок) или программы расширенной иммунизации (ПРИ). В основном в странах региона используется двухдозовая стратегия иммунизации. Программы дополнительной иммунизации (ПДИ) детей против кори, начиная с 2002 г., выполнили ряд государств: Япония, Лаос, Вьетнам, Филиппины, Монголия, Камбоджа, Папуа-Новая Гвинея, Китай. Начиная с 2005 г. ситуация по кори в ЗТР значительно улучшилась, к 2012 г. показатель заболеваемости в целом снизился до 5,9 на 1 млн населения. В последние годы в странах ЗТР наблюдалось снижение уровня охвата вакцинацией декретированных групп населения, и в 2013–2015 гг. в ЗТР распространилась очередная эпидемия кори, в которую в той или иной степени были вовлечены почти все страны региона. В Китае показатель составил 19,6 на 1 млн. Наиболее высокие показатели (на 1 млн заболевших) были отмечены на Филиппинах (548,0), в Папуа-Новой Гвинее (345,9), во Вьетнаме (182,8) и др. Среди заболевших преобладали не вакцинированные по возрасту дети младшего возраста, а также подростки и молодые взрослые. Вспышки кори были связаны с наличием «пропусков» иммунизации и увеличением количества восприимчивых к кори лиц. В группе риска дети до года, подростки и молодые взрослые, которые не получили 2 дозы вакцины. Анализ эпидемической ситуации в регионе показывает, что требуют решения проблемы отказа родителей от вакцинации детей, неосведомленность населения о преимуществах вакцинации; недостаточный уровень охвата иммунизацией мигрантов, путешественников и лиц, меняющих место жительства, работников медицинских и образовательных учреждений. В 2017–2018 гг. в регионе определялись генотипы: D8 — Австралия, Новая Зеландия, Республика Корея, Сингапур, Япония; H1 — Китай; B3 — Филиппины, Австралия и Япония; D9 — Сингапур, Австралия, Макао (Китай), Малайзия и Япония. Штаммы генотипа H2 эндемичны для Вьетнама. По данным ВОЗ в Бруней-Даруссаламе, Камбодже и Японии эндемичная трансмиссия кори прекращена; Австралия, Макао, Монголия и Республика Корея находятся на этапе верификации элиминации кори; Гонконг (Китай) и Сингапур (по имеющейся информации) готовы к верификации элиминации кори. Таким образом, элиминация кори в ЗТР ВОЗ достижима при решении существующих проблем — повышения и поддержания высокого уровня рутинной вакцинации и проведения кампаний дополнительной иммунизации эпидемически значимых групп населения.

Ключевые слова: *корь, Западно-Тихоокеанский регион, заболеваемость, программа элиминации, календарь прививок, генотип.*

Introduction

The strategic plan for measles elimination by 2020 (WHO, 2012) calls for elimination in at least five WHO regions, with the exception of the South-East Asia [1, 2, 3, 23].

The Western Pacific Region (WPR) includes 37 continental and island states. Of these, 21 countries are known as the Pacific Island Countries and Territories (PICTs). PICTs are characterized by active migration of residents between the islands and towards urban centers. Geographical environment differs markedly from that of most other countries, and extreme weather conditions upset regular medical care for many months. Due to these peculiarities, PICTs are united into one group for the epidemiological surveillance of measles [18, 27].

The population of the Western Pacific Region is 1.85 billion people. The largest country in the region, both in terms of area and population, is China (1.4 billion people). The population of Papua New Guinea is 7 million people. About 3 million people live in PICTs. Other WPR countries include Australia, Vietnam, Mongolia, Republic of Korea, Japan, and others [29].

The measles surveillance system in the countries of the WHO Western Pacific Region

In the Western Pacific Region, particularly large outbreaks of measles have developed on the islands. In 1875, 27 to 50 thousand Fiji residents died as a result of importing the measles virus into a nonimmune population. In 1893, the twentieth of the population of Tonga died [18].

The fight against measles in the WPR began in the second half of the 20th century, but until 2000 the incidence rate remained high. For example, in the

Marshall Islands measles epidemics occurred every 10 years — in 1968, 1978, and 1988 [18].

In 2003, the Regional Committee formally declared the goal of eliminating measles in the WPR by 2005 (Western Pacific Regional Plan of Action for Measles Elimination). It was not achieved, however. A new goal for the countries of the region has become the elimination of measles by 2012, according to the Regional Strategy and Plan of Action for Measles and Rubella Elimination in the Western Pacific [5, 20, 22]. National plans were developed to achieve this goal. For example, in 2006, the Chinese Ministry of Health developed a plan of action for eliminating measles in 2006–2012. The elimination strategy included immunization, epidemiological surveillance and infection control [8]. This goal was also not achieved by 2012, so the WPR countries joined the WHO Measles Elimination Plan and the Global Measles Strategic Plan purporting the measles elimination by 2020. A detailed plan of action to achieve the goals was outlined in the Regional Framework for Implementation of the Global Vaccine Action Plan in the Western Pacific [20].

National systems of measles surveillance are currently being improved. In addition to the guidelines of the WHO Regional Office, the WPR states and territories have national regulatory documents governing the activities on the elimination of measles. In China, they have a National Measles Surveillance Guideline (1998, 2003) [8]. Japan has a guideline of the Ministry of Health, Labor and Welfare (Social Security) (Special Infectious Disease Prevention Guidelines for Measles) and the Infectious Diseases Control Law (2008) [11, 13].

The virological surveillance has become an integral part of the surveillance system for measles in all countries of the region. For example, in China a measles laboratory network operates since 2001, including 331 prefecture laboratories in 31 provinces. The National Measles Laboratory of China became the regional reference laboratory in the WPR in 2003 [5]. In the WPR countries (including Japan (since 2008) and China (since 2009)), a system of measles surveillance was launched based on the investigation of each suspected measles case supported by laboratories [8, 13]. In China, clinical samples for measles diagnosis are sent to measles laboratories, and samples for genotyping are sent to the National Measles Laboratory [8]. In Japan, upon registration of a suspected measles case, a medical institution sends clinical samples to prefecture and municipal health institutions for the isolation and genotyping of the measles virus and to a commercial laboratory for the detection of IgM antibodies to the virus (through national health insurance).

Data representation and analysis systems are being improved. In China, measles is subject to registration since the 1950s. In 2005, the National Disease Reporting System was implemented. Epidemiological data from hospitals and regional Centers for Disease Control and Prevention are transmitted over a computer network to the National Center for Disease Control and Prevention. The mandatory registration of all suspected measles cases is carried out in Japan. If the diagnosis is confirmed by a laboratory examination, then a confirmed measles case is recorded. In 2008, only 38% of suspected measles cases underwent laboratory examination and were confirmed. In 2014, such cases made up 90%, and genotyping was performed in 78% of cases [13, 26]. In Vietnam, epidemiological data are collected from four measles laboratories, where the registration forms for investigating measles cases are filled in monthly [14]. These data are then sent to the EPI National Institute of Hygiene and Epidemiology.

Preventive vaccination of measles

All WPR countries carry out vaccination against measles as part of routine immunization (national vaccination schedules) or the Expanded Program on Immunization (EPI). Investigations into a number of major outbreaks in the region involving people vaccinated against measles have justified a two-dose immunization strategy [10], which has been adopted in almost all countries of the region.

Different vaccination schemes are used. In China, a single dose of measles vaccine to children at the age of 8 months was included in the national vaccination schedule in 1978 [5, 8]. The second dose of the vaccine was recommended in 1986 at the age of 7 years, and in 2005 the age of administration was reduced to 18–24 months. In Japan, routine immunization has been carried out since 2006 using measles- and rubella-containing vaccines at the age of one year (first dose) and before entering school (second dose) [13]. In Australia, the first and the second doses are administered to children aged 12 and 18 months respectively [9]. In Vietnam, vaccination against measles with a single dose was introduced into the routine immunization schedule in 1982. Since 2001, vaccination of children from 9 months to 6 years has been accepted as part of the National Expanded Program on Immunization. Since 2006, scheduled vaccination against measles with first and second doses was introduced for children aged 9–11 months and 18 months respectively [4, 14, 17]. In PICTs, immunization schedules vary considerably. The first dose of measles-containing vaccine is intended for children aged 12–15 months. The age of the second dose varies from 13 months to 6 years. In 2015, 20 PICTs adopted a two-dose vaccination strategy [10, 18].

In order to eliminate measles according to WHO recommendations, it is necessary to maintain a vaccine coverage rate of at least 95%. In addition to routine immunization, 7 of the 16 non-island states performed supplementary immunization activities (SIAs) or campaigns against measles. For example, in various parts of Vietnam, SIAs for the population aged between 9 months and 10 years were executed in 2002–2003 and for the population aged between 6 and 20 years — in 2004, 2007 and 2008 [17]. In the Lao People's Democratic Republic, SIAs were performed in 2011 and 2014; in the Philippines, children born in 2002–2010 and 2009–2013 were vaccinated in 2011

and 2014 respectively. SIAs were also performed in Mongolia (2012), Cambodia (2013), and Papua New Guinea (2015–2016).

In Japan, from 2008 to 2012, a vaccination session was performed for students of the first grade of middle school (13 years old) and the third grade of high school (18 years). Two doses of vaccine were administered to children born in 1990–1999 [13].

An unprecedented SIA session was carried out in China. The coverage rate of the primary measles vaccination in China was not sufficient, although increased from 80.4% in 2000 to 91.1% in 2009. The coverage rate for the booster vaccination in 2009 was 84.3%. In order to stop the spread of measles virus among children under the age of 14, who were most involved in the epidemic process, 27 out of 31 provinces of China initiated SIAs against measles independently between 2003 and 2009, getting 185.7 million children vaccinated. However, despite the success of individual territories, a new increase in the incidence of measles has been observed since 2008. In September 2010, a national program for supplementary immunization using measles-containing vaccine was performed, which covered the children from decreed groups regardless of their vaccine status. Within three weeks, more than 100 million children were vaccinated; vaccination coverage was 97.5% [5, 14].

In 2014, mass vaccination sessions against measles and rubella were conducted in the Federated States of Micronesia, the Lao People's Democratic Republic, the Philippines, the Solomon Islands and Vietnam.

Thanks to SIAs, the vaccination coverage in the region has increased significantly. For example, in Vietnam in the period between 1994 and 2009, the primary vaccination coverage was 93–97%, with the exception of 2007 (83%) [17]. In Japan, the first dose vaccination coverage in 2010–2013 was at least 95% of children, who were subject to routine vaccination. The coverage rate for the second dose in 2013 was 93%.

In 2014 in Japan, when studying the population immunity to measles, it was shown that 73% of children under 5 months had (maternal) antibodies; at 6–11 months, 12% of children were seropositive, and at least 95% of people in age groups older than 2 years had IgG antibodies to measles virus [13].

Among Australian students, 20.9% were vaccinated, while 51% had unknown vaccine status. The risk group contained all people born after 1966, who did not receive two doses of the vaccine.

In recent years, there has been a tendency in the WPR countries to reducing or stagnating coverage of the decreed population groups by vaccination. For example, a study of the intensity of immunity against measles virus in Australia showed that the vaccination coverage rate was 93.3% in children at the age of five [9]. During the measles outbreak in Vietnam in April 2014, the absolute majority of affected children (86%) were not immunized or no vaccination data were available [4].

To achieve the goal of eliminating measles in the WPR countries, it is necessary to have a large number of doses of a thermostable vaccine, as it should be administered in a tropical climate. In close cooperation with Japan (Kitasato Institute, Tokyo), PolyVac vaccine was developed and tested in Vietnam (Military Academy of Medicine, Hanoi) on the basis of AIK-C vaccine strain (Kitasato Institute, Tokyo). This is the first vaccine that was produced in Vietnam by the Center for Research and Production of Vaccines and Biology (Ministry of Health) as part of the project launched in 2013 that included the transfer of POLYVAC technology and was funded by the Japan International Cooperation Agency (JICA). The project aims to create a combined measles and rubella vaccine corresponding to WHO standards. In March 2016, with the assistance of Japanese experts from the Japanese technology transfer unit (Kitasato Daiichi Sankyo Vaccine) and the Center's staff, a clinical trial was carried out for the measles and rubella vaccine, which proved to be safe and effective. With a current capacity of 7.5 million doses per year, the Center can fully meet domestic demand for vaccines and even export them in future. Currently, Vietnam is among the 25 states in the world that can produce vaccines and four Asian states that can produce a measles and rubella vaccine (after Japan, India and China) [24].

The incidence of measles in the WHO Western Pacific Region

In 1974, at the beginning of the Expanded Program on Immunization, 3 381 826 cases of measles were registered in the WHO Western Pacific Region, with 33 818 children's deaths due to measles. By 1990, vaccination coverage in the region reached 93%, the number of measles cases was reduced to 155 000 and the number of deaths up to 1561 [18, 20]. However, the data reported through the WHO/UNICEF joint reporting form (JRF) could be understated due to weak surveillance systems, especially in countries and areas with the greatest burden of diseases. WHO estimates that in 2002 the number of measles cases in the region could reach 6.7 million; measles could be the cause of 30 000 deaths. For example, in 2003, a large measles outbreak in the Marshall Islands ended after more than 35 000 from 51 000 people were vaccinated, despite a reported high enough measles vaccination coverage (80–93%) before the outbreak [10, 18].

As a result of the implementation of the Regional Strategy and Plan of Action for Measles and Rubella Elimination in the Western Pacific, the situation of measles in the WPR has improved significantly since 2005. From 2009 to 2012, the number of laboratory-confirmed cases of measles in the WPR decreased by 84%; the incidence rate as a whole decreased to 5.9 per 1 million people. The highest incidence of measles (confirmed cases) was in Malaysia (63.7 per million of population), Philippines (15.9 per million of population), and New Zealand (12.3 per million of population) [16].

However, in 2013–2015, another measles epidemic began in the WPR, which involved all countries of the

region in varying degrees. In 12 countries, the incidence rate significantly exceeded 10 per 1 million people. The peak incidence in most countries fell in 2014, when 80 576 cases of measles were recorded in the region (fig.) [31].

The highest rates were recorded in the Philippines — 548.0 per 1 million population (54 669 cases) and Papua New Guinea — 345.9 (2589 cases), and in Vietnam, the rate was 182.8 per 1 million (16 741 cases). In 2014, 834 confirmed cases were registered in Thailand [9]. In the island states (PICTs), 279 cases of measles (85.3 cases per 1 million of population) were recorded, including 257 in the Federated States of Micronesia, where the incidence rate was 2473.5 per 1 million people. In Mongolia in 2015, there were 20 374 cases of measles and 28 813 cases in 2016 [21, 27].

The Solomon Islands experienced a measles epidemic in June 2014 after the return of travelers from Papua New Guinea with 4654 suspected measles cases, including 38 serologically confirmed [18, 27]. To stop the outbreak on the islands, an additional immunization session was carried out. All residents between the ages of 6 months and 30 years were vaccinated first in the epicenter of the outbreak, then in the remaining territories [18]. Measles was also recorded in New Zealand (274 cases, 60.2 per 1 million of population). In Australia outbreaks were associated with the importation of the virus from other countries [9]. The majority of the diseased was constituted by children who had not been vaccinated according to age, adolescents and young adults .

Note should be made of the situation with measles in the mainland countries of the region. China has the bulk of all cases of measles in the WPR, so a decrease in the incidence in the region is associated with control over this infection in China. The average annual incidence rate per 100 000 of population in 1960–1969 was 572.0 and 355.3 in 1970–1979 [8]. During 1995–2004, as a result of successful vaccination sessions, the incidence rate dropped to 6 per 100 000 population [5]. Despite the successes, large increases in measles incidence were noted in 2003, 2005–2007, 2009, 2011–2012. In total, from 2005 to 2012, there were 569 948 measles cases registered in the country (59.9% among males), including 344 deaths. Children under 1 year and people aged from 15 to 35 were most affected [5, 8]. Nevertheless, the number of registered measles cases was declining steadily, and in 2012 the incidence rate was 4.6 per 1 million of population, with 98.3% of cases being confirmed in laboratory [16].

However, in 2013, another epidemic increase in the incidence rate was registered in China, when the figure was 19.4 per 1 million of population and 26 883 cases of measles were registered. The maximum was noted in 2014 (52 628 cases of measles). Two peaks of incidence were observed, one in April-May and another in August–October. Among the diseased, children prevailed; the average age of the patients was 11 months. Twenty-four deaths were recorded, 13 of them in children less than 8 months old, 8 at the age of 8–23 months, and 3 cases in children in the 24–48 month age group. The rise in incidence was due to a decrease in the coverage with primary vaccinations; in the 8–23 month age group, 72% of children were not vaccinated in 2013 [5, 8]. In 2016 and 2017 there were 25 584 and 5999 confirmed cases of measles in China and the incidence rate was 18.5 and 4.3 per 1 million of population respectively [24, 25].

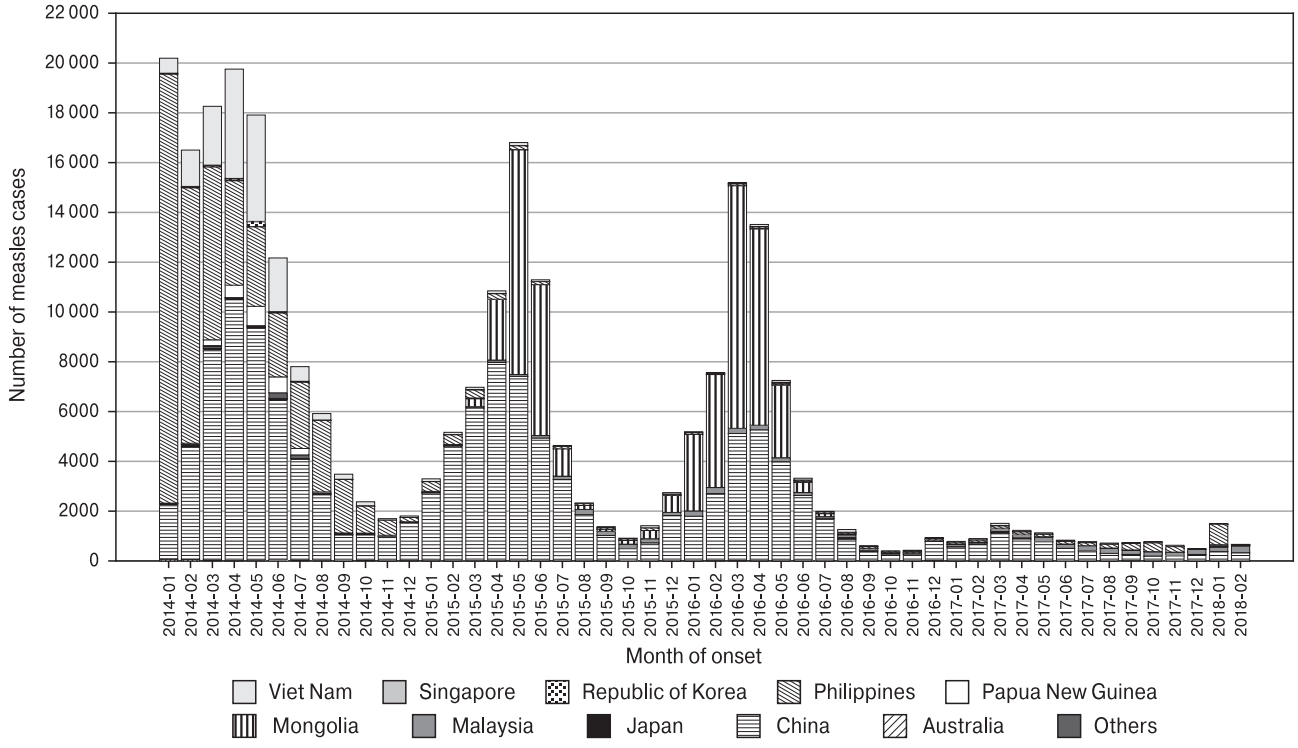

Figure. Measles case distribution (WPR), 2014–2018

Japan is a measles-free country, despite its large and dense population. However, in 2014, as a result of the importation (from the Philippines, in particular), a measles outbreak began in the country, involving employees of educational institutions and medical personnel. Due to active supervision, the outbreak ended in about one week. Of the diseased, 47% were not vaccinated, 19% were vaccinated once, 7% were vaccinated twice, and 27% did not know their vaccine status. The proportion of children under the age of 1 year was 20%, of which 83% were not vaccinated. The share of adults (≥ 20 years) constituted 70% of measles cases that occurred in 2013, compared to 33% in 2008. Among the diseased who were older than 6 years and should have received two doses of the vaccine, 70 of 142 (49%) were not vaccinated [12, 13]. After stopping the outbreak, the incidence rate in Japan was 0.3 per 1 million of population in 2015 [13, 27]. In 2017, 187 confirmed cases were registered, the incidence rate being 1.46 per 1 million of population [24, 25].

In Vietnam, the high incidence is also due to the shortcomings of preventive vaccination of measles and to the presence of a large number of people susceptible to measles. From 2004 to early 2008, there were isolated outbreaks of measles in Vietnam, mainly in the mountainous areas of the Northern Region of the country. In October 2008, measles outbreaks were reported in three provinces of the Northern Vietnam. In Vĩnh Phúc province, 17 college students fell ill with measles. In Thanh Hóa province, measles had spread among children under the age of 15, but the outbreak ended after mopping up immunization in December 2008. The highest number of cases was in Hanoi province. The first rash case was registered on October 20 in a 19-year-old student of one of Hanoi educational institutions. By the end of 2008, 84 confirmed measles cases had been reported, 68 of them among 18–26-year-olds. In total, 184 measles cases were reported in Vietnam in 2008. From October 2008 to January 2010, there were 7948 confirmed measles cases in 60 of the 63 Vietnam provinces, the incidence rate being 93 cases per 1 million of population [17, 30]. High incidence rates were noted in two age groups — children under 12 months and at the age of 1–4 years (318 and 328 cases per 1 million of population respectively). Among those who fell ill, 53% were older than 15 years. These were mainly students of colleges and universities [17]. Only 30% of patients were vaccinated. The 2013–2015 measles epidemic in Vietnam affected 24 cities and provinces, including major urban centers like Hanoi and Ho Chi Minh City. In 2013, 1233 confirmed measles cases were registered and 7580 in 2014 [4, 30]. In 2014, the coverage rate of the first dose of measles vaccine was 85.6% [6]. Among those who fell ill in 2014, only 22.4% of children over 1 year were vaccinated once, 1.3% were vaccinated twice, and 76.3% had no information about vaccination [14]. In 2014–2015, an additional immunization session against measles was organized in Vietnam for children born between 2000 and 2013 [20]. In 2016, 368 cases of measles were registered, the incidence rate being 3.9 per 1 million of population; in 2017, 588 cases of measles with an incidence rate of 6.22 per 1 million [25].

The Philippines is one of the countries most heavily afflicted by measles. About 60 000 cases of measles were registered between 2011 and 2014, with a peak in 2014 after Typhoon Haiyan [9], the incidence rate being 548 per 1 million. In 2017, there were 1602 cases of measles [24, 25].

The measles outbreak did not stop in Malaysia, where the number of cases increased from 22 in 2014 to 1964 in 2017, the incidence rate being 61.7 per 1 million of population [25, 27].

The criterion for measles elimination is the absence of circulation of endemic measles virus within 36 months from the date of registration of the last case, provided that an adequate system of measles surveillance is in place.

During the 2009–2012, measles viruses of D9 (Philippines, Malaysia and Singapore), D8 (Malaysia) and H1 (China, identified since 1993) genotypes were most frequently detected in the WPR. Until May 2010, measles viruses of D5 genotype circulated in Japan. B3, D4 and G3 genotypes, which were found in the WPR during this period, were imported from other regions [10, 16]. In 2009–2012, measles viruses of D4, D8, D9 and D11 genotypes were also found in China. Since 2003, measles viruses of H1 and H2 genotypes have been detected in the central (Nha Trang) and northern (Hanoi) parts of Vietnam [7]. H2 strains are endemic and are circulating constantly in the capital of Vietnam. Viruses of H1 genotype were detected in 2008–2010 (Northern and Southern Regions) and in 2014 (Hanoi). Both endemic transmission and importation from China are probable [17]. Since 2014, measles viruses of D8 genotype have been isolated in South Vietnam, which have at least three unique amino acid sequences in the N gene and form a separate D8-VNM cluster. The strain of measles virus from India is probably the closest one to the ancestral form of this genovariant [14, 20, 27]. In 2014–2015, the H1 genotype prevailed in the WPR (China, Mongolia); B3 and D8 were detected, but there was no D9 genotype [31]. In 2017–2018, the following genotypes were identified in the region: D8 in Australia, New Zealand, Republic of Korea, Singapore, Japan; H1 in China; B3 in Philippines, Australia and Japan (including those imported from the Philippines); D9 in Singapore, Australia, Macau (China), Malaysia and Japan [5, 8, 13, 27, 28].

The Regional Verification Commission for Measles Elimination in the Western Pacific Region found in 2015 that endemic transmission of measles virus was stopped in Brunei, Cambodia and Japan. Australia, Macau, New Zealand and the Republic of Korea are at the stage of verifying measles elimination; Hong Kong (China) and Singapore (according to available information) are ready to verify the elimination of measles. New Zealand and the Republic of Korea were also the first to confirm the elimination of rubella in 2015 [9, 21, 22]. In 2015, a measles outbreak began in Mongolia. The majority of confirmed cases were in children under 9 months of age, but there

were more suspected measles cases in those aged 15–24 years; 80% of the diseased were not vaccinated [31].

Despite the fact that in 2017 the incidence of measles in the region decreased to 11.8 per million of population, the spread of the infection did not stop. In January 2018 330 confirmed cases of measles from 708 in the region were registered in the Philippines and 290 cases were registered in China [27]. Measles outbreaks were also registered in Malaysia, Papua New Guinea and Vietnam [9, 21, 22].

Conclusion

Despite the success of preventive vaccination, measles outbreaks are associated with "skipped" immunizations and an increase in the number of people susceptible to measles. Risk groups are children under one year, adolescents and young adults who have not received two doses of the vaccine. [5, 8]. It is essential that a high level of vaccination coverage is maintained. Special attention should be paid to persons, who were not included in the vaccination programs and were not covered by the second dose of measles-containing vaccine, not to mention the first one.

Parents' refusal to vaccinate children prevents achievement of measles elimination. It is noted that the development of the epidemic of the disease in Vietnam was facilitated by insufficient awareness of parents and loss of public confidence in the government vaccination program [6, 15]. Many parents stopped vaccinating their children, leaving them susceptible to measles. For many countries in different WHO regions it has been shown that the level of people's awareness of the benefits and risks of vaccination corresponds to the level of education. Governments use different ways of convincing people about the advantages of immunization. For example, in Australia, a "No Jab, No Pay" policy has been introduced, related to financial incentives for parents [9].

The vaccination of travelers and people changing their place of residence is an important part of preventive measles vaccination program. In Australia, the most popular tourist routes include Indonesia (Bali), Thailand, India and China. In 2014–2015 about 9.2 million people traveled abroad. Studies have shown that only 1.6% of Australian travelers were vaccinated against measles prior to travel. Among the measles cases, 80% of people have repeatedly traveled outside of Australia [9]. It was also shown that in Australia, migrants are the key risk group to be vaccinated against measles.

In China more than 30% of measles cases were registered in 2005–2007 among the "floating population", that is, people without a permanent residence permit in the area where they actually lived. Routine vaccination is carried out at the place of official registration. The number of "floating" people is constantly growing due to the increased migration of rural population to urban centers [4]. It is necessary to increase the level of vaccination coverage among travelers.

Timely diagnosis of measles and compliance with the anti-epidemic regime remains a major problem. During a measles outbreak in Hanoi, parents often brought sick children to the hospital, which soon became the source of infection of measles-contact children and the center for transmission of infection [4, 19].

To sum up, seven countries in the Western Pacific Region achieved measles elimination by 2017: Australia, Brunei, Cambodia, Macau (China), Mongolia, Republic of Korea and Japan. Thus, the elimination of measles in the WHO Western Pacific Region is achievable if current problems are addressed, that is, the increase and maintenance of a high level of routine and supplementary immunization.

Список литературы/References

1. Антипова А.Ю., Бичурина М.А., Лаврентьева И.Н., Тотолян Арег А. Корь в странах Африканского региона ВОЗ на этапе элиминации инфекции // Актуальные инфекции в Гвинейской Республике: эпидемиология, диагностика и иммунитет / под ред. А.Ю. Поповой. СПб.: ФБУН НИИЭМ имени Пастера, 2017. С. 264–278. [Antipova A.Yu., Bichurina M.A., Lavrentieva I.N., Totolian Areg A. Kor' v stranakh Afrikanskogo regiona VOZ na etape eliminatsii infektsii // Aktual'nye infektsii v Gvineiskoi Respublike: epidemiologiya, diagnostika i immunitet / pod red. A.Yu. Popovoi. [Measles in countries of the WHO African Region during the elimination phase. In: Actual infections in the Republic of Guinea: epidemiology, diagnosis and immunity. Ed. A.Yu. Popova]. *St. Petersburg: St. Petersburg Pasteur Institute, 2017, pp. 264–278.*]
2. Попова А.Ю., Бичурина М.А., Лаврентьева И.Н., Железнова Н.В., Антипова А.Ю., Щербакова С.А., Буаро М.Й., Тотолян А.А. Изучение уровня иммунитета к вирусу кори в отдельных группах населения Гвинейской Республики в рамках глобальной программы элиминации кори. Сообщение 1 // Инфекция и иммунитет. 2016. Т. 6, № 4. С. 353–358. Popova A.Yu., Bichurina M.A., Lavrentyeva I.N., Zheleznova N.V., Antipova A.Yu., Shcherbakova S.A., Boiro M.Y., Totolian A.A. Measles virus immunity level study in particular population groups of the Republic of Guinea within the framework of Global measles elimination program. Report 1. *Infektsiya i immunitet = Russian Journal of Infection and Immunity, 2016, vol. 6, no. 4, pp. 79–84. doi: 10.15789/2220-7619-2016-4-353-358 (In Russ.)*]
3. Попова А.Ю., Бичурина М.А., Лаврентьева И.Н., Железнова Н.В., Антипова А.Ю., Щербакова С.А., Буаро М.Й., Тотолян А.А. Изучение уровня иммунитета к вирусу кори в отдельных группах населения Гвинейской Республики в рамках глобальной программы элиминации кори. Сообщение 2 // Инфекция и иммунитет. 2017. Т. 7, № 1. С. 79–84. [Popova A.Yu., Bichurina M.A., Lavrentyeva I.N., Zheleznova N.V., Antipova A.Yu., Shcherbakova S.A., Boiro M.Y., Totolian A.A. Measles virus immunity level study in particular population groups of the Republic of Guinea within the framework of Global measles elimination program. Report 2. *Infektsiya i immunitet = Russian Journal of Infection and Immunity, 2017, vol. 7, no. 1, pp. 79–84. doi: 10.15789/2220-7619-2017-1-79-84 (In Russ.)*]
4. Hang L.K.N., Do L.P., Van T.T.T., Nguyen S.V., Hoang P.V.M., Pham H.T., Le T.T., Tran H.T.T., Vuong C.D., Mai T.Q.L. Viral co-infections among children with confirmed measles at hospitals in Hanoi, Vietnam, 2014. *Asian Pac. J. Trop. Med., 2017, vol. 10, no. 2, pp. 171–174.*

5. Ji Y., Xu S., Zhang Y., Zhu Z., Mao N., Jiang X., Ma C., Lu P., Wang C., Liang Y., Zheng H., Liu Y., Dai D., Zheng L., Zhou J., Wang S., Zhang Z., Wu S., Nan L., Li L., Liang X., Featherstone D. A., Rota P. A., Bellini W. J., Xu W. Genetic characterization of wild-type measles viruses isolated in China, 2006–2007. *Virol. J., 2010, vol. 7, pp. 105.*
6. Kien V. D., Minh H.V., Giang K.B., Mai V.Q., Tuan N.T., Quam M.B. Trends in childhood measles vaccination highlight socio-economic inequalities in Vietnam. *Int. J. Public Health., 2017, vol. 62, no. 1, pp. S41–S49.*
7. Kremer J.R., Nguyen G.H., Shulga S.V., Nguyen Ph.H., Nguyen U.T., Tikhonova N.T., Muller C.P. Genotyping of recent measles virus strains from Russia and Vietnam by nucleotide-specific multiplex PCR. *J. Med. Virol., 2007, vol. 79, pp. 987–994.*
8. Ma C., Hao L., Zhang Y., Su Q., Rodewald L., An Z., Yu W., Ma J., Wen N., Wang H., Liang X., Wang H., Yang W., Lia L., Luo H. Monitoring progress towards the elimination of measles in China: an analysis of measles surveillance data. *Bulletin of the World Health Organization, vol. 92, no. 5, pp. 340–347. doi: 10.2471/BLT.13.130195*
9. MacIntyre C.R., Karki S., Sheikh M., Zwar N., Heywood A.E. The role of travel in measles outbreaks in Australia — an enhanced surveillance study. *Vaccine, 2016, vol. 34, no. 37, pp. 4386–4391. doi: 10.1016/j.vaccine.2016.07.023*
10. Marin M., Nguyen H.Q., Langidrik J.R., Edwards R., Briand K., Papania M.J., Seward J.F., LeBaron C.W. Measles transmission and vaccine effectiveness during a large outbreak on a densely populated island: implications for vaccination policy. *Clin. Infect. Dis., 2006, vol. 42, no. 3, pp. 315–319. doi: 10.1086/498902*
11. NIID. Measles in Japan, 2012. *Infectious Agents Surveillance Report, 2013, vol. 34, no. 2.*
12. NIID. Measles in Japan (IDSC). *Infectious Agents Surveillance Report, 2014, vol. 35, pp. 278–280.*
13. NIID. Measles in Japan, as of March 2015. *Infectious Agents Surveillance Report, 2015, vol. 36, no. 4, pp. 51–53.*
14. Pham V.H., Nguyet D.P.H., Mai Kh. N. H., Truong Kh.H., Huynh L.V., Measles epidemics among children in Vietnam: genomic characterization of virus responsible for measles outbreak in Ho Chi Minh city, 2014. *EBioMedicine, 2014, vol. 1, pp. 133–140.*
15. Roberts L. In Vietnam, an anatomy of a measles outbreak. *Science, 2015, vol. 348, iss. 6238, pp. 962. doi: 10.1126/science.348.6238.962*
16. Schluter W.W., Xiaojun W., Mendoza-Aldana J., Jee Y., Diorditsa S. Progress Toward Measles Elimination — Western Pacific Region, 2009–2012. *MMWR Morb. Mortal. Wkly Rep., 2013, vol. 62, no. 22, pp. 443–447.*
17. Sniadack D.H., Mendoza-Aldana J., Huyen D.T.T., Van T.T.T., Cuong N.V., Olive J.M., Toda K., Hien N.T. Epidemiology of a measles epidemic in Vietnam 2008–2010. *J. Infect. Dis., 2011, vol. 204, no. 1, pp. S476–S482.*
18. Soakai T.S., Sadr-Azodi N., Ozturk M., Clements C.J. Measles control in pacific island countries and territories. *Ann. Virol. Res., 2016, vol. 2, no. 3, pp. 1022.*
19. Tran D. Respiratory viral coinfection and clinical disease severity. *J. Pediatr., 2013, vol. 89, no. 5, pp. 421–423.*
20. WHO. Draft measles and rubella elimination in the Western Pacific: regional strategy and plan of action. *URL: http://www.wpro.who.int/about/regional_committee/68/documents/wpr_rc68_4_annex_measles_rubella_elimination.pdf*
21. WHO. Brunei Darussalam, Cambodia, Japan verified as achieving measles elimination. Western Pacific Region achieves progress towards measles elimination, but challenges remain. News release, 2015. *URL: http://www.wpro.who.int/mediacentre/releases/2015/20150327/en*
22. WHO. Expanded programme on immunization. Regional and country profiles of measles elimination. *URL: http://www.wpro.who.int/immunization/documents/measles_regional_country_profile/en*
23. WHO. Global measles and rubella strategic plan: 2012–2020. *Geneva, Switzerland: World Health Organization, 2012. URL: http://www.who.int/immunization/newsroom/Measles_Rubella_StrategicPlan_2012_2020.pdf*
24. WHO. Global Measles and Rubella Update. April 2018. Measles. *URL: http://www.who.int/immunization/monitoring_surveillance/burden/vpd/surveillance_type/active/Global_MR_Update_April_2018.pdf?ua=1*
25. WHO. Immunization, Vaccines and Biologicals. Measles and Rubella surveillance data. *URL: http://www.who.int/immunization/monitoring_surveillance/burden/vpd/surveillance_type/active/measles_monthlydata/en*
26. WHO. Manual for the laboratory diagnosis of measles and rubella virus infection. Second edition. *URL: https://globalhealthlaboratories.tghn.org/site_media/media/articles/measles_procedures.pdf*
27. WHO. Measles-Rubella Bulletin. 2018, vol. 12, no. 2, pp. 1–10. *URL: http://www.wpro.who.int/immunization/documents/measles_rubella_bulletin/en*
28. WHO. Measles and Rubella status report: progress, challenges and lessons. Meeting of the WHO Strategic Advisory Group of Experts. 7 November, 2012. *URL: http://www.who.int/immunization/sage/meetings/2012/november/2_Measles_Figueroa.pdf*
29. WHO. Regional health leaders target elimination of: measles and rubella; HIV, hepatitis B and syphilis in babies. *News release. URL: http://www.wpro.who.int/mediacentre/releases/2017/20171011/en*
30. WHO. Reported measles cases and incidence rates by WHO States 2013, 2014 as of 11 February 2015. *URL: http://www.who.int/immunization/monitoring_surveillance/burden/vpd/surveillance_type/active/measlesreportedcasesbycountry.pdf*
31. Zhang Y. An update for the western pacific region including an update on the current outbreak in mongolia. *WHO. Report on the 13th global measles and rubella laboratory network meeting, WHO/HQ Geneva, 29 June – 1 July 2015, pp. 18–19. URL: http://www.who.int/immunization/monitoring_surveillance/burden/laboratory/13th_GMRLN_Draft_Report_final.pdf*

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РЕЗУЛЬТАТЫ МОЛЕКУЛЯРНОЙ ДЕТЕКЦИИ И ХАРАКТЕРИСТИКА ВИРУСОВ ГРИППА И ДРУГИХ ВОЗБУДИТЕЛЕЙ РЕСПИРАТОРНЫХ ИНФЕКЦИЙ В РОССИИ, СЕЗОН 2017–2018 ГГ.

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Резюме. Активизация вирусов гриппа в сезон 2017–2018 гг. началась значительно позже в сравнении с пятью предшествующими сезонами. Эпидемия гриппа продлилась в течение 12 недель (с 6 по 17 неделю), имела среднюю интенсивность с вовлечением 10,4% населения страны. Дети возрастных групп 0–2 и 3–6 лет, как обычно, болели наиболее часто. Средняя частота госпитализации пациентов с гриппом и ОРВИ составила 2,6% и была наиболее высокой (5,4%) в младшей возрастной группе (0–2 лет). Число смертельных исходов при гриппе в этом сезоне было в 2 раза выше, чем в сезон 2016–2017, что может быть объяснено распространением вирусов гриппа A(H1N1)pdm09, которые, по-прежнему, являются основной причиной летальных исходов при гриппе в стране. Всего за сезон в 55 сотрудничающих региональных базовых лабораториях было обследовано с помощью ОТ-ПЦР в реальном времени 72 759 пациентов. Грипп был лабораторно подтвержден с помощью ПЦР в 12 149 (20,7%) случаях, из числа которых 39,3% составили вирусы гриппа A(H1N1)pdm09, 29,6% — A(H3N2) и 31,1% — вирусы гриппа B (Ямагатской линии). Первые случаи гриппа были зарегистрированы в самом начале сезона (недели 40–45.2017), однако отчетливое увеличение частоты их детекции было установлено лишь на 2 неделе 2018 г. с пиком на 13–14 неделях и последующим постепенным снижением вплоть до конца сезона. Выявлены определенные отличия в этиологии гриппозной заболеваемости между федеральными округами. Определена роль вирусов гриппа и других возбудителей ОРВИ на разных стадиях эпидемического процесса. Так, в предэпидемический период рост заболеваемости был обусловлен, в основном (около 32,7% случаев) не гриппозными вирусами, в особенности риновирусами и респираторно-синцитиальным вирусом (10,2 и 8,0% случаев соответственно), тогда как лабораторно подтвержденные случаи гриппа (ЛПГ) регистрировались лишь в 3,4%. В период эпидемии частота ЛПГ увеличилась до 29,2% при одновременном снижении частоты заболеваний парагриппозной, аденовирусной, бокавирусной, коронавирусной и, особенно, риновирусной этиологии, в меньшей степени снизилась частота инфекции, вызванной респираторно-синцитиальным вирусом. В постэпидемический период роль вирусов гриппа A(H1N1)pdm09, A(H3N2) и B снизилась до 6,1; 6,9 и 3,6% соответственно, с увеличением значимости риновирусной инфекции

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(до 9,5% заболеваний). Антигенный и генетический анализ вирусов гриппа A(H1N1)pdm09 и A(H3N2), циркулировавших в сезон 2017–2018 гг., показал, что все проанализированные вирусы гриппа А, в отличие от вирусов гриппа В, по структуре поверхностных генов, соответствовали штаммам, введенным в состав вакцин для Северного полушария на сезон 2017–2018 гг. Вместе с тем, во внутренних генах циркулирующих вирусов был обнаружен ряд замен. Контроль чувствительности 316 вирусов гриппа А и В к противовирусным препаратам показал, что абсолютное большинство из них (99.7%) сохранили свою чувствительность к ингибиторам нейраминидазы.

Ключевые слова: *молекулярная диагностика, грипп, ОРВИ, генетический анализ, антигенные свойства, антивирусные препараты.*

SUMMARY OF INFLUENZA AND OTHER RESPIRATORY VIRUSES DETECTED AND CHARACTERIZED IN RUSSIA DURING 2017–2018 SEASON

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Abstract. The influenza season 2017–2018 started significantly later compared to the five previous seasons. Influenza epidemic lasted for 12 weeks (weeks 6–17), was of moderate intensity and 10,4% of the population of the country was involved with children aged 0–2 and 3–6 years being the most affected groups as usually. The average hospitalization rate of patients with ILI and ARI was 2,6% and was the highest in infants aged 0–2 years (5,4%). The number of influenza-associated deaths was two times higher this season compared to 2016–2017 which can be attributed to the circulation of A(H1N1)pdm09 viruses that still is the major cause of lethal influenza outcomes in the country. A total 72 759 patients were investigated by RT-PCR in 55 collaborating RBLs. Laboratory confirmed influenza (LCI) was detected in 12 149 (20.7%) cases, of which 39.3% were influenza A(H1N1)pdm09 viruses, 29.6% were A(H3N2) and 31.1% influenza B (Yamagata lineage) viruses. The first cases of influenza viruses were detected at the very beginning of the season (weeks 40–45.2017), however a distinct increase in the rate of detection was registered only from the week 2.2018 with the peak on the week 13–14.2018 and subsequent gradual decline up to the end of the season. The certain differences in the etiology of morbidity between Federal Districts were registered. The impact of influenza and other ARI agents in different stage of epidemic was determined. In the pre-epidemic period, the incidence growth was occurred mainly due to ARI agents (about 32,7%), especially due to rhinoviruses (RhV) and RSV (10.2 and 8.0% cases, respectively) while LCI were registered in 3.4% only. During the epidemic, the rate of LCI detection increased up to 29.2% at simultaneous decrease in frequency of parainfluenza, adenovirus, bocavirus, coronavirus and, especially, rhinoviruses, to a lesser extent RSV infection. In the post-epidemic period, the role of influenza A(H1N1)pdm09, A(H3N2) and B viruses decreased up to 6.1; 6.9 and 3.6%, respectively, with increase of rhinoviruses (9.5% of diseases). Genetic analysis of influenza A(H1N1)pdm09 and A(H3N2) viruses circulating in 2017–2018 season showed that all analyzed viruses by the structure of surface genes encoding antigenic determinants, in difference from influenza B viruses, corresponded to the vaccine strains recommended by WHO for the Northern Hemisphere for 2017–2018 epidemic season. However, significant changes in the internal genes of circulating viruses were revealed. The control of the susceptibility of 316 influenza A and B viruses to antiviral drugs showed that the absolute majority of them (99.7%) retained their susceptibility to neuraminidase inhibitors.

Key words: *molecular diagnostics, influenza, ARVI, genetic analysis, antigenic properties, antivirals.*

Введение

В отличие от контролируемых вирусных инфекций, таких как оспа, полиомиелит, корь, краснуха, паротит, эпидемии гриппа продолжают свирепствовать в мире, вызывая ежегодно до 440 000–553 000 смертельных исходов [8], при этом около 28 000–111 000 смертей регистрируется среди детей в возрасте до 5 лет [13].

В целях снижения ущерба от гриппа ВОЗ рекомендует ежегодную вакцинацию людей из «групп риска» инфицирования и развития тяжелых острых респираторных инфекций (ТОРИ) с опасностью смертельных исходов от гриппа. К их числу в последние годы отнесены беременные женщины, дети младшего возраста (6–59 месяцев), пожилые люди, больные с определенной хронической патологией, а также работники здравоохранения, социальных служб и сферы образования [20].

Очевидно, что для формирования новых решений по проблеме гриппа необходим комплексный подход с учетом значимости разных составляющих эпидемического процесса, включая сроки начала и продолжительность эпидемии, ее интенсивность, тяжесть, степень вовлеченности разных возрастных групп населения, показатели госпитализации и смертности, наряду с анализом структурных и биологических особенностей возбудителей, гете-

рогенности вирусной популяции, и, главное, определение соответствия циркулирующих вирусов штаммам, введенным в состав вакцин. Необходимо ежегодно определять наиболее уязвимые возрастные группы населения в отношении доминирующих вирусов гриппа и других, наиболее значимых, возбудителей ОРВИ. Важно оценивать роль изменений уровня популяционного иммунитета к современным штаммам вируса гриппа в регуляции этиологии надвигающихся эпидемий, а также контролировать чувствительность вирусов к лицензированным в РФ антивирусным препаратам.

Для выполнения этих задач в последние годы в Российской Федерации разработана мультиплексная система надзора за гриппом и ОРВИ с усовершенствованной инфраструктурой традиционного эпидемиологического надзора и новой системой сигнального надзора за ТОРИ среди госпитализированных больных, а также гриппоподобными заболеваниями и острыми респираторными инфекциями (ГПЗ/ОРИ) среди больных, находящихся на амбулаторном лечении.

Целью исследований был интегрированный анализ эпидемиологических данных и результатов молекулярной диагностики гриппа и других ОРВИ в разных регионах РФ в целях определения первичного старта эпидемии, оценки ее интенсивности, географии распространения и определения доминирующих возбудителей, а также углубленный анализ эволюции вирусов гриппа на основании определения антигенных и генетических особенностей циркулирующих вирусов гриппа, в том числе с использованием антигенной картографии, филогенетического анализа и полногеномного NGS-секвенирования.

Основные итоги исследований в сезон 2017–2018 гг. представлены в настоящей статье.

Материалы и методы

Организационная структура традиционного надзора (ТН) и сигнального надзора (СН) за гриппом и другими респираторными инфекциями в России была описана ранее [1, 5, 19]. ТН осуществлялся силами двух Национальных центров по гриппу ВОЗ (НЦГ) в Санкт-Петербурге и Москве в сотрудничестве с 60 региональными базовыми лабораториями (РБЛ) в системе Роспотребнадзора, СН за ТОРИ и ГПЗ/ОРИ — в 10 РБЛ расположенных в разных федеральных округах (ФО) России. Все данные аккумулировались в электронной базе данных НИИ гриппа для быстрой автоматизированной обработки поступающей информации. Результаты анализа эпидемиологических и лабораторных данных в системе ТН и СН представлялись еженедельно в Минздрав России, Роспотребнадзор и обратно в РБЛ на протяжении всего сезона. Одновременно данные вводили в базы данных ЕвроВОЗ (TESSy) и ВОЗ (FluNet) и публиковались в бюллетене FluNews Europe (http://flunewseurope.org) и на сайте FluNet (http://www.who.int/influenza/gisrs_laboratory/flunet/en), а также на сайте Института (www.influenza.spb.ru).

Анализ заболеваемости. Относительные показатели заболеваемости из расчета на 10 000 населения (в целом и по возрастным группам 0–2, 3–6, 7–14, 15–64 года и более), определялись автоматически (с использованием специальных Госпрограмм обработки данных «INFLUBASE», разработанных в Институте) на основе сообщений из РБЛ, поступающих через интернет в базу данных НИИ гриппа. Показатели заболеваемости для каждого города сопоставлялись с регулярно обновляемыми еженедельными эпидемическими порогами (ЭП) [2]. Кроме того, еженедельно учитывалось число случаев госпитализации с гриппом и ОРВИ по тем же возрастным группам и число смертельных исходов с ПЦР-подтвержденным гриппом. Показатель летальности выражали в виде отношения числа случаев смерти от лабораторно подтвержденного гриппа к числу зарегистрированных случаев гриппа и ОРВИ из расчета на 100 000 заболеваний.

Критерии определения начала эпидемии гриппа. Резкое увеличение уровня заболеваемости с превышением еженедельных порогов более чем на 20% и выявлением ПЦР-подтвержденных случаев гриппа, было основным критерием определения начала эпидемий в отдельных городах. В целях определения старта эпидемии в стране усредненные показатели заболеваемости по всем городам сопоставлялись с ЭП для страны в целом и базовой линией заболеваемости (БЛ) [20]. Превышение БЛ, сопровождающееся ростом частоты детекции вирусов гриппа, было сигналом начала эпидемии в России.

Лабораторный надзор включал детекцию специфических последовательностей вирусов гриппа и семи других возбудителей ОРВИ в ОТ-ПЦР с использованием наборов «АмплиСенс» (Интерлабсервис, Россия), выделение, антигенный и генетический анализ вирусов гриппа, тестирование их на чувствительность к противовирусным препаратам (озельтамивир, занамивир, римантадин). В целях поддержания системы глобального надзора за гриппом (GISRS) проводился обмен репрезентативными и дрейф-вариантами вирусов гриппа между двумя НЦГ в России и Сотрудничающими центрами ВОЗ по гриппу в Атланте (США) и Лондоне (Великобритания).

Выделение вирусов и антигенный анализ. В работе применяли стандартные операционные

процедуры выделения вирусов с использованием клеток MDCK или MDCK-SIAT в соответствии с действующими Методическими рекомендациями [3] и Руководством ВОЗ [10].

Вкратце, клинические образцы от пациентов с гриппом и ОРВИ, суспендированные в транспортной среде (UTM-RT, Copan, США), использовали для заражения клеток MDCK, выращенных в среде Игла МЕМ с 5% фетальной сыворотки после двукратной промывки монослоя и введения свободной от сыворотки среды, содержащей трипсин TPCK (2 мкг/мл). Инфицированные клетки, инкубированные в CO_2 термостате, регулярно исследовали в инвертированном микроскопе (UNICO, США). При наличии ЦПД среду собирали и использовали для определения гемагглютининов.

Антигенный анализ вирусов проводили в РТГА или микронейтрализации с использованием набора специфических антисывороток к референс-штаммам и российским изолятам. Кроме того, для антигенного анализа новых изолятов использовали постинфекционные сыворотки хорьков, любезно предоставленные СЦ ВОЗ в Лондоне, и диагностические сыворотки в составе набора, полученного из СЦ ВОЗ в Атланте (США), за что авторы выражают им свою благодарность.

Генетический анализ вирусов. Выделение вирусной РНК проводили с использованием набора реагентов Qiagen RNeasy mini kit (Qiagen, Германия) в соответствии с рекомендациями производителя. Полногеномную амплификацию генетического материала исследуемых вирусов проводили методом одноступенчатой обратной транскрипции — полимеразной цепной реакции (ОТ-ПЦР) с использованием набора реагентов SuperScript III High Fidelity RT-PCR kit (Life Technologies, США). Специфическую амплификацию последовательностей, кодирующих поверхностные антигены исследуемых вирусов проводили методом одноступенчатой ОТ-ПЦР с использованием набора реагентов Ambion AgPath-ID с оригинальными праймерами. Для филогенетического анализа полученных последовательностей использовалось программное обеспечение MEGA6.

Секвенирование нового поколения. Определение генетических последовательностей проводилось на секвенаторе Illumina MiSeq (Illumina, США) с набором реагентов Illumina MiSeq Reagent kit v3 600 cycle.

Для оценки результатов секвенирования, извлечения консенсуных последовательностей и оценки гетерогенности вирусных популяций использовалось программное обеспечение Iterative Refinement Meta-Assembler.

Результаты

ПЦР-диагностика гриппа в НЦГ и РБЛ. В сезон 2017–2018 (период с 40 недели 2017 г. по 20 неделю 2018 г.) проведено изучение вклада вирусов гриппа А и В и других возбудителей в заболеваемость острыми респираторными вирусными инфекциями (ОРВИ) по результатам ПЦР-детекции вирусов в образцах от 72 759 больных, полученным двумя НЦГ в сотрудничестве с 55 РБЛ. Частота детекции вирусов гриппа в среднем за весь сезон по стране в целом составила 20,7%. Всего было диагностировано 12 149 случаев гриппа, из которых 39,3% составили вирусы гриппа A(H1N1) pdm09, 29,6% — вирусы гриппа A(H3N2) и 31,1% — вирусы гриппа типа В (табл. 1).

Таблица 1. Результаты ПЦР-диагностики гриппа в городах Российской Федерации

Table 1. Influenza PCR testing conducted in various cities of the Russian Federation

Референс-центры по мониторингу гриппа Reference centers for influenza monitoring	**Число лабораторий-участников** Number of laboratories involved	**Число обследованных больных** Number of patients examined	**Число ПЦР-положительных случаев гриппа** Number of PCR-positive influenza cases			
			A(H1N1)pdm09	**A(H3N2)**	**B**	**Всего** Total
При ФГБУ НИИ гриппа им. А.А. Смородинцева At Smorodintsev Research Institute of Influenza	46	61 183	3790	2910	3146	9846 (16,1%)
При ФГБУ НИЦЭМ им. Н.Ф. Гамалеи At FSBI "N.F. Gamaleya NRCEM"	10	11 576	987	683	633	2303 (19,9%)
Частота детекции вирусов гриппа Frequency of detected influenza viruses			8,9%	6,6%	5,2%	20,7%
Процентное соотношение возбудителей A proportion of influenza viruses			39,3%	29,6%	31,1%	

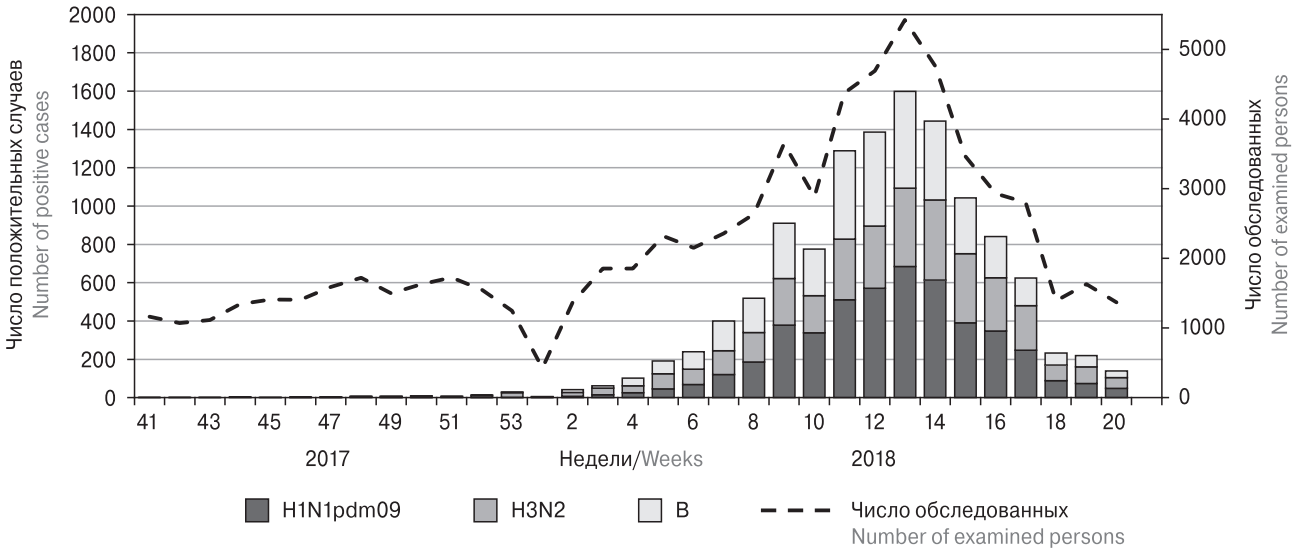

Рисунок 1. Мониторинг распространения вирусов гриппа по результатам ПЦР-диагностики
Figure 1. PCR used in monitoring influenza viruses spread

Мониторинг распространения вирусов гриппа. Первые случаи гриппа A(H1N1)pdm09, а также вирусов A(H3N2) и B, были диагностированы в самом начале сезона (40–45 недели 2017 г.), однако отчетливый, прогрессивный рост частоты детекции вирусов гриппа зарегистрирован лишь в первой декаде января 2018 г. с пиком диагностирования на 13 неделе и последующим постепенным снижением числа выявляемых случаев вплоть до 20 недели 2018 г. В развитии эпидемии с самого ее начала активно участвовали все 3 возбудителя, но с 8–9 недели и до 17 недели 2018 г. по данным ПЦР-мониторинга наметилось преобладание вируса гриппа A(H1N1) pdm09 (рис. 1).

Сравнение ПЦР-данных с показателями эпидемической заболеваемости показало опережающую (на 4 недели) роль диагностической информации, поскольку старт эпидемии, определенный по превышению базовой линии и эпидемического порога заболеваемости для страны, был зарегистрирован лишь на 6 неделе 2018 г., тогда как повышение частоты диагностирования гриппа — со 2 недели года (рис. 2).

По сравнению с предыдущими сезонами в 2017–2018 гг. зарегистрировано более позднее начало эпидемии и время достижения пика заболеваемости, а также снижение показателей заболеваемости (в среднем по всем возрастным группам переболело 9,7% населения по сравнению с 14,2% в 2016–2017гг.). Как обычно, заболеваемость гриппом и ОРВИ среди детей младшего возраста (0–2 и 3–6 лет) в десятки раз превышала аналогичные показателя для взрослых и составляла 51,1 и 56,2% от численности соответствующих возрастных групп против 4,8% в возрастной группе 15–64 лет.

Установлены выраженные отличия в показателях заболеваемости между федеральными округами, которая была наиболее высокой в Уральском, Северо-Западном и Приволжском ФО и наименьшей в Южных районах Европейской части России (рис. 3).

Продолжительность эпидемии в разных федеральных округах варьировала от 7 до 11 недель и, как правило, была на несколько недель короче, чем в предыдущем сезоне.

Госпитализация. Увеличение показателя госпитализации больных с клиническим диагнозом «грипп» было зарегистрировано на 3–4 неделях. В отличие от заболеваемости его значения на пике (13 неделя) были выше по сравнению с предшествующим сезоном (1516 и 1097 случаев за неделю, соответственно) (рис. 4).

География распространения вирусов гриппа представлена в табл. 2, которая показывает социркуляцию всех 3 возбудителей в разных регионах страны с небольшими отличиями в этиологии между Федеральными округами. Так грипп A(H1N1)pdm09 несколько чаще диагностировали в Центральном, Сибирском и Приволжском ФО, A(H3N2) — в Северо-Западном и Центральном ФО, вирусы гриппа B — в Северо-Западном и Северо-Кавказском ФО, где частота ПЦР-детекции указанных вирусов была достоверно выше средней по РФ в целом.

Летальность. Всего за отчетный период из РБЛ поступили сообщения о 53 летальных исходах от ПЦР-подтвержденного гриппа, что было в 2,1 раза больше по сравнению с предыдущей эпидемией. Вирус гриппа A(H1N1)pdm09 был основной причиной летальных исходов (41 из 53 смертельных случаев). 9 случаев было связано с вирусом гриппа A(H3N2) и 3 случая —

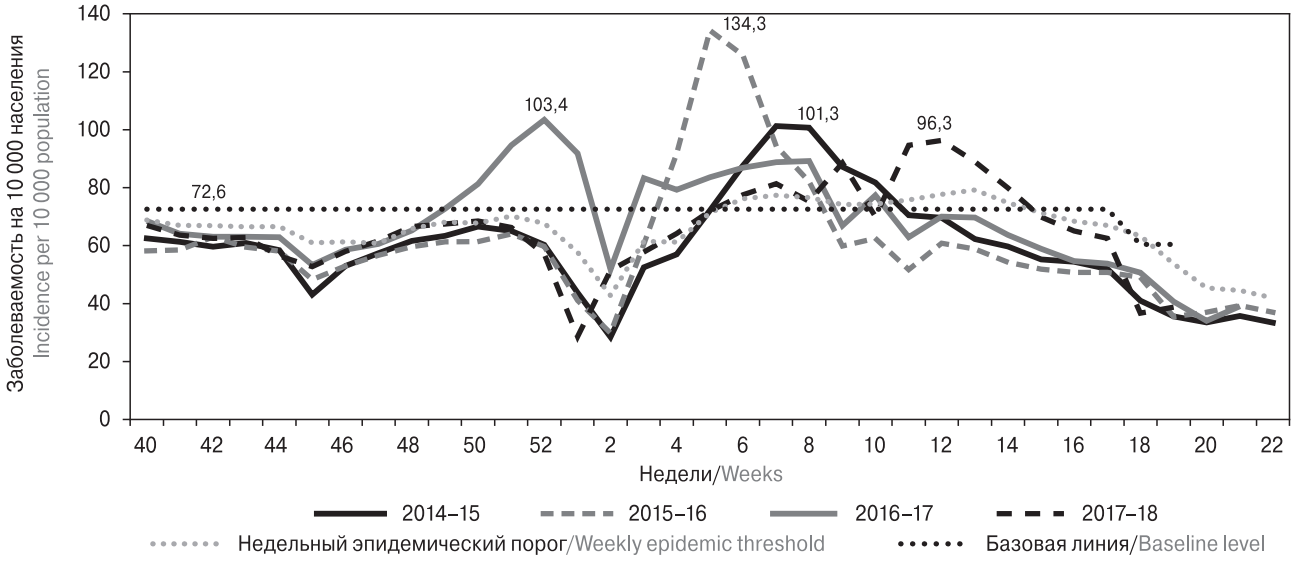

Рисунок 2. Сравнительные данные по динамике развития эпидемий гриппа за последние четыре сезона по показателям заболеваемости гриппом и ОРВИ в 60 городах РФ

Figure 2. Comparative data assessing dynamic changes in developing influenza epidemics during the four previous seasons based on influenza and ARVI incidence in the 60 cities of the Russian Federation

с вирусом гриппа В. Основная масса смертельных исходов зарегистрирована в эпидемический по гриппу период, о 5 случаях смерти было сообщено до начала эпидемии, в том числе — 3 случая от гриппа A(H1N1)pdm09, 1 — от гриппа A(H3N2) и 1 — от гриппа В (рис. 5).

Большая часть пациентов, умерших от гриппа, была из старших возрастных групп и имела сопутствующую патологию. В последнем сезоне отмечено увеличение числа смертельных исходов среди больных с диабетом, ожирением, сердечно-сосудистой патологией и болезнями внутренних органов. Умершие больные не были вакцинированы от гриппа, за исключением одного пациента с ХБЛ. Четыре случая смерти от гриппа зарегистрировано среди детей. Беременных среди умерших от гриппа не выявлено. Показатель летальности от гриппа за период эпидемии 2017–2018 составил 0,93 на 100 000 переболевших гриппом и ОРВИ и был в 2,7 раза выше по сравнению с эпидемией прошлого года (0,34 на 100 000 переболевших), когда циркулировали только вирусы гриппа A(H3N2) и В.

Оценка ущерба от гриппа и других возбудителей ОРВИ. В целях дифференцированного выяснения ущерба от гриппа и других возбудителей ОРВИ проведено сравнение частоты их детекции в материалах от больных на разных

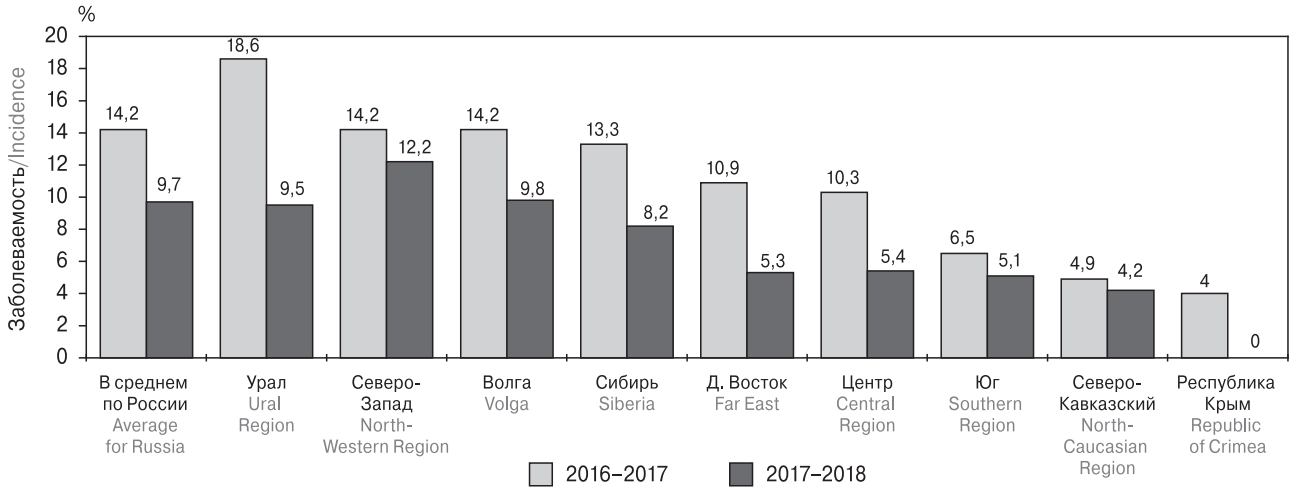

Рисунок 3. Сравнение показателей заболеваемости гриппом и ОРВИ по федеральным округам в эпидемические периоды 2017–2018 и 2016–2017 гг.

Figure 3. Comparison of influenza and ARI incidence rate among Federal Districts of the Russian Federations during 2017–2018 and 2016–2017 epidemic periods

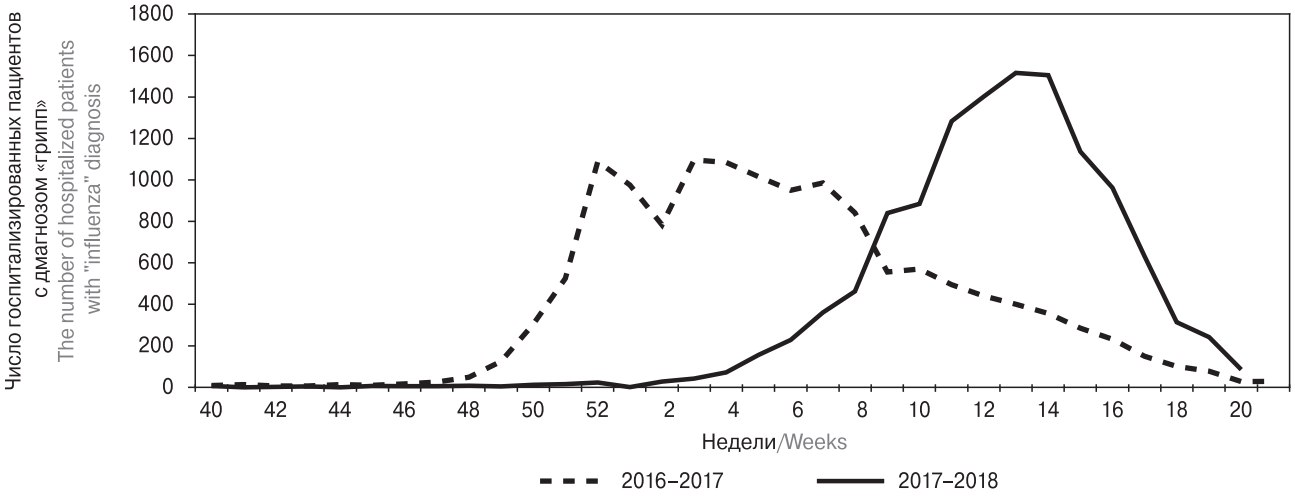

Рисунок 4. Мониторинг госпитализации больных с клиническим диагнозом «грипп» в эпидемии 2016–2017 и 2017–2018 гг.

Figure 4. Monitoring hospitalization rate of patients clinically diagnosed with influenza during the 2016–2017 and 2017–2018 epidemics

Таблица 2. Особенности циркуляции вирусов гриппа в разных федеральных округах в сезон 2017–2018 гг.

Table 2. Features of circulating influenza viruses found in various Federal Districts during 2017–2018 season

Федеральный округ Federal District	**Частота диагностирования гриппа (%)** Prevalence of verified influenza virus infection (%)		
	A(H1N1)pdm09	**A(H3N2)**	**B/Ямагата** B/Yamagata
Центральный/Central	13,3	8,9	4,5
Южный/Southern	8,2	1,4	2,7
Северо-Западный/North-Western	8,4	9,3	8,1
Дальневосточный/Far Eastern	2,1	5,5	4,7
Сибирский/Siberian	11,7	6,0	2,7
Уральский/Ural	0,6	1,6	4,1
Приволжский/Privolzhsky	10,6	6,1	5,3
Северо-Кавказский/North-Caucasian	8,2	4,1	10,1
Российская Федерация (усредненные данные) Russian Federation (averaged data)	8,9	6,6	5,2

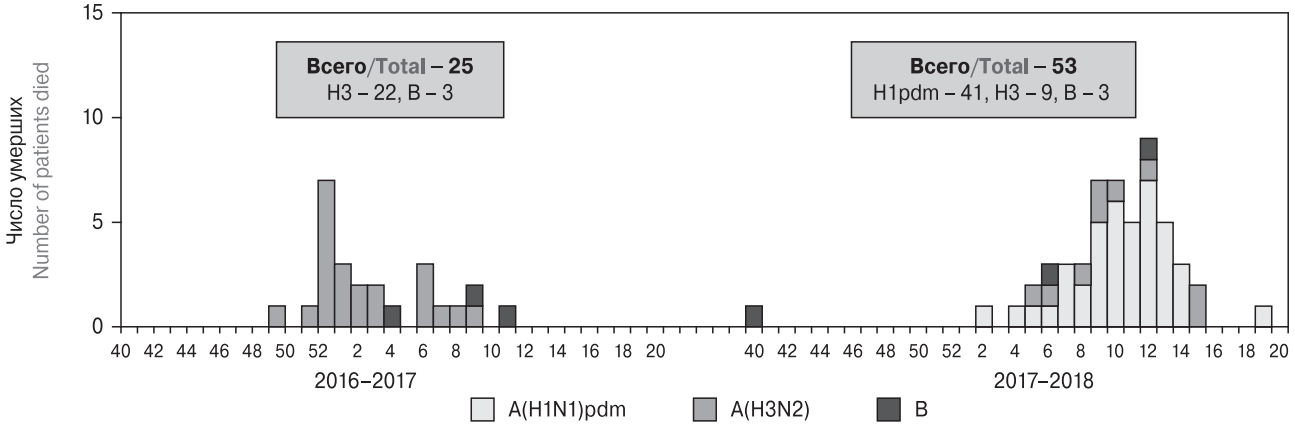

Рисунок 5. Этиология летальных исходов от лабораторно подтвержденного гриппа, зарегистрированных в региональных базовых лабораториях в сезоны 2017–2018 и 2016–2017 гг.

Figure 5. Etiology of laboratory-confirmed influenza fatal cases registered in Regional Base Laboratories during 2017–2018 and 2016–2017 seasons

Таблица 3. Частота ПЦР-детекции вирусов гриппа и других возбудителей ОРВИ в разные периоды эпидемического процесса

Table 3. Frequency of PCR-detected influenza viruses and other ARVI causative agents found in various periods of epidemic process

Вирусы/Viruses	**Периоды**/Periods							
	Предэпидемический (40 нед. 2017 – 5 нед. 2018) Pre-epidemic (Week 40.2017 — week 5.2018)		**Эпидемический (6–17 нед. 2018)** Epidemic (6–17 weeks 2018)		**Постэпидемический (18–20 нед. 2018)** Post-epidemic (18–20 weeks 2018)		**Всего (40 нед. 2017 – 20 нед.2018)** Total (Week 40.2017 — week 20.2018)	
	Число ПЦР(+) случаев Number of PCR (+) cases	%	**Число ПЦР(+) случаев** Number of PCR (+) cases	%	**Число ПЦР(+) случаев** Number of PCR (+) cases	%	**Суммарное число ПЦР(+) случаев** Total number of PCR (+) cases	%
Грипп A(H1N1)pdm09/Influenza A(H1N1)pdm09	106	0,8	4460	11,8	211	6,1	4777	8,9
Грипп A(H3N2)/Influenza A(H3N2)	235	1,8	3134	8,2	224	6,9	3593	6,6
Грипп B/Influenza B	149	0,6	3473	8,2	157	3,6	3779	5,2
Парагрипп/Parainfluenza	777	3,9	282	1,1	51	1,8	1110	2,3
Аденовирус/Adenovirus	930	4,7	443	1,8	69	2,4	1442	3,0
РС вирус/RS virus	1583	8,0	1501	6,0	97	3,4	3181	6,7
Бокавирус/Bocavirus	393	2,7	85	0,4	13	0,5	491	1,4
Метапневмовирус/Metapneumovirus	163	1,1	404	1,9	77	3,1	644	1,7
Коронавирус/Coronavirus	321	2,1	284	1,3	25	1,0	630	1,6
Риновирус/Rhinovirus	1890	10,2	1155	4,9	263	9,5	3308	7,3

стадиях развития эпидемии. Установлено, что в предэпидемический период рост заболеваемости был обусловлен, в основном, риновирусами и РС-вирусом (10,2 и 8,0% случаев соответственно) при меньшем участии аденовирусов, вирусов парагриппа, бокавирусов, коронавирусов и метапневмовирусов (1,1–4,7%). В сумме разные возбудители ОРВИ негриппозной этиологии вызвали в предэпидемический период около 32,7% заболеваний, тогда как вирусы гриппа — лишь около 3,4% инфекций.

В период эпидемии (6–17 недели 2018 г.) частота диагностирования гриппа возросла до 29,2%, в том числе A(H1N1)pdm09 — до 11,8%, A(H3N2) — до 8,2%, гриппа B — до 8,2%. При этом, как и в прошлом году, наблюдали снижение частоты заболеваний парагриппозной, аденовирусной, бокавирусной, коронавирусной и, особенно, риновирусной этиологии.

В постэпидемический период, когда заболеваемость гриппом и ОРВИ пошла на спад и частота обнаружения вирусов гриппа A(H1N1) pdm09, A(H3N2) и B снизилась до 6,1; 6,9 и 3,6%, основную роль вновь стали играть риновирусы (9,5% заболеваний). В целом (за весь сезон 2017–2018) по частоте диагностирования РСВ и риновирусы уступали только вирусам гриппа A(H1N1)pdm09 (табл. 3).

Мониторинг циркуляции возбудителей ОРВИ негриппозной этиологии подтвердил, что в начале сезона наибольшую активность проявляли риновирусы. Непосредственно перед эпидемией (за 3 недели до ее начала) доминирующую роль приобрел РСВ, значительно превысив уровень циркуляции других респираторных агентов. В постэпидемический период на первый план вновь вышли риновирусы, которые достаточно активно циркулировали на протяжении всего сезона (рис. 6).

Выделение и антигенная характеристика вирусов гриппа. За период эпидемии в системе НЦГ и РБЛ было выделено 1374 вируса гриппа, в том числе 430 штамма A(H1N1)pdm09, 282 штамма A(H3N2) и 662 штамма вируса гриппа B Ямагатской линии. В НИИ гриппа антигенно охарактеризовано 535 штаммов в РТГА или реакции микронейтрализации, используемой, в основном, для вирусов гриппа A(H3N2) с низкой гемагглютинирующей активностью.

Антигенное картирование вирусов гриппа показало, что вирусы гриппа A(H1N1)pdm09 сохраняли свое антигенное единообразие, о чем свидетельствовала равная удаленность современных изолятов от референс вирусов A/Калифорния/07/2009 и A/Мичиган/45/2015, хотя в последнем сезоне наметилось формирование двух явно различимых групп изолятов, что свидетельствовало о постепенно намечающемся дрейфе возбудителя.

Вирусы гриппа A(H3N2) прошедшего сезона равномерно группировались вместе с вирусами гриппа сезона 2016–2017. Антигенные различия вирусов между отдельными генетическими подгруппами внутри группы 3C.2a не обнаружены, однако очевидны их антигенные отличия от группы 3C.3a.

Немногочисленные вирусы гриппа B Викторианской разновидности группировались в единый кластер с вирусами гриппа B прошлых лет выделения в силу их тесного антигенного родства. Та же закономерность выявлена и для вирусов гриппа B Ямагатской разновидности, которые широко распространились в последнюю эпидемию, но группировались вместе со штаммами 2015–2017 гг. выделения (рис. 7, III обложка).

Таким образом, результаты выделения вирусов подтвердили активное участие всех трех возбудителей гриппа A(H1N1)pdm09, A(H3N2) и B в этиологии эпидемии 2017–2018 гг. Активность всех трех вирусов стала устойчиво повышаться со 2 недели 2018 г. и их циркуляция продолжалась вплоть до конца сезона, включая постэпидемический период.

Генетический анализ вирусов гриппа сезона 2017–2018 гг. показал, что по структуре генов, кодирующих антигенные детерминанты, все анализированные вирусы гриппа A соответствовали вакцинным штаммам, рекомендованным ВОЗ для производства вакцин в Северном полушарии на эпидемический сезон 2017–2018 гг. Генетически было охарактеризовано 59 вирусов гриппа A(H1N1) pdm09. Все они относились к филогенетической группе 6B (референс-штамм — A/Южная Африка/3626/2013) с характерными заменами аминокислотных остатков в гемагглютинине (HA) — K163Q (антигенный сайт Sa) и A256T — и принадлежали к подгруппе 6B.1, характеризующейся дополнительными заменами аминокислотных остатков S162N (появление потенциального сайта гликозилирования) и I216T (рис. 8).

Согласно филогенетическому анализу гена HA все вирусы гриппа A(H3N2) 2017–2018 гг. относились к клайду 3C.2a (65 вирусов) и разделялись на 3 генетические подгруппы: 3C.2a3 (5 вирусов), 3C.2a1b (19 вирусов) и 3C.2a2 (41 вирус) (рис. 9).

Подгруппа 3C.2a3 характеризуется заменами N121K и S144K в HA (антигенный сайт A). HA1 некоторых вирусов данной подгруппы также кодирует T135K (что приводит к потере потенциального сайта гликозилирования), R150K и R261Q.

Большинство секвенированных вирусов относились к подгруппе 3C.2a2 и характеризовались заменами T131K, R142K (антигенный сайт A) и R261Q в HA. Некоторые из вирусов в этой подгруппе имели замену S144R (вирусы из Самары), другие вирусы — замены P21S и K92R (антигенный сайт E). Были идентифицированы 2 вируса (A/Санкт-Петербург/RII-334S/2018 и A/Санкт-Петербург/RII-449S/2018) с заменами в стебле (H311Q) и трансмембранном домене (I550V).

Подгруппа 3C.2a1b в целом характеризовалась заменами K92R (антигенный сайт E) и H311Q. Некоторые вирусы данной подгруппы имели в HA замены, приводящие к утрате потенциального сайта гликозилирования — N122D (антигенный сайт A), T135K (антигенный сайт A) и T128A. В результате замены T135N также в антигенном сайте A произошло смещение потенциального сайта гликозилирования из положения 133 в положение 135.

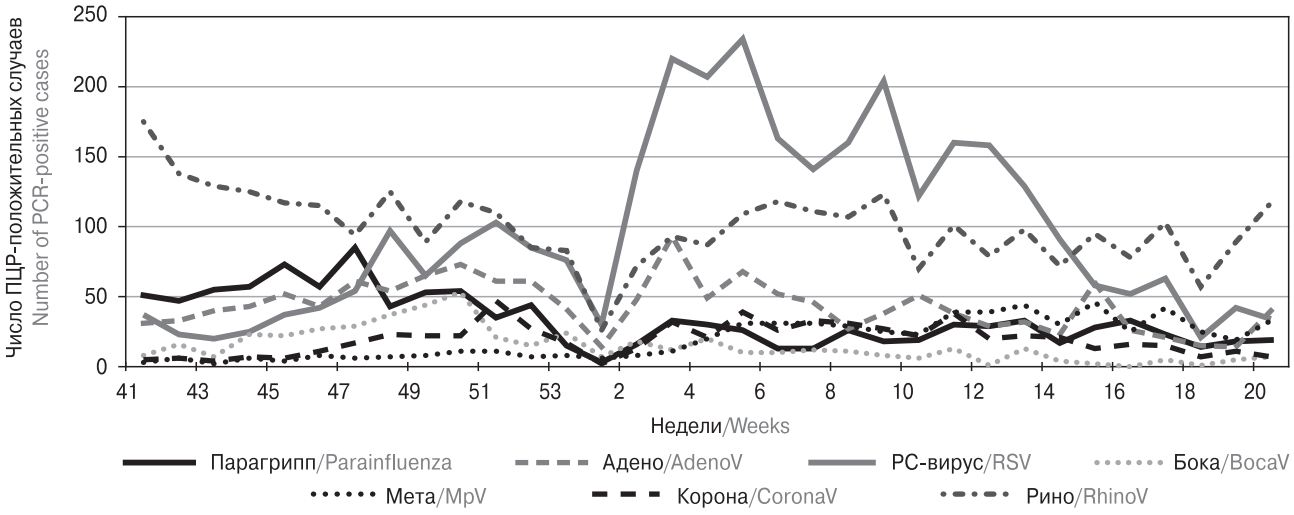

Рисунок 6. ПЦР-мониторинг циркуляции возбудителей ОРВИ негриппозной этиологии в сезон 2017–2018 гг.

Figure 6. PCR-monitoring of circulating non-influenza acute respiratory pathogens during 2017–2018 season

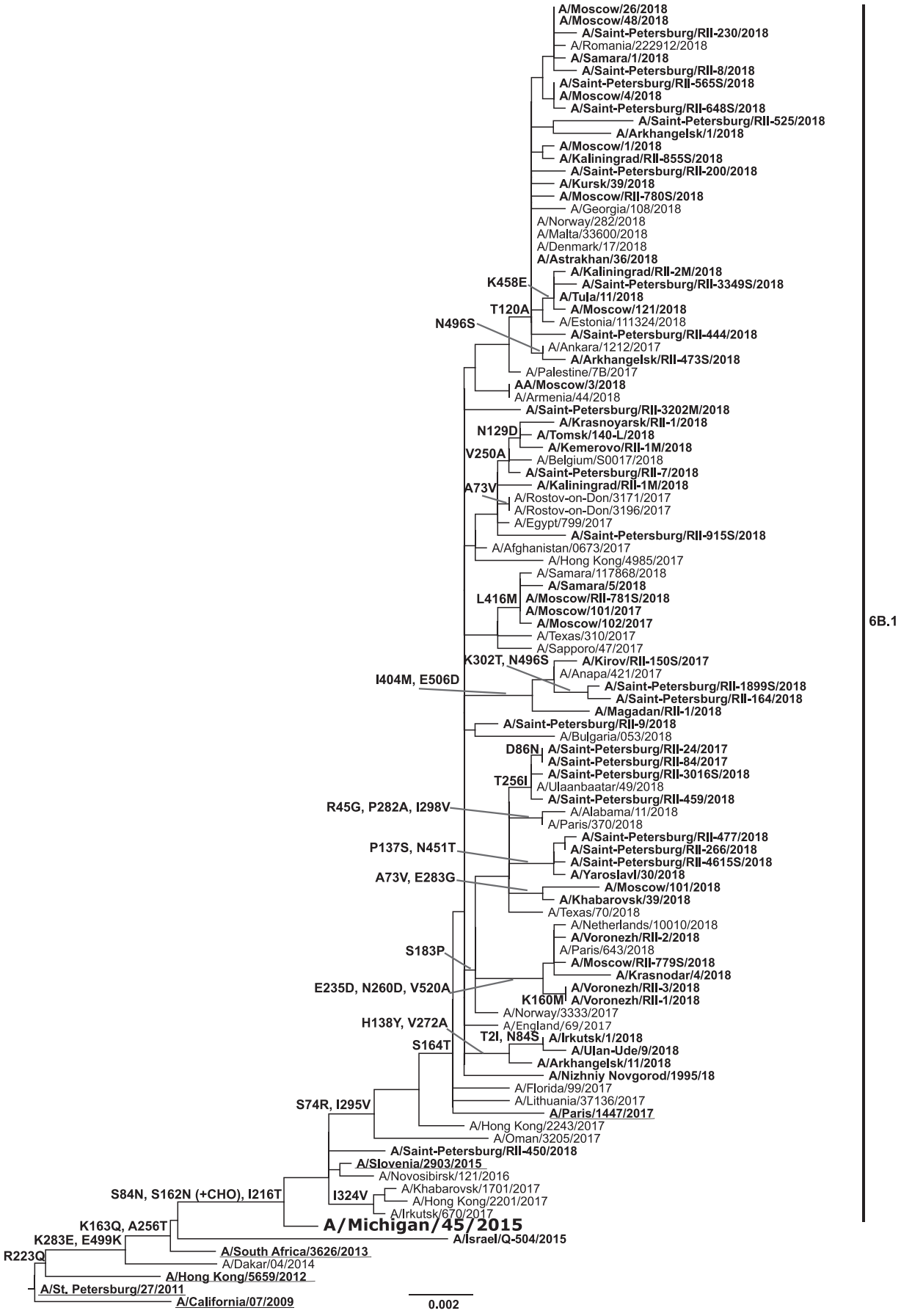

Рисунок 8. Филогенетическое дерево по гену НА вирусов гриппа A(H1N1)pdm09

Figure 8. A phylogenetic tree for influenza A(H1N1)pdm09 hemagglutinin gene

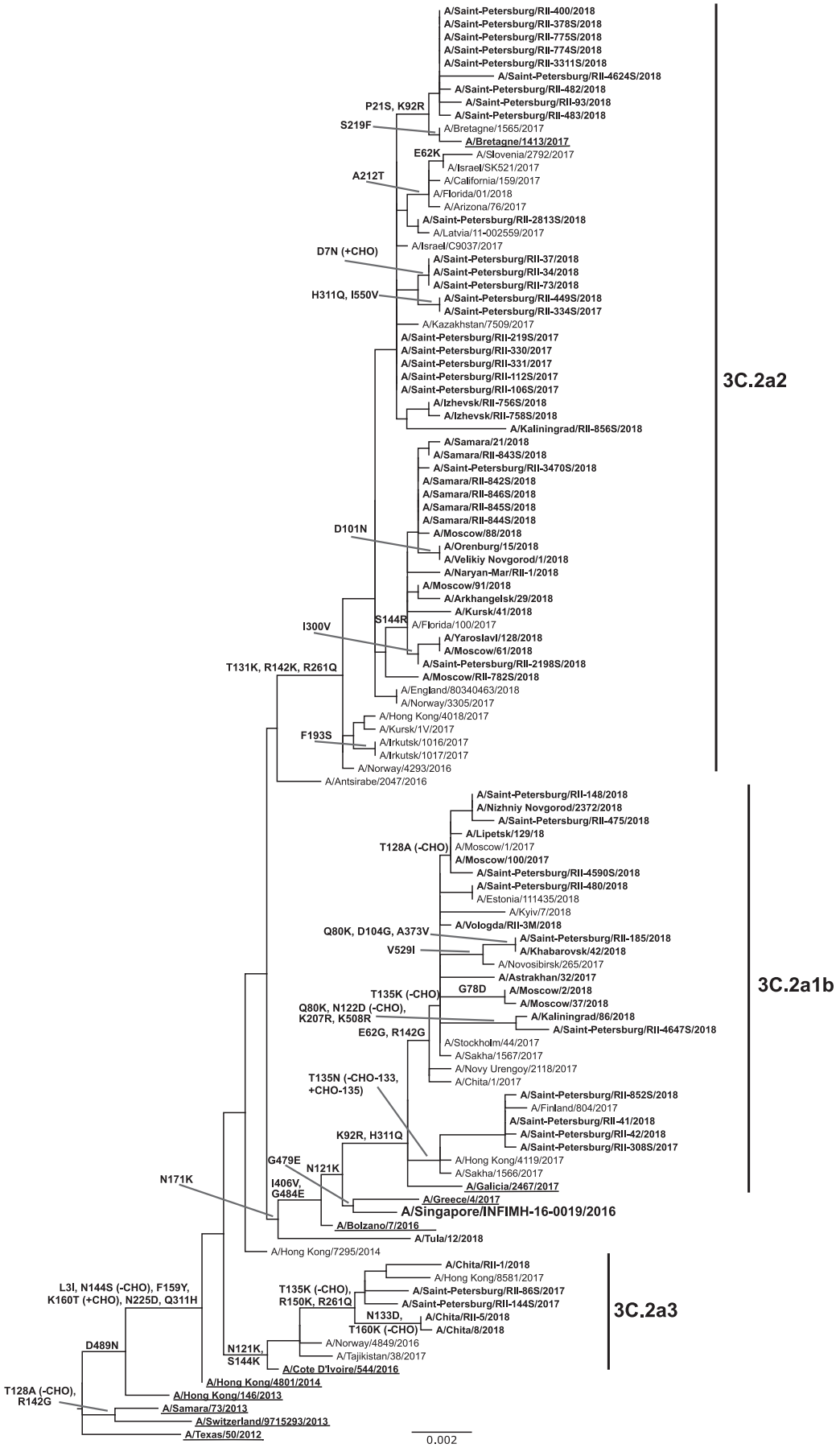

Рисунок 9. Филогенетическое дерево по гену НА вирусов гриппа A(H3N2)

Figure 9. A phylogenetic tree for influenza A(H3N2) hemagglutinin gene

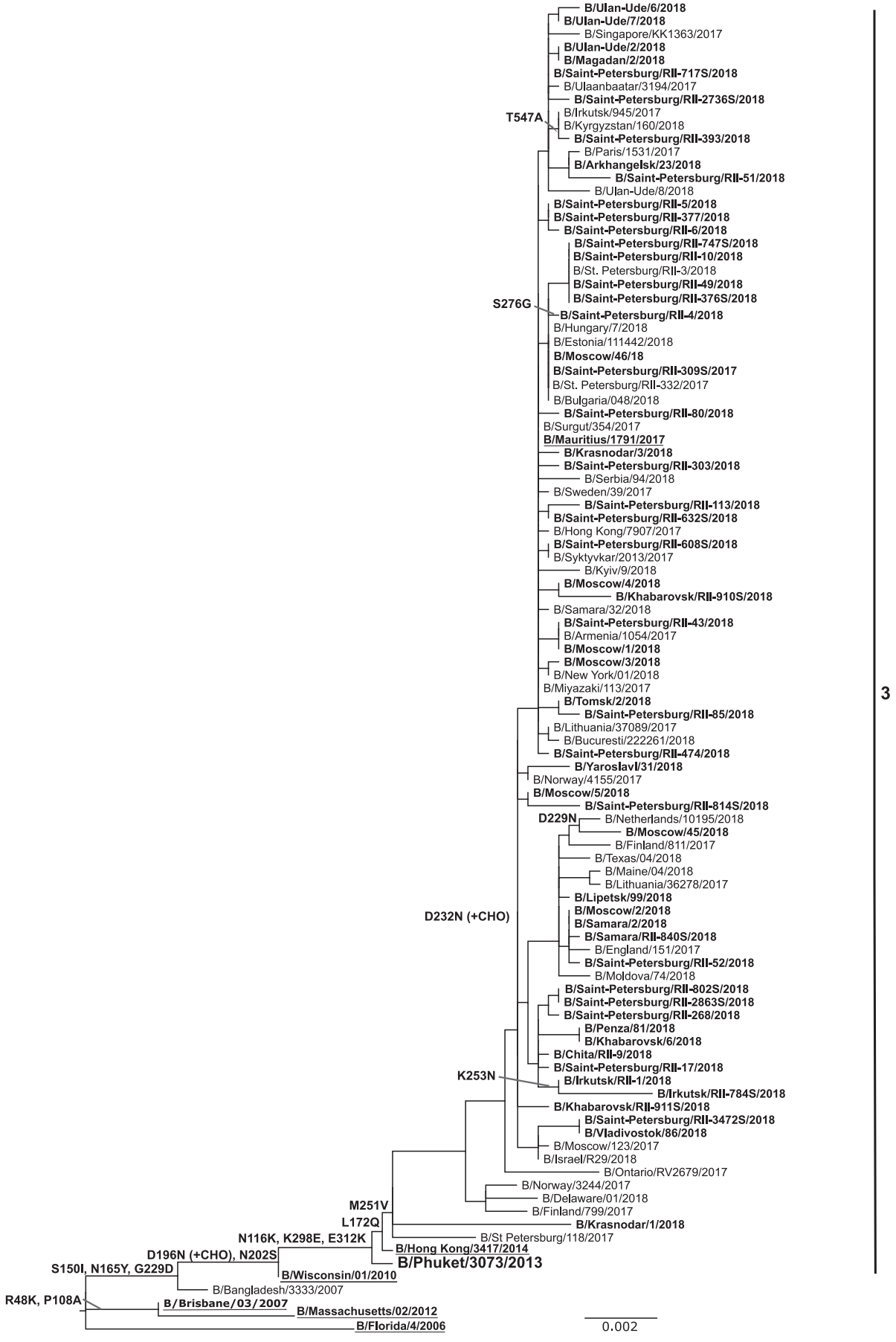

Рисунок 10. Филогенетическое дерево по гену HA вирусов гриппа B Ямагатской линии

Figure 10. A phylogenetic tree for Yamagata lineage influenza B hemagglutinin genes

В сезон 2017–2018 гг. произошли значительные изменения во внутренних генах циркулирующих вирусов. Возросло общее количество аминокислотных замен относительно референс-вируса по сравнению с двумя предыдущими годами. При этом более высокая степень изменчивости установлена для вирусов гриппа A(H1N1)pdm09 по сравнению с подтипом A(H3N2).

Необходимо отметить полное несоответствие антигенных свойств циркулировавших в 2017–2018 гг. вирусов гриппа В Ямагатской линии штамму B/Брисбан/60/08 (Викторианской линии) в составе вакцин, что не могло не сказаться на показателях их эффективности. Абсолютное большинство секвенированных вирусов гриппа В (55 штаммов) относились к клайду 3, были подобны штамму B/Пхукет/3073/2013 и несли ряд характерных замен в антигенных сайтах BC, BA, BB и BD. Три вируса (B/Москва/2/2018, B/Самара/2/2018 и B/Самара/RII-840S/2018) имели замену D232N, что привело к усилению потенциального сайта гликозилирования. У двух вирусов из Иркутска наблюдали замену в позиции 253 в антигенном сайте HA BD (K253N) (рис. 10).

Контроль чувствительности 316 вирусов гриппа A(H1N1)pdm09, A(H3N2) и B к лицензированным в России противовирусным препаратам показал, что абсолютное большинство из них (99,7%) сохранили свою чувствительность к ингибиторам нейраминидазы (озельтамивир, занамивир) и резистентность к ремантадину.

Обсуждение

Прошедший эпидемический сезон по гриппу в России отличался более поздним стартом и меньшей продолжительностью. Эпидемия 2017–2018 гг. была средней по интенсивности и по своим параметрам была сопоставима с эпидемией 2014–2015 гг., также связанной с участием всех трех возбудителей сезонного гриппа. В течение сезона велась непрерывная работа, направленная на реализацию комплексного подхода к решению проблемы гриппа на основе изучения эпидемиологических особенностей современных эпидемий за счет внедрения количественных критериев оценки интенсивности эпидемии по показателям заболеваемости, госпитализации и смертности. Расчет и применение базовых линий заболеваемости для отедельных Федеральных округов и страны в целом позволил четко определить начало эпидемии, которая началась в прошедшем сезоне значительно позже, чем в странах Северной Америки и Западной Европы, а также в предшествующие 4 сезона в РФ. Помимо этого, были выявлены отличия между федеральными округами по срокам начала эпидемии, а также по показателям заболеваемости и продолжительности периода эпидемического подъема заболеваемости. Традиционно наиболее интенсивной эпидемия была в Северо-Западном, Приволжском и Уральском ФО. Указанные ФО на протяжении многих сезонов являются лидирующими по показателям заболеваемости. Четкой связи с особенностями этиологии заболеваемости в данных регионах не выявлено, что определяет необходимость выяснения причинно-следственных связей с точки зрения климатогеографических, социальных, демографических и иных факторов. Частота госпитализации с гриппом и ОРВИ в прошедшем эпидемическом сезоне была несколько выше сезона 2016–2017 гг. (2,6 против 2,4%), когда доминировал вирус гриппа A(H3N2), и совпадала с таковой для сезона 2015–2016 гг., когда в стране активно циркулировал вирус гриппа A(H1N1)pdm09. При этом, число летальных исходов в 2,1 раза превышало таковое для сезона 2016–2017 гг., что, несомненно, связано с активным участием вирусов гриппа A(H1N1)pdm09 в этиологии последней эпидемии. Вирусы гриппа А данного подтипа по-прежнему являются основным фактором летальных исходов от гриппа в России, в отличие от стран Северной Америки, где важную роль в смертности играет также вирус гриппа A(H3N2) (21% в структуре детской смертности от гриппа в США в сезон 2017–2018) [12]. Интересно, что в России по показателям заболеваемости в последние годы растет интенсивность эпидемий гриппа, связанных с участием вируса гриппа A(H3N2) [1] на фоне выраженной гетерогенности циркулирующих популяций этого вируса с регистрацией новых генетических субклайдов 3C.2a3, 3C.2a1b и 3C.2a2 с характерными заменами в гене HA. В то же время до сих пор не определены детерминанты, которые объясняли бы тяжелое течение инфекции, вызванной A(H1N1)pdm09, с опасностью летальных исходов у ряда категорий больных с сопутствующей патологией. Наши исследования [7] позволили установить, что в последние годы появился ряд замен во внутренних генах вируса. Исследования коллег, проведенные при анализе 109 вирусов гриппа A(H1N1)pdm09, выделенных из материалов от умерших в сезоне 2015–2016 гг. выявили более частую встречаемость замены D222G [6] в гемагглютинине, роль которой активно обсуждалась в научной литературе ранее [16]. Однако для выявления связи мутаций в геноме вируса с тяжестью вызываемого ими заболевания необходимо проводить множественные систематизированные исследования с детальной клинической картиной на каждого пациента, отобранного в исследование. В настоящее время такие исследования ведутся в рамках Глобального проекта по госпитальному надзору [9, 14].

Анализ антигенных и генетических свойств вирусов гриппа, циркулировавших в эпидемический сезон 2017–2018 гг. показал, что вирусы гриппа A(H1N1)pdm09 и A(H3N2) соответствовали по своим свойствам штаммам, введенным в состав тривалентных вакцин. Однако ситуация по гриппу В была противоположной, поскольку в России доминировали вирусы гриппа В Ямагатской разновидности, в то время как в вакцину был включен штамм В/Брисбен/60/2008 Викторианской разновидности. Несовпадение вакцинного компонента по гриппу В является частой проблемой при использовании тривалентных вакцин: за последние эпидемические сезоны такое несоответствие наблюдали в 2014–2015, 2015–2016 и 2017–2018 гг. Введение и широкое использование квадривалентных вакцин призвано устранить такие несоответствия [15]. До настоящего времени в нашей стране не обнаружены делеционные варианты, антигенно отличающиеся от вируса В/Брисбен/60/2008. В то же время такие вирусы широко циркулировали в прошедшем сезоне в странах Северной Америки, в Китае, Южной Африке и некоторых Европейских странах. На предстоящий эпидемический сезон в состав тривалентных вакцин эксперты ВОЗ рекомендовали штамм В/Колорадо/06/2017, относящийся к делеционным вариантам. По нашим данным население России не имеет выраженного иммунитета к подобным вирусам, в связи с чем роль своевременной вакцинации в преддверии наступающего эпидемического сезона особенно велика.

В настоящей работе впервые проведен сравнительный мониторинг циркуляции вирусов гриппа и других возбудителей ОРВИ на разных стадиях эпидемического процесса в целях оценки их относительного ущерба. При этом показана важная роль респираторно-синцитиального вируса непосредственно в предэпидемический период и в период эпидемии, тогда как риновирусы активно циркулировали на протяжении всего сезона. Более детальный анализ в рамках госпитального надзора показал важнейшую роль РСВ и риновирусов в развитии ТОРИ у детей младшего возраста [19], которая подтверждается многочисленными исследованиями [4, 11, 17]. Удельная значимость других возбудителей (метапневмовирусы, аденовирусы, парагриппозные, корона- и бокавирусы) в развитии эпидемического процесса была намного ниже, но в совокупности все они вызывали более 30% острых респираторных инфекций.

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Список литературы/References

1. Карпова Л.С., Волик К.М., Столяров К.А., Поповцева Н.М., Столярова Т.П., Соминина А.А., Бурцева Е.И. Особенности эпидемического процесса при гриппе A(H1N1)pdm09 и A(H3N2) в России с 2009 по 2017 годы // Вопросы вирусологии. 2018. Т. 63, № 4. С. 177–184. [Karpova L.S., Volik K.M., Stolyarov K.A., Popovtseva N.M., Stolyarova T.P., Sominina A.A., Burtseva E.I. Features of the epidemic process for influenza A (H1N1) pdm09 and A (H3N2) in Russia from 2009 to 2017. *Voprosy Virusologii = Problems of Virology, 2018, vol. 63, no. 4, pp. 177–184. doi: 10.31631/2073-3046-2018-17-1-13-19 (In Russ.)*]
2. Методика расчета эпидемических порогов по гриппу и острым респираторным вирусным инфекциям по субъектам Российской Федерации: метод. рекомендации МР 3.1.0005-10. М., 2010. 86 с. [The method of calculation of the epidemic threshold for influenza and acute respiratory viral infections by the subjects of the Russian Federation: methodological recommendations MR 3.1.0005-10. Moscow: 2010. 86 p.]. *URL: http://www.rospotrebnadzor.ru/documents/details.php?ELEMENT_ID=6288 (20.09.2018)*
3. Выделение вирусов гриппа в клеточных культурах и куриных эмбрионах и их идентификация: метод. рекомендации № 0100/4430-06-34 (утв. Федеральной службой по надзору в сфере защиты прав потребителей и благополучия человека от 18 апреля 2006 г.). 15 с. [Isolation of seasonal and potentially pandemic influenza viruses in cell cultures and chick embryos and their identification: methodological recommendations No. 0100/ 4430-06-34. (approved by Federal Service for Supervision of Consumer Rights Protection and Human Welfare of April 18, 2006). 15 p.]
4. Михайлова Е.В., Чудакова Т.К., Ярушкина М.С., Романовская А.В., Хворостухина Н.Ф. Респираторно-синцитиальная вирусная инфекция: клинические особенности и лабораторные показатели интоксикации // Детские инфекции. 2017. Т. 16, № 2. С. 23–25. [Mikhailova E.V., Chudakova T.K., Yarushkina M.S., Romanovskaya A.V., Khvorostukhina N.F. Respiratory syncytial viral infection: clinical features and laboratory indicators of intoxication. *Detskie infektsii = Children's infections, 2017, vol. 16, no. 2, pp. 23–25. (In Russ.)*]
5. Соминина А.А., Смородинцева Е.А., Столяров К.А., Мельникова А.А. Совершенствование системы надзора за гриппом в Российской Федерации: основные результаты сигнального надзора за гриппом и другими острыми респираторными вирусными инфекциями // Эпидемиология и вакцинопрофилактика. 2017. Т. 16, № 1. С. 7–15. [Sominina A.A., Smorodintseva E.A., Stolyarov K.A., Melnikova A.A. Improving the system of influenza surveillance in the

Russian Federation: the main results of sentinel surveillance for influenza and other acute respiratory viral infections. *Epidemiologiya i vaktsinoprofilaktika = Epidemiology and Vaccine Prevention, 2017, vol. 16, no. 1, pp. 7–15. doi: 10.31631/2073-3046-2017-16-1-7-15 (In Russ.)*]

6. Ilyicheva T., Durymanov A., Susloparov I, Kolosova N., Goncharova N., Svyatchenko S., Petrova O., Bondar A., Mikheev V., Ryzhikov A. Fatal cases of seasonal influenza in Russia in 2015–2016. *PLoS ONE, 2016, vol. 11, no. 10: e0165332.*
7. Komissarov A., Fadeev A., Sergeeva M., Petrov S., Sintsova K., Egorova A., Pisareva M., Buzitskaya Z., Musaeva T., Danilenko D., Konovalova N., Petrova P., Stolyarov K., Smorodintseva E., Burtseva E., Krasnoslobodtsev K., Kirillova E., Karpova L., Eropkin M., Sominina A., Grudinin M. Rapid spread of influenza A(H1N1)pdm09 viruses with a new set of specific mutations in the internal genes in the beginning of 2015/2016 epidemic season in Moscow and Saint Petersburg (Russian Federation). *Influenza Other Respir. Viruses, 2016, vol. 4, pp. 247–253. doi: 10.1111/irv.12389*
8. Lozano R., Naghavi M., Foreman K., Lim S., Shibuya K., Aboyans V., Abraham J., Adair T., Aggarwal R., Ahn S.Y., Alvarado M., Anderson H.R., Anderson L.M., Andrews K.G., Atkinson C., Baddour L.M., Barker-Collo S., Bartels D.H., Bell M.L., Benjamin E.J., Bennett D., Bhalla K., Bikbov B., Bin Abdulhak A., Birbeck G., Blyth F., Bolliger I., Boufous S., Bucello C., Burch M., Burney P., Carapetis J., Chen H., Chou D., Chugh S.S., Coffeng L.E., Colan S.D., Colquhoun S., Colson K.E., Condon J., Connor M.D., Cooper L.T., Corriere M., Cortinovis M., de Vaccaro K.C., Couser W., Cowie B.C., Criqui M.H., Cross M., Dabhadkar K.C., Dahodwala N., De Leo D., Degenhardt L., Delossantos A., Denenberg J., Des Jarlais D.C., Dharmaratne S.D., Dorsey E.R., Driscoll T., Duber H., Ebel B., Erwin P.J., Espindola P., Ezzati M., Feigin V., Flaxman A.D., Forouzanfar M.H., Fowkes F.G., Franklin R., Fransen M., Freeman M.K., Gabriel S.E., Gakidou E., Gaspari F., Gillum R.F., Gonzalez-Medina D., Halasa Y.A., Haring D., Harrison J.E., Havmoeller R., Hay R.J., Hoen B., Hotez P.J., Hoy D., Jacobsen K.H., James S.L., Jasrasaria R., Jayaraman S., Johns N., Karthikeyan G., Kassebaum N., Keren A., Khoo J.P., Knowlton L.M., Kobusingye O., Koranteng A., Krishnamurthi R., Lipnick M., Lipshultz S.E., Ohno S.L., Mabweijano J., MacIntyre M.F., Mallinger L., March L., Marks G.B., Marks R., Matsumori A., Matzopoulos R., Mayosi B.M., McAnulty J.H., McDermott M.M., McGrath J., Mensah G.A., Merriman T.R., Michaud C., Miller M., Miller T.R., Mock C., Mocumbi A.O., Mokdad A.A., Moran A., Mulholland K., Nair M.N., Naldi L., Narayan K.M., Nasseri K., Norman P., O'Donnell M., Omer S.B., Ortblad K., Osborne R., Ozgediz D., Pahari B., Pandian J.D., Rivero A.P., Padilla R.P., Perez-Ruiz F., Perico N., Phillips D., Pierce K., Pope C.A. 3rd, Porrini E., Pourmalek F., Raju M., Ranganathan D., Rehm J.T., Rein D.B., Remuzzi G., Rivara F.P., Roberts T., De León F.R., Rosenfeld L.C., Rushton L., Sacco R.L., Salomon J.A., Sampson U., Sanman E., Schwebel D.C., Segui-Gomez M., Shepard D.S., Singh D., Singleton J., Sliwa K., Smith E., Steer A., Taylor J.A., Thomas B., Tleyjeh I.M., Towbin J.A., Truelsen T., Undurraga E.A., Venketasubramanian N., Vijayakumar L., Vos T., Wagner G.R., Wang M., Wang W., Watt K., Weinstock M.A., Weintraub R., Wilkinson J.D., Woolf A.D., Wulf S., Yeh P.H., Yip P., Zabetian A., Zheng Z.J., Lopez A.D., Murray C.J., AlMazroa M.A., Memish Z.A. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet, 2012, vol. 380, no. 9859, pp. 2095–2128. doi: 10.1016/S0140-6736(12)61728-0*
9. Lopez-Labrador X., Natividad-Sancho A., Pisareva M., Komissarov A., Salvatierra K., Fadeev A., Moya A., Grudinin M., Diez-Domingo J., Afanasieva O., Konovalova N., Sominina A., Puig-Barbera J. Genetic characterization of influenza viruses from influenza-related hospital admissions in the St. Petersburg and Valencia sites of the Global Influenza Hospital Surveillance Network during the 2013/14 influenza season. *J. Clin. Virol., 2016, vol. 84, pp. 32–38.*
10. Manual for the laboratory diagnosis and virological surveillance of influenza. *WHO, 2011. 139 p.*
11. Moe N., Stenseng I.H., Krokstad S., Christensen A., Skanke L.H., Risnes K.R., Nordbø S.A., Døllner H. The burden of human metapneumovirus and respiratory syncytial virus infections in hospitalized norwegian children. *J. Infect. Dis., 2017, vol. 216, no. 1, pp. 110–116.*
12. Morbidity and Mortality Weekly Report. Influenza activity in the United States during the 2017–18 season and composition of the 2018–19 influenza vaccine. *MMWR, 2018, vol. 67, no. 22, pp. 634–642.*
13. Nair H., Brooks W.A., Katz M., Roca A., Berkley J.A., Madhi S.A., Simmerman J.M., Gordon A., Sato M., Howie S., Krishnan A., Ope M., Lindblade K.A., Carosone-Link P., Lucero M., Ochieng W., Kamimoto L., Dueger E., Bhat N., Vong S., Theodoratou E., Chittaganpitch M., Chimah O., Balmaseda A., Buchy P., Harris E., Evans V., Katayose M., Gaur B., O'Callaghan-Gordo C., Goswami D., Arvelo W., Venter M., Briese T., Tokarz R., Widdowson M.A., Mounts A.W., Breiman R.F., Feikin D.R., Klugman K.P., Olsen S.J., Gessner B.D., Wright P.F., Rudan I., Broor S., Simões E.A., Campbell H. Global burden of respiratory infections due to seasonal influenza in young children: a systematic review and meta-analysis. *Lancet, 2011, vol. 378, no. 9807, pp. 1917–1930. doi: 10.1016/S0140-6736(11)61051-9*
14. Puig-Barberà J., Tormos A., Trushakova S., Burtseva E., Sominina A., Pisareva M., Ciblak M.A., Badur S., Yu H., Cowling B.J., Burtseva E. The Global Influenza Hospital Surveillance Network (GIHSN): a new platform to describe the epidemiology of severe influenza. *Influenza Other Respir. Viruses, 2015, vol. 9, no. 6, pp. 277–286. doi: 10.1111/irv.12335*
15. Ray R., Dos Santos G., O Buck P., Claeys C., Matias G., Innis B., Bekkat-Berkani R.. A review of the value of quadrivalent influenza vaccines and their potential contribution to influenza control. *Human Vaccines & Immunotherapeutics, 2017, vol. 13, no. 7, pp. 1640–1652. doi: 10.1080/21645515.2017.1313375*
16. Ruggiero T., De Rosa F., Cerutti F., Pagani N., Allice T., Stella M., Milia M.G., Calcagno A., Burdino E., Gregori G., Urbino R., Di Perri G., Ranieri M.V., Ghisetti V. A(H1N1)pdm09 hemagglutinin D222G and D222N variants are frequently harbored by patients requiring extracorporeal membrane oxygenation and advanced respiratory assistance for severe A(H1N1)pdm09 infection. *Influenza Other Respir. Viruses, 2013, vol. 7, no. 6, pp. 1416–1426.*
17. Shi T., McAllister D.A., O'Brien K.L., Simoes E.A.F., Madhi S.A., Gessner B.D., Polack F.P., Balsells E., Acacio S., Aguayo C., Alassani I., Ali A., Antonio M., Awasthi S., Awori J.O., Azziz-Baumgartner E., Baggett H.C., Baillie V.L., Balmaseda A., Barahona A., Basnet S., Bassat Q., Basualdo W., Bigogo G., Bont L., Breiman R.F., Brooks W.A., Broor S., Bruce N., Bruden D., Buchy P., Campbell S., Carosone-Link P., Chadha M., Chipeta J., Chou M., Clara W., Cohen C., de Cuellar E., Dang D.A., Dash-yandag B., Deloria-Knoll M., Dherani M., Eap T., Ebruke B.E., Echavarria M., de Freitas Lázaro Emediato C.C., Fasce R.A., Feikin D.R., Feng L., Gentile A., Gordon A., Goswami D., Goyet S., Groome M., Halasa N., Hirve S., Homaira N., Howie S.R.C., Jara J., Jroundi I., Kartasasmita C.B., Khuri-Bulos N., Kotloff K.L., Krishnan A., Libster R., Lopez O., Lucero M.G., Lucion F., Lupisan S.P., Marcone D.N., McCracken J.P., Mejia M., Moisi J.C., Montgomery J.M., Moore D.P., Moraleda C., Moyes J., Munywoki P., Mutyara K., Nicol M.P., Nokes D.J., Nymadawa P., da Costa Oliveira M.T., Oshitani H., Pandey N., Paranhos-Baccalà G., Phillips L.N., Picot V.S., Rahman M., Rakoto-Andrianarivelo M., Rasmussen Z.A., Rath B.A.,

Robinson A., Romero C., Russomando G., Salimi V., Sawatwong P., Scheltema N., Schweiger B., Scott J.A.G., Seidenberg P., Shen K., Singleton R., Sotomayor V., Strand T.A., Sutanto A., Sylla M., Tapia M.D., Thamthitiwat S., Thomas E.D., Tokarz R., Turner C., Venter M., Waicharoen S., Wang J., Watthanaworawit W., Yoshida L.-M., Yu H., Zar H.J., Campbell H., Nair H., RSV Global Epidemiology Network. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. *Lancet, 2017, vol. 390, no. 10098, pp. 946–958. doi: 10.1016/S0140-6736(17)30938-8*

18. Sominina A., Burtseva E., Eropkin M., Karpova L., Zarubaev V., Smorodintseva E., Konovalova N., Danilenko D., Prokopetz A., Grudinin M., Pisareva M., Anfimov P., Stolyarov K., Kiselev O., Shevchenko E., Ivanova V., Trushakova S., Breslav N., Lvov D., Klimov A., Moen A., Cox N. Influenza surveillance in Russia based on epidemiological and laboratory data for the period from 2005 to 2012. *Am. J. Infect. Dis., 2013, vol. 9, no. 3, pp. 77–93. doi: 10.3844/ajidsp.2013.77.93*
19. Sominina A.A., Pisareva M.M., Grudinin M.P., Eropkin M.Y., Konovalova N.I., Sukhovetskaya V.F., Afanasieva O.I., Voloshuk L.V., Komissarov A.B., Buzitskaya Z.V., Fadeev A.V., Stolyarov K.A., Smorodintseva E.A., Afanasieva V.S., Yanina M.A., Gonchar V.V., Osidak L.V., Go A.A. Two years of experience in hospital surveillance for the severe influenza like illnesses in St. Petersburg: etiology, clinical characterization of diseases, antigenic and genetic properties of isolated influenza viruses. *Microbiol. Mol. Biol. Rev, 2015, vol. 2, no. 1, pp. 44–55. doi:10.18527/2500-2236-2015-2-1-44-55*
20. Vega T., Lozano J., Meerhoff T., Snaken R., Mott J., Ortiz de Lejarazu, Nunes B. Influenza surveillance in Europe: establishing epidemic thresholds by the moving epidemic method. *Influenza Other Respir. Viruses, 2013, vol. 7, no. 4, pp. 546–558. doi: 10.1111/j.1750-2659.2012.00422x*
21. World Health Organization. Vaccines against influenza WHO position paper — November 2012. *Wkly Epidemiol. Rec., 2012, vol. 87 (47), pp. 461–476.*
22. Zheng X.Y., Xu Y.J., Guan W.J., Lin L.F. Regional, age and respiratory-secretion-specific prevalence of respiratory viruses associated with asthma exacerbation: a literature review. *Arch. Virol., 2018, vol. 163, no. 4, pp. 845–853.*

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NEW OPPORTUNITIES TO IDENTIFY AND TYPE *STAPHYLOCOCCUS* spp. BY USING MALDI-TOF MASS SPECTROMETRY

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Abstract. Mass spectrometry profiles of microorganisms obtained by time-of-flight matrix-associated laser desorption/ionization (MALDI-TOF) mass spectrometry are a source of information about peptide profiles can be used for microbial identification and typing. A variety of technical and bioinformational solutions complicate developing of a united mass-spectro-profile database. *Staphylococcus* spp. strains are good studied objects for identification by MALDI-TOF mass spectrometry, frequently resulting in nosocomial infections, especially in immunocompromised patients. Rapid differentiation of nosocomial, multiresistant and highly virulent isolates of *Staphylococcus* spp. allows to reduce the lethality in weakened and immunocompromised patients. The study was aimed at assessing comparability and reproducibility of identification and typing results for Staphylococcus spp by MALDI-TOF mass spectrometry. Comparing 292 *Staphylococcus* spp. isolates in clinical specimens obtained fron the multidisciplinary hospital at the NWSMU im. I.I. Mechnikov was carried out by using MALDI-TOF mass spectrometer BactoSCREEN ID (Litech, Russia) and Bruker Biotyper 3.1 (Bruker GmbH, Germany). Comparability of *Staphylococcus* spp. identification showed that 95.9%; 12 isolates (4.1%) by "Bruker Biotyper 3.1" and 3 isolates (1.1%) by using "BactoSCREEN ID" were incorrectly identified. Repeated identification leveled the differences between the systems used. In addition, it was shown that the method of protein extraction did not affect reliability of *Staphylococcus* spp. species identification by using databases (χ^2, $p > 0.05$) compared to intraspecific typing (χ^2, $p < 0.0001$). Using different extraction protocols showed that *Staphylococcus* spp. mass-spectra differed by peak intensity level within the mass range up to 4000 m/z, 5300±600 m/z and 6500±500 m/z, as well as higher than 7000 m/z. Peaks of low-molecular weight peptides were detected under full protein extraction compared to sample preparation on plate extraction. To develop a unified protocol for mass-spectrometry profile processing, a reliability of the basic statistical variables (mode, median, maximum, minimum and arithmetic mean) was evaluated. Analysis of the median mass spectrometry profiles is recommended for *Staphylococcus* spp. intraspecific typing by using MALDI-TOF mass spectrometry as the most reproducible and consistent approach. As a result, two systems for MALDI-TOF mass spectrometry reliably identify *Staphylococcus* spp., but standardization of sample preparation and bioinformation analysis is required for *Staphylococcus* spp. typing.

Key words: *Staphylococcus spp., MALDI-TOF mass spectrometry, typing, mass-spectra, cluster analysis, identification.*

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НОВЫЕ ВОЗМОЖНОСТИ ДЛЯ ИДЕНТИФИКАЦИИ И ТИПИРОВАНИЯ *STAPHYLOCOCCUS* spp. МЕТОДОМ MALDI-TOF МАСС-СПЕКТРОМЕТРИИ

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Резюме. Масс-спектро-профили микроорганизмов, получаемые с помощью времяпролетной матрице-ассоциированной лазерной десорбции/ионизациии (MALDI-TOF) масс-спектрометрии являются источником информации о пептидных профилях, которая может быть использована для идентификации и типирования. Разнообразие технических и биоинформационных решений затрудняет формирование единой базы масс-спектро-профилей. Бактерии рода *Staphylococcus* являются одними из наиболее изученных объектов для идентификации с помощью MALDI-TOF масс-спектрометрии, а также являются частыми возбудителями внутрибольничных инфекций, в особенности среди иммунокомпрометрированных пациентов. Методы быстрой дифференцировки нозокомиальных, полирезистентных и высоковирулентных изолятов *Staphylococcus* spp. позволят снизить летальность среди ослабленных и иммунокомпрометированных пациентов. Целью исследования была оценка сопоставимости и воспроизводимости результатов идентификации и типирования *Staphylococcus* spp. с помощью MALDI-TOF масс-спектрометрии. Сравнительные исследования 292 изолятов *Staphylococcus* spp., выделенных из клинических образцов на базе многопрофильного стационара СЗГМУ им. И.И. Мечникова проводили с помощью MALDI-TOF масс-спектрометрометров «BactoSCREEN ID» (ООО «Литех», Россия) и Bruker Biotyper 3.1 (Bruker GmBH, Германия). Сопоставимость результатов видовой идентификации *Staphylococcus* spp. составляла 95,9%; причем среди неправильно идентифицированных изолятов изолятов 12 (4,1%) составляли идентифцированные с помощью Bruker Biotyper 3.1 и 3 изолята (1,1%) *Staphylococcus* spp. с помощью BactoSCREEN ID. Повторная идентифкация нивелировала различия между используемыми системами. Выявили, что способ экстракции белков не влиял на надежность видовой идентификации *Staphylococcus* spp. с использованием сравниваемых библиотек данных (χ^2, $p > 0,05$) в отличие от внутривидового типирования (χ^2, $p < 0,0001$). Масс-спектры *Staphylococcus* spp. при использовании различных протоколов экстракции различались по уровню интенсивности пиков диапазонов масс до 4000 m/z, 5300±600 m/z и 6500±500 m/z и более 7000 m/z. Пики низкомолекулярных пептидов выявляли при полной экстракции белка в отличии от пробоподготовки на поверхности мишени. Для формирования унифицированного протокола обработки масс-спектро-профилей проводили оценку надежности базовых статистических величин (мода, медиана, максимум, минимум и среднее арифметическое). Анализ медианы масс-спектро-профилей рекомендуется использовать для воспроизводимости и стабильности результатов внутривидового типирования *Staphylococcus* spp. с помощью MALDI-TOF масс-спектрометрии. В результате сравнительных исследований выявили, что две системы для MALDI-TOF масс-спектрометрия надежно идентифицируют *Staphylococcus* spp., а для типирования требуется унификация пробоподготовки и биоинформационного анализа.

Ключевые слова: *Staphylococcus spp., MALDI-TOF масс-спектрометрия, типирование, масс-спектр, кластерный анализ, идентификация.*

Introduction

Staphylococcus spp. are important causative agents of human infections especially in intensive care units (ICU) [2]. The spread of methicillin-resistant isolates of *Staphylococcus* spp. is the cause of the patients condition burden in hospital media [1, 6].

MALDI-TOF mass spectrometry is an effective method for identifying *Staphylococcus* spp. Nevertheless, increasing of the number of nosocomial infections associated with *Staphylococcus* spp. requires rapid and reliable identification and obtaining additional information about resistance potential and epidemiological significance [10, 18]. The problem solution is impossible without unified typing protocol development and a multicentral comparison of a MALDI-TOF mass spectrometry results, but to the date MALDI-TOF mass spectrometers are represented by several independent technical solutions with independent mass spectrometry libraries [18]. Methods of sample preparation, technological features of devices and approaches to bioinformatic analysis among manufacturing companies are different, which interfere obtaining of a multicentral data analysis. Moreover, the proposed methods for typing *Staphylococcus* spp., in particular MRSA, do not have united approaches [14, 19]. Data about reproducibility of MALDI-TOF mass spectrometry as a method of typing are contradictory [3, 5, 17]. Thus, to form a unified system for identification and typing of *Staphylococcus* spp. where is necessary to evaluate the reliability, reproducibility and comparability of the results of identification and

typing conducted using various technical solutions and data libraries.

At present, MALDI-TOF mass spectrometers are presented as technical solutions from manufacturers: "Bruker" (Germany), "bioMerieux" (France), "Shimadzu" (Japan), "Litech" (Russia). "Bruker", "bioMerieux" and "Shimadzu" use mass-spectra databases "Saramis" and "MS RUO" for identification each are very close [11], but "Litech" uses an original database, different from previously noted. For the identification of microorganisms cultures, manufacturers sugest different methods of sample preparation, which is due to the features of obtaining mass-spectro-profiles included in the data libraries [11]. Due to the libraries of MALDI-TOF mass spectrometers are separated, where is difficult to compare the effectiveness of MALDI-TOF mass spectrometry for the typing of *Staphylococcus* spp. and screening for methicillin resistance [11].

Thus, the study purpose was a comparative assessment of the comparability of the results of species identification and typing of *Staphylococcus* spp. using various libraries, sample preparation protocols and technical solutions in the field of MALDI-TOF mass spectrometry.

Materials and Methods

Staphylococcus spp. isolates (n = 292) were obtained from samples of patients from a multidisciplinary hospital in St. Petersburg. First inoculation was carried out on a set of media: Colombia agar with 5% sheep erythrocytes (Biomedia, Russia), yolk-salt agar (NITF, Russia). Plates were incubated for 24 hours at 37°C.

MALDI-TOF mass spectrometry was performed using Bruker AutoFleX Speed mass spectrometers (Bruker GmBH, Germany) and BactoSCREEN ID (Litech, Russia). Incubation of *Staphylococcus* spp. on Colombia agar with 5% sheep erythrocytes for 24 hours at 37°C. Extraction of peptides was carried out by the following methods:

1. Direct extraction on the target using a 70% solution of formic acid, drying in air for 2 minutes [4].
2. Total extraction by alcohol-acid method [15] with purification by acetonitrile solution.
3. Short protocol of alcohol-acid extraction without precipitation of cells components and acetonitrile purification [13].

After this, α-cyano-4-hydroxycinnamic acid was added and dried in air.

Using a Bruker AutoFleX Speed mass spectrometer, mass spectra were collected using a linear TOF protocol with a laser frequency of 20 Hz and an estimate in the mass range of 2000 to 20 000 m/z. The voltage on the acceleration was 20 kV, the voltage IS2 was 18.6 kV. For total spectrum, 1200 separate spectra were collected from the entire target area. The identification of cultures was carried out according to the protocol "Bruker Taxonomy", included in the software package "Bruker Real Time Classification 3.1". In the case of "Score" above 2.0, the identification was considered good, for isolates that had "Score" from 1.4 to 1.9 were re-identified. To increase the reliability, this procedure was repeated twice for each isolate.

Sampling and analysis of mass spectra obtained with the MALDI-TOF mass spectrometer "Bruker AutoFleX Speed" was carried out using the "R 3.3.3" software package with the MaldiQuantForeign extension package [7]. The Savitzky-Golay protocol was used to smoothing, and the baseline level was performed using a statistically-dependent non-linear cross-referenced algorithm (SNIP) [12]. Noises were excluded using the "Friedman's Super Smoother" method included in the R 3.3.2 MaldiQuantForeign software package. Analysis of variance was used to estimate the variability of peak intensities on the obtained mass spectra in the R-commander software package.

Using the BactoSCREEN ID mass spectrometer, identification of *Staphylococcus* spp. provided accordance with manufacturer recomendations. Identification was carried out twice for each culture in order to improve reliability. ATCC 29213 *S. aureus* strain was used as a control sample. A preliminary grouping of the results of MALDI-TOF mass spectrometry using the mass spectrometer data was carried out using software package R 3.3.3 by the original program code.

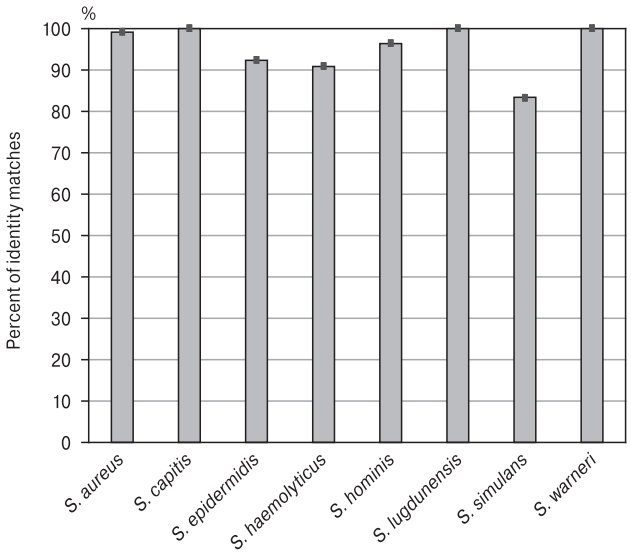

Figure 1. *Staphylococcus* spp. identification results using Bruker Autoflex Speed and BactoSCREEN ID (Litech Ltd.)

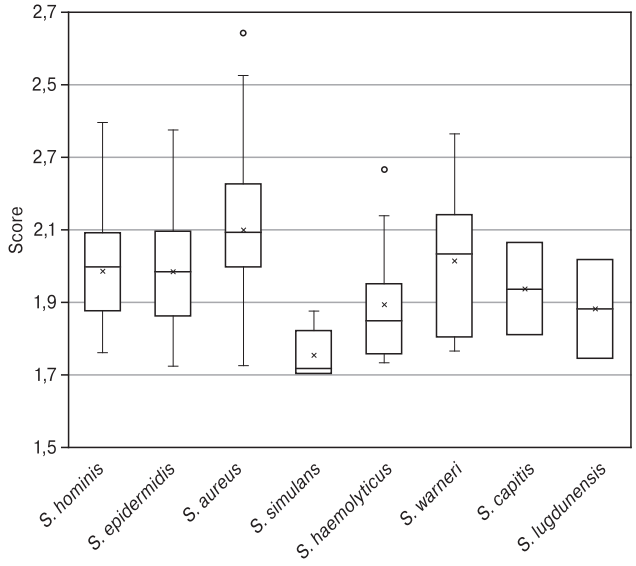

Figure 2. Reliability of *Staphylococcus* spp. identification using "Bruker AutofleX Speed" (Biotyper 3.1) library

Results

Identification of *Staphylococcus* spp. 292 isolates using MALDI-TOF mass spectrometer "BactoSCREEN ID" and "Bruker AutofleX Speed" have revealed a high level of comparability (fig. 1).

Identification of 12 *Staphylococcus* spp. isolates (4.1%) was unreliable using Bruker AutofleX Speed (Biotyper 3.1): five *S. epidermidis*, one *S. aureus*, two *S. auricularis*, one *S. haemolythicus*, three *S. simulans*, but the differences have been eliminated by repeated identification.

Using the database of the "BactoSCREEN ID" device, 3 isolates (1.1%) were not identified: two *S. simulans* and one *S. epidermidis*, which were reliably identified by Bruker Biotyper 3.1. The isolates were identified correctly after second round of identification.

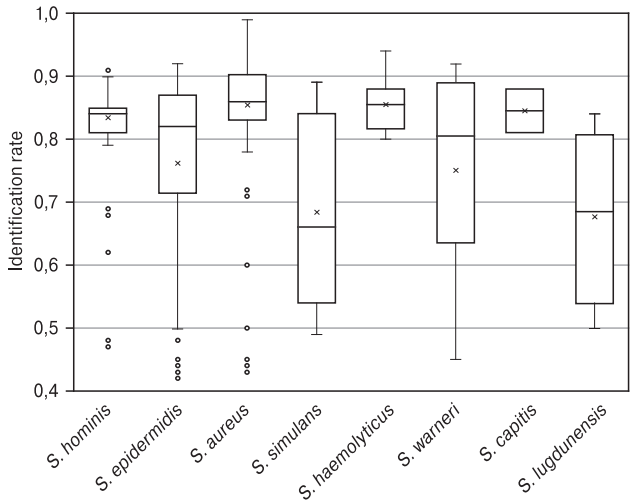

Figure 3. Reliability of *Staphylococcus* spp. identification using the "BactoSCREEN ID" library

When using "Bruker AutofleX Speed" (Biotyper 3.1), the lowest reliability of identification was revealed for *S. simulans* and *S. haemolythicus* (fig. 2).

Analyzing the stock of *Staphylococcus* spp. using the database of the "BactoSCREEN ID", the low reliability of the *S. simulans* species identification and atypical spectra of *S. hominis*, *S. epidermidis* and *S. aureus* was revealed, which were lay outside the general population of values (fig. 3).

Evaluating the effect of extraction protocols on the identification reliability, where was estimated the level of reliability of identification using databases "Bruker Biotyper 3.1" and "BactoSCREEN ID" (fig. 4) did not depend on the extraction protocol (Fisher criterion, $p > 0.05$). Statistically significant differences in the reliability of identification were revealed between the databases during the on-plate extraction (Fisher criterion, $p < 0.001$).

To assess the differences in mass spectra profiles obtained with different extraction protocols, the differences in the structure of the mass spectrometry profiles of *S. epidermidis* (n = 82) were compared; linear and diagonal discriminatory analysis were used. Total extraction is characterized by a rather low intensity of peaks in the regions: 5300±600 m/z and 6500±500 m/z and higher than 7000 m/z. On the other hand, the intensity of signals of low molecular weight peptides (3500±100 m/z) was higher at complete extraction (fig. 5). The combined extraction method was characterized as an intermediate, including the positive sides of both full extraction and direct application.

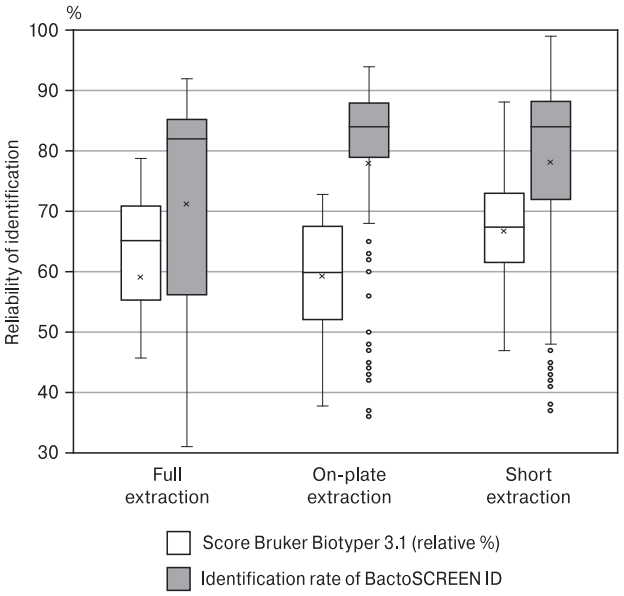

Figure 4. Dependence of the "Score" BrukerBiotyper 3.1 and identification reliability using the database of the device "BactoSCREEN ID" from the extraction protocol

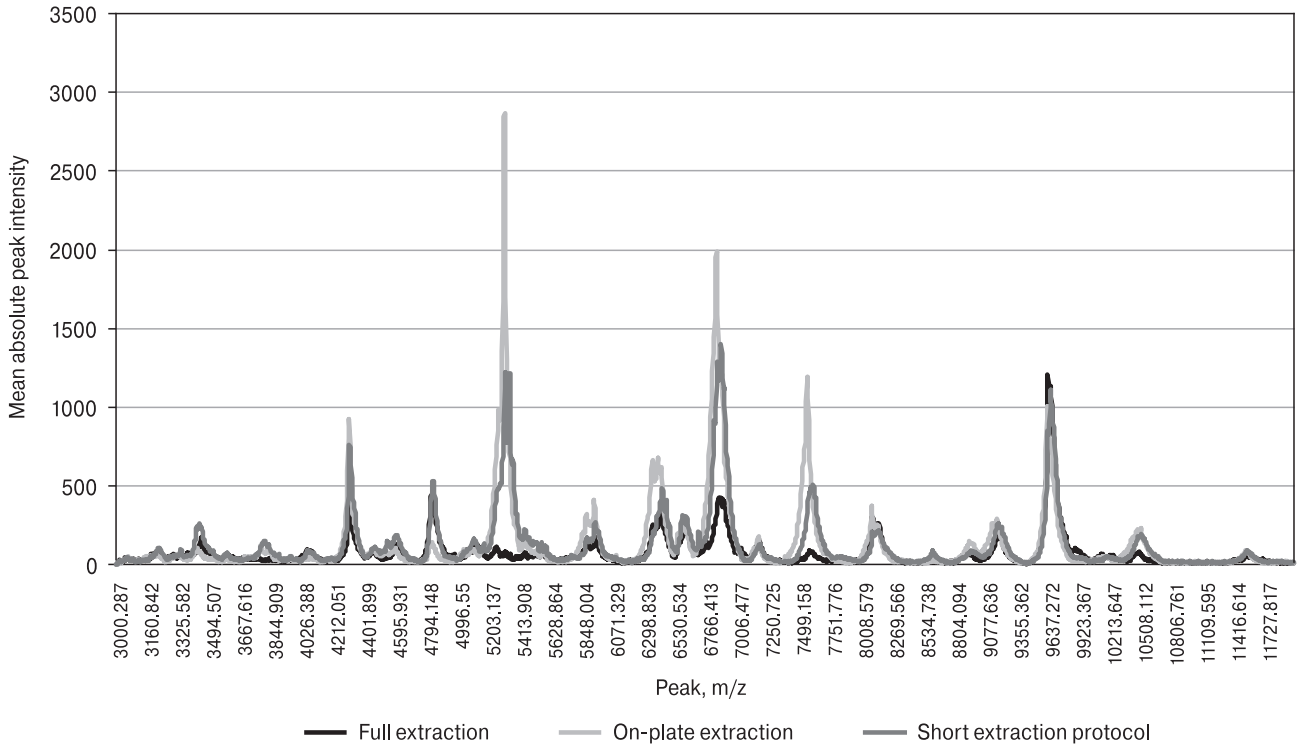

Figure 5. Comparison of mass spectrometry profiles of *S. epidermidis*, depending on the method of extraction of peptides

The structure of the mass spectrometry profiles of other *Staphylococcus* spp. species also differed depending on the method of protein extraction: the intensity of the median mass range peak peptides was the most intensive at direct application and extraction on the target, while the use of a complete extraction protocol allows more effective detection of peaks of low molecular weight proteins (up to 4000 m/z) .

To estimate the effect of peptide extraction on the identification result, the possibilities of harmonizing the sample of mass spectra based on main statistical parameters of the mass spectrometer profile were evaluated. In contrast to the previously proposed harmonization methods for high-intensity stable sites, median harmonization was used [18]. Since the scatter of the peaks in the 6800–9500 m/z ranges was significant and the harmonization was not universal for all species (fig. 6).

Comparing the structure of the *S. epidermidis* population by analysis of harmonized and unharmonized mass-spectrometry profiles, revealed statistically significant differences in the intraspecific clusters structure (fig. 7).

The discrimination of *S. epidermidis* into clusters was characterized by high stability: only 10% of the isolates were assigned to different clusters at the re-identification and mass spectrometer profile evaluation.

Intraspecific structure of other *Staphylococcus* spp. also differed depending on using of mass spectra harmonization protocols. Based on bootstrap analysis, where was obtained the harmonization allow to discriminate only two general clusters in all *Staphylococcus* spp. with stapility during 100-fold repetitions (alpha > 0.95). Nevertheless, the protein extraction method had a statistically significant effect on the intraspecific *Staphylococcus* spp. differentiation despite the harmonization using (χ^2, $p < 0.0001$).

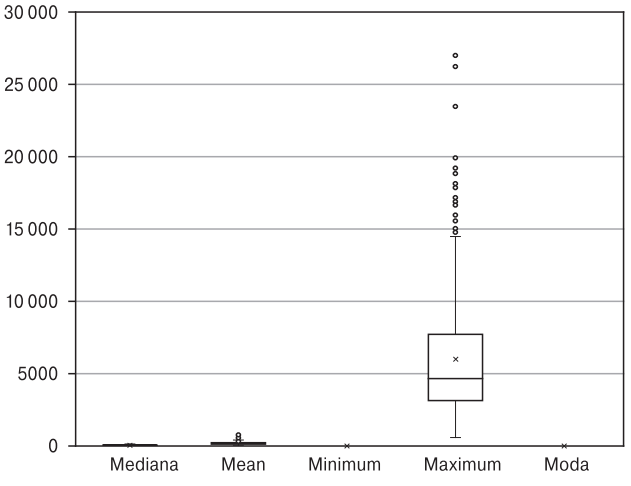

Figure 6. Evaluation of the stability *Staphylococcus* spp. mass-spectra profiles

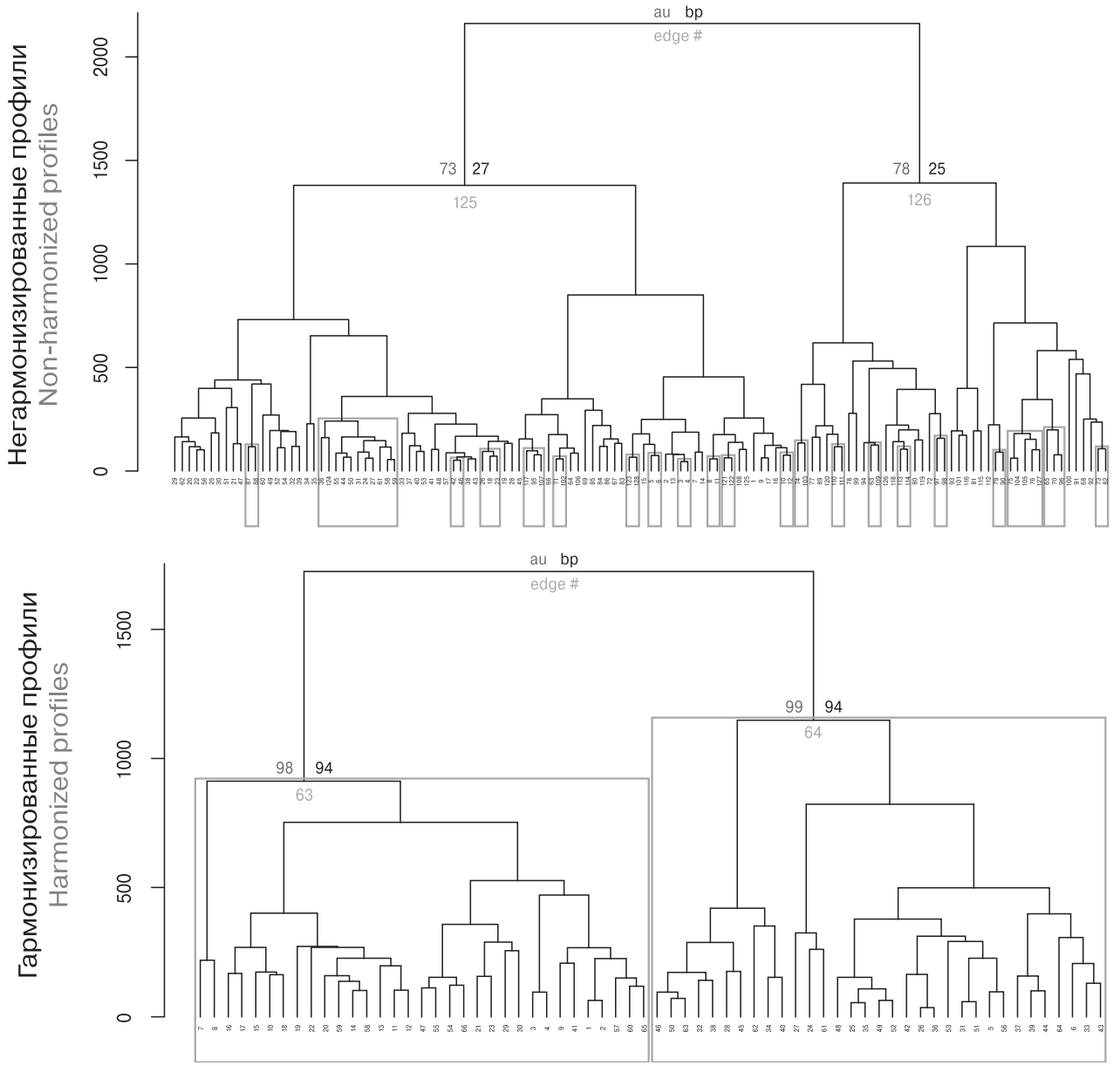

Figure 7. Subspecies structure of *S. epidermidis*

Discussion

The comparison of the identification results 292 *Staphylococcus* spp. isolates revealed 95.9% of the coincidence of species identification using both the Bruker "Biotyper 3.1" databases and the "BactoSCREEN ID" devices. The lowest rating of identification reliability was revealed for *S. simulans* using both instrument solutions.

Reliability of species identification, expressed as "Score" (Bruker Biotyper 3.1) and "Identity Reliability" (BactoSCREEN ID), did not differ significantly depending on the protocol of protein extraction, but statistically significant differences in the intensity of individual peaks of mass spectra were found. Moreover, when using extraction on the target, some peaks of low molecular weight proteins were not detected, which can be used for typing or evaluating resistance [16]. Similar results of bacterial identification were obtained by comparing the instruments of Vitek MS (bioMerieux, France) and Bruker Microflex: for gram-positive bacteria, the comparability of identification results between instruments was 97.4% [9], for Gram-negative bacteria — 99.4% [8]. Thus, despite the differences in bioinformation and technical solutions, the species identification using MALDI-TOF mass spectrometry is reliable, and the results are comparable. The differences obtained by comparing the identification results can be explained by the library structure features of mass-spectrometer, which are formed on the basis of the complete extraction protocols of the protein (Bruker Biotyper 3.1) and extraction on-plate extraction (BactoSCREEN ID).

The results of our research show that to compare the results of typing on different systems for MALDI-TOF mass spectrometry, where is necessary to carry out mass-spectrometry harmonization based on the mass-spectrum median estimate similary to the Savitzky–Golay protocols [7]. Using of previously proposed calibrations of peaks intensity on the range 6800±300 m/z [14] is less reliable due to the high variability of the absolute intensity of these peaks.

The structure of the obtained clusters in the case of *S. epidermidis* was stable for 90% isolates during MALDI-TOF mass-spectrometry repitition. However, statistically significant effect of used extraction protocol on the results of intraspecific typing of all *Staphylococcus* spp. was observed.

Thus, the use of MALDI-TOF mass spectrometry for *Staphylococcus* spp. identification was characterized by high reproducibility of results using various technical solutions in the field of mass spectrometry, extraction protocols and various data libraries. However, quantitative analysis of peaks for intraspecific typing requires not only a single bioinformational approach, but also unification of sample preparation protocols.

Список литературы/References

1. Гостев В.В., Калиногорская О.С., Черненькая Т.В., Науменко З.С., Ворошилова Т.М., Захарова Ю.А., Хохлова О.Е., Круглов А.Н., Сидоренко С.В. Антибиотикорезистентность метициллинорезистентных Staphylococcus aureus, циркулирующих в Российской Федерации // Антибиотики и химиотерапия. 2015. Т. 60, № 1–2. С. 3–9. [Gostev V.V., Kalinogorskaya O.S., Chernenkaya T.V., Naumenko Z.S., Voroshilova T.M., Zakharova Yu.A., Khokhlova O.E., Kruglov A.N., Sidorenko S.V. Antibiotic resistance of MRSA in Russian Federation. *Antibiotiki i khimioterapiya = Antibiotics and Chemotherapy, 2015, vol. 60, no. 1–2, pp. 3–9. (In Russ.)*]
2. Козлова Н.С., Баранцевич Е.П., Баранцевич Н.Е. Антибиотикорезистентность стафилококков, выделенных из крови // Научное обозрение. 2014. № 3. С. 184–189. [Kozlova N.S., Barantsevich E.P., Barantsevich N.E. Antibiotic resistance of staphylococci isolated from blood. *Nauchnoe obozrenie = Scientific Review, 2014, no. 3. pp. 184–189. (In Russ.)*]
3. Albrethsen J. Reproducibility in protein profiling by MALDI-TOF mass spectrometry. *Clin. Chem., 2007, vol. 53, no. 5, pp. 852–858. doi: 10.1373/clinchem.2006.082644*
4. Böhme K., Fernández-No I.C., Barros-Velázquez J., Gallardo J.M., Cañas B., Calo-Mata P. Comparative analysis of protein extraction methods for the identification of seafood-borne pathogenic and spoilage bacteria by MALDI-TOF mass spectrometry. *Analyt. Methods, 2010, vol. 2, no. 12, pp. 1941–1947. doi: 10.1039/C0AY00457J*
5. Camoez M., Sierra J.M., Dominguez M.A., Ferrer-Navarro M., Vila J., Roca I. Automated categorization of methicillin-resistant Staphylococcus aureus clinical isolates into different clonal complexes by MALDI-TOF mass spectrometry. *Clin. Microbiol. Infect., 2016, vol. 22, no. 2, pp. 161.e1–161.e7. doi: 10.1016/j.cmi.2015.10.009*
6. Chen X.-P., Li W.G., Zheng H., Du H.Y., Zhang L. Extreme diversity and multiple SCCmec elements in coagulase-negative Staphylococcus found in the Clinic and Community in Beijing, China. *Ann. Clin. Microbiol. Antimicrob., 2017, vol. 16: 57. doi: 10.1186/s12941-017-0231-z*
7. Gibb S., Strimmer K. MALDIquant: a versatile R package for the analysis of mass spectrometry data. *Bioinformatics (Oxford, England), 2012, vol. 28, no. 17, pp. 2270–2271. doi: 10.1093/bioinformatics/bts447*
8. Jamal W., Albert M.J., Rotimi V.O. Real-time comparative evaluation of bioMerieux VITEK MS versus Bruker Microflex MS, two matrix-assisted laser desorption-ionization time-of-flight mass spectrometry systems, for identification of clinically significant bacteria. *BMC Microbiology, 2014, vol. 1, no. 14: 289. doi: 10.1186/s12866-014-0289-0*
9. Lee M., Chung HS., Moon HW., Lee SH., Lee K. Comparative evaluation of two matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) systems, Vitek MS and Microflex LT, for the identification of Gram-positive cocci routinely isolated in clinical microbiology laboratories. *J. Microbiol. Methods, 2015, vol. 113, pp. 13–15. doi: 10.1016/j.mimet.2015.03.020*
10. Patel R. MALDI-TOF MS for the diagnosis of infectious diseases. *Clin. Chem., 2015, vol. 61, no. 1, pp. 100–111.*
11. Ryan C.G., Clayton E., Griffin W.L., Sie S.H., Cousens D.R. SNIP, a statistics-sensitive background treatment for the quantitative analysis of PIXE spectra in geoscience applications. *Nucl. Instrum. Methods Phys. Res. B., 1988, vol. 34, pp. 396–402. doi: 10.1016/0168-583X(88)90063-8*
12. Schulthess B., Brodner K., Bloemberg G.V., Zbinden R., Böttger EPP., Hombach M. Identification of Gram-positive cocci by use of matrix-assisted laser desorption ionization–time of flight mass spectrometry: comparison of different preparation methods and implementation of a practical algorithm for routine diagnostics. *J. Clin. Microbiol., 2013, vol. 51, no. 6, pp. 1834–1840. doi: 10.1128/JCM.02654-12*
13. Schulthess B., Bloemberg G.V., Zbinden R., Böttger E.C., Hombach M. Evaluation of the Bruker MALDI Biotyper for identification of gram-positive rods: development of a diagnostic algorithm for the clinical laboratory. *J. Clin. Microbiol., 2014, vol. 52, no. 4, pp. 1089–1097. doi: 10.1128/JCM.02399-13*
14. Schuster D., Josten M., Janssen K., Bodenstein I., Albert C., Schallenberg A., Gajdiss M., Sib E., Szekat C., Kehl K., Parčina M., Hischebeth G.T.R., Bierbaum G. Detection of methicillin-resistant coagulase-negative staphylococci harboring the class A mec complex by MALDI-TOF mass-spectrometry. *Int. J. Med. Microbiol., 2018, vol. 308, no. 5, pp. 522–526. doi: 10.1016/j.ijmm.2018.05.001*
15. Seng P., Fournier PE., La Scola B., Drancourt M., Raoult D. MALDI-TOF-mass spectrometry applications in clinical microbiology. *Future Microbiol., 2010, vol. 5, no. 11, pp. 1733–1754. doi: 10.2217/fmb.10.127*
16. Staphylococcus aureus subtyping for MRSA detection. *URL: https://www.bruker.com/products/mass-spectrometry-and-separations/maldi-biotyper-for-microbiological-research/mbt-subtyping-software-module/staphylococcus-aureus-subtyping-for-mrsa-detection.html (21.11.2017)*

17. Wang H.-Y., Lee T.Y., Tseng Y.J., Liu T.P., Huang K.Y., Chang Y.T., Chen C.H., Lu J.J. A new scheme for strain typing of methicillin-resistant Staphylococcus aureus on the basis of matrix-assisted laser desorption ionization time-of-flight mass spectrometry by using machine learning approach. *PLoS One, 2018, vol. 13, no. 3, pp. e0194289. doi: 10.1371/journal.pone.0194289*
18. Westblade L.F., Garner O.B., MacDonald K., Bradford C., Pincus D.H., Mochon A.B., Jennemann R., Manji R., Bythrow M., Lewinski M.A., Burnham C.-A.D., Ginocchio C.C. Assessment of reproducibility of matrix-assisted laser desorption ionization-time of flight mass spectrometry for bacterial and yeast identification. *J. Clin. Microbiol., 2015, vol. 53, no. 7, pp. 2349–2352. doi: 10.1128/JCM.00187-15*
19. Wolters M., Rohde H., Maier T., Belmar-Campos C., Franke G., Scherpe S., Aepfelbacher M., Christner M. MALDI-TOF MS fingerprinting allows for discrimination of major methicillin-resistant Staphylococcus aureus lineages. *Int. J. Med. Microbiol., 2011, vol. 301, no. 1, pp. 64–68. doi: 10.1016/j.ijmm.2010.06.002*

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TIME-KILL ASSAY: AN EFFICACY OF SYNERGY BETWEEN CARBAPENEMS AND CLODRONIC ACID

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Abstract. Currently, a search for augmenting antibiotics activity is still crucial due to elevated frequency of detecting carbapenem-resistant Gran-positive bacterial isolates. To resolve this, it might be reasonable to combine carbapenems metal-β-lactamase (MβL) inhibitors. Unfortunately, no MβL inhibitors approved for treatment of carbapenem-resistant infections are currently available. Pathogenic bacteria may survive antibiotic attack, exert tolerance and persistence accompanied with the ongoing infectious process. In connection with this, determining dependence between antimicrobial-related bactericidal effect and exposure time on microbes at 4, 8, 12 and 24 hours after the onset, a so called time-kill assay, is necessary. A synergy between both agents was noted upon reduced microbial population by ≥ 3 $\log_{10}$. A checkerboard array followed by seeding the microplate well contents onto a dense nutrient medium at various time points were used to assess a synergistic efficacy of carbapenems applied together with clodronic acid against MβL-producing VIM-genotype *P. aeruginosa* 532/14 clinical isolate obtained from patients with infectious complications (minimal inhibitory concentrations [MIC] for imipenem or meropenem were 512 μg/ml), microbial burden 10^6 CFU/ml. Optical density was measured at two wavelengths (490 and 630 nm) in ELx800 reader, within 4–24 hour exposure time to determine time of logarithmic growth phase emerging in test culture. It is noteworthy that magnitude of optical density is a difference between two bichromatic measurements resulting in remarkably reduced inaccuracy due to scratches or fingerprints left on the plate. It was found that clodronic acid exhibited a synergic bactericidal effect with carbapenems against a clinically resistant MβL-producing VIM-genotype *P. aeruginosa* 532/14 strain. Upon that, imipenem-related antimicrobial activity was evident as early as 8 hours after the onset decreasing cell growth down to 1.4 $\log_{10}$ compared to control, whereas 12 hours later it resulted in total inhibition of test strain by decreasing growth of the test strain by 6 $\log_{10}$. Meropenem in combination with clodronic acid showed a more pronounced activity: complete absence of *P. aeruginosa* 532/14 growth by 8 hours of incubation, growth suppression by 3.2 $\log_{10}$, which reached 6 $\log_{10}$ 12–24 hours after the onset. Time-kill assay allows to identify efficient combinations of carbapenems and MβL inhibitors, which is of great importance for increasing therapeutic efficacy of patients with severe purulent-septic complications.

Key words: *clodronic acid, carbapenem-resistant Gram-negative bacteria, metal-β-lactamase inhibitor, carbapenems, time-kill assay.*

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TIME-KILL ASSAY: ЭФФЕКТИВНОСТЬ СИНЕРГИДНОГО ДЕЙСТВИЯ КАРБАПЕНЕМОВ И КЛОДРОНОВОЙ КИСЛОТЫ

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Резюме. В связи с возросшей частотой выделения изолятов грамотрицательных микроорганизмов, резистентных к карбапенемам, важным остается поиск способов усиления действия этого класса антибиотиков. Одним из возможных способов решения этой проблемы является комбинация карбапенемов с препаратами — ингибиторами металло-бета-лактамаз (МБЛ). К сожалению, в настоящее время разрешенных для применения в клинике ингибиторов МБЛ карбапенемрезистентных микроорганизмов. Патогенные микроорганизмы могут переживать антибиотическую атаку, проявлять свойства толерантности и персистенции, при этом инфекционный процесс продолжается. В связи с этим важно определение зависимости между бактерицидным действием антимикробных средств и временем экспозиции их воздействия на микроорганизм не в одной временной точке через 24 ч инкубации, а в нескольких — через 4, 8, 12 и 24 ч, так называемая кривая зависимости «время — летальное действие» (time-kill assay). Синергидный эффект двух препаратов отмечается при условии снижения уровня микробной популяции на ≥ 3 $\log_{10}$. Для оценки эффективности синергидного действия карбапенемов и клодроновой кислоты использовали микрометод перекрестного титрования («шахматной доски») с последующим высевом содержимого ячеек на плотную питательную среду в разные временные промежутки. Исследования проводили в отношении клинического штамма *Pseudomonas aeruginosa* 532/14, выделенного от пациентов с инфекционными осложнениями, продуцирующего МБЛ генотипа VIM и характеризующегося высокой степенью резистентности к карбапенемам (МПК имипенама или меропенема 512 мкг/мл); микробная нагрузка 10^6 КОЕ/мл. На ридере ELx800 проводили измерение оптической плотности при двух длинах волн (490 и 630 нм) при экспозициях от 4 до 24 ч и выявляли время появления логарифмической фазы роста тест-культуры. Следует отметить, что при бихроматическом измерении значение оптической плотности является разницей двух измерений, при этом значительно снижается погрешность результатов, вызванная царапинами или отпечатками пальцев на планшете. В исследовании показана способность клодроновой кислоты проявлять синергидный бактерицидный эффект с карбапенемами в отношении клинического резистентного штамма *P. aeruginosa* 532/14, продуцирующего металло-бета-лактамазу генотипа VIM. При этом в таком сочетании антимикробное действия у имипенема начинается с 8 часов инкубации до уровня 1,4 $\log_{10}$ по сравнению с контролем, а с 12 ч отмечено полное подавление роста тест-культуры. При этом снижение роста тест-штамма составило 6 $\log_{10}$. Меропенем в комбинации с клодроновой кислотой проявлял более выраженную активность: полное отсутствие роста *P. aeruginosa* 532/14 к 8 часам инкубации, подавление роста на 3,2 $\log_{10}$. Этот показатель через 12–24 ч составил 6 $\log_{10}$. Получение кривой зависимости «время — летальное действие» позволяет выявлять эффективные комбинации карбапенемов и ингибиторов металло-бета-лактамаз грамотрицательных бактерий, что имеет большое значение для повышения эффективности лечения тяжелых гнойно-септических осложнений у пациентов.

Ключевые слова: *клодроновая кислота, карбапенемрезистентные грамотрицательные микроорганизмы, ингибитор металло-бета-лактамазы, карбапенемы, «time-kill assay».*

Introduction

Due to the increased frequency of isolation of isolates of gram-negative microorganisms resistant to carbapenems, it is still important to find ways to enhance the action of this antibiotics class. One of the possible ways to solve this problem is combining carbapenems with agents that are metal-beta-lactamases (MBL) inhibitors. Unfortunately, at present no MBL inhibitors for carbapenem-resistant microorganisms are approved for clinical use [9]. Beta-lactam inhibitors are known and used in public health practice: tazobactam, sulbactam and clavulanic acid. In addition, a number of chemical compounds are studied as inhibitors of beta-lactamases [11].

In the sphere of activities performed by microbiological laboratories, the determination of dependence between the bactericidal action of antibiotics and the time of exposure of a microbe to their effect (time-kill assay) is widely recognized. In general, it is a question of determining the bactericidal or fungicidal action of antibiotics or their combinations not at a single time point (usually after 24 hours), but in dynamics. Indications for the application of this technique relate mainly to in-depth studies of new antimicrobial agents or their properties, as well as

to the effects of bacterial resistance that haven't been yet studied in everyday practice of microbiological laboratories (so-called "tolerance" of microorganisms, "persistence", "minor colonies", etc.) [3, 4, 6].

Earlier, we showed the ability of etidronic acid (a medicinal agent from the group of bisphosphonates) to enhance the action of carbapenems on clinical strains of gram-negative bacteria that are resistant to carbapenems and produce MBL [1]. This study originated from the search for new MBL inhibitors for carbapenem-resistant microorganisms approved for clinical use and enhancing the action of carbapenems.

Materials and Methods

To assess the effectiveness of the synergistic action of carbapenems and clodronic acid, we used the cross-tapering micromethod (chessboard method) intended for testing the sensitivity of microorganisms to the combined effect of two antibiotics [12, 13], followed by inoculation of well contents onto Müller–Hinton solid medium. To study the dependence between the antimicrobic action of test articles combination with the time of exposure of a microorganism to their effect (time-kill assay), we determined the bactericidal activity of the mixture of medicinal agents not at a single time point after 24 hours of incubation, but at several time points: after 4, 8, 12 and 24 hours of incubation.

The study used antibiotics (imipenem and meropenem) diluted during standard titration in Müller–Hinton medium [5]. The initial clodronic acid concentrate for preparing an intravenous solution containing 60 mg/ml was diluted in Müller–Hinton medium by successive two-fold dilutions. 95 μl of clodronic acid dilution were added to the wells of a polystyrene 96-well plate containing 95 μl of the antibiotic dilution, so that the volume of the mixture made up 190 μl. We studied the *Pseudomonas aeruginosa* 532/14 clinical strain isolated in patients with infectious complications that produces MBL of the VIM genotype and is characterized by a high degree of resistance to carbapenems (MIC of imipenem and meropenem being 512 μg/ml). The microbial burden was 10^6 CFU/ml. 10 μl of microbial suspension were added to each well. Thus, the final microbial burden of the test strain in each well ran to 5×10^4 CFU in 200 μl.

Table. Suppression of *P. aeruginosa* 532/14 growth over time in the presence of a combination of carbapenems with clodronic acid

Test articles and their doses	The CFU amount in 1 μl of medium, when inoculated onto a solid growth medium (n = 5, P < 0.05)				
	before incubation	4 hours of incubation	8 hours of incubation	12 hours of incubation	24 hours of incubation
***P. aeruginosa* 532/14 culture growth control**	102±1.5	116±0.9	$1.64 \times 10^3 \pm 2.4$	$8.64 \times 10^5 \pm 9.4 \times 10^3$	$1.9 \times 10^6 \pm 3.3 \times 10^4$
Imipenem ½ MIC	101±2.3	112±1.5	$1.56 \times 10^3 \pm 8.8$	$7.8 \times 10^5 \pm 5.7 \times 10^3$	$1.1 \times 10^6 \pm 3.3 \times 10^4$
Decrease in the CFU number in $\log_{10}$ relative to the control	–	–	–	–	–
Clodronic acid ½ MIC	92±0.9	96±1.2	$1.1 \times 10^3 \pm 57.7$	$7.7 \times 10^5 \pm 8.8 \times 10^3$	$1.26 \times 10^6 \pm 1.2 \times 10^4$
Decrease in the CFU number in $\log_{10}$ relative to the control	–	–	–	–	–
½ MIC of imipenem + ½ MIC of clodronic acid	87±8.8	90±8.8	60±8.8	0	0
Decrease in the CFU number in $\log_{10}$ relative to the control	–	–	1.4	6	6.3
Meropenem ½ MIC	96±2.4	105±2.8	$1.5 \times 10^3 \pm 2.3$	$6.9 \times 10^5 \pm 4.8 \times 10^3$	$1.2 \times 10^6 \pm 1.9 \times 10^4$
Decrease in the CFU number in $\log_{10}$ relative to the control	–	–	–	–	–
Clodronic acid ½ MIC	92±2.1	96±1.4	$1.4 \times 10^3 \pm 2.4$	$6.8 \times 10^5 \pm 5.1 \times 10^3$	$1.4 \times 10^6 \pm 2.1 \times 10^4$
Decrease in the CFU number in $\log_{10}$ relative to the control	–	–	–	–	–
½ MIC of meropenem + ½ MIC of clodronic acid	100±2.2	84±2.6	0	0	0
Decrease in the CFU number in $\log_{10}$ relative to the control	–	–	3.2	6	6.3

After every 4 hours of incubation, we performed inoculation out of wells with the test article concentration of ½ MIC, as well as combinations of the corresponding carbapenem and clodronic acid in doses of ½ MIC. During inoculation, 1 μl of the respective well contents was introduced into 1 ml of Müller–Hinton medium and then 1 μl was plated onto the surface of the Müller–Hinton agar and spread evenly with a spreader. Incubation took 24 hours at 37°C.

Using the ELx800 reader (Bio-Tek Instruments Inc., USA), we measured the optical density at two wavelengths (490 and 630 nm) at exposures from 4 to 24 hours and revealed the start time of the testing culture logarithmic growth phase. It should be noted that in bichromatic measurement, the optical density value is the difference between two measurements, which reduces significantly any possible errors in results caused by scratches or fingerprints on a plate.

The obtained results for 5 replications were subjected to statistical processing using Microsoft Excel 2007 and Statistica 6.0 by methods of parametric and nonparametric statistics.

Results and Discussion

Our preliminary studies based on the chessboard method defined both the intrinsic antimicrobic action of clodronic acid and its ability in sub-bactericidal concentration to increase the effect of carbapenems on the test strains of antibiotic-resistant gram-negative bacteria producing MBL. Then we modeled a system showing the ability of bisphosphonates to inhibit the activity of MBL and prevent an increase in the level of resistance to carbapenems in test strains of gram-negative microorganisms that were previously sensitive to them [2].

Table shows the results of inoculating the *P. aeruginosa* 532/14 clinical strain onto a solid growth medium at test time intervals. Literature data register the synergistic effect of two agents provided that the microbial population is reduced by ≥ 3 $\log_{10}$ [8].

The data in Table indicate that the combined use of sub-bactericidal concentrations of carbapenems and clodronic acid as the MBL inhibitor has a "lethal effect" on the resistant *P. aeruginosa* 532/14 strain in the period from 8 to 24 hours. The reduction of the CFU number in the presence of a combination of imipenem and clodronic acid ran to 6 $\log_{10}$ by 12–24 hours of incubation. In the presence of a mixture of meropenem with clodronic acid, this effect was registered after 8 hours of incubation, when the test strain growth decreased by 3.3 $\log_{10}$ in comparison with the control. These data indicate a synergistic bactericidal effect of the combinations used [10].

The determination of the time-kill assay for the combination of imipenem or meropenem with clodronic acid against *P. aeruginosa* 532/14 was based on the optical density of the substrate at various exposures. The data are presented in Fig. 1 and Fig. 2.

The logarithmic phase of the test strain growth in the control began after 4 hours of incubation. The culture growth curves in the presence of sub-bactericidal doses of the corresponding carbapenem or clodronic acid on both graphs run in parallel up to 12 hours. Then, the rate of *P. aeruginosa* 532/14 growth slowed by 24 hours. In the presence of a combination of sub-bactericidal concentrations of imipenem or meropenem with clodronic acid, the absence of a logarithmic growth phase of the test microorganism was observed.

When it comes to the matter of life and death of a patient with an infection caused by multiresistant microorganisms, it is important to determine the bactericidal action of combinations of antibiotics, rather than the bacteriostatic one, since the patient's weakened immunity does not complement the action of an antibiotic. Pathogenic microorganisms survive an antibiotic attack and show tolerance and persistence,

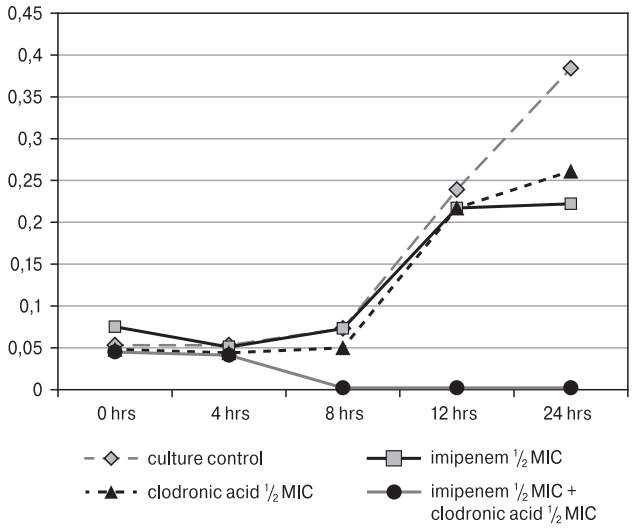

Figure 1. Phases of *P. aeruginosa* 532/14 growth in the presence of imipenem and clodronic acid

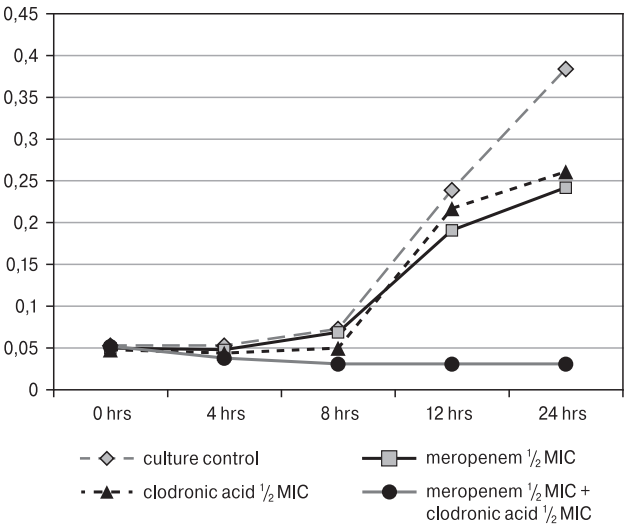

Figure 2. Phases of *P. aeruginosa* 532/14 growth in the presence of meropenem and clodronic acid

while the infectious process continues. In this regard, it is important to determine the dependence between the bactericidal action of antimicrobial agents and the time of exposure of a microorganism to their effect (the so-called time-kill assay) [6].

Our preliminary studies based on the chessboard method have made it possible to identify promising MBL inhibitors for gram-negative bacteria from among the medicinal agents, i.e. bisphosphonates already approved for clinical use [1].

The present study shows the ability of clodronic acid to exhibit a synergistic bactericidal effect with carbapenems against a clinical resistant *P. aeruginosa* 532/14 strain producing MBL of the VIM genotype*. In this combination, the antimicrobic action of imipenem starts after 8 hours of incubation up to 1.4 $\log_{10}$ level as compared with the control. After 12 hours, the total suppression of testing culture growth is registered. The decrease in growth of the test strain ran to 6 $\log_{10}$. Meropenem in combination with clodronic acid showed more pronounced activity, i. e. complete absence of *P. aeruginosa* 532/14 growth by 8 hours of incubation and suppression of growth by 3.2 $\log_{10}$. In 12–24 hours, this rate ran to 6 $\log_{10}$.

The results of our study are consistent with foreign authors's methodical approach to the estimation of the synergistic effect of antimicrobial combinations [7, 8, 10, 14]. The derivation of the time-kill assay allows us to identify effective combinations of carbapenems and promising inhibitors of metal-beta-lactamases for gram-negative bacteria, which is of great importance for the increase in treatment efficacy for patients with severe purulent-septic complications.

* Patent no. RU 2618433 has been obtained based on the research findings.

Список литературы/References

1. Афиногенова А.Г., Ворошилова Т.М., Афиногенов Г.Е., Мадай Д.Ю. Оценка эффективности нового ингибитора металло-бета-лактамазы в условиях модельной системы in vitro // Инфекция и иммунитет. 2016. Т. 6, № 4. С. 335–344. [Afinogenova A.G., Voroshilova T.M., Afinogenov G.E., Maday D.Y. The new metall-beta-lactamase's inhibitor efficacy in a model system in vitro. *Infektsiya i immunitet = Russian Journal of Infection and Immunity, 2016, vol. 6, no. 4, pp. 335–344. doi: 10.15789/2220-7619-2016-4-335-344 (In Russ.)*]
2. Афиногенова А.Г., Ворошилова Т.М., Афиногенов Г.Е., Родионов Г.Г. «Метод шахматной доски» как тест для оценки снижения уровня резистентности грамотрицательных микроорганизмов к карбапенемам в присутствии бисфосфоната // Клиническая микробиология и антимикробная химиотерапия. 2015. Т. 17, № 1. С. 24–32. [Afinogenova A.G., Voroshilova T.M., Afinogenov G.E., Rodionov G.G. «Checkerboard array» as a test for evaluation of decrease of microbial resistance decrease to capbapenems in the presence of bisphosphonate. *Klinicheskaya mikrobiologiya i antimikrobnaya khimioterapiya = Clinical Microbiology and Antimicrobial Chemotherapy, 2015, vol. 17, no. 1, pp. 24–32. (In Russ.)*]
3. Борисова М.И., Лазакович Д.Н., Сидорова Н.А., Савушкин А.И. Биопленкообразующая активность и феномен персистенции микроорганизмов // Journal of Biomedical Technologies. 2015. № 2. С. 28–35. [Borisova M.I., Lazakovich D.N., Sidorova N.A., Savushkin A.I. Biofilm-forming activity and the phenomenon of persistense in microorganisms. *Journal of Biomedical Technologies, 2015, no. 2, pp. 28–35. doi: 10.15393/j6.art.2015.3382 (In Russ.)*]
4. Бухарин О.В. Персистенция бактериальных патогенов как физиологический феномен // Вестник Московского Университета. Серия 16. Биология. 2008. № 1. С. 6–13. [Bukharin O.V. Persistence of bacterial pathogens as a physiological phenomenon. *Vestnik Moskovskogo universiteta. Seriya 16. Biologiya = Herald of the Moscow State University. Series 16. Biology, 2008, no. 1, pp. 6–13. (In Russ.)*]
5. Определение чувствительности микроорганизмов к антимикробным препаратам: методические указания. М.: Федеральный центр госсанэпиднадзора Минздрава России, 2004. 91 с. [Determination of the sensitivity of microorganisms to antimicrobial drugs: methodological guidelines. Moscow: Federal Center for State Sanitary and Epidemiological Supervision of the Russian Ministry of Health, 2004, 91 p.]. *URL: http://fcgie.ru/download/elektronnaya_baza_metod_dokum/muk_1890-04.pdf* (19.10.2018)]
6. Поляк М.С. Лабораторное обеспечение антибиотикотерапии. СПб.: ООО «Анатолия», 2012. 256 с. [Polyak M.S. Laboratornoe obespechenie antibiotikoterapii [Laboratory provision of antibiotic therapy]. SPb: LLC "Anatoliya", 2012. 256 p.]
7. Aiyegoro O.A., Afolayan A.J., Okoh A.I. In vitro time kill assessment of crude methanol extract of Helichrysum pedunculatum leaves. *African J. Biotechnol., 2008, vol. 7, no. 11, pp. 1684–1688. doi: 10.5897/AJB08.055*
8. Basri D.F., Khairon R. Pharmacodynamic interaction of Quercus infectoria galls extract in combination with vancomycin against MRSA using microdilution checkerboard and time-kill assay. *Evid. Based Complement. Alternat. Med., 2012, vol. 2012: 493156. doi: 10.1155/2012/493156*
9. Bedenic B., Vraneš J., Sviben M., Beader N., Kalenić S. Postantibiotic and post-beta-lactamase inhibitor effect of carbapenems combined with EDTA against Pseudomonas aeruginosa strains producing VIM-metallo beta-lactamases. *Chemotherapy, 2008, vol. 54, pp. 188–193. doi: 10.1159/000140461*
10. Bhardwaj M., Singh B.R., Sinha D.K., Kumar V., Prasanna Vadhana O.R., Varan Singh S., Nirupama K.R., Pruthvishree and Archana Saraf B.S. Potential of herbal drug and antibiotic combination therapy: a new approach to treat multidrug resistant bacteria. *Pharm. Anal. Acta, 2016, vol. 7, no. 11. doi: 10.4172/2153-2435.1000523*
11. Drawz S.M., Bonomo R.A. Three decades of β-lactamase inhibitors. *Clin. Microb. Rev., 2010, vol. 23, no. 1, pp. 160–201. doi: 10.1128/CMR.00037-09*
12. Eliopoulos G.M., Moellering R.C. Antimicrobial combinations. In: Antibiotics in Laboratory Medicine; 4th edition. Ed. V. Lorian. *Baltimore, MD, USA: Williams & Wilkins, 1996, pp. 330–396.*

13. Leber A. Synergism testing: broth microdilution checkerboard and broth macrodilution methods. In: Clinical Microbiology Procedures Handbook; 4th edition. *Washington, DC: ASM Press, 2016, pp. 5.16.1-5.16.23. doi: 10.1128/9781555818814.ch5.16*
14. Rani R., Sharma D., Chaturvedi M., Yadav J.P. Antibacterial activity of twenty different endophytic fungi isolated from Calotropis procera and time kill assay. *Clin. Microbiol., 2017, vol. 6, no. 3. doi: 10.4172/2327-5073.1000280*

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Молекулярные основы эпидемиологии, диагностики, профилактики и лечения актуальных инфекций

Международная конференция, посвященная 110-летию со дня основания Санкт-Петербургского института эпидемиологии и микробиологии имени Пастера и 95-летию со дня присвоения Институту имени Пастера

Санкт-Петербург, 4–6 декабря 2018 года

Тезисы докладов

Molecular bases of epidemiology, diagnostics, prevention and treatment of infectious diseases

International conference, dedicated to the 110th anniversary of St. Petersburg Pasteur Institute and to the 95th anniversary of naming the Institute after Pasteur

December 4–6, 2018, St. Petersburg

Abstracts

1. RESULTS AND DIRECTIONS OF ACTIVITIES TO ENSURE THE EPIDEMIOLOGICAL SAFETY OF THE POPULATION IN MODERN CONDITIONS

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FEATURES OF HIV EPIDEMIC SITUATION AMONG CHILDREN AND TEENAGERS OF THE FAR EASTERN FEDERAL DISTRICT

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The objective of the research was to evaluate the fraction of children and teenagers in the structure of the HIV-positive people subjected to regular medical check-ups in the Far Eastern Federal District (FEFD) during 2013–2017.

Three age groups were analyzed during the study: 0–7 years old, 8–14 years old, teenagers (15–17 years of age). The data of the official reporting form No. 61 was utilized.

An increase in the fraction of HIV-infected teenagers by 6.5 times from 0.04±0.02% in 2013 to 0.3±0.05% in 2017 ($p = 10^{-6}$) was detected. The percent of children aged 0–7 years rose by 38.29% from 0.47±0.07 to 0.65±0.07% ($p = 0.07$). A decline of the index by 45.9% was registered in children aged 8–14 years from 0.61±0.08 to 0.33±0.05% ($p = 0.003$). A statistically significant decline of the index by 43.21% from 0.81±0.12% down to 0.46±0.08% ($p = 0.014$) in the age group of 8–14 years as well as an increase of the fraction of HIV-positive teenagers by 4.8 times from 0.05±0.03% up to 0.29±0.06% ($p = 0.0007$) in 2013–2017 was registered only in the Primorsky Region. In 2017, the Magadan Region children and teenagers were free of HIV-infection. No cases of HIV-positive children aged 8–14 years were registered in Jewish Autonomous District and Kamchatka Region while in the Chukotka Autonomous District children aged 8–14 years and teenagers were free of HIV. That said HIV-positive children aged 0–7 years were registered almost in all constituent entities of the FEFD. The fraction of specified HIV-positive children was higher compared to the mean rate in the FEFD (0.65±0.07%) in Amur Region (2.4±0.79, $p = 0.011$) and Republic Sakha (Yakutia) (1.65±0.37%, $p = 0.03$).

The increase of the fraction of HIV-positive children aged 0–7 years necessitates strengthening of preventive measures against mother-to-child transmission of HIV. The rise of the teenagers' proportion in total structure of HIV-positive people can indicate on their low HIV awareness and highlights the need to improve the preventive measures against HIV in this age group.

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MODERN CHARACTERISTICS AND TENDENCIES OF DIARRHEAL INFECTIONS EPIDEMIC PROCESS IN RUSSIA

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In recent years diarrheal infections enhance their leading role in the structure of population infectious diseases. The global trend towards the expansion of viral pathogens range and laboratory diagnostics development have changed the etiological structure of pathogens. At present nearly 70% of the reported diarrheal infections cases with identified etiology in Russia caused by viral pathogens. The results of molecular-genetic and epidemiological analysis indicated an increase of circulating pathogens genetic diversity, as well as strengthening of norovirus infection etiological significance. Since the implementation of registration system norovirus incidence rate has increased by 15 times. In Moscow, noro — and rotavirus infections outbreak incidence was 34% (2011) and 38% (2016). The maximum number of infection cases (414 people) was detected in outbreaks caused by norovirus.

Every fourth etiologically identified case of diarrhoeal infection in Russia belongs to food zoonoses. Among them, salmonellosis holds the leading positions. In recent years its incidence rate has a weak decrease tendency with 25–36 cases per 100 000. Salmonellosis outbreaks are second by registration frequency only to viral etiology outbreaks and are recorded among adults mainly. In Moscow, the share of children in the salmonellosis outbreak structure was 18.6%.

Despite the evident decrease in shigellosis incidence in Russia (with an average annual rate of 17.2%), this infection remains relevant for several regions. Among them are not only regions with water supply quality problems (Republics of Tuva, Dagestan, Khakassia, Karachay-Cherkessia, Astrakhan region, etc.), but also megacities. In Moscow, where incidence rate doesn't exceed 5 cases per 100 000, shigellosis leads by the focal index (27.3). Shigellosis keeps the second place in terms of the outbreak morbidity, having a large proportion of children (76%) in its structure.

Finally, one of the significant trends is a comorbidity growth, which reveals itself by the increase in the number of outbreaks with multiple etiology.

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ON THE TIMELINESS OF VACCINATION IN CHILDREN'S OUTPATIENT DEPARTMENT

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Vaccination is an effective preventive measure, aimed at reducing the morbidity, lethality and mortality from many infectious diseases. However, the full effect of vaccination is provided only when the immunization is high, at least 95% of population.

The purpose of this study was to assess the completeness and timeliness of children immunization in children's outpatient department within the time frames regulated by National calendar of preventive vaccination.

During the study, the history of development (f.112/y) and preventive vaccination records (f.063/y) were analyzed for 631 children under the age of 18 months.

It was found that vaccination coverage of children in decreed age groups for any vaccination regulated by

the law did not meet the benchmark of 95% recommended by the World Health Organization.

Only 81.3% ($\phi = 7.79$, $p < 0.01$) were timely vaccinated for the first time against hepatitis B, only 35.5% ($\phi = 19.92$, $p < 0.01$) received the third vaccination on time; and only 77.9% ($\phi = 9.25$, $p < 0.01$) were vaccinated against tuberculosis. The proportion of children timely vaccinated against whooping cough, diphtheria, tetanus and polio, was 45.5% ($\phi = 19.80$; $p < 0.01$), and those who have completed the full vaccination set until 6 months accounted for 22.3% ($\phi = 22.42$, $p < 0.01$).

The coverage of children with vaccination against measles, rubella and mumps in the decreed period (12 months) was also insufficient and represented 42.5% ($\phi =$ of 17.03, $p < 0.01$) which does not guarantee epidemiological welfare of the territory, and in case of introduction of infection it can lead to its spread among the unvaccinated population.

According to the vaccination documentation, parents refusal to vaccinate and medical conditions (prematurity, low weight at birth, respiratory distress, neonatal jaundice, maternal HIV-infection) were causes of failure to immunize children against tuberculosis and hepatitis B in maternity. Temporary medical exemptions to immunization (acute respiratory infection, intestinal infection, allergic dermatitis), delayed appearance to the vaccination, and parents refusal to vaccinate were the leading causes of violation of the timing for subsequent vaccination in the outpatient department.

Thus, the examination of outpatient medical records revealed serious shortcomings in terms of preventive vaccination regulated by National calendar and timing of vaccination in young children, which forms a group of people susceptible to infection which is sufficient for activation of the epidemic process.

1.4 doi: 10.15789/2220-7619-2018-4-1.4

REGISTRATION OF DYSENTERY SONNEI CASES IN SPECIALIZED MEDICAL INSTITUTIONS OF THE LENINGRAD REGION

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We conducted an epidemiological investigation of infectious disease group focus that emerged in January 2018 in one of the specialized hospitals in the Leningrad region.

Within eight days in one of hospital departments nine patients and the department nurse developed similar symptoms: abdominal pain, fever and diarrhea. In all cases, "Acute gastroenteritis of mild severity" was initially diagnosed. Subsequently every case had *S. sonnei* positive result of feces examination. In blood sera specific antibodies were detected in diagnostic titres.

The presumed source was the patient admitted to the hospital in the incubation stage. Numerous violations of the hospital sanitary and epidemiological regime as well as personal hygiene by patients and staff made it possible to implement a contact-household transmission route and the emergence of a group illness. Patients were transferred to the clinic of infectious diseases, preventive and anti-epidemic measures in the hospital allowed to quickly stop the outbreak. Strains of *S. sonnei* characterized by identical enzymatic properties and antibiogram. The strains were resistant to ampicillin, tetracyclines, chloramphenicol, sulfonamides, trimethoprim, and were characterized by a low level resistance to fluoroquinolones. Sensitivity persisted in the expanded spectrum of cephalosporins (ceftazidime, cefatoxime, cefepime) and carbapenems (merapenem), aminoglycosides (gentamicin, tobramycin, amikacin), nitrofurans.

The genotyping of isolated strains using RAPD-PCR, like the phenotypic methods, showed their identity.

During laboratory and clinical examination of medical and technical personnel of the department, as well as workers of the food unit, shigelosis patients and carriers of *S. sonnei* were not identified.

A shigellosis outbreak in a specialized hospital with a long stay of patients, caused by *S. sonnei*, arose as a result of the introduction of infection to the department, and had nosocomial spreading through a contact-household transmission route.

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ZIKA FEVER IN THE WORLD AND THE RISK OF ITS DISTRIBUTION IN THE RUSSIAN FEDERATION

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Zika fever is an arbovirus transmissible disease caused by Zika virus and transmitted by mosquitoes Aedes aegypti and Aedes albopictus. Discovered for the first time in 1947 in Uganda, the Zika virus led to diseases among people in the 1960s–1980s in Africa and Asia, then to outbreaks in 2007 on the island of Yap in the Western Pacific and in 2013–2014 in French Polynesia. The epidemic, which began in the fall of 2015 in South and Central America, reached 70 countries and led to more than half a million cases. The aim and objectives of the work are to analyze the dynamics of the incidence of Zika fever and to identify tendencies in the distribution in the regions of the world, to identify the features of mosquito biology and to assess the factors affecting the spread of these diseases and the incidence among humans, and to determine the risks of spreading the fever to other areas.

With the help of GIS, an epidemiological analysis of the incidence and spread of Zika fever was carried out, and the features of mosquito biology were determined. Information about the incidence is obtained from the information messages of the WHO and Rospotrebnadzor. Statistical and graphical methods of investigation were used to process the results.

The epidemic process of Zika fever in South and Central America has come to an end. The analysis of morbidity, which was carried out, showed that the outbreak was uneven in time and space, with 6 phases of the epidemic process identified. The obtained data for biological characteristics of populations of two species of mosquitoes allowed us to attribute *Aedes aegypti* to the group of more important species for epidemic spread — to the main carriers, and *Aedes albopictus* to less significant, secondary carriers. The information obtained does not give an accurate prediction of the further spread of Zika fever in different regions. But it can be assumed that there can be a region in the Russian Federation — the Black Sea coast of the Russian Federation (the Caucasus and the Crimea), which should be assessed at present as unfavorable for the formation of a focal point for augmented transmission of infection. However, with changing climatic conditions, the situation may change. The causative agents of some fevers may expand the regions of distribution and this is associated with the same types of mosquito vectors as in the case of Zika fever.

The expansion of the geography of mosquito vector regions and their adaptation to colder and drier climatic conditions with the potential for transmission of infection gradually expand the area and require scientific study and monitoring of hemorrhagic fever viruses and Zika virus in particular.

1.6 doi: 10.15789/2220-7619-2018-4-1.6

MODERN PROBLEMS OF INFECTIOUS DISEASES PREVENTION IN PUBLIC HEALTH OF REPUBLIC OF GUINEA

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Republic of Guinea is one of the developing countries of West Africa. Of the whole GDP only 400–500 USD are on one habitant annually. More than half habitants live down the poverty level. The country carries a heavy burden of many infectious diseases.

The aim of the study was to describe the actual problems of infectious diseases prevention in public health of Republic of Guinea.

Statistical data from public health institutions of the country were used. Methods — descriptive and analytical.

Population size of Guinea increases and numbers now more than 12 millions habitants. The public health structure of the country includes: 925 first-aid posts, 410 prefecture and regional centers of health, 8 communal health centers, 26 prefecture, 7 regional and 3 national hospitals. Though medical aid is below of the regional norms of WHO. In Guinea 74 nosoforms of infectious and non infectious diseases are registered. The part of infectious diseases in the morbidity structure was 38–44% (1.9–2.5 millions cases annually). The most widespread infections were malaria, acute respiratory (ARI) and intestinal infections. In the mortality structure ARI occupied 12.5%, malaria — 10%, acute intestinal infections — 6%, HIV infection consists 5%. The sexual transmissible infections are widespread: 200 thousand cases in a year. The outbreaks of measles, meningitis, cholera, Yellow Jack are registered too. Prevention measures are actively conducted. All little children are vaccinated obligatory against: poliomyelitis (4th time), BCG, kombi (diphtheritic, tetanus and pertussis), measles, Yellow Jack. The pregnant women receive anti malaria drugs as prevention and anti tetanus vaccine. The HIV positive pregnant women receive antiretroviral therapy. One realizes health education programs, particulary among the youth. One popularizes the use of contraceptives, organizes centers of family planning. However this activity meets some difficulties because of the low education level, some religious and socio-cultural customs of population, what was visually revealed during Ebola fever outbreak. The vaccination program meets such difficulties as cold chain, lack of qualified medical personnel, lack of medicaments and technical equipment of diagnostics laboratories, which number is insufficient. In order to dissolve these problems of diagnostics, control and prevention of infectious diseases in 2018 the 3-years program "National strategy on medical biology" was elaborated.

In spite of difficulties the public health of Guinea goes on to develop. The realization of the "National strategy" will help in strengthening of health and welfare of population of Guinea.

1.7 doi: 10.15789/2220-7619-2018-4-1.7

FECAL-ORAL MECHANISM IN THE GROUP AND EPIDEMIC STRUCTURES ON THE TERRITORY OF THE ROSTOV REGION WITHIN 10 YEARS

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Infections with the fecal-oral transmission take one of the leading places in the infection pathology of population including economically developed counties, going only after respiratory infections among mass infectious diseases.

In Rostov region according to the form of statistic observation No. 23–17 "Information about outbreaks of infectious diseases", within 10 years from 2008 until 2017th 104 cases of group and outbreak morbidity was reported, with 1853 caseload, including 1130 children under 17 years (61.0% of the total number of cases).

In structure the main gravity in the region lies on the fecal-oral mechanism (76.9%) and also on aspiration (10.6%) and others (12.5%).

The spread was occurred by food — in 47 cases (45.2%), by water — in 14 (13.5%), by household contacts — in 19 (18.3%), airborne-in 11 (10.5%) and other — 13 (12.5%)

Etiological factors in group and outbreak morbidity were bacterial pathogens of infection: *Salmonella* Enteritidis (11), *Salmonella* Typhimurium (3), *Salmonella* Seegefeld (1), *Salmonella* Muenchen (1), *Salmonella* Isangi (1), *Salmonella* spp. (1), *Shigella sonnei* (6), opportunistic pathogenic microflora (7) *(Staphylococcus aureus, Proteus vulgaris, Citrobacter, Escherichia coli, Enterobacter aerogenes)*, viral aetiology: group A rotaviruses (15), 2 genotype Noroviruses (18), hepatitis a virus (9), Enteroviruses (14), mixed etiology (1), undetermined etiology (4), measles virus (5), Crimean-Congo hemorrhagic fever (1), parvovirus in 19 (1), influenza (1), tuberculosis (1), chickenpox (1), community-acquired pneumonia (1) and epidparotite (1).

Improvement of decoding of acute intestinal infections (ОКИ) and further development of laboratory diagnostics in etiological structure dramatically changes were noticed: the increasing epidemiological importance is given to "intestinal" viruses, the intensity of circulation of which has increased in recent years (enteroviruses, rotaviruses and noroviruses).

The changed structure of the epidemic process including acute intestinal infections, the growth of viral infections requires new approaches in the improvement of activities and diagnosis, anti-epidemic supply and prevention activity.

1.8 doi: 10.15789/2220-7619-2018-4-1.8

THE CHARACTER OF MICROBIOTA IN THE INTENSIVE CARE UNIT OF THE CHILDREN'S HOSPITAL AND ITS EPIDEMIOLOGICAL SIGNIFICANCE

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As is known, the risk of infections associated with medical care is highest in intensive care units, especially in the department of pathology of newborns and premature infants. The aim of the study was to study the composition and characteristics of bacteria circulating in the intensive care unit of the children's hospital.

Material for the study: 637 sputum samples from 171 children of the intensive care unit, including from the department of pathology of newborns and premature infants; scrapes from the throat, nose and hands of 53 medical staff; 86 scrapes from the surfaces of the environment. Research methods: bacteriological and MALDI-TOF/MS (Bruker Daltonics).

It was found that 30 species of bacteria were isolated from children, including: *Staphylococcus aureus* (10%), *Staphylococcus epidermidis* (8%), *Klebsiella pneumoniae* (7%), *Streptococcus pneumoniae* 7%), *Enterococcus faecium* (2%). *S. aureus* (45%), *S. epidermidis* (90%), *S. pneumoniae* (17%), *K. pneumoniae* (6%) were isolated from medical personnel serving these children. Staphylococci of different species with the highest proportion of *S. epidermidis* (21%) were isolated from 50% of environmental objects. Most often they were isolated in newborns (16%), less often in children aged 1 month to 1 year (10%), in children 1–3 years (8%), in children older than 3 years (1%). These data indicate a contact-household route of transmission of infection caused by *S. epidermidis*. The frequent occurrence of *S. aureus* and *S. pneumoniae* in children and medical personnel in the absence of these bacteria in the environment indicates the role of resuscitation department staff as a source of these infections. Bacteria *K. pneumoniae* and *E. faecium* are isolated from both children, medical personnel and environmental objects. All of the above bacteria were well subjected to destruction by disinfectants. After reorganization activities in the department, the proportion of positive bacterial seeding decreased significantly.

However, the study found that *Bacillus cereus* strains isolated from newborn infants (6%) and from medical and general-purpose equipment are resistant to all disinfectants used in the department. As a result of the research, the drugs to which the isolated bacilli are sensitive were selected.

Thus, continuous monitoring of the microbiota, the study of its characteristics and the epidemiological approach to assessing the situation can significantly reduce or avoid the development of infections associated with the provision of medical care.

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ETHICS OF VACCINATION AS THE CRITERIA OF THE SCIENTIFIC AND HUMANISTIC APPROACH

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The aim of this research was to analyze the unique role of the vaccination from its successful and problematical sides concerning the developing, distribution and using the vaccines in different epidemiological situation in the mirror of the global bioethics. The role of the balance between ethics and success of vaccination was clearly illustrated and based on examples from the history of vaccination and our own experience connected with amazing contribution of Leningrad/St. Petersburg Pasteur Institute in the history of vaccination. It was clear shown that the particular importance has the harmonization and solidarity of all persons and structures involved in the process of vaccine prevention within local, national, regional, and global levels. In order to reach the goal in protection of the infections diseases by vaccination, it is necessary to follow in reality and to demonstrate to the society such universal ethical standards as scientific honesty, social expediency, justice, non-discrimination, transparency and overcoming of the conflict of interests. Detailed description how these ethical principals particularly work in the stage of R&D vaccines, its distribution, using for vulnerable population and in the frame of WHO program for eradication of poliomyelitis, measles, and rubella was done. The specific relevance of the presented study was to highlight the key role of ethical principles of the adversarial position to the increasing anti-vaccination lobby. It is well known that the success of vaccination is associated not only with effective and safe vaccines, but directly depends on the society adherence to vaccination acceptance and realization. A negative influence of anti-vaccination movement, which promotes mistrust to vaccination, is playing an essential role in this process. The objective analysis and identification of ethical errors in the process of vaccination demonstrated in this research show the way any unethical action is creating a basis for the anti-vaccination movement. The maintaining of ethical standards balance and building partnerships and interaction with society as well as the implementation the ethical elements in legal regulation of the vaccination process on national and international levels are crucial for achieving optimum results in countermeasures against evolving the anti-vaccination lobby and protection against infections by vaccination in the present time and future perspective.

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ETHICAL CONSIDERATION IN CONCEPTION OF INFECTIOUS DISEASES ERADICATION

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The goal of this study was to show the universal role of ethical conception in the realization WHO global initiative on the eradication of infectious diseases: polio, measles, rubella and congenital rubella. The analysis of the WHO Strategic Plan activities was done in the light of UNESCO Declaration "On Bioethics and Human Rights", 2005. During implementation of WHO Strategic Plan for eradication measles, rubella and congenital rubella at the national, regional and global levels was happened the obligatory need to twice postpone the deadline from 2010 to 2015 and from 2015 to 2020. The reason of this event was connected with the lack of solidarity during preventive measures and clearly demonstrates the importance for joint actions and control over the epidemic process. The acceptation and following of these measures could help to achieve the effect, which corresponds to the ethical principals from article 13 "Solidarity and Cooperation" and article 24 "International Cooperation" of UNESCO Declaration. The diversity of situations and conditions in the implementation of the WHO program in different regions of the world, or in relation to the different cultural, social, religious, economic and psychological status of contingents requires adherence to the principles laid down in articles 8, 9, 12: "Recognition of human vulnerability and respect for personal integrity", "Equality, justice and equality" and "Respect for cultural diversity and pluralism". The whole system of administration and management of the WHO activity at each individual level of implementation should be based on the ethical principles decelerated in articles 3, 5, 14–17: "Human dignity and human rights", "Independence and individual responsibility", "Social responsibility and health", "Sharing benefits", "Protecting future generations" and "Protecting the environment, the biosphere and biodiversity." In general all elements of the implementation the global goals both in the field improving vaccine and vaccination pro-

cess and in laboratory practice for diagnostic and control of the elimination program could be achieved only on the basis of following guidelines mentioned in article 21 "Transnational practices", which facilitate the exchange of new technologies and Article 23 "Education, training and information in the field of bioethics" which give the universal orientation for ethical cooperation.

1.11 doi: 10.15789/2220-7619-2018-4-1.11

EPIDEMIOLOGICAL CHARACTERISTICS, ETIOLOGICAL STRUCTURE AND MODERN METHODS OF DETECTION OF PATHOGENS OF ACUTE INTESTINAL VIRAL INFECTIONS IN ORGANIZED GROUPS

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In organized collectives of the Armed forces of the Russian Federation, acute intestinal infections (AII) occupy one of the leading rank places, which is associated with the level of morbidity and with large labor losses. Diagnostics is carried out taking into account the requirements of documents of the Sanitary legislation of the Russian Federation, using modern methods of laboratory diagnostics. Recently, specific laboratory studies indicate the dominance in the etiological structure of acute intestinal viral infections of unknown etiology (AIVIUE) of intestinal viruses, the most significant of which are viruses that cause enteritis and gastroenteritis: rotaviruses, caliciviruses, including noroviruses and related viruses, astroviruses, adenoviruses, enteroviruses, etc. Special place is occupied by the group incidence of acute intestinal viral infections (AIVI). In recent years, for these purposes are embedded devices, styling, diagnosticums and test systems made in Russia, some of them are tested in the army now.

The aim of the work was to assess the epidemiological significance of AIVI in military personnel, the etiological structure of viruses and diagnostic value, means of sample preparation and their detection. Detection of markers of rotaviruses, adenoviruses and noroviruses in feces was carried out by methods of enzyme immunoassay (ELISA), real-time PCR with multiplex test system "OKA-screen", in addition, the method of latex agglutination using domestic test systems "Rota-screen, "Adeno-screen", "Noro-screen" was used. Enteroviruses (ECHO, Coxsackie A, Coxsackie) was determined in the feces of a classical virological method of neutralization. It is established that a leading place among the causative agents of viral etiology OKA occupied: rotaviruses — 49%, noroviruses 12%, adenoviruses — 9%, astroviruses — 1%, enteroviruses (ECHO — 18%, Coxsackie A — 8%, Coxsackie B — 3%) — 29%.

Thus, complete etiological decoding of AIVI with the help of specific laboratory tests allows to evaluate both epidemiological and clinical features of acute intestinal viral infections in organized groups.

1.12 doi: 10.15789/2220-7619-2018-4-1.12

COMPLIANCE OF HEALTH CARE WORKERS WITH VACCINATION AS THE FACTOR OF FORMATION OF POSITIVE ATTITUDE TOWARDS VACCINATION IN THE POPULATION

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Despite the importance of preventive vaccination to eradicate infectious diseases, in recent years, there were those who doubt about the need to continue mass immunization.

The aim of the study is to evaluate the role of health care workers in shaping the attitude towards vaccination among the population.

In the research, an anonymous questionnaire was designed and 865 parents were surveyed about their attitude towards vaccination. 78.2% of respondents believe that vaccination is necessary, 6.7% are convinced that vaccination is not needed, and 15.1% did not answer the question about the advisability of vaccination.

When assessing the attitude towards vaccination among people of different age and education level, no significant differences have been revealed ($\phi < 1.64$, $p > 0.05$). When analyzing the gender structure of the respondents, the most reluctant parents were found among men ($\phi > 2.31$, $p < 0.01$).

A negative attitude to vaccination among parents was linked with the uncertainty of their safety (45.2%). In the second place, it was observed that the vaccinated children could also get sick (16.0%) and then there were doubts about the quality of modern vaccines (11.5%).

The majority of parents (76.8%) stated that they often received information about vaccinations from health care professionals and less often from relatives, friends, Internet, and television ($\phi > 2.31$, $p < 0.01$).

To assess the opinion of health care workers about vaccination, a survey of 1325 employees of five various hospitals was conducted. It has been established that the majority (85.9%) had a positive attitude towards vaccination, and 4.5% were negative and 9.6% were unable to formulate their answer.

Among 187 employees who do not trust the vaccination there were 41 physicians, 79 nurses, 14 paramedical personnel and 53 employees of non-medical specialties. Among physicians of different specialties the greatest number of opposed employees were among dentists, surgeons, pediatricians, intensive care workers and laboratory workers. A negative attitude towards vaccination was most often found among employees with work experience over 20 years ($\phi > 2.31$, $p < 0.01$), while no significant gender differences were revealed ($\phi < 1.64$, $p > 0.05$).

Thus, to improve the system of preventive vaccination, it is necessary to raise the awareness of health care professionals in matters of vaccination, because they are the most important and authoritative sources of information about vaccination for the population.

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CLINICAL-EPIDEMIOLOGICAL CHARACTERISTIC OF THE INFLAMMATORY BOWEL DISEASES IN SAINT PETERSBURG

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One of the leading problems in the structure of diseases of the digestive system are the Inflammatory bowel disease (IBD), which includes the Ulcerative colitis (UC) and the Crohn's disease (CD). In recent years, a steady increase in the incidence of UC and CD has been noted in industrialized countries. The peak incidence falls on the age of 15–35 years. The aim of our research was to study the incidence and prevalence of the UC and CD among adults, and also to analyze the sex and the age features of their course in Saint Petersburg. We evaluated the incidence of UC and CD among adults in two districts (Frunzensky and Vyborgsky) in Saint Petersburg

in 2017. The total number of patients with IBD was 459 people. The results of the research showed that the incidence of UC and CD is higher in the Vyborgsky district than in the Frunzensky district ($p < 0.05$), the prevalence is almost the same in both regions ($p < 0.05$). The incidence of UC is 9.05 per 100 000 people in the Frunzensky district in 2017, in Vyborgsky it is 12.58 per 100 000 people. The incidence of CD is 3.21 per 100 000 people in the Frunzensky district in 2017, in Vyborgsky it is 6.29 per 100 000 people. The prevalence of UC is 40.6 per 100 000 people in the Frunzensky district in 2017, in Vyborgsky district it is 38.9 per 100 000 of the population. The prevalence of the CD is 20.4 per 100 000 population in the Frunzensky district, in Vyborgsky district 23.9 per 100 000 population. Among patients with UC, women predominate in a ratio of 1:2 ($p < 0.05$), CD is almost the same in men and women ($p < 0.05$). The incidence of UC prevails over the incidence of CD, as well as higher incidence rates among female patients ($p < 0.05$). An analysis of the age structure showed that about 50% of cases of UC occur at the age of 20–49 years, about 40% of patients with UC are in the age group over 60 years, no more than 10% of cases occur at the age of 18–19 and 50% 59 years old. More than 60% of patients with CD are in the age group of 20–49 years and about 25% of cases of CD are in patients older than 60 years, about 15% in the group of 50–59 years and no more than 5% of cases in the 18–19 age group. Given that the incidence of UC and CD are higher among women and more than 50% of patients are in the most able-bodied age of 20–49 years, inflammatory bowel disease is a group of highly relevant and socially significant diseases. The revealed differences in morbidity between districts should be taken into account when organizing preventive, therapeutic and diagnostic activities.

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THE USE OF CORRELATION ANALYSIS ON THE EXAMPLE OF INFLUENZA VACCINATION ON THE TERRITORY OF THE ROSTOV REGION

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Vaccination company is held every year in Rostov region before the epidemic increase of influenza infection. For immunization, influenza vaccines of domestic and foreign production are used, included for the Northern hemisphere on the recommendation of the world health organization of actual strains of influenza A(H1N1), A(H3N2) and B for the current season.

To assess the vaccination effect in the Rostov region, sings of influenza infection were analyzed during the epidemic seasons and coverage of preventive vaccinations during the 15 seasons (season 2003–2004 up to 2017–2018 years). Precautionary coverage was reevaluated on accordance to the form No. 5 of the state statistic observe data.

During the research period precautionary coverage from influenza has raised from 17.1% of communities in the 2003–2004 seasons up to 45% in 2017–2018 years.

Correlation rate was calculated with non-parametric correlation rate of Spirman's degrees, and it is equal to 0.67 in the current research. To estimate the zero-hypothesis the target value of the criteria should be compared with the tabled value criteria. According to the Spirman's table of crucial correlation criteria $n = 15$ and along with the rate of statistic value 0.05 the crucial point for ρ was 0.521. Target value (0.67) is more than crucial, discovered association between the precautionary coverage of influenza prevention and virulence is statistically valued. In addition, the correlation coefficient value and sign with which it turned out, we can suggest about the strength and direction of the connection.

This correlation coefficient is equal to +0.67, that indicates a direct and average dependence, on this basis, it is proved, that there is an increase in vaccination coverage against influenza groups at risk (children over 6 months, people suffering from chronic diseases, pregnant women, as well as persons from occupational risk groups — health workers, teachers, students, service and transport workers) leads to a decrease in the incidence of this infection, prevent complications and reduce the number of deaths.

1.15 doi: 10.15789/2220-7619-2018-4-1.15

EPIDEMIOLOGICAL CHARACTERISTICS OF INCIDENCE OF CHRONIC VIRAL HEPATITIS B INFECTION AMONG HEALTHCARE WORKERS IN ST. PETERSBURG IN 2013–2017

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The aim of the study was to determine the clinical and epidemiological features of viral hepatitis B (HBV) in medical personnel newly identified in St. Petersburg under the conditions of vaccine prevention.

In St. Petersburg vaccination of health care workers began in 1996. Coverage with 3-fold of hepatitis B vaccine among medical workers are expressed in the following figures: in 2013 — 94.7%, in 2014 — 96.7%, in 2015 — 96%, in 2016 — 96.6%, in 2017 — 96.7%.

160 registered cases of viral hepatitis B in healthcare organizations in St. Petersburg, including 141 cases of chronic HBV, 5 cases of acute HBV, and 14 cases of HBV carriage. Out of 160 cases, 112 cases were medical workers 112 (70%) and 48 (30%) were not medical staff.

The incidence rate of HBV per 100 000 medical workers was 38.2 in 2013; 50.8 in 2014; 24.3 in 2015; 22.7 in 2016; and 4.5 in 2017.

The group of medical workers consists of 99 cases of chronic HBV (88.4%), two cases of acute HBV (1.8%) and 11 cases of HBV carriage (9.82%) ($P < 0.05$)

Women predominate with 74.1% of cases while men comprise 25.9% ($P < 0.05$).

Age structure: 20–24 years — 2.7%, 25–29 years — 7.1%, 30–34 years — 7.1%, 35–39 years — 5.4%, 40–44 years — 8.9%, 45–49 years — 9.8%, 50–54 years — 10.7%, 55–59 years — 20.6%, and from 60 and over — 27.7%.

In the socio-occupational structure of HBV cases in 2013–2017, the main share is occupied by the middle medical personnel — 42%, professional medical personnel — 33% and junior medical personnel — 25%. The socio-occupational risk groups for HBV according to the type of departments are: surgical — 23.2%, outpatient polyclinic — 17%, dental — 8%, therapeutic — 6%, staff of clinical diagnostic and biochemical laboratories — 5.4%.

Thus, the incidence of HBV in St. Petersburg in the conditions of vaccine-preventive maintenance of infection tends to decrease, over the five-year span by 8.5 times — from 38.2 in 2013 to 4.5 in 2017. The preponderance of persons over 55 years old among healthcare workers affected by HBV old and the dominance of chronic

forms of infection indicates the imperfection of activities to identify hidden forms of the disease. The socio-professional structure corresponds to the risk groups specified in the guidelines. The study showed that the incidence of viral hepatitis B in health care workers is still an actual problem, despite the high efficiency of the activities and programs.

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THE PREVALENCE AND MOLECULAR EPIDEMIOLOGY OF *STENOTROPHOMONAS MALTOPHILA* IN THE INTENSIVE CARE UNITS

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Stenotrophomonas maltophilia is a ubiquitous aerobic nonfermentative Gram-negative bacillus that exists in humid environments, water sources, soil, and plants. *S. maltophilia* has the ability to colonize epithelial cells of the respiratory tract and surfaces of medical devices. This nosocomial pathogen that is highly antibiotic resistant and associated with high morbidity and mortality, particularly in immunocompromised or critically ill patients. Episodes of fever and neutropenia are common complications of treatment for cancer. The use of prophylactic and early empirical antibiotics has reduced mortality but decreases the sensitivity of diagnostic tests based on culture. It seems obvious importance of early identification of *S. maltophilia*.

This study was aimed to determine the prevalence of *S. maltophilia* in the intensive care units.

The material was *S. maltophilia* isolates, collected from 392 samples, including patient's blood, medical devices, surfaces, beds and the surroundings of patients in wards. Genotyping was performed using sequencing of 16S rRNA gene. The 16S rRNA sequence of each strain was aligned with 16S rRNA gene sequences from the GenBank sequence database using the BLAST algorithm.

A total of 47 (11.9%) isolates of *S. maltophilia* were recovered from patients and environmental samples. Most of the isolates were not genetically related. However some isolates found from the surroundings of patients in wards were genetically similar to those obtained from patients. So some evidence of clonal dissemination was found, indicating the occurrence of cross-transmission of antibiotic-resistant strains within the hospital.

The presence of *S. maltophilia* in the hospital environment indicates that it can act as a reservoir of this microorganism. This underscores the need for effective control and prevention measures in hospitals. Using sequencing 16S sequence reduces time of identification bacteria, including allowing the identification of the object directly from the patient's blood.

2. MODERN METHODS OF MOLECULAR DIAGNOSTICS OF INFECTIOUS DISEASES

2.1 doi: 10.15789/2220-7619-2018-4-2.1

GENOMIC DIVERSITY OF NON-TOXIGENIC *VIBRIO CHOLERAE* EL TOR STRAINS AND METHOD FOR DIFFERENTIATION OF CHOLERA VIBRIOS WITH DIFFERENT EPIDEMIC SIGNIFICANCE, USING PCR

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In the territory of the Russian Federation over the period of 2008–2017 in the course of surface water bodies monitoring, 725 non-toxigenic strains of cholera vibrios with *ctxA*$^-$ *tcpA*$^-$ and *ctxA*$^-$*tcpA*$^+$ genotypes were isolated. The question regarding the origins of non-toxigenic strains and their genomic diversity remains an open one. In this context, objective of the study was to investigate genomic diversity of non-toxigenic *V. cholerae* El Tor strains, evaluate their epidemic significance, using a designed multiplex PCR.

We applied conventional microbiological and molecular-genetic methods, as well as whole-genome sequencing tools.

The analysis included isolated natural and experimentally obtained non-toxigenic strains. For non-toxigenic strains the presence of a complete set of mobile genetic elements (MGE), responsible for pathogenic (CTXϕ, TLCϕ, RS1, VPI-1, VPI-2) and epidemic (VSP-I и VSP-II) potential was characteristic. Non-toxigenic strains turned out to be genetically heterogeneous and were divided into three groups. The first group of *ctxA*$^-$*tcpA*$^-$ strains lacked CTXϕ, TLCϕ, RS1, VPI-1, VSP-I, and VSP-II elements. Pathogenicity island, VPI-2, had the deletions the size of 33–49 kb, depending upon the strain. The second group of *ctxA*$^-$*tcpA*$^+$ strains was devoid of CTXϕ, RS1, VSP-I, VSP-II, but preserved pathogenicity islands, VPI-1 with *tcpA,* and VPI-2. The latter one had the deletions the size of approximately 34 kb. The third group consisted of experimentally obtained non-toxigenic strains that lost CTXϕ prophage in aqueous medium, but retained pandemicity islands, VSP-I and VSP-II. Among the studied non-toxigenic strains isolated in the territory of Russia, this type of strains was not found. According to the international NCBI GenBank database, such non-toxigenic strains were detected in endemic as regards cholera regions. Genome analysis of the mentioned strains showed that they were deprived of CTXϕ prophage only, but contained all other MGEs with genes of virulence and epidemicity. The data gathered suggest that natural non-toxigenic *ctxA*$^-$*tcpA*$^+$ strains may be derivatives of toxigenic ones. Thus, in the territory of the Russian Federation two main groups of non-toxigenic strains with *ctxA*$^-$*tcpA*$^-$ and *ctxA*$^-$*tcpA*$^+$ genotypes circulate. Heterogeneity of non-toxigenic *ctxA*$^-$*tcpA*$^+$ strains by the structure of the genome and epidemiological significance pointed to the necessity of PCR construction for their differentiation. We designed multiplex PCR which simultaneously separates toxigenic from non-toxigenic strains by the presence/absence of *ctxA* and *tcpA* genes, and differentiates the latter ones into potentially epidemically hazardous and epidemically safe ones by the presence/absence of pandemicity islands' genes, VSP-I (*VC0180*) and VSP-II (*VC0514*).

Non-toxigenic *V. cholerae* El Tor strains with *ctxA*$^-$ *tcpA*$^+$ genotype are genetically inhomogeneous group with varying epidemiological significance. The strains of *ctxA*$^-$ *tcpA*$^+$VSP$^+$ can pose a potential epidemic threat and circulate only in endemic territory. *ctxA*$^-$*tcpA*$^+$VSP$^-$ strains circulating in the territory of Russia are epidemically safe due to the loss of considerable genome regions.

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DETECTION AND ANALYSIS OF CRISPR-CAS SYSTEMS IN PLASMIDS OF DIFFERENT *BACILLUS THURINGIENSIS* STRAINS

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Bacillus thuringiensis (Bt) is a gram-positive spore-forming bacteria capable of producing toxic proteins (Cry, Cyt or Vip) some of which are used against insects, nematodes and human-cancer cells. CRISPR loci absent in chromosome sequences of Bt strains available in public databases. In 2017 an acting CRISPR-Cas system was detected in its plasmid. The presence and structure of CRISPR-Cas systems in other plasmids of Bt has not yet been studied. The analysis of these systems is basis for research phage resistance in industrially and medically important strains of Bt.

The aim of the study is to perform a search and comparative analysis of CRISPR-Cas systems in plasmids of different strains of Bt using bioinformatic methods.

Nucleotide sequences and protein profiles of all available in NCBI databases (in June 2018) plasmids of Bt have been analyzed by bioinformatic software tools.

We identified the genomic loci of CRISPR-Cas system in 16 circular plasmids ranging in size from 94695 to 761374 bp. 10 plasmids have genes of insecticidal proteins: Cry1Aa, Cry2Aa, Cry2Ab, Cry2Ac. All detected CRISPR loci belong to the class 1, type I, subtype C and vary in length from 3495 to 12188 bp. CRISPR-Cas systems with complete set of *cas*-genes were found in 2 of 16 plasmids. The genes of adaptation module absent in 14 plasmids, therefore an acquisition of new spacers does not occur. One plasmid does not contain CRISPR arrays and gene of *endonuclease Cas3* which cleave foreign genetic elements. CRISPR arrays of 15 plasmids comprise the repeats (32 bp) separated by 3–17 short spacers (32–35 bp). The presence of CRISPR loci in the plasmids confirms a possible transfer of CRISPR locus from the nucleoid to plasmids. The results of this study provide new information about the degradation of CRISPR-Cas system in some Bt strains.

2.3 doi: 10.15789/2220-7619-2018-4-2.3

INDEL TYPING OF *VIBRIO PARAHAEMOLYTICUS* STRAINS ISOLATED DURING OUTBREAKS IN THE RUSSIAN FEDERATION

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The use of molecular methods for intraspecific typing of bacteria allows to analyze and predict the spread

of clones with increased virulence, resistance to environmental factors and antibiotics. One of the new molecular methods is INDEL-typing which is based on the search for spontaneous inserts/deletions of several nucleotides that differ in length in different clones.

Vibrio parahaemolyticus is a common and important pathogen that causes human gastroenteritis worldwide. We have developed a method for typing *V. parahaemolyticus* strains based on the analysis of six INDEL-locus (S.O. Vodop'yanov et al., 2016). The INDEL analyses of the *V. parahaemolyticus* collection revealed that strains of different INDEL-genotypes circulate in environment. The discriminating power of INDEL-typing for environmental strains was 0.95. However, to date, there is no information about the INDEL-genotypes of clinical strains.

The aim of this work was to study INDEL-markers of *V. parahaemolyticus* strains isolated during two foodborne disease outbreaks in the Russian Federation.

It was investigated 29 clinical strains of *V. parahaemolyticus* isolated in July-October 2012 in the Primorsky region of the Russian Federation. The study was performed on INDEL loci Vp967, Vp08, Vp619, Vp2256, VpA472, Vp506. The result showed that all 29 studied *V. parahaemolyticus* strains had identical INDEL genotype with the formula Vp967 — 112, Vp08 — 89, Vp619 — 114, Vp2256 — 111, VpA472 — 95 and Vp506 — 79 base pairs. Thus, both outbreaks were caused by one clone of the pathogen. At the same time, strains with other INDEL genotypes circulated in the environment.

In our opinion, the INDEL-typing method of *V. parahaemolyticus* strains can be useful in carrying out epidemiological investigation of outbreaks of food gastroenteritis.

2.4 doi: 10.15789/2220-7619-2018-4-2.4

EXPRESSION OF RECOMBINANT NS1 PROTEINS OF WEST NILE, DENGUE AND ZIKA FEVER VIRUSES IN *NICOTIANA TABACUM* FOR FUTURE USE IN DIAGNOSTICS

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In connection with the increasing frequency of infectious diseases outbreaks caused by arboviruses, the monitoring of the epidemiological situation in the Russian Federation requires development of immunological diagnostic kits for differential diagnosis. These kits could be developed using individual recombinant antigen proteins of selected viruses. Standard eukaryotic systems, for example insect cells, have a number of limitations in terms of productivity and costs. In our work, we used plants for the production of flavivirus antigens which are an ideal biofabric system because of their ability to generate large amounts of proteins with low cost and to produce an appropriate post-translational modification of recombinant proteins. Protein targets for expression were NS1 non-structural proteins of flaviviruses which were described in the literature as reliable serological markers.

The sequences of the NS1 proteins of Zika virus (ZIKV), West Nile virus (WNV) virus and the two serotypes of Dengue virus (DNV1 and DNV3), have been optimized for expression of the target proteins in the *Nicotiana tabacum*. The resulting DNA sequences were submitted in the GenBank database under accession numbers: MH134590, MH134591, MH134592, MH134593 for ZIKV, DNV1, DV3 and WNV respectively. Sequences were synthesized *de novo* using oligonucleotides by the enzymatic "Two step PCR" method. Expression cassettes containing 35S CaMV promoter and tNOS terminator for strong constitutive expression of the target were constructed on the base of the pBI121 plasmid. Four binary vector systems for the expression of NS1 proteins in plants were developed. Tobacco leaf discs were transformed using *Agrobacterium tumefaciens* Ti-plasmids of strain AGL0 and further regeneration of tobacco plants was carried out. For each expression structure, 10 independent transgenic lines were obtained and were transferred to rooting media for further transfer to conditions of closed soil, which would enable the collection of the necessary amount of biomass to isolate antigen proteins, for their further use in the creation of diagnostic systems. The target gene insertions in each line were confirmed by PCR.

Thus, the plant expression system of West Nile, Dengue and Zika virus antigens was developed and our future studies would include purification of target antigens and their verification as serological markers in diagnostic systems (ELISA, immunochip).

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2.5 doi: 10.15789/2220-7619-2018-4-2.5

WHOLE-GENOME SEQUENCING AS A TOOL FOR COMPREHENSIVE ASSESSMENT OF THE PATHOGENIC POTENTIAL OF ANCIENT ARCTIC MICROBIOMES

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Arctic permafrost is a natural reservoir of ancient prokaryotic mobile genetic elements (MGE) associated with pathogenicity or resistance to antimicrobials. It has been shown that ancient MGE have possibility to integration and effective expression in the genomes of modern bacteria. For example, an ancient *Psychrobacter* sp. pKLH80 plasmid from strain isolated in the Pleistocenic permafrost, contains blaRTG-6 β-lactamase gene, able to be mobilized in the modern epidemic *Acinetobacter baumannii* (Petrova M. et al., 2014). Horizontal genetical transfer of virulence and antibiotic resistance determinants from ancient microorganisms can lead to the appearance of genotypes with high epidemic potential. Thus the process of removal of paleomicroorganisms or their genetic material by degradation of permafrost due the global climatic changes is associated with the risk of emergence of new pathogenes or activation of forgotten infectious diseases. An effective monitoring of the pathogenic potential of the polar microbiota should be implemented.

In our opinion, one of the most promising approaches to the study of the pathogenic characteristics of bacteria found in permafrost is the whole genome sequencing. As a result of our team's studies several bacterial genomes isolated from Pleistocenic mammoth fauna were annotated. In particular, the ancient genomes of *Enterococcus* sp. (GenBank Acc. No. LGAN000000000000, NZ_LGAO00000000), *Arthrobacter* sp. ((Acc. No. QDAE00000000), *Clostridium perfringens* (Bac. No. QDAE00000000, QDAF00000000), *Serratia* spp. (Acc. No. MQRH00000000, MQML00000000), *Acinetobacter lwoffii* (Acc. No. LZDF00000000) were described.

The presence of of the modern epidemic clones markers in the genomes of Arctic paleobacteria was found. For example, IS16 genetic element characteristic for modern vancomycin-resistant enterococci in the ancient *E. faecium*

58m strain (GenBank Acc. No. LGAN00000000) genome was detected. Also blaOXA-335 beta-lactamase gene was identified in the genome of the strain *A. lwoffii* 51m (Acc. No. LZDF00000000) from mammoth intestinal tract (Goncharov A. et al., 2016). Based on this data we believe that the formation of epidemic clones in opportunistic bacteria is more likely determined by the natural selection of strains that carry genetic determinants of pathogenicity or resistance to antimicrobial drugs from natural populations (including in the polar regions of our planet), than by their formation in human society *de novo*.

2.6 doi: 10.15789/2220-7619-2018-4-2.6

THE RESULTS OF THE RESEARCH OF USING A COMMERCIAL KIT FOR DETENTION THE RABIES VIRUS`S RNA IN THE COURSE OF EVALUATION OF THE INFECTION OF THE FIELD MATERIAL

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There are 3000 animal diseases of the Rabies registered annually in Russia (Poleshchuk et al., 2013). The monitoring of this disease helps to reduce it for animals and people. Forward-looking molecular genetic methods are used more and more often in order to diagnose the disease (Iovleva et al., 2012; Devyatkin et al., 2014; Dedkov et al., 2016).

"The kit for the detection of the Rabies virus`s RNA in a complete set" produced by Ltd "Fractal Bio" (St. Petersburg) is a set of reagents for the outflow of the nucleic acid and the realization of the real-time PCR with hybridization-fluorescent registration, it was used to analyze the Rabies of 210 samples: 153 field material and 57 samples of bioprobes.

The initial material included samples of foxes, racoon dogs, corsac foxes, minks, Siberian striped weasels, martens, ferrets, sables, ermines, bats and men as a 10% suspension of a cerebrum based on Henx solution. There were also the samples of bioprobes of not purebred white mice that died in the course of the experiment. The samples were collected from 2–11 passages and were presented by 10% suspensions of the cerebrum. The apparatus used during the research was RotorGene6000.

With the use of test-system, the presence of Rabies virus`s RNA was confirmed in all positive samples of the initial material of foxes (n = 14), cattle (n = 6) and men (n = 2). None was detected in all the negative samples. If negative probes, they registered the formation in the reaction of the product of the amplification of the internal control sample (genomic DNA of mammals).

A different selection of the field material revealed drawbacks of the set when using the internal control sample (ICS). Therefore, ICS did not work with the initial samples of racoon dogs (n = 10), bats (n = 12) and all the mice samples. When researching the initial material of foxes (n = 35) and corsac foxes (n = 3), there was no reaction of the ICS or it was very weak (from the 30^{th} — 36^{th} cycle) in more than 50% cases. The dilute of the nucleic acid, repeated several times, did not improve the result of the reaction with the ICS. That means it lacks the universality of the amplifiable fragment. Provided that there is high reliability of identification of the Rabies virus`s RNA, the negative results of the ICS make the interpretation of the result more difficult.

Therefore, the above-mentioned test-system needs refining in part of the optimization of the ICS.

2.7 doi: 10.15789/2220-7619-2018-4-2.7

COLLISION OF CRISPR-CAS SYSTEMS WITH THE POTENTIAL OF VIRULENCE OF *ESCHERICHIA COLI* STRAINS THAT PRODUCE SHIGA-TOXINS

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Shiga toxin-producing *Escherichia coli* (STEC) strains are a diverse group of *E. coli* strains belonging to over 400 *E. coli* serotypes, some of which cause outbreaks and sporadic cases of food-borne illnesses ranging from diarrhea to hemorrhagic colitis and the hemolytic-uremic syndrome (HUS). It was long believed that bacteria could not resist phage attacks, but in 1987 a strange region was discovered in the *E. coli* genome that consisted of multiple repeats. The discovered structures were termed CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats — CRISPR-associated proteins). CRISPR is a chronological record of infectious assault on a bacterium from viral and other genetic elements. According to Delannoy S. et al., It has been shown that CRISPR polymorphisms in *E. coli* strongly correlate with both the serotype of the microorganism and the presence of virulence factors in its genome (*stx* and *eae* genes).

Genomic sequences (GenBank databases) of *E. coli* isolates of different serogroups (n = 658) were analyzed for the presence of CRISPR-Cas systems and stx-genes. Of the 658 *E. coli* isolates, 60.5% of the loci of CRISPR-Cas systems were found. At the same time, according to the structural organization of CRISPR-Cas, the systems of the strains studied were of type I-E in 92.7% and type I-F of 7.3%. Analysis for the presence of genes of shiga toxins 1 and 2 types showed that 14.4% of isolates having a CRISPR-Cas system of type I-E were positive. The genes *stx1A* and *stx1B* were registered in 6.1 and 5.9% of cases. The frequency of registration of *stx2* subtypes was 2 times higher than *stx1* (6.1 and 12.3%). *Stx2B* was detected in 9.9% of cases.

Stx2A, which according to the literature is more often associated with HUS than other subtypes, was detected in 9.7%. According to the CRISPR-Cas data, the I-E subtype system is associated with the *stx1A* (10.1%), *stx1B* (9.8%) *stx2A* (16.1%) and *stx2B* (16.3%) genes. Further linkage between CRISPR elements and the pathogenicity of the isolate will allow us to determine the causal relationships that stimulate the acquisition of the isolate of both the CRISPR-Cas system and the genes coding for pathogenicity.

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NGS CAPABILITIES FOR THE STUDY OF ENTEROAGGREGATIVE *E. COLI*

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Enteroaggregative *E. coli* (EAggEC) are the causative agents of such intestinal diseases as acute and chronic diarrhea, inflammation of the intestine in children and

adults, and growth deficiency in children. EAggEC are among the most frequent pathogens of nosocomial infections, often leading to the death of patients. EAggEC isolates are characterized by the presence of a wide range of virulence factors and resistance to the standard spectrum of antibiotics.

The goal of this work was to determine possibilities of the massive-parallel sequencing (NGS) for the entoreaggregative *E. coli* study in comparison with standard *in vitro* tests.

We examined 8 strains of *E. coli* isolated from children under 2 years of age with the diagnosis of "intestinal dysbiosis". Determination of sequences of isolate genomes was carried out using massive parallel sequencing on a MiSeq (Illumina) instrument using MiSeq reagent kit v2 reagents (500 cycles).

Phenotypic resistance to antibiotics was determined by the disc-diffusion method. The detection of drug resistance genes in the resulting genomic sequences was carried out by the ResFinder program. Virulence factors were determined by identifying the virulence genes EAggEC by PCR in a multiplex format followed by electrophoretic detection, as well as the VirulenceFinder software, which searches for the corresponding sequences in the genomic sequences.

Most often, the isolates were resistant to β-lactams (6 isolates), aminoglycosides (6 isolates) and sulfonamides (5 isolates). In most cases (29 of 34), the results obtained from the analysis of the full genomic sequencing data coincided with the results of the disco diffusion test, even in cases of a low average coverage of the reference genome, which indicates the applicability of NGS for the study of bacterial isolates for resistance to antibacterial drugs.

The VirulenceFinder program on average found 12 (from 5 to 18) virulence factors for each isolate. The most common genes encoding virulence factors, such as *aap* (encodes dispersin, 7 isolates) and ORF4 (6 isolates). Genes coding for toxins, *astA* and *sat*, met in the genomes of 3 and 2 isolates, respectively. When compared with PCR data, the results were the same in 88% of cases (35 of 40).

Moreover, analysis of NGS data gave information on genome structure, MLST and serotype phenotypes. Thus, NGS can extend results of *in vitro* tests of enteroaggregative *E. coli* without loss of other significant information.

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ALGORITHM OF EXPRESS LABORATORY DIAGNOSTICS IN THE STUDY OF DIPHTHERIA

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In recent years, against the background of active migration processes, negative attitude to vaccination in part of the population, as well as the loss of attention to some infections, there is a risk of foci and the spread of diphtheria. In Western Europe, diphtheria cases are associated with the importation of infection from Africa and the Middle East. In Russia, migration flows are directed from the countries of Central Asia, which border on the regions with high incidence of diphtheria (the countries of the Indian Peninsula). Therefore, the aim of the work was to develop a method for rapid and effective screening for diphtheria and to create an algorithm for laboratory diagnosis.

The study used bacteriological, molecular, mass spectrometric (MALDI-TOF/MS) methods and technology "lab on a chip". The method was tested on 400 strains of *Corynebacterium* of different species, including 180 strains of *Corynebacterium diphtheriae*.

As a result, we have developed a method of rapid growth of bacteria and their identification in 3 hours. The biological sample is sown on a porous membrane with a pore diameter of 5 μm filled with agarose gel according to an improved formulation. This makes it possible to grow all the bacteria at once in a "clean" culture, and, in just 3 hours. Next, the video sensor registers the image of the emerging colonies of bacteria, and a special computer program processes the images and determines the type of microbes. The specificity of this method in determining the genus of bacteria is 95%, in determining the species — 85%.

The development of an express method of cultivation and identification of bacteria allowed the authors to propose an algorithm for rapid laboratory diagnosis of diphtheria. Previously, A. Berger et al. proposed an algorithm that allows to obtain a result on the presence of a toxigenic strain of *C. diphtheriae* in a biological sample at least 48 hours. Algorithm offered by us allows to identify the bacteria *C. diphtheriae* and determine their toxicity using previously presented techniques for 3.5 hours. This will allow rapid and qualitative screening of large groups of the population, including migration centers, for diphtheria, which will help to identify the patient or carrier of *C. diphtheriae* in time and prevent the spread of infection.

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MODERN LABORATORY DIAGNOSTICS OF ESCHERICHIOSIS

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For many years, for differentiation of *E. coli* phenotypic methods based on biochemical identification and O-serotyping (agglutination with antiserum) were used. But these methods are insufficient as the majority of biochemical properties and serogroups are common for both pathogenic and non-pathogenic *E. coli*. Currently the molecular methods allow obtaining useful information about O- and H-antigens, virulence genes and other genetic markers.

400 strains of *E.coli* isolated in 2014–2017 were investigated. The strains belonged to five serogroups: O1 (200 strains), O144 (125), O26 (52), O111 (17) and O55 (6), and were official registered as the pathogens of acute enteric infections.

In *E. coli* O1, the antigenic formula O1:K1:H7 was determined by molecular serotyping. The strains hadn't virulence genes of diarrheagenic *E.coli* but had the genes typical for ExPEC *(pap, sfa, hly, cnf, aer)*. According to the data of literature, the strains with this antigenic formula and virulence genes are highly virulent for birds, and are capable of causing UTI in human.

The strains of *E. coli* O144, isolated from healthy people but officially registered as EIEC, belonged to the biovar 2 and hadn't invasive genes *(ial, ipaH)*.

According to modern data, *E. coli* of serological groups O26, 55 and 111 can refer to two pathogens: EPEC and EHEC, and are subject for epidemiological surveillance, since EHEC infection can occur with HUS and renal failure. The molecular serotyping has showed that all strains of *E. coli* O26 belonged to the same serovar

O26:H11 (*rfb*O26, *fliC*11); *E. coli* O111 — to 2 serovars: O111:H8 (5 strains *rfb*O111, *fliC*8) and O111:H2 (12 strains of *rfb*O111, *fliC*2). *E. coli* O55 also belonged to 2 serovars: O55:H7 (one strain, *rfb*O55, *fliC*7) and O55:H6 (five strains of *rfb*O55, *fliC*6). According to our data, 10 strains of O26:H11, 5 strains of O111:H8 and 1 strain of O55:H7 had *stx1* gene (encoding the production of shiga-like toxin 1) in combination with *eae* gene (the adhesion factor, intimin) and could be consider as EHEC. All strains *E. coli* O111:H2 and *E. coli* O55: H6 and 42 *E. coli* O26: H11 had only *eae* gene, indicating that these strains belonged to the EPEC.

The introduction of molecular methods of serotyping and detection of virulence factors in laboratory diagnostics makes it possible to confirm the true pathogenicity of *E. coli* strains and to minimize the diagnostic errors in etiological interpretation of acute enteric infections.

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COMPARISON OF PHENOTYPIC AND MOLECULAR-GENETIC PROPERTIES OF THE STRAINS *NEISSERIA MENINGITIDIS* ISOLATED FROM PATIENTS WITH GENERALIZED FORMS OF MENINGOCOCCAL INFECTION AND CARRIERS

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Characterization of isolates of *Neisseria meningitidis* obtained from patients with meningococcal disease or from nasopharyngeal swabs of asymptomatic carriers can be achieved by several methods which provide different levels of discrimination.

A total of 42 gram-negative, oxidase-positive diplococcus strains isolated from individuals with meningococcal disease in 2009–2018 years and 65 isolates from 1075 nasopharyngeal carriers in 2016–2018 years were examined by three approaches: serological typing by agglutination, determination of the serogroups by real-time PCR, multilocus sequence typing (MLST). Each strain from patients with meningococcal disease was also determined sensitivity to antibiotics by dilution in broth.

The majority of strains isolated from patients belonged according to the results of agglutination and real-time PCR data to serogroup B (50 and 40.5% respectively), C (16.7 and 11.9% respectively) and W (14.3% by results of both methods). Among the isolates from carriers according to the results of agglutination and real-time PCR data were dominated serogroup W (37.0 and 30.8% respectively) and B (32.3 and 26.2% respectively). Invasive isolates of serogroup B were resistant to penicillin (28.6%), levofloxacin (33.3%), chloramphenicol (27.3%), rifampicin (14.3%), invasive isolates of serogroup W135 to chloramphenicol (9.1%).

MLST established the genetic relationships of the isolates from patients and identified members of known hypervirulent lineage CC11 (n = 4).

Six isolates of *N. meningitidis* (invasive and from nasopharyngeal swab) were additionally investigated by whole genome sequencing.

The results are included in the GenBank international database: SRR7352647 SAMN09435696 Nm-146 blood 16-Apr-2018, SRR7352646 SAMN09435695 Nm-105 nasopharyngeal swab Aug-2016 (https://www.ncbi.nlm.nih.gov/sra/SRP150714)

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MOLECULAR TYPING IN RESEARCH OF EPIDEMICAL CHOLERA MANIFESTATION

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The causative agent of El Tor cholera evolved adaptive mechanisms to ensure its' preservation and accumulation in certain ecological niches and provide existence of its population in various climatic and geographical zones. Considering this, epidemics development mechanism differs in endemic and non-endemic areas. Siberian and the Far East regions are non-endemic territories for cholera. The last epidemic complications in this region were reported in 1990s and had a form of certain infection importation cases and acute outbreaks, associated with importations. Along with this, strains of the *Vibrio cholerae* El Tor, devoid of the pathogenicity determinants, are found annually in environmental objects.

The aim of this work is to elucidate the patterns of cholera epidemiological manifestations in Siberia and the Far East, based on a complex molecular genetic analysis of *V. cholerae* El Tor strains.

In complex assay, using amplification profiling, MLVA-, PFGE-, MLST-, and wgSNP-typing, we found, that *V. cholerae* El Tor strains isolated in epidemic complications, homogenous in basic pathogenicity, pandemicity, persistence determinants along with nucleotide context of housekeeping genes, are characterized by diversity in the associated with pathogenicity genomic loci structure, macrorestriction patterns, SNP-profiles, and structure of variable tandem repeats loci. At the same time, closely related subclones of one clonal variant were identified within the individual outbreaks. Considering the typing data, we can conclude that the outbreaks genesis in the non-endemic territories of the region is determined by the hyperinfectious clone importation. Circulation of closely related subclonal vibrio variants during the outbreak can be caused by environmental factors during the implementation of the water or the contact-household transmission routes.

V. cholerae O1 El Tor isolates from surface watercourse in a cholera free period are characterized by a significant polymorphism of MLVA profiles and PFGE genotypes; that indicates a high heterogeneity of the water populations of the microorganism and the probability of microevolutionary changes during persistence in surface watercourse, as well as the possible periodic introduction of new clones into aquatic ecosystems.

Thus, molecular approaches in the analysis of cholera manifestations provides an understanding of the epidemic complications development patterns in the territory and *V. cholerae* persistence in the environment.

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PCR FOR DIAGNOSIS OF GONOCOCCAL INFECTION: PANACEA OR ESCAPE FROM REALITY

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The aim of this study was to provide an analytical assessment of the role and place of PCR in gonococcal infection (GI) monitoring. Clinical guidelines define a set of laboratory tests for its diagnosis, including molecular genetic techniques (MGT). It is considered, that MGT have the highest diagnostic sensitivity, in contrast to the traditional procedures (microscopic, bacteriolo-

gical, immunological). Moreover, the latter do not allow to establish the disease etiology in 20–40% of cases due to the variability of morphological, cultural properties and antigenic structure of the pathogen [Gushchin A.E. et al., 2014]. The use of MGT had to improve the laboratory diagnostics quality and, as a consequence, to provide a high level of GI detection. According to official statistics, in the Perm region there is a stable reduction of GI incidences. Meanwhile, PCR is used no more than in 25% cases of GI and is carried out exclusively by commercial laboratories. The PCR modifications used in such institutions do not exclude the appearance of both false-negative and false-positive results due to a number of objective and subjective reasons. Thus, a high degree of nucleotide sequence homology and frequent genetic exchange between gonococci and other species of the genus *Neisseria*, as well as a high level of genetic polymorphism of different strains of *Neisseria gonorrhoeae* are the serious problem of molecular diagnosis of GI [Palmer H.M. et al., 2003; Whiley D.M. et al., 2006; Tabrizi S.N. et al., 2011]. The cross-reaction with other microorganisms leads to a false positive results. MGT, which does not involve analysis of strain characteristics (presence of "pathogenicity islands", genetic markers of resistance, etc.), does not provide qualitative monitoring of *N. gonorrhoeae*. In addition, the detectable fragments of nucleic acids do not always belong to living pathogens. Thus, microbiological diagnostics of GI, including the use of PCR, remains an unsolved problem. The needs of practical health care require the improvement of existing approaches and the development of new ones.

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EXPERIENCE OF EXPANDED PRIMER SET USING FOR DETECTION OF THE PATHOGENIC POTENTIAL OF *ESCHERICHIA COLI*

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The purpose of the study to reveal the pathogenic potential of *E.coli* in routine intestinal dysbiosis diagnostics in children are under regular medical check-up because of chronic conditions by detection of genes encoding pathogenicity factors by polymerase chain reaction (PCR).

9–17 years old children were examined with standard procedure of bacteriological diagnostics of intestinal dysbiosis and subdivided into groups: 1) with chronic gastroduodenitis — 22 patients; 2) with atopic dermatitis (AtD) — 19; 3) with bronchial asthma — 7. *E.coli* DNA was recovered with assay reagent set "Ribo-prep" (Russia). Specific gene sequences of *ETEC, EPEC, EHEC, EAEC* and *EIEC* were found by real-time PCR (CFX 96, BioRad, USA) with assay reagent set "Amplisens-Escherichioses" (Russia). Also, individual primers (BioBeagle, Russia) were used to identify genes of pathogenicity factors of *E. coli*, specifically *eaeA, bfpA* — of *EPEC*, *st1, lt* — of *ETEC*, *cnf1* — *NMEC*, *afa* — *DAEC*, *ipaH*, *ial* — *EIEC*, *aggA*, *east1* — *EAEC*, *stx1*, *stx2* — *EHEC*, *chuA* — of *AIEC* (so-termed Adherent-Invasive *E. coli*, associated with Crohn's disease) correspondingly.

In the group of chronic gastroduodenitis *E. coli* stains with pathogenicity coding genes were detected in 7 of 22 tested strains (genes *eaeA, bfpA, aggA, afa, chuA*), in the group with AtD — 3 of 19 (genes *eaeA, bfpA, aggA, east*). Among the children with bronchial asthma, pathogenic *Escherichia* was not found. In routine intestinal dysbiosis diagnostics virulence coding genes of diarrheagenic *E. coli* were detected in 11 cases (23.9%).

In order to reveal the etiological significance of *Escherichia* in children with chronic diseases, as well as to determine their probabilistic role in the manifestation of diseases, the infectious component of which remains poorly studied, the multiplex real-time PCR should be introduce in the laboratory procedure specifications at very least, as well as the creation of primer set based on updated data on diarrheagenic *E. coli* or sighting of genes, as in the case of Crohn's disease.

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HEPATITIS B VIRUS IDENTIFICATION IN THE ENSURING INFECTIOUS SAFETY OF BLOOD TRANSFUSIONS

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Ensuring the infectious safety of blood transfusions during planned and urgent surgical operations is a topical medical problem and should be implemented first of all in order to prevent the transmission of viruses. Hepatitis B virus (HBV) is one of the most common hepatotropic viruses that can cause both acute and chronic course of the disease. One form of chronic viral hepatitis B is occult hepatitis B, characterized by the presence of HBV DNA in the liver and undetectable levels of HBsAg and HBV DNA in the peripheral blood. Then in most cases, virus replication and gene expression can be suppressed so much that the viral load in the peripheral blood of the patient is extremely low, up to the impossibility of detecting HBV DNA by standard methods, but no elimination of the virus.

The aim of our study was the identification and genotyping of HBV in blood donors.

The material was blood plasma of 1003 blood donors from two transfusion centers. A method for detecting HBV DNA with a low viral load based on a two-step PCR, followed by sequencing was used.

HBV was detected in 6.14% of donors. In the region with a high prevalence of HBV, the incidence of occult HBV in blood donors was 9.4%. In the region with a relatively lower prevalence of HBV, the incidence of occult HB in blood donors was 4.23%. In phylogenetic analysis among the HBV samples obtained from HBsAg-negative blood donors in a region with a high prevalence of HBV, the following subgenotypes are represented: D1 — 46.8%, D2 — 17.05%, D3 — 31.9%, A2 — 4.25%, respectively. In the region with a relatively lower prevalence of HBV subgenotypes are presented: D1 — 22.73%, D3 — 72.73%, C — 4.54%. In the case of donors with detected HBV DNA, HBcor IgG antibodies were detected in 34.7% of cases. At the same time, in the analysis for serological markers of the whole group, HBcor IgG antibodies were detected in 21.2% of cases, of which HBV DNA was detected only in 11.3%, which is in agreement with the data on the hyperdiagnosticity of this marker.

The use of molecular diagnostic methods in the blood safety algorithm to detect HBV with low viral load can ensure the viral safety of blood transfusions, especially where viral hepatitis is widespread.

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THE USE OF RAPD-PCR FOR GENOTYPING OF *S.* ENTERITIDIS ISOLATED DURING THE OUTBREAK

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For a long time *Salmonella enterica* serovar Enteritidis remains the main pathogen of salmonellosis in Russia. Traditionally, different typing methods are used for outbreak investigation to establish the identity of isolates, to identify the way and factors of transmission. Despite of all their advantages, pulse filed gel electrophoresis (PFGE) and whole genome sequencing, which are the "gold typing standards", are currently unavailable for the majority of the bacteriological laboratories because of their complexity and high requirements for the staff qualification.

The aim of this work was to assess the possibility to use the RAPD-PCR for the rapid genotyping of *S.* Enteritidis strains isolated during outbreaks. 27 strains of *S.* Enteritidis isolated from patients involved in two unrelated *Salmonella* outbreaks were studied. 13 strains were isolated during the first outbreak, 14 strains — during the second. For RAPD-PCR four oligonucleotide primers were used: mnv-45, mnv-3-1, mnv-3-2 and RAPD4. Amplified fragments were separated by electrophoresis in a 1.5% agarose gel in 0.5 x TBE-buffer. Analysis of electrophoresis pictures was carried out using Bio-Rad Universal Hood II Gel Doc System.

All experiments with *Salmonella* strains were performed in three repeats to confirm the result reproducibility. Primer mnv-3-2 had the best discriminating ability and allowed to separate the strains belonging to different outbreaks by the number and length of amplified fragments. Primers mnv-3-1, mnv-45 and RAPD4 gave the almost identical profiles of amplified fragments, and mnv-45 also required a special electrophoretic conditions, since the amplified fragments differed slightly (by 20–30 base pairs).

The obtained results allow us to recommend the use of RAPD-PCR method with mnv-3-2 primer for operative genotyping of *S.* Enteritidis isolates, which reveals the individual characteristics of epidemic strains. If the laboratory is equipped with PCR equipment with electrophoretic detection, the strain typing can be performed within one working day.

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A NEW VISION AT THE IDENTIFICATION OF THERMOTOLERANT CAMPYLOBACTERS

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Earlier in the bacteriological laboratories the identification has always been considered as the easiest step in Campylobacter diagnostic, since thermotolerant Campylobacters have a typical morphology, if the strains have been isolated on special media and in the microaerobic atmosphere. It allowed to use only two key tests for differentiation of *C. jejuni*, *C. coli* and *C. lari*: hippurate hydrolysis and indoxyl acetate.

In case of impossibility to carry out the species identification, it was enough to establish that the isolate belonged to thermotolerant *Campylobacter*s, since all of them were considered as etiological agents of the acute diarrhea.

Currently, the discovery of new types of *Campylobacters* (*C. avium* in 2009 and *C. hepaticus* 2016) requires a revision of the approach to the identification of thermotolerant *Campylobacters*. They are also thermotolerant and capable of hippurates hydrolysis like *C. jejuni*. The pathogenicity of *C. avium* is not identified, and *C. hepaticus* cause the spotty liver disease of chickens, but do not cause disease in humans. Currently known ecological features of *C. avium* made it unlikely their frequent isolation in the routine laboratory practice. As concern of *C. hepaticus*, there can be a problem with the interpretation of the results during the examination of both clinical material and food products.

The emergence of new *Campylobacter* species requires the revision of phenotypic identification algorithms and extensive use of previously little used tests: the reduction of selenite and nitrates, the growth in the presence of 2.3,5 TTC, the hemolytic activity. Commercial test systems (API Campy) do not currently allow to identify *C. avium* and *C. hepaticus*. It is also necessary to update the databases of MALDI TOF mass spectrometry and to develop the specific primers for identification the new types of *Campylobacter* in PCR.

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IDENTIFICATION OF ROTAVIRUS I- AND E-GENOTYPES BY MULTIPLEX PCR METHOD

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In recent years, the sudden appearance of reassortant rotaviruses was reported in many countries (Australia, Hungary, Vietnam, Spain, Thailand, Japan). Their rate sometimes amounted to 46.7%. These strains cannot be detected using the binary classification (G[P]-genotype) only. Identification of reassortants is possible with complementary analysis of gene segments encoding the inner capsid protein VP6 and enterotoxin NSP4. The aim of this work was the development of multiplex PCR methods for determination of rotavirus I (VP6) and E (NSP4) genotypes.

Rotavirus-positive fecal samples from children accommodated with acute gastroenteritis in the infectious hospital of Nizhny Novgorod were used. The nucleotide sequences of the VP6 and NSP4 genes were determined on the Beckman Coulter CEQTM 8000 (Beckman Coulter, USA). The phylogenetic analysis was carried out using the BEAST 1.8 software package. MEGA 6.0, UGENE, and OligoCalc were used for the primers design.

At the first step, the variety of rotaviruses in Nizhny Novgorod was studied on the basis of the VP6 and NSP4 genes of 16 strains. Two I-genotypes (I1 and I2) and three E-genotypes (E1, E2 and E3) were determined. Three alleles of the VP6 gene (I1-1, I2-IV, and I2-VII) and four ones of the NSP4 gene (E1-I, E2-X, E2-XII, and E3) were shown. Additionally, 128 nucleotide sequences of the VP6 and NSP4 genes of rotaviruses from other 23 countries were also analyzed. Next, sequences of reverse primers specific for the most common among human rotaviruses I- and E-genotypes were selected. In combination with the forward primers, they flanked the regions of the VP6 and NSP4 genes with lengths of 195 bp (I3), 273 bp (I1), 368 bp (I2) and 233 bp (E3), 305 bp (E2), 443 bp (E1), respectively, which could be detected by agarose gel electrophoresis. The optimal annealing temperatures for primers

(57°C and 55°C) and the concentration of magnesium ions in the reaction mixes (2.5 mM) were determined. The PCR conditions and primers specificity were tested using a sample of 16 previously characterized specimens. The results of genotyping by the developed methods fully corresponded to the data of sequencing.

Thus, these methods can be used for routine determination of I- and E-genotypes in rotavirus-positive samples and for identification of reassortant rotavirus strains.

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CXCL10 GENE PROMOTER POLYMORPHISM A-1447G MODULATES PROTEIN EXPRESSION IN SERUM AND ASSOCIATED WITH INVASIVE ASPERGILLOSIS IN FEMALE ONCOHEMATOLOGICAL PATIENTS

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Invasive aspergillosis (IA) — is life-threatening invasive infection, especially in immunocompromised hosts, most of which are oncogematological patients. The key components of fungal infections pathogenesis are disturbances of the immune system. Chemokine CXCL10, also known as interferon gamma-induced protein 10 (IP10), is a member of CXC chemokines. CXCL10 is an inflammatory mediator, which stimulates the directional migration of Th1 cells as well as increasing T-cell adhesion to endothelium. *CXCL10* gene promoter single nucleotide polymorphisms (SNPs) affects protein expression via NF-κB transactivation.

The purpose of this study is to investigate of allelic variants A-1447G (rs 4508917) and G-135A (rs 56061981) effect on the amount of CXCL10 protein in serum and risk of development IA in oncohematological patients in St. Petersburg.

171 oncogematological patients on the background of cytostatic polychemotherapy with symptoms of lung injury were recruited to participate this study. 75 oncogematological patients (44.5%) either developed proven or probable IA as defined by criteria of EORTC/MSG 2008 (median age 43±14, 57% males) whereas controls (96 oncogematological patients (55.5%) without IA comparable in age and sex) did not fulfill these criteria.

SNPs was analyzed by the method of restriction fragment length polymorphism analysis. Chemokine CXCL10 amount was determined with the use of commercial ELISA kit sets (Cloud-Clone Corp, USA). Statistical analysis was performed using SPSS 21 (IBM, USA).

The heterozygous AG rs 4508917 and homozygous GG rs 56061981 genotypes prevailed in the both studied groups and there were no significant differences in genotype distribution of A-1447G and G-135A between oncogematological patients with probable IA and without IAL. However, when dividing patients by sex in a female group G allele rs 4508917 was significant associated with the occurrence of IA ($\chi^2 = 3.853$, $p < 0.50$, OR 3.13 95% CI (1.196–8.204).

There were no differences in serum CXCL10 levels between –135 GG and GA genotypes. However, individuals with –1447G allele had significantly higher serum levels of CXCL10 than those with –1447(A/A) genotype ($p = 0.022$).

Further increase in the number of patients included in the study will allow to make conclusions about the prospect of typing the studied polymorphic variant of the gene CXCL10 as a predictive marker of the risk of mycosis development with a strong significance.

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DEVELOPMENT OF A PANEL OF MONOCLONAL ANTIBODIES FOR STUDYING OF LOCAL PRODUCTION OF CYTOKINES IN CHRONIC RHINOSINUSITIS

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Cytokines as key regulators of inflammation play a central role in the pathophysiology of chronic rhinosinusitis (CRS). CRSs are divided into CRS with and without polyps of the nasal mucosa, but this difference is not sufficient for a clear definition of subgroups with the same pathophysiology and production of cytokines. This area remains open for more detailed studies. The purpose of our work was the development of a panel of monoclonal antibodies for studying the characteristics of local production of cytokines in CRS. For studies, monoclonal antibodies to human cytokines were obtained using hybridoma technologies. One or more clones producing antibodies to cytokines (IL-1β, IL-4, IL-6, IL-8, IL-17, TNFα, GM-CSF, IFNα, IFNγ) were obtained. Specificity of antibodies was proved in ELISA: direct and sandwich method. To create a panel, the antibodies were tested by indirect immunohistochemistry using the avidin-biotin-alkaline phosphatase system. Isolated peripheral blood mononuclear cells from three donors were stimulated LPS 500 μg/ml or PHA 20 μg/ml overnight at +37°C at 5% CO_2.The cell smears on the glasses were fixed with 4% PF. The antibodies studied were used as the first antibodies, dilutions were selected in preliminary experiments. Under the optical microscope, the numbers of lymphocytes or monocyte having red staining in the cytoplasm were counted; the result was expressed as a percentage. The reaction intensity was expressed in points (1-moderate, 2-medium, 3-intensive reaction). As a result, clones were selected that produce antibodies that best detect cytokines in human cells. After induction LPS, IL-1β was detected in 41.0±19.3% of lymphocytes (intensity 2 points) and 90.7±1.3% of monocytes (3 points); IL-6 in 4.0±1.53% of lymphocytes (2 points) and 78.7±8.4% of monocytes (2–3 points), IL-8 in 10.0±4.6% of lymphocytes 1–2 points) and 48.0±10.6% monocytes (1–2 points); TNFα was rarely detected in lymphocytes, in 46.7±18.9% of monocytes (1–3 points); IFNα was detected mainly in monocytes (77.7±10.0%, 2–3 points); The weak but distinct GM-CSF production was determined in 56.7±18.6% monocytes (1–2 points). After PHA induction IL-4 was detected in 6.0±2.5% of lymphocytes (1–2 points), 47.3±2.0 monocytes (1–2 points); IL-17 — was determined in 38.67±15.4% monocytes (1–2 points); IFNγ — in 16.7±11.6% of lymphocytes (1 point) and 32.3±6.38% of monocytes (1–2 points). Thus, it was shown that the obtained antibodies reliably detect the corresponding cytokines in human cells. This panel of antibodies will be used by us to assess the specific features of local production of cytokines in CRS, as well as a number of other inflammatory processes.

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ISLAND RND FOUND IN A STRAIN OF *VIBRIO CHOLERAE* ISOLATED IN THE RUSSIAN FEDERATION

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Genetic islands, VPI-I, VPI-II, VSP-I, VSP-II, ICE, VcB played an important role in *Vibrio cholerae*, providing virulence and pathogen survival. However, the authors' efforts are primarily aimed at the analysis of genetic islands in toxigenic *(ctx$^+$)* strains. Meanwhile, in the Russian Federation for many years, non-toxic *(ctx$^-$)* strains of *Vibrio cholerae* have been consistently isolated from the objects of the environment. The causes of stable residence *(ctx$^-$)* strains are unknown, and the possible role of genetic elements in this process has not been investigated.

The aim of this work is to analyze a new genetic element identified by us as the island of RND detected in a nontoxigenic strain *V. cholerae* O1 El Tor 278 isolated in August 2017 from the Temernik river.

To achieve this task, we have conducted whole genome sequencing of strain 278 using sequencers MySeq and MinION.

The RND island is localized to the second chromosome, has a size of 43.596 base pairs and contains 51 open reading frames. On two sides it is limited by the genes of glycine cleavage system aminomethyltransferase T and threonine-tRNA ligase. Four genes VCA0281, VCA0284, VCA0285 and VCA0286, described earlier in the composition of the island VcB in *tcpA*$^+$ strains, are also included in the island of RND.

In strains presented in GenBank, RND island was detected in three non-toxic strains of *V. cholerae* (1154–74, Env-390, 2012Env-9). Given that the first strain of 1154–74 with the island of RND belonged to the serogroup O49 and was isolated in India in 1974, and two other strains of serogroup O1 found in Haiti in 2012, it can be assumed that the island of RND is capable of horizontal transport, which may partly explain the global spread of this genetic structure. In our opinion, additional research is needed to study the prevalence of the island of RND and the possible function of genes in its composition.

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APPLICATION OF A COMPLEX OF METHODS IN LABORATORY DIAGNOSTICS OF WEST NILE FEVER

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The aim of the study was to assess the feasibility of two methods, commonly used in laboratory diagnostics of West Nile fever (WNF). We used 148 blood samples obtained from presumably WNV-infected patients to identify WNV markers (IgG, IgM, RNA WNV). All samples were tested by a reference laboratory for WNF between 2015–2017. Based on MUK 4.2.3009-12 normative guidelines numerous laboratory tests, including RT-PCR ("AmpliSens WNV-FL", Central Research Institute of Epidemiology, Russia), ELISA ("Anti-West Nile Virus ELISA (IgM)", "Anti-West Nile Virus ELISA (IgG)", Euroimmun Ltd., Germany), were carried out to confirm the diagnosis of WNF. WNV-specific antibodies were detected in 65 (44%) samples. Immune responses to WNV without a specific viral RNA were identified in 63 (42.5%) samples. Specific IgM were identified in 23 (15.5%) samples, while IgG — in 10 (6.7%) samples. Viral RNA was detected in 2 (1.3%) samples. There were no cases of identifying WNV RNA without immune response in all blood samples. Our findings indicate that ELISA has more diagnostic utility than RT-PCR in WNF laboratory diagnostics. Conversely, a low percentage of positive PCR results can be explained by untimely examination of patients and a short period of viremia. The reliability of WNF diagnosis can be enhanced by simultaneous identification of several specific markers, therefore at any stage of the disease it is necessary to use a set of basic immuno- and genodiagnostic methods.

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THE USE OF MOLECULAR GENETIC METHOD TO DETERMINE THE ETIOLOGY OF COMMUNITY-ACQUIRED PNEUMONIA IN SERVICEMEN

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Community-acquired pneumonia is extremely relevant for conscripts because of the high level of morbidity of military personnel, the severity of the clinical course with the threat of deaths, the danger of serious complications such as exudative pleurisy and myocarditis, an increase in the frequency of protracted forms and repeated diseases, the tendency to epidemic spread in the troops with coverage in a short time (December-February) a significant proportion of the recruits. For the use of adequate means of prevention and treatment of pneumonia in servicemen it is important to take into account their etiology.

The aim of the work was to study the etiology of community-acquired pneumonia in military conscripts by polymerase chain reaction (PCR diagnosis).

The results of PCR diagnostics of sputum and swabs from the throat of patients with pneumonia of conscripts admitted for treatment at the Military medical academy and the district clinical military hospital in St. Petersburg in 2014–2017 are analyzed.

The frequency of determining DNA *S. pneumoniae* in patients with pneumonia was the highest — 56.3%. *Haemophilus influenzae* DNA was detected in 16.2% of cases. DNA *Mycoplasma pneumoniae* and DNA *Chlamydophila pneumoniae* were found in 13.4 and 8.1% of cases, respectively. Among agents of the viral nature adenoviruses were the leaders. The detection rate of *adenovirus* DNA was 35.9%. RNA of *rhinoviruses* was found in 23.5% of patients with pneumonia. RNA of *influenza A and B viruses* were detected in 7.6 and 4.0% of cases, respectively. RNA of *RS*-virus were detected in 3.0%, RNA *virus parainfluenza* — in 2.1%, RNA *metapneumovirus* — in 3.4%, DNA *bocavirus* — in 1.9%, *Legionella pneumophila* DNA — in 1.6%, RNA of *enteroviruses* — in 9.3% of patients with pneumonia. Most of the pneumonia — 56.1% — was of mixed, mainly of viral-bacterial etiology, more often — of adenovirus-pneumococcal etiology.

During the PCR diagnosis in the period from 2014 to 2017 the preservation of the leading role of pneumococci and adenoviruses in the etiology of pneumonia in conscripts was revealed. Mixed viral-bacterial infection was dominated.

3. VIRAL INFECTIONS MANAGED BY MEANS OF VACCINATION AT THE STAGE OF DESTRUCTION AND ELIMINATION

3.1 doi: 10.15789/2220-7619-2018-4-3.1

PROCESS FOR IMPLEMENTING POLIOVIRUS ENVIRONMENTAL SURVEILLANCE IN COTE D'IVOIRE FROM DECEMBER 2016 TO DECEMBER 2017

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Poliomyelitis surveillance underpins the entire Global Polio Eradication Initiative. Two major surveillance strategies are essential and complementary. Surveillance of acute flaccid paralysis in areas with continued transmission and environmental monitoring in countries declared polio-free and in endemic countries. Environmental monitoring is a tool to provide evidence of the absence of vaccine-related viruses after cessation of the use of oral polio vaccine and to demonstrate the circulation of polio viruses and non-polio enteroviruses in the vaccine environment. It aims to detect the silent circulation of wild polioviruses, vaccines and circulating viruses derived from oral polio vaccine. An initial meeting with the actors involved allowed the identification of probable sites. The capacity building of the Polio laboratory was initiated by the development of a specific room followed by a staffing of laboratory equipment and reagents. The surveillance team was formed. Sites were selected with 2 sites in Yopougon Health District and 1 site in Adjamé Plateau-Attecoubé District. Collector training and validation of the technical procedures for sample analysis and dissemination of results were made. The collection was done between 6am and 7.30am twice a month per site with immediate delivery to the laboratory. From December 2016 to December 2017, 78 wastewater samples were received treated in the laboratory. 71 samples were positive with 32 vaccine strains, 17 non-polio enteroviruses and 22 vaccine strains and enteroviruses. These results confirm the proper implementation of environmental monitoring with the support of WHO.

3.2 doi: 10.15789/2220-7619-2018-4-3.2

ANALYSIS OF MEASLES CASES IN CHILDREN DURING THE OUTBREAK IN MONGOLIA, 2015

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The incidence of measles declined dramatically worldwide due to the introduction of the measles vaccine and similarly, measles incidence in Mongolia went down, resulted in elimination in 2014 comparing to an average incidence 93.4 (39.9–218.3) per 10 000 population in pre-immunization period, 1960–1972. We investigated severe measles cases admitted into intensive care unit of the national center for communicable diseases in period of March-July, 2015, made analysis of demographics, clinical and epidemiological characteristics of the patients. In addition, periodic nationwide measles supplemental immunization activities were implemented in 1994, 1996, 2000, 2007, 2012. Coverage of two doses of measles-containing vaccine (MCV1 and MCV2) was constantly reported ≥ 95% since 2001 and measles cases were not registered since 2011 in Mongolia.

Median age of 305 patients stayed in ICU with measles was 6.2 months. Of those, 243 (79.7%) cases were aged below 9 months (before the age of eligibility for MCV1), 62 (20.3%) were aged 9 months — 9.2 years. Therefore, 283 (92.8%) were unvaccinated against measles, 8 (2.62%) had received 1 dose, 5 (1.63%) had received 2 doses and 9 (2.95%) had unknown vaccination status. 174 (57%) were male. 84 (27.5%) were exposed at home, 146 (48%) in healthcare facility and 75 (24.5%) were not aware of exposure. 41 (13.44%) patients were aged over 10 months and among them 25 (61%) were unvaccinated and 8 (19.5%) had unknown immunization status. In addition, 16 of those had an underlying background disease, however, there was not found relationship between presence of background disease and missing vaccination ($p = 0.7326$).

Although Mongolia has kept a high level of immunity against measles in the whole population of the country, there still remain unvaccinated population of children in the community who can cause measles outbreak through the contact with infected ones and can increase risk of severe measles complication requiring Intensive care.

3.3 doi: 10.15789/2220-7619-2018-4-3.3

DETECTION OF PRIMATE ERYTHROPARVOVIRUS 1 DNA IN BLOOD SERUM OF PATIENTS WITH ERYTHEMA

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Primate Erythroparvovirus 1 (Parvovirus B19, B19V) is a pathogen of human. The virus is causative agent of wide range of illness: infectiosum erythema, arthrolgy, aplastic crisis, myocarditis, fetal hydropsis and others. There are two usual methods for diagnostic of parvovirus B19 infection, PCR and ELISA. In Russian Federation PCR is popular because it's more available then ELISA.

The aim of the study was the analysis of Primate erythroparvovirus 1 DNA prevalence in blood serum of patients with erythema. Blood sera (n = 124) were collected in 2015–2017 from patients with erythema and fever (t ≥ 38.5°C). All samples were negative for IgM to measles and IgM to rubella and were positive for IgM-PVB19. DNA was extracted by "RIBO-prep" (InterLabService Ltd., Russia). The diagnostic PCR test "AmpliSens® Parvovirus B19-FRT" (InterLabService Ltd., Russia) was used. Parvovirus B19 DNA was detected in 86 of 124 samples (69.4%). DNA positive sera were collected from 3 to 45 days from the moment when erythema has been manifested. Among these 86 sera 66 were collected in the first week from erythema appearance. Viral loads were: 10^6 copies of DNA PVB19/ml — in 14% cases; 10^5 — in 30% blood serums; 10^4 — 28% samples; 10^3 copies of DNA PVB19/ml — in 14% cases, thereover, viral loads of 47.7% samples was higher than 10^5 copies of DNA PVB19/ml. One sample was detected with 10^9 copies DNA PVB19/ml.

Our results indicate that the combination of ELISA and PCR methods is optimal for diagnosis and choice of the treatment of parvovirus B19 infection.

3.4 doi: 10.15789/2220-7619-2018-4-3.4

STUDY ON HERD IMMUNITY AGAINST INFLUENZA VIRUS AMONG THE POPULATION OF ALMATY REGION IN 2017–2018

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Influenza is one of the infections registered on all continents. Despite the obvious scientific achievements pertaining to anti-epidemic measures, ARVI and influenza still present a major problem in medicine. Timely diagnosis of the causative agents for influenza and acute respiratory infections in humans allows for adjusting the treatment regimen and determining the correct vaccination tactics and choosing the appropriate vaccine variant during the interepidemic period. The arsenal of diagnostic methods still preserves serological techniques, which ensure the detection of specific antibodies in the blood in the disease dynamics and provide indirect evidence of the influenza virus circulation among humans.

The purpose of this study was to carry out serological analysis of the circulation of influenza A and B viruses in the 2017–2018 epidemic season. The level of antibodies specific for the influenza virus hemagglutinins in blood serum was determined in the hemagglutination inhibition assay (HAI) and enzyme immunoassay (EIA).

60 serums collected from patients diagnosed with ARVI, influenza, ARD, and pneumonia in health care facilities located in the Almaty region during the 2017–2018 epidemic period were used for serological studies. EIA data showed that antibodies in 23.3% of cases (14 serums) were detected in high titers (1:80–1:320) against the A/H3N2 virus serosubtype, in 18.3% (11 samples) against influenza A/H1N1 viruses, and in 1.6% (1 sample) against the causative agent of influenza type B virus.

Antibodies against influenza virus were detected by HAI assay in 31.6% of cases (19 samples), of which 16.6% (10 samples) were classified as A/H1N1 subtype and 13.3% (8 samples) as A/H3N2 subtype. Antibodies against influenza type B virus were found in 1.6% (1 sample).

The results from serological studies of serums thereby indicate that in the 2017–2018 epidemic season the simultaneous circulation of influenza A (H1N1 and H3N2) and B viruses was observed in the Almaty region.

3.5 doi: 10.15789/2220-7619-2018-4-3.5

CIRCULATION OF INFLUENZA VIRUSES AMONG HUMANS AND SWINE IN THE REGIONS OF NORTHERN AND WESTERN KAZAKHSTAN IN 2017–2018

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The influenza viruses of genus A are unique among the agents of infectious diseases in both humans and a large number of mammals (horses, swine, whales, seals, etc.) and birds (Lvov D.K., 1987; Webster R.G. et al., 1992). To predict the influenza epidemics and timely preventive measures, an important stage consists in tracking the spread of the infection in various regions of the world, including the Republic of Kazakhstan.

The aims and objectives were to examine the circulation of influenza viruses among humans and swine in the regions of Northern and Western Kazakhstan in 2017–2018.

In the 2017–2018 epidemic and inter-epidemic periods 274 nasopharyngeal swabs were collected in the Northern and Western Kazakhstan: 94 human and 180 swine samples from livestock farms.

Primary screening of nasopharyngeal swabs was performed in real-time polymerase chain reaction (RT-PCR) using AmpliSens reagent kits (Moscow, Russia).

Primary screening in RT-PCR of 94 biological samples collected from humans showed the presence of genetic material of the influenza virus in 32 swabs (34.04% of the total number of samples examined). Influenza type A virus RNA was detected in 19 samples (20.21%), influenza type B virus RNA in 13 samples (13.83%). Subtyping of influenza type A positive samples revealed influenza A/H1 virus RNA in 3 samples (3.19%), A/H3 virus RNA in 16 samples (17.02%).

RT-PCR screening of biological materials obtained from swine showed the presence of influenza virus RNA in 28 swabs (15.56% of the total number). A/H1 virus RNA was detected in 26 samples (14.44%), A/H3 virus RNA in two samples (1.11%).

The results from the primary screening of nasopharyngeal swabs collected from humans and swine in RT-PCR indicate the co-circulation of A/H1N1 and A/H3N2 influenza viruses in humans and swine during the period 2017–2018 in the regions of Northern and Western Kazakhstan. In this regard, the monitoring of the spread of infection among humans and swine, as well as timely diagnosis of the infectious agent and prevention of the disease are extremely important.

3.6 doi: 10.15789/2220-7619-2018-4-3.6

CHARACTERIZATION OF ENTEROVIRUSES BY NEXT-GENERATION SEQUENCING

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Next generation sequencing methods constitute a powerful tool for the study of viruses. By allowing concomitant sequencing of millions of DNA fragments, they allow rapid sequencing of a great number of samples and in-depth characterization of minority genomic variants.

Our laboratory has developed different technics suitable for the characterization of enteroviruses in different types of samples. By specifically targeting enterovirus genomes, these technics reduce the number of reads from non-virus origin: thus, more than 90% of the reads that are generated through sequencing are relevant.

However, generating full-length genomic sequences remains a challenge in case of samples containing complex mixtures of viruses, particularly when mixed viruses share common sequences because of recombination. We are currently trying to address this limitation.

3.7 doi: 10.15789/2220-7619-2018-4-3.7

EPIDEMIC RISE OF INFLUENZA IN ST. PETERSBURG IN JANUARY-MARCH 2018

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Influenza and acute respiratory viral infections remain one of the most urgent medical and socio-economic problems. Almost every year in autumn and winter there

are epidemic rises of the incidence of influenza and acute respiratory viral infections. In St. Petersburg, in 2018 the epidemic reached its peak in the 7th week (February), when the epidemic threshold was exceeded, the maximum number of cases was registered during the 11th week (16 365 patients on 12.03.18), then after the 15th week the number of cases was below the epidemic threshold. The average daily number of cases of influenza and respiratory viral infections in January was 5270, in February it was 8127, in March — 9018, in April — 6330. The highest incidence was among children 3–6 years of age. In total, more than 20 000 patients were hospitalized from the beginning of the year till May, among whom 20% were adults. Among children, The majority of hospitalized children (55.3%) were the children under the age of two.

In January-May 2018 the laboratory received 111 nasopharyngeal samples from patients of St. Petersburg hospitals 18–70 years of age. Samples were examined by real-time PCR using test systems from "InterLabService", Moscow, 50 samples proved to be positive of the influenza virus and 7 samples — to other respiratory viruses (rhinoviruses, adenoviruses). In 20 samples out of 50 RNA of virus of influenza B was detected, which makes 40%, the samples with RNA of influenza A(H3N2) virus constituted 24%, in 14.0% of the samples we identified RNA of virus of influenza A(H1N1) while in 22.0% of samples we did not identify RNA of the influenza virus. From the positive in PCR samples we isolated 26 strains of influenza viruses by two passages on the culture of MDCK cells. The typing of isolates with diagnostic sera to reference and epidemic strains of influenza viruses showed that in February-March 2018 all three serotypes of the influenza virus circulated in St. Petersburg. In 50% of cases we identified viruses of influenza B of the Yamagata Line, antigenic related to the strain B/Phuket/3073/13; 30.8% of isolates belonged to serotype A (H3N2) and were antigenic related to the strain A/HK/4801/14, and 19.2% of isolates were closely related to the strain A(H1N1)pdm09. During this epidemic rise we did not find influenza viruses of the Victorian Line which prevailed in circulation for many previous years.

3.8 doi: 10.15789/2220-7619-2018-4-3.8

MEASLES AND RUBELLA IN NORTH-WEST REGION OF RUSSIA AT THE STAGE OF ELIMINATION

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The WHO strategic plan to eliminate measles, rubella and congenital rubella is aimed to the global elimination of these infections by 2020. The National program to eliminate measles and rubella is developed and carried out in Russia. In this study, ELISA test was used to detect the IgM and IgG antibodies to measles virus (MV) and rubella virus (RV) in blood serum samples from patients suspected to have the MV infection.

Within the last decade in the North-West Region (NWR) of Russia the highest measles incidence was registered in 2012 (1.11 per 100 000). In the following years the measles morbidity declined to even none of measles cases in 2016 and then 3 measles cases were registered in 2017. Molecular genetic studies of the biological material from patients with measles revealed the D8 genotype, MVs/Frankfurt Main. In January-June, 2018 the measles incidence significantly increased — 24 cases were notified on 5 territories of NWR of Russia. In St. Petersburg 17 measles cases were laboratory confirmed in this period: 5 of 17 cases were revealed in the City Hospital and 2 cases were the contacts with this epidemiological focus. The epidemic cluster of 3 cases was notified in St. Petersburg among patients from the Republic of Moldova. The B3 genotype, Kabul MeaNS-4298 was genetically confirmed.

Rubella incidence in NWR of Russia remains at low level: one family focus of 2 rubella cases was notified on one territory in 2016 and no cases were registered in 2017. In the first half of 2018 three rubella cases were laboratory confirmed in St. Petersburg and Leningrad Oblast'. Besides, 13 blood serum samples from pregnant women suspected to have the RV infection were studied. The lack of the specific IgM antibodies to RV, high levels of the IgG antibodies titers of high avidity were revealed by ELISA in serum samples of all examined pregnant women thus evidencing the absence of the acute RV infection.

The presented data on low measles and rubella morbidity in 2016–2017 and the absence of the local MV circulation within more than 12 years period evidenced the possibility of certifying the NWR of Russia for the absence of the endemic MV circulation. However the worsening of the epidemic situation on measles was observed in NWR as well as in Russia as a whole in the first half of 2018.

3.9 doi: 10.15789/2220-7619-2018-4-3.9

EVOLUTION OF THE VP1 REGION TYPE 2 VACCINE-DERIVED POLIOVIRUS SHEDDING FROM AN IMMUNOCOMPROMISED ALGERIAN CHILD

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Both live attenuated oral poliomyelitis virus vaccines (OPV) and inactivated poliomyelitis virus vaccines (IPV) are effective in providing individual protection against poliomyelitis and have been used widely. However, in rare incidences the attenuated virus used in OPV reverts to neurovirulence results in transmissible vaccine-derived poliovirus (VDPV) strains. Here, we describe the accumulation of mutations in gene coding the VP1 protein of Sabin 2 Poliovirus (PV), suggested a prolonged replication of the vaccine, occurring in an infant with severe combined immunodeficiency.

The analyses of stool specimens were conducted from 2011 to 2012 in a 6-month-old boy, with severe combined immunodeficiency. Viral isolation is carried out according to the standard methods recommended by the WHO. Extracts were cultured on human rhabdomyosarcoma cell line (RD), and mouse L cells expressing the human PV receptor (L20B). All isolates routinely characterized by VP1 sequencing. Analysis of the full nucleotide VP1 region was performed.

The 12 samples were positive for PV. The rRT-PCR confirms the vaccine-like. 10 isolates of type 2 exhibit significant nucleotide variations in the VP1 protein and were collinear with the Sabin 2 strain.

A detailed molecular analysis VP1 region showed accumulates mutations. The substitution Ile143Thr which restores the consensus residue for the prototype wild type 2 PV strains is found in all type 2 polioviruses isolates.

Polio eradication requires not only complete absence of circulating wild PV but also absence of VDPV the emergence of genetically divergent vaccine-derived polioviruses

(iVDPVs) during prolonged infection in persons with primary immunodeficiency disorders seems to be one probable source of poliovirus infection and these individuals are a potential reservoir for infection in which the virus can evolve into neurovirulent forms and become transmissible circulating vaccine-derived PV.

3.10 doi: 10.15789/2220-7619-2018-4-3.10

EPIDEMIOLOGICAL CHARACTERISTICS OF MEASLES

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Tremendous progress has been made to decrease childhood death caused by measles. Before the introduction of measles vaccine in 1963 major epidemics occurred every 2–3 years and caused 2.6 million deaths per year. In 2012 the WHO endorsed a plan to eliminate measles by 2020. The aim of this study was to reveal epidemiological characteristics and trends of measles.

Measles is a highly contagious airborne infectious disease caused by the measles virus. Although the impressive achievements in eliminating measles with a low record in 2016 with 5273 cases in Europe region it affected 21 315 people and caused 35 deaths in 2017. There were reported about 4400 cases in Italy from January to August 2017 with median age 27 years, 88% of the cases were unvaccinated. Over 41 000 people in Europe have been infected in 2018 with at least 37 deaths. Over 23 000 people affected in Ukraine but the highest number of deaths 14 was reported by Serbia. Also a large number of cases were registered in Italy, France, and Georgia, Serbia and Moldova. There were 5004 confirmed measles cases, including 68 deaths, reported in the American region this year with 3545 cases and 62 deaths in Venezuela and 1237 cases, 6 deaths in Brazil.

Russian Federation reported about 1717 infections in children and adults this year (127 cases for the same time in 2017). Overall, there were increasing from 178 cases in 2016 to 721 last year. The most affected areas were the Republic of Dagestan (3.3 cases per 100 000), Moscow (2.7 cases per 100 000) and the Chechen Republic (2.3 cases per 100 000). Almost 9 of 10 of the affected people were not vaccinated. Ratio adults to children about 6:4. The majority of cases were caused by genotype "Dublin B-3" that is endemic for the Europe. There were several household outbreaks of measles as a recent case in Chita with 15 members of one family infected.

This situation caused by declining vaccination rates. To prevent outbreaks, at least 95% immunization coverage in every country; timely detection of all suspected cases and provide laboratory conformation; strengthen epidemiological surveillance in border areas and vaccination one month ahead of a trip to any of the European countries the WHO list; adequate intra-hospital management to avoid nosocomial transmission.

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INTRATYPIC DIFFERENTIATION OF POLIOVIRUSES IN THE INTER-POLIO LABORATORY OF THE INSTITUT PASTEUR OF COTE D'IVOIRE IN 2002–2017: WHAT EVOLUTION?

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Laboratory analysis of cases of acute flaccid paralysis is one component of the four polio eradication strategies. This analysis consisted in isolating the viruses and characterizing them by the technique of intra-typical differentiation (ITD).This study proposed to take stock of the evolution of the different techniques of ITD used from 2002 to 2011.

The stools are treated with chloroform and inoculated to L20B and RD cells. The identification of isolated viruses and their characterization was carried out by evolutionary methods: seroneutralization typing with an antibody pool, conventional RT-PCR coupled with an enzyme-linked immunosorbent assay (Elisa) and finally real-time PCR. From 2002 to 2006, the identification of 370 strains of poliovirus was made by serum neutralization. It identified 258 polio type 1, 102 polio type 2 and 206 type 3. The wild or vaccine nature was determined in South Africa. From 2007 to 2010: 492 strains identified by conventional RT-PCR/ELISA were given: 256 wild polio (241 PV1, 15 PV3) and 259 poliovirus type vaccines, with dual reactions limiting the separation of virus mixtures of different type. From 2011 to 2016, 1034 strains of poliovirus tested by real-time PCR showed 300 wild-type PV3, 02 VDPV2 and many vaccine strains type 1, 2, and 3 or mixed serotypes with readily available results and The possibility of processing several samples especially with the advent of version 5.0 since October 2016.

The evolution of the techniques of differentiation allowed the increase of the capacities of the laboratory and the reliability of the results. Adaptation to new techniques (sequencing) is essential to continue to offer better services.

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EPIDEMIOLOGICAL MONITORING OF POLIOMYELITIS IN THE CENTRAL AFRICAN REPUBLIC FROM 2004 TO 2017 AND IMPLEMENTATION OF POLIOVIRUS ENVIRONMENTAL SURVEILLANCE IN BANGUI IN 2017

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Central African Republic (CAR) joined the Polio Eradication Initiative (PEI) in 1996. Despite the fact that the last autochthone wild poliovirus was isolated in 2000, the country experienced several episodes of wild poliovirus importations between 2003–2011. Nevertheless, since 2003 CAR is ongoing numerous political-military crisis that affects the health system including the PEI performance.

The aims of the study were the analysis of key performance indicators of active acute flasks paralysis (AFP) surveillance in CAR from 2004 to 2017 (14 years period) and to describe the introduction of Poliovirus Environmental Surveillance (ES) in Bangui, the capital of CAR.

We conducted a retrospective analysis of data available at the Institut Pasteur de Bangui, the Department of Health and Population and WHO to evaluate the polio eradication program in CAR from 2004 to 2017. The rationale, steps and first results of Poliovirus Environmental Surveillance implementation are described.

During the study period we listed 1803 notified cases of (AFP). Out of 3920 stools samples collected from AFP cases, contacts and internally displaced population, 64.4% (2524/3920) were transported at the laboratory within three days of collection and 76.4% (2997/3920) of the stool samples were considered to be adequate. We isolated 225 vaccine polioviruses, 803 non-polio enterovirus, and 51 wild polioviruses of which the last one in November 2011. ES was implemented at the week 52 of 2017 in Four (4) sites selected in Bangui. We received 46 ES samples from the implementation to the 30th of June 2018, among which we isolated 7/42 (16.6%) non-polio enterovirus. (No) Any poliovirus was not isolated in these 46 ES samples. The routine vaccine coverage was particularly low in the country with an average of 49%. The quality of SIA's is still poor and part of the CAR territory is inaccessible for security reasons.

The recurrent civil and military unrest have considerably affected the surveillance system of AFP which must be reinforced by the ES extension to the former districts that experienced wild poliovirus importation, coupled to an improvement of the routine vaccine coverage to attend the WHO standards.

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CIRCULATION OF THE EPIDEMIC VARIANT OF NOROVIRUS GII.4_SYDNEY2012 IN NIZHNY NOVGOROD, RUSSIA

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Noroviruses (NoVs) are a major cause of gastroenteritis. The epidemic process of NoV infection in the last two decades is characterized by the dominance and periodic replacement of variants belonging to genotype GII.4. Currently more than 10 epidemic variants of NoVs GII.4 identified. Dominance period of most of these variants did not exceed 2–4 years. However, the variant GII.4_ Sydney2012 prevails in many countries of the world for the last 5–6 years.

The aim of this work was to analyze the dynamics of circulation of NoVs GII.4_Sydney2012 in the territory of Nizhny Novgorod in 2013–2018.

NoVs were detected by reversed transcription polymerase chain reaction in fecal specimens obtained from patients with acute diarrhea. Genotyping of NoVs was performed by partial sequencing of the genome regions encoding capsid protein and RNA-dependent RNA polymerase using the genetic analyzer Beckman Coulter CEQ8000 (USA). The nucleotide sequences were analyzed using a web based NoV Genotyping Tool 2.0 and program MEGA6.

From July 2013 to June 2018 7018 children under 14 years hospitalized in the infectious diseases hospital of Nizhny Novgorod were examined. NoVs were detected in 17.5% of cases, the genotype was determined for 189 isolates. Distribution of genotypes: GII.2 — 19.0%, GII.3 — 1.6%, GII.4 — 45.5%, GII.6 — 20.6%, GII.7 — 0.5%, GII. 13 — 1.1%, GII.14 — 0.5%, GII.17 — 10.6%.

Variant GII.4_Sydney2012 predominated in all years, with the exception of 2014–15 season, when its share in the spectrum of NoVs genotypes decreased to 9.8%, and the genotype GII.6 came out on top. In autumn 2016 there was a sharp increase in the frequency of NoVs detection, which coincided with the replacement of previously circulating recombinants GII.Pe-GII.4_Sydney2012 to recombinants GII.P16-GII.4_Sydney2012. The share of the latter was 60.0% in 2016–17 and 55.3% in 2017–18.

Phylogenetic analysis of recombinants showed the presence of clusters corresponding to the specificity of the polymerase, with the absence of significant differences in the capsid protein.

Thus, the prolonged circulation of NoVs GII.4_Sydney2012 may be associated with the acquisition of genes of non-structural proteins that provide virus with selective advantages. However, the impact of minor changes in the capsid protein on the antigenicity of the virus and its ability for successful spreading can not be ruled out.

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INFLUENCE OF VAGINAL MICROBIOTA ON THE ACTIVITY OF HUMAN PAPILLOMAVIRUS

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The prevalence increases in women with cervical pathology in proportion to the severity of the lesion and reaches about 90% in the contingent with a third degree of cervical intraepithelial neoplasia and invasive cervical cancer. The severity of these changes depends not only on the duration of the persistence of pathogens, but also on their activity. This determines the need to identify the factors influencing on viral activity.

Therefore, the aim of this study was to compare the viral load of pathogens in women with bacterial vaginosis and with vaginal normocenosis.

40 women aged 23–32 were selected for the study. Diagnosis of bacterial vaginosis was based on microscopy, genetic study (polymerase chain reaction in real time, PCR-RT) of the vaginal discharge and clinical features. Control group consisted of 40 patients aged 25–34 without disorders of vaginal microbiota. HPV of phylogenetic groups A5, A6, A7 and A9, most often affecting the epithelium of urogenital tract and the perianal zone and low oncogenic risk of type 6 and type 11, were identified by PCR-RT.

HPV of low oncogenic risk of type 6 and type 11 were not detected in any of the patients. Clinically, the infection manifested as small papillary formations in the vagina and vulvae 23 (57.5%) patients from control group and 28 (70%) from study group. In 5 (12.5%) patients from control group and 9 (22.5%%) from study group changes were revealed only in colposcopy as planar formations in the thickness of the mucous membrane of the vaginal part of the cervix. The presence of flat warts correlated with mild dysplasia of 1 steppe revealed by cytological study. The viral load evaluation demonstrated that it was significantly higher in study group than in the control group (lg 5.24±0.18 and log 4.30±0.26 respectively, $p < 0.001$).

The results suggest that the presence of bacterial vaginosis in patients with concomitant papillomavirus infection (PVI) of the urogenital tract can support and stimulate the activity of HPV, which contributes to more frequent formation of manifest forms of viral infection in such patients. This indicates the need to assess the microbiota of the genital tract in all patients with an identified oncogenic HPV with an obligatory correction when finding violations. This should be considered as a main part of therapeutic measures in the therapy of PVI of the urogenital tract in women.

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EMERGENCE OF VACCINE-DERIVED POLIOVIRUSES DURING EBOLA VIRUS DISEASE OUTBREAK IN GUINEA, 2014–2015

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From December 2013 to May 2016, 3.351 laboratory-confirmed cases of EVD occurred in Guinea, resulting in 2.083 deaths and reaching a peak of 509 confirmed cases in October 2014. During this outbreak,13 type 2 circulating vaccine-derived polioviruses (cVDPVs) were isolated from 6 polio patients and 7 healthy contacts. To clarify the genetic properties of cVDPVs and their emergence, we combined epidemiologic and virologic data for polio cases in Guinea.

Patients with paralytic poliomyelitis were identified through Guinea's AFP surveillance system according to WHO guidelines. During September 2015–December 2016, additional fecal samples were collected from contacts of most AFP patients.

Polioviruses were isolated from fecal samples according to WHO standard procedures and subjected to intratypic differentiation by reverse transcription PCR targeting the VP1 region. Typing of non-polio enterovirus isolates was performed by RT-snPCR targeting part of the 3'-VP3 and the 5'-VP1 regions. Isolates with discordant intratypic differentiation results were sent to the National Institute for Communicable Diseases, Johannesburg, South Africa, for entire VP1 sequencing according to WHO guidelines

To assess epidemiologic factors associated with the outbreak of Ebola Virus Disease (EBV), field investigations were conducted during December 17–28, 2015, in Siguiri and Kankan Prefectures, Guinea.

In September 2014, a case of laboratory-confirmed type 2 cVDPV infection was identified in Guinea (Siguiri Prefecture). During October 2014–March 2015, collection of fecal samples from AFP patients in Guinea was interrupted because of the outbreak of EVD. On September 4, 2015, type 2 cVDPV was isolated from a fecal sample of a child living in the Kankan region of Guinea. Subsequently, type 2 cVDPV isolates were recovered from 5 other AFP patients and 7 healthy contacts. The 7 healthy type 2 cVDPV-positive contacts were epidemiologically linked to 3 of the AFP case-patients. Most (12/13) poliovirus-positive case-patients were incompletely vaccinated children. All 13 type 2 cVDPV strains were isolated from persons in the Kankan region in eastern Guinea, most (12/13) persons were from Siguiri Prefecture. During the first semester of 2015, the coverage of routine OPV3 vaccination in Siguiri Prefecture was 31%. In 2014, the official national OPV3 routine coverage in Guinea was 42%.

All 13 VDPV isolates showed discordant intratypic differentiation results and were further characterized by sequencing the VP1 capsid-coding region. All isolates diverged > 0.6% nt from the type 2 OPV strain, and classified as type 2 VDPVs. The 13 strains clustered in a monophyletic group with a high (96%) bootstrap value

Deviation of public health resources to the Ebola outbreak disrupted polio vaccination programs and surveillance activities, which fueled the spread of neurovirulent VDPVs in an area of low vaccination coverage and immunity. Genetic properties of cVDPVs were consistent with their capacity to cause paralytic disease in humans and capacity for sustained person-to-person transmission. Circulation ceased when coverage of oral polio vaccine increased.

A polio outbreak in the context of the Ebola virus disease outbreak highlights the need to consider risks for polio emergence and spread during complex emergencies and urges awareness of the challenges in polio surveillance, vaccination, and diagnosis.

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ENTEROVIRUS INFECTION IN THE RUSSIAN FEDERATION IN 2008–2018

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In the XXI century in Europe (including Russia), Asia and the Pacific region occur a significant increase in the incidence of enterovirus infection (EVI). In Russia, the rates of registered incidence of EVI and enteroviral meningitis (ASM) are included in official statistical reporting in 2006.

The Reference Center for Monitoring EVI, established in 2008, studies the molecular epidemiology of enteroviruses (EVs) and EVI. The work is carried out in close cooperation with the Directorates of Rospotrebnadzor and the Centers for Hygiene and Epidemiology in the subjects of the Russian Federation (RF) and the scientific centers.

EV-positive samples from 7940 patients with various clinical manifestations and 656 environmental objects collected in different regions of Russia in 2007–2018 were studied using partial sequencing of the region of the VP1 genome.

Nonpolio EVs were typed in 4323 cases. 53 types of viruses were identified: *sp. Enterovirus A*: CVA2–8, CVA10, CVA14, CVA16, EV-A71, EV-A76, EV-A120; *sp. Enterovirus B*: CVB1–5, CVA9, E1–7, E9, E11, E13–21, E25, E29, E30, E31, E33; *sp. Enterovirus C*: CVA1, CVA13, CVA17, CVA19, CVA20–22, CVA24, EV-C99, EV-C113, EV-C116. Annually, about 30 types of nonpolio EVs were detected, and many types of viruses were characterized by the presence of several simultaneously circulating genovariants.

During the observation period, all peaks of enteroviral meningitis incidence in the RF registered in 2008–2009, 2013 and 2016–2017, were associated with E30. The E30 of the genotype eC2 circulated in 2007–2009. In 2013, E30 of genotype h has become more active and continues to circulate until now. Among the causative agents of ASM, in addition to E30, during this period the most active viruses were E9, E6, CVA9, CVB2–5 and other members of the sp. Enterovirus B.

The increase in the incidence of HFMD in the Russia was associated with an increase in the circulating activity of viruses of the sp. Enterovirus A (primarily CVA6 of new genotype), which began in 2010.

The current situation in the RF on the incidence of EVI requires continuous molecular monitoring of the circulation of epidemic variants of enteroviruses, and in case of its aggravation, the development of specific means of prevention.

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MEASLES IN YEKATERINBURG: THE HISTORICAL PATH FROM THE PERIOD BEFORE VACCINATION TO THE STAGE OF ELIMINATION OF THE INFECTION

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Measles is still salient due to the reports of its outbreaks in many regions of the world, including in the Russian Federation.

The aim of the study is to characterize the manifestations of the epidemic process of measles in a large industrial center with different strategies of vaccination.

The research is based on statistical reports on the incidence of measles in Yekaterinburg from 1950 to 2016. The manifestations of the epidemic process were analyzed for 6 periods: the period before vaccination (1950–1961), the period of selective immunization (1962–1965), routine vaccination of children under the age of 8 years (1966–1972), prolongation of the age for measles vaccination to 14 years (1973–1986), the revaccination of children and adolescents (1987–2001) and the period of elimination of infection (2002–2016).

In the period before vaccination the mean annual incidence was 1381.7±162.9 ‰, the seasonal rise was in December-May, children prevailed in the structure of cases. The incidence was anti-persistent with the Hurst index (H) 0.472. In the period of selective vaccination, the incidence decreased to 1082.8±189.1 ‰.

During routine vaccination of children under the age of 8 years, the incidence reduced to 219.8±110.8 ‰, with the annual decline rate of –53.0%, the trend stability of the incidence is confirmed by the Hurst index (0.529). The incidence reduced in almost all age groups, seasonality was similar to the previous periods.

While the vaccinated population under the age of 14 years increased, the incidence decreased to 89.9± 39.1 ‰. However, 2 outbreaks (in 1979 and 1984) were reported in this period. The incidence of measles in this period was anti-persistent (H = 0.381).

The revaccination against measles led to a significant decrease in incidence to 5.7±1.6 ‰, offset of seasonal rises to February-May, while cases among adolescents and adults prevailed.

In the period of elimination of infection (2001–2015), the morbidity was sporadic (0.06±0.02 ‰), due to introduction of measles from other regions without spread. In 2016, there was an outbreak of infection in the city with 72 cases.

Thus, in the historical context the strategy of vaccination determined the situation with measles. However, at the stage of elimination of infection, the possibility of outbreaks of measles remains among unvaccinated children and adults, which requires rethinking of evaluation criteria of epidemiologic safety and their constant adjustment.

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GENERATION AND CHARACTERIZATION OF GENETIC REASSORTANTS BETWEEN POTENTIALLY PANDEMIC VIRUSES (A/H9N2 OR A/H5N8) AND THE A/HONG KONG/1/68/162/35 (H3N2) MASTER DONOR VIRUS

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In this work, reassortants based on potentially pandemic viruses (A/H9N2 or A/H5N8) and the A/Hong Kong/1/68/162/35 (H3N2) (A/HKca) master virus were generated and characterized. The A/HK/HK/6:2/2016 (H9N2) (RA-52) and A/UNL/HK/2:6/2017 (H5N8) (RA-54) strains were obtained by genetic reassortment of wild viruses (A/Hong Kong/1073/99 (H9N2) or A/Common tern/Uvs-Nuur Lake/26/2016 (H5N8)) and A/HKca attenuated high yield virus, which serves as a donor of internal protein gene segments [Tsybalova, 2012]. The RA-52 reassortant was obtained after 10 passages in 10–12 day-old embryonated chicken eggs (CE); the RA-54 was obtained after 7 passages. The reassortants inherited 2 surface proteins genes from wild viruses and 6 internal protein genes from the donor strain. Genetic compositions were confirmed using restriction fragment length polymorphism analysis. The antigenic identities of reassortants and wild-type strains was confirmed by haemagglutinin inhibition reaction. Full-genome sequencing showed one amino acid substitution in the PB2 protein sequence (M475I) in RA-54, in comparison with the donor strain. RA-52 had no amino acid substitutions. Both reassortants are high virus yield in CE. The RA-52 yield was 8.5 log EID_{50}/0.2 ml; the RA-54 yield was 7.75 log EID_{50}/0.2 ml. To confirm that both were cold-adapted and temperature sensitive, the reproductive capacities at different temperatures were measured at 26°C and 39°C. The RCT_{26} for RA-52 was 1.0 log EID_{50}; for RA-54, it was 2.25 EID_{50}. The RCT_{39} values were 7.0 log EID_{50}, and 7.5 log EID_{50}, respectively. These properties were retained after 5 passages (CE), which indicates their stability.

The results show that the reassortants are antigenically identical to wild-type strains and that they inherited high yields, cold adaptation, and temp. sensitivity from the master strain. They can be used for development of both inactivated and live influenza pandemic vaccines. Both reassortants were deposited in the State Collection of Viruses under the numbers 2884 (RA-52) and 2885 (RA-54). Nucleotide sequences have been deposited in GISAID under numbers EPI_ISL_321099 (RA-52) and EPI_ISL_321098 (RA-54).

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FURTHER DEVELOPMENT OF "IgY-TECHNOLOGY": AN ELISA SYSTEM BASED ON SPECIFIC ANTIBODIES FROM EGG YOLKS AS A SURROGATE VARIANT OF THE NEUTRALIZATION TEST

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Neutralization test (NT) remains the "gold standard" in seroepidemiological and diagnostic studies of infections associated with poliovirus (PV). This is due to functional nature of the NT, which reflects the real immune status of the test serum. However, the NT uses cell cultures and live viruses, which requires compliance with relevant biosafety requirements. In accordance with the plans of the WHO Polio Eradication Initiative, work with PV (wild or Sabin strains) in the near future will be sharply limited (and is already limited for PV Sabin type 2) by the requirements of the containment and will be possible only in a small number of specially accredited institutions.

In this report, we present the results of the development of blocking ELISA as a surrogate variant of NT for the detection of antibodies to PV based on the use of specific IgY antibodies isolated from egg yolks of chickens immunized with PV and inactivated standard poliovirus antigen.

The results of comparison of two tests (NT and blocking ELISA) are presented when titrating 90 blood serums of children who received 2 doses of IPV following 3 doses

of OPV. according to National vaccination schedule. Mean values (arithmetic mean) of NT titers/blocking ELISA were for PV type 1 — 128/60; type 2 — 131/20 and type 3 — 54/25. In this case, the sensitivity of the ELISA relative to the NT for PV type 1 was 98%, type 2 — 100%, type 3 — 98%. Correlation coefficient *r* for PV type 1 — 0.67, type 2 — 0.61, type 3 — 0.76, which corresponds to the definitions "mean correlation" (for types 1 and 2) and "high correlation" (for type 3). Thus, the presented results demonstrate the further development of "IgY-technology" in combination with certain serological technique — surrogate version of NT (blocking ELISA) for use in large-scale seroepidemiological studies of poliomyelitis. Blocking ELISA does not require the use of live PV, which allows it to be used in laboratories of different levels in PV containment conditions. The duration of the test (24 hours) is its additional advantage compared to the NT (5–7 days), which opens the prospect of its use for rapid diagnosis of poliovirus infection.

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REALIZATION OF POLIO ERADICATION PROGRAM IN THE RUSSIAN FEDERATION: CURRENT STATUS AND CHALLENGES OF THE PERIOD AFTER CERTIFICATION OF THE EUROPEAN REGION, 2003–2017

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In 2002 Russia, as part of WHO European Region, was certified as "polio-free country" and has successfully maintained this status for more than 15 years. This is guaranteed by a high (97–99%) polio vaccine coverage and effective epidemiological surveillance of acute flaccid paralysis (AFP). Nevertheless, Russia faced a number of challenges, expected at the final stage of polio eradication: importation of wild poliovirus (WPV) from endemic regions, vaccine-derived PV (VDPV), poliomyelitis cases associated with using of oral poliovirus vaccine (OPV).

In 2010, Russia was involved in a large-scale outbreak of poliomyelitis in Tajikistan caused by WPV1. Newly arrived migrants from countries bordering Tajikistan as well as unvaccinated citizens of several Russian regions were affected. The outbreak was interrupted by vigorous vaccine interventions.

After certification, VDPV strains were not found through routine AFP surveillance and special studies of persons with immunodeficiency undertaken in Russia. At the same time, VDPVs2 were detected during supplementary surveillance for PV. In 2015, highly divergent (17.6% nucleotide substitutions in VP1 region) VDPV2 was isolated from the wastewater. The divergence rate of the virus is most likely indicative of its long-term (> 15 years) excretion by the immunodeficient person. In 2016, after "switch" from tOPV to bOPV, two VDPV2 were isolated from healthy unvaccinated children of different regions of Russia. The genetic association of the isolates and their origin from the PV2 Sabin was confirmed by the presence of 10 and 13 nucleotide substitutions compared to vaccine PV2, 10 of which were common. Epidemiological investigation revealed family ties and contact children in one household. This "event" in the period after the cessation of use of PV2 in oral polio vaccine required an assessment of risk of spread, large-scale organizational and vaccination activities.

The occurrence of polio cases associated with vaccine (VAPP) is particularly unacceptable at the final stage of eradication. During 2003–2017, there were 76 cases of VAPP in Russia. The introduction of IPV in the National Immunization Schedule in 2008 did not lead to a complete elimination of VAPP, among 26 cases registered in 2008–2017, VAPP in unvaccinated children prevailed.

This experience allows conclude the necessity to continue both AFP and supplementary poliovirus surveillance following the global certification of poliomyelitis eradication.

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ENVIRONMENTAL AND HUMAN SURVEILLANCE OF POLIOVIRUSES AND OTHER ENTEROVIRUSES IN MADAGASCAR. IMPACT OF THE TRIVALENT TO BIVALENT ORAL POLIO VACCINE SWITCH

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Poliomyelitis has been a major public health concern and currently, efforts are being made towards eradicating poliovirus type 2 (PV2). A global switch from trivalent oral poliovirus vaccine (tOPV) to bivalent oral poliovirus vaccine (bOPV without PV2) has been organized by the World Health Organization (WHO) to prevent epidemics of recombinant type 2 pathogenic circulating vaccine-derived polioviruses (cVDPVs). In an attempt to monitor the decline of OPV2 following the switch and the possible effect on other enteroviruses in the human population, environmental and human surveillance was conducted before, during, and after the switch in three regions of Madagascar. The developed WHO "gold standards" for detecting PV consists of isolation on cell lines and characterization by rRT-PCR assays. Other enterovirus isolates can be identified using sequencing. These methods are poorly conducive to large environmental studies, investigating multiple enteroviruses in one sample. Therefore, we developed an RT-PCR assay where we designed degenerate primers for conserved regions of the genome capable of sequencing the whole genome for all enteroviruses (A, B, C, and D). For this study, stool samples from healthy children (> 200) and sewage samples (> 400) were collected, concentrated, and inoculated on RD and L20B cells.

To date, the results from sewage and stool samples collected indicate that prior to the switch from tOPV to bOPV in April 2016, all Sabin strains were detected until July 2016. After which, Sabin 2 was no longer isolated in either sample sets analyzed. For stool samples, the majority of the enteroviruses detected were EV-B (81%) followed by EV-C (12%) and EV-A (7%). For sewage samples, EV-B (98.6%) was detected in the majority followed by EV-C (1.2%), and EV-A (0.2%). EV-D was not detected in any samples collected in this study. We were successful in detecting and characterizing, in a single sequencing run, all enteroviruses present in mixtures containing up to five different serotypes. We were able to confirm the results concerning PV obtained with the classical WHO method thus, validating the presence/absence of polioviruses and other enteroviruses using a metagenomics methodology. Additionally, this study allowed us to gain a deeper understanding of the enterovirus ecosystem and diversity and opened the way to study the genetic interactions among viruses that favor the emergence of pathogenic recombinant cVDPVs in Madagascar.

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ENTEROVIRUSES ISOLATED FROM CHILDREN FROM MIGRANTS' FAMILIES IN THE NORTH-WEST OF RUSSIA

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The goal of the study was to compare non-polio enteroviruses isolated from children from migrants' families and from resident children in the North-West of Russia. Isolation of viruses was performed according to the Polio Laboratory Manual, WHO, 2005. The genome region VP1 of certain isolated enterovirus strains was partially sequenced in order to identify virus serotype. WHO Subnational Poliomyelitis Laboratory in St. Petersburg is responsible for 14 administrative territories with the population of more than 20 million people. Annually we investigate nearly three hundred samples from children with acute flaccid paralysis, enterovirus infection, healthy resident children and healthy children under five who arrive in the North-West of Russia from unsafe territories, mostly from Central Asia.

The percentage of enterovirus isolation from healthy children from migrants' families was practically the same as from resident patients with enterovirus infection (about 10%). Enteroviruses frequently circulating in the North-West of Russia are Echoviruses 6, 9, 11, 13, 30, Coxsackieviruses A4, A6, A10, A16 and Coxsackieviruses B1–6. We found all these enteroviruses in the samples from children from migrants' families. In addition to these enteroviruses we also detected enteroviruses which had not been previously isolated in the North-West of Russia, such as Coxsackieviruses A13, 17, 24, Enteroviruses 75, 99 and 120, Echoviruses 18 and 29. Phylogenic analysis showed that serotypes of enteroviruses which were isolated from migrants' children had a different origin when compared to the viruses of the same serotype regularly detected in the North-West of Russia. Nucleotide sequences of Coxsackieviruses A13 and A17 strains isolated from the children from Tajikistan differed dramatically from sequences presented in GenBank. This may indicate that Coxsackieviruses A strains circulating in the countries of Central Asia have their specific genotype.

Our study proved that the spectra of nonpoliomyelitis enteroviruses isolated from children from migrants' families and from resident children were significantly different. In order to prevent the circulation of imported new enterovirus serotypes and genotypes it is necessary to examine systematically the groups at risk, such as children under 5 who arrive in the North-West of Russia from unsafe territories.

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DYNAMICS OF MORBIDITY OF THE WEST NILE FEVER IN THE ASTRAKHAN REGION

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The variety and wide prevalence of arbovirus infections, the possibility of adverse outcomes determine the relevance of their study. In the territory of the Astrakhan region the epidemic focus of West Nile Fever is registered. The purpose of this study was to analyze the dynamics of the morbidity of the West Nile Fever in the Astrakhan region from 2014 to 2017. The analysis of "Data on infectious and parasitic diseases" (Form 1) in the Astrakhan region was carried out.

As our research has shown, West Nile Fever in the Astrakhan region is currently characterized by a low intensity of the epidemic process. 5 people in the Astrakhan region were affected by the West Nile Fever in 2014, the morbidity rate per 100 000 of the population was 0.5. The number of cases increased by 3.0 and 4.8 times respectively in 2015 and 2016. 15 people fell ill with West Nile Fever in 2015, and 24 people — in 2016. The mortality rate per 100 000 of the population was equal to 1.5 and 2.4 respectively. It should be noted that the number of people with West Nile Fever in Russian Federation as a whole increased by 1.5 and 4.9 times in 2015 and 2016, compared to 2014. The source of infection in West Nile Fever is mainly wild birds. The increase in the incidence rate in 2015 and 2016 in the Astrakhan region and in Russia may be associated with increased infection of migratory birds during their seasonal migration from the natural foci of West Nile Fever. Only one case of West Nile Fever was registered in the Astrakhan region in 2017, the mortality rate per 100 000 of the population decreased in 24 times compared to the previous year and amounted to 0.1. Children under the age of 14 years were 11.1% of all the patients with this arbovirus infection from 2014 to 2017.

Thus, the natural focus of the West Nile Fever remains in the Astrakhan region, which activity depends on both the sources of infection and its vectors influenced by the intensity of the epidemic process in endemic foci, seasonal migration of sources of infection and climatic conditions.

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DETECTION OF PARVOVIRUS INFECTION MARKERS IN RISK GROUPS

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Parvovirus infection (PVI) caused by Parvovirus B19 (B19V) is transmitted by airborne, parenteral and vertical routes. The virus affects the precursor cells of erythrocytes. PVI can cause serious complications, up to a lethal outcome, in people at risk who are hematological patients, patients with immunosuppression, people requiring blood derived product etc. Despite the high prevalence data on the incidence of B19V in people at risk and the clinical manifestations of the disease is not enough.

The aim of the study was to investigate the frequency of occurrence of B19V markers and the effect of PVI on the clinical course and the outcome of initial disease in patients with hematologic profile — malaria patients, children after hematopoietic stem cell transplantation (allo-HSCT).

Plasma/serum samples of malaria patients (n = 316) and patients who underwent allo-HSCT (n = 54) at the age of 0.6–19 years were examined for the presence of B19V DNA by PCR and IgG-antibodies to B19V by ELISA method.

In patients with co-infection with B19V and *P. falciparum*, the rates of complications and mortality were significantly higher: observed in 72.7±2.7% of cases compared to 37.9±3.0% in the group of malaria patients without PVI. Moreover the disease led to death in 6 (10.9±4.4%) cases within the first group and in 2 (0.8±0.5%) cases in the second group. Most of cases of complicated malaria with PVI-coinfection falls on patients under 5 years. Important that 6 out of 8 deaths occur in the same group, that is significantly higher than in the absence of PVI.

In patients with allo-HSCT, a high incidence of detecting PVI markers in plasma was demonstrated. IgG antibodies to B19V were detected in 68.5–80.4% of cases, which is 2 times higher than among healthy population of the same age. Non-zero viral load values were observed in 28–30.4% of cases. The B19V DNA detection in the blood by the 30th day was associated with febrile neutropenia in these terms in 100% of cases versus 68% in patients with no DNA.

Parvovirus infection B19V is widespread in people at risk and can cause a complicated course of the underlying disease.

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CYTOKINE PROFILE IN ADULTS WITH RESPIRATORY SYNCYTIAL VIRAL INFECTION

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Respiratory syncytial viral (RSV) infection often has a complicated course. Studies show the possibility of viral persistence which leads to chronic bronchitis and asthma. It is extremely important to predict development of complications in patients with this infection, especially in risk groups: HIV-infected, hematological patients and elderly people.

48 patients with RSV infection aged 15 to 59 years were enrolled in the study. The diagnosis was confirmed by immunofluorescence or immunochromatography. All patients underwent interferon and cytokine status studies with determination of serum IFNα, IFNγ, IL-1, IL-2, IL-4, IL-5, IL-6, IL-12, TNFα.

Cytokine status of patients with RSV infection reflects high pathogenic role of pro-inflammatory cytokines and predominance of cytokine profile of the humoral immune response. Anti-inflammatory cytokine IL-4 and pro-inflammatory IL-1 have been shown to be the most important in the course of a complicated RSV infection. From the second week of the illness, a significant increase in the levels of IL-4 and IL-1 in the serum indicates a favorable trend in the development of the infection.

The progression of bronchitis is indicated by the growth of the cytokine coefficient (IL-1/IL-4) in the second week of the disease (above 0.5). The increase in IL-5 levels above 50 pg/ml after first 3 days of the illness indicates development of acute tonsillitis as a complication. During the first days of the disease high levels of IL-12 (more than 2000 pg/ml) indicate a higher possibility of tonsillitis and bronchitis. While after the 9th day it accompanies resolution of pneumonia. The level of induced production of IL-6 by leukocytes above 20 000 pg/ml in the first 3 days of the illness indicates risk of complications, and its growth in the second-third week coincides with the onset of recovery in pneumonia.

Cytokine levels in blood serum of patients with RSV infection have significant deviations. In the first days of the disease, a high level of induced IL-12 and IL-6, an increase in the level of IL-5 indicate a high risk of complications. From the 10th day of the illness, high values of induced IL-12 and IL-6, serum IL-4 and IL-1, as well as a decrease in IL-5 values, indicate the onset of the recovery. Imbalance of the cytokine profile in RSV infection has an important pathogenetic and prognostic value in the development of complicated forms of the disease.

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SURVEILLANCE OF POLIOMYELITIS AND ACUTE FLACCID PARALYSIS IN THE SOUTH OF THE RUSSIAN FEDERATION IN 2013–2017

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The main indicators of sensitive surveillance of poliomyelitis and acute flaccid paralysis (AFP) on the administrative territories of the South of Russia which are under responsibility of Stavropol Regional Center for epidemiological surveillance of poliomyelitis and acute flaccid paralysis in 2013–2017 corresponded to the recommended level. The morbidity index for AFP among children under 15 were 1.6–2.2 for 100 000 of these children. The predominant diagnosis among AFP cases (71.4%) was polyneuropathy. The percentage of AFP cases with two stool samples was 100%, the percentage of samples examined not later than 14 days after onset of paralysis was 97.9%. The samples of good quality constituted 96.1%, they arrived at the laboratory during 72 hours in 97.9% of cases. The virological investigation showed that from 313 patients we isolated 12 polioviruses (3.8%) and 13 nonpoliomyelitis enteroviruses (4.2%). Half of polioviruses belonged to type 3, the 3 mixed samples contained polioviruses of type 2 and type 3, polioviruses of type 1 were isolated from two samples and poliovirus type 2 from one sample (in 2013–2014). Enteroviruses were represented in 30.7% by enterovirus 71, 15.4% of viruses belonged to Coxsackieviruses B1–6, 7.7% to Coxsackievirus A4, Echoviruses 3 and 29, 30% of enteroviruses were not identified. In order to search wild polioviruses on the territories which did not reach the appropriate number of revealed AFP we investigated 310 samples from healthy children under 5 and confirmed the absence of wild polioviruses. In the frame of supplementary surveillance 1625 samples from groups at risk were examined, vaccine derived or wild polioviruses were not found. The percentage of poliovirus and enterovirus detection varied during the years from 3.6% till 6.8%. Vaccine polioviruses of three types were isolated in 1,7% of cases and the majority of them (53.6%) belonged to type 3. Nearly half of isolated enteroviruses were represented by Coxsackieviruses B1–6 (49%), Coxsackieviruses A constituted 7.5%, Echoviruses were detected in certain cases and nearly 40% of enteroviruses were not identified.

The system of sensitive epidemiological surveillance combined with good quality virological surveillance allowed to confirm polio free status of the territories in the South of Russia.

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OPTIMIZATION OF EXPRESSION, PURIFICATION, AND STABILIZATION CONDITIONS FOR FLG-HA2-4M2E, A RECOMBINANT PROTEIN IN UNIVERSAL INFLUENZA VACCINE

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The aim of this work was to increase the yield of Flg-HA2-4M2e recombinant protein, which is the main component of a broadly protective (universal) intranasal influenza vaccine. Flg-HA2-4M2e includes a hemagglutinin stalk (aa76-130) consensus fragment of influenza A viruses belonging to phylogenic group 2 (HA2-2) joined with 4 tandem copies of M2e (human influenza viruses A M2 protein ectodomain). Those fragments were sequentially linked to the C-terminus of flagellin and a 6-histidine tag was added to the N-terminus. The sequence was cloned into pQE30, transformed into *E. coli DLT1270*, and cells were grown in LB medium at 37°C. When an OD_{600} of ~0.5–0.7 was reached, the culture was cooled rapidly and IPTG (1 mM final) was added. During optimization, the expression temperature was reduced from 37 to 28°C and the duration was increased from 4 to 18 hrs. Because of these changes, we obtained the protein in soluble form, thus avoiding refolding during further purification. Cells were collected by centrifugation and frozen at –20°C overnight, it was not destroyed immediately as before. The lysis method was changed from lysozyme to sonication. Densitometry showed that the level of expression increased from ~5 to ~25% of total protein. Protein purification was carried out using metal affinity chromatography with a Ni-sorbent. Due to the protein's expression in soluble form, it was purified using native buffers (without urea). Column elution was carried out using a linear imidazole gradient, which yielded a cleaner product than stepwise elution. Flg-HA2-4M2e was evaluated by SDS-PAGE, which indicated a single band (*MW* ~74 kDa) of ~95% purity. Western Blot, using antibodies specific to flagellin and M2e, confirmed the presence of those proteins. Protein stabilization conditions were compared; L-arginine, Tween-80, sucrose, and polyglukin were tried as stabilizers. L-arginine was chosen according to the results of densitometry, and the stability of the protein during 4 months of storage was verified by SDS-PAGE.

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THE ROLE OF MORAXELLA CATARRHALIS IN THE DEVELOPMENT OF COMPLICATIONS AFTER INFLUENZA AND OTHER ACUTE RESPIRATORY DISEASES

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Annually in the world from flu and its complications die from 200 to 500 thousand people. However, it is not possible to identify the causative agent of community-acquired pneumonia in 50–60% of patients. According to domestic authors, 0 to 15% of cases of acute respiratory diseases (ARD) are etiologically associated with *M. catarrhalis*, while in foreign publications *M. catarrhalis* is associated with 20 to 35% of cases. Cause of low detection of strains *M. catarrhalis* is a laborious cultural method of isolating microorganisms and the lack of regulatory documents for the study of genetic and phenotypic markers of bacterial virulence. The aim of the study: to determine the role of *M. catarrhalis* in the development of complications of influenza and other ARD.

The study involved 339 patients aged 18 to 48 years with influenza and other acute respiratory infections, of which 299 patients with complications. The control group consisted of 320 healthy individuals. The methods used were bacteriological, virological, mass spectrometric analysis, methods of detection of genetic and phenotypic markers of virulence, methods of statistical analysis.

It was found that the most significant in the structure of bacterial complications in patients with influenza and ARD are community-acquired pneumonia (29% of cases) caused by *M. catarrhalis* (31%), which is present in monoculture and in combination with gram-positive coccal flora (38%). We studied the genetic and phenotypic markers of virulence in *M. catarrhalis* strains isolated from patients with angina, bronchitis, sinusitis and pneumonia for the presence of the *mcaP* gene that encodes the production of McaP protein. He takes part in adhesion of *Moraxella* to the cells of the mucous epithelium. As a control, we investigated *M. catarrhalis* strains isolated from healthy individuals. It was shown that in 84% of cases *M. catarrhalis* strains isolated from patients had the *mcaP* gene, while in healthy individuals bacteria had it in 14% of cases. In addition, the etiological role of *M. catarrhalis* strains in the development of pneumonia was proved by the presence of the highest adhesion index to buccal epithelial cells (17.3±2.7) in contrast to (4.5±0.6) in strains isolated from healthy individuals.

In the case of isolation of *M. catarrhalis* from patients with complicated flu and other ARD, it is necessary to carry out genetic typing of strains to detect the gene of virulence of *mcaP*. In phenotypic confirmation of *mcaP* gene expression, it is necessary to consider this pathogen as an etiological factor of the disease.

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THE IMPORTANCE OF PATHOGENICITY FACTORS OF SOME SPECIES OF *STAPHYLOCOCCUS, STREPTOCOCCUS* AND *KLEBSIELLA* IN DETERMINING THEIR ETIOLOGICAL ROLE IN THE DEVELOPMENT OF INFLAMMATORY PROCESSES OF THE RESPIRATORY TRACT

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Currently, the greatest difficulty in the diagnosis of infectious diseases of the respiratory tract is to determine the etiological role of the isolated microorganisms, especially if they belong to the group of opportunistic or kommensals. More than half of these diseases remain unencrypted, because they are allocated conditionally pathogenic bacteria are not subject to etiological accounting because of species. The aim of the study was to characterize the virulence of opportunistic bacterial infectious disease of the respiratory tract and to improve the methodology of the etiological decryption.

We studied 100 strains of *Staphylococcus epidermidis*, 220 strains of *Streptococcus* spp., 125 strains of *Klebsiella*

spp. We used the following methods: bacteriological methods of isolation of microorganisms from clinical material, methods of detection of phenotypic virulence markers, methods of detection of virulence genes, mathematical methods of data processing.

The result revealed that the strains of *S. epidermidis* isolated from patients with chronic rhinitis and sinusitis had the virulence gene *icaA* responsible for adhesive properties, 5 times more frequently than in strains isolated from healthy individuals. In the phenotypic test in buccal cells, the strains of *S. epidermidis* had an index of adhesion is 3 times higher than strains isolated from healthy individuals. Some species of *Streptococcus* such as *S. mitis, S. anginosus, S. oralis*, isolated from patients with high frequency. In the study of strains of these species for the presence of virulence genes *sagA, lmb, fap1, ply, lytA* was found that the strains isolated from patients, were often several times than the strains isolated from healthy individuals. The adhesion index of strains isolated from patients were 2–4 times higher than that of strains isolated from healthy individuals. Strains of *Klebsiella oxytoca* isolated from humans with sinusitis, had virulence genes *MrkD, magA, kfu,* which are characteristic for strains of *Klebsiella pneumoniae*. In phenotypic tests it was found that the adhesion index in *K. oxytoca* strains isolated from patients was 4 times higher than in strains of this species isolated from healthy individuals. To confirm the etiological role of the opportunistic microorganism in the development of the infectious process, it is necessary to be guided by data on genetic and phenotypic markers of virulence of the isolated strain. This will help to prescribe adequate therapy in the early stages of the infectious process and prevent the development of complications.

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PARVOVIRUS INFECTION: DISSEMINATION, MEDICAL-SOCIAL SIGNIFICANCE

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The clinical importance of parvovirus infection (PVI) is associated with the teratogenic effect of the virus, as well as its ability to disrupt erythropoiesis. Currently, in a number of countries, including Russia, there is no systematic registration of PVI cases, therefore the true extent of its spread is unknown. Also, the problems are not solved of preventing congenital PVI in children, as well as of viral safety at transfusion of blood and its components. However, the detection of cases of PVI in the Russian Federation has increased significantly during the rubella integration into the measles elimination program, which involves a laboratory examination of patients with exanthematous diseases. Identification of cases of PVI on the territories of the North-West Federal District (NWFD) of the Russian Federation in the period 2014–2017 allows to obtain new information about the spread of infection in the region. 1044 blood sera of patients with maculopapular rash and fever were received from 11 territories of the NWFD. The presence of serum IgM antibodies to parvovirus B19 was assessed as sign of acute PVI. Specific IgM antibodies were detected using the Anti-Parvovirus B19 ELISA IgM test system (EUROIMMUN, Germany). IgG antibodies were used to examine 733 sera from clinically healthy men and women aged 18 to 60 years. The assay was performed with the Anti-Parvovirus B19 ELISA IgG test system (EUROIMMUN, Germany). It has been established that PVI is continuously identified in 10 of the 11 districts of the region, mainly in the areas close to borders. The prevalence was observed among patients of children of 3–6 years (25.3% of cases) and 7–14 years (33.3% of cases). A high proportion of seropositive among the examined donors was found (75.4–88.9%). On the contrary, a low proportion of seropositive (56.7%) pregnant women was detected. A high proportion of errors in the primary diagnosis of PVI is shown. The main erroneous clinical diagnosis was "rubella".

These results are in agreement with those obtained earlier (2009–2012) and indicate a wide spread of PVI in the NWFD. Population immunity is actively formed in organized collectives. In the main risk group (pregnant women), the proportion of people who are sensitive to infection is high (43.3%). At the stage of elimination of measles and rubella, it is necessary to carry out differential diagnosis with parvovirus infection in each case of exanthematous disease.

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SENSITIVITY OF 2018 KAZAKHSTAN INFLUENZA TYPE B VIRUSES TO ANTIVIRAL DRUGS

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As it's known, a characteristic feature of the influenza virus is its high variability, manifested by a wide variety of properties of circulating seasonal strains. One of the characteristics of epidemically relevant viruses which is most important to clinical practice is their sensitivity to chemotherapy drugs.

To examine the drug sensitivity of three influenza type B viruses isolated in 2018 from clinical samples collected in health care facilities located in Almaty region, etiotropic anti-influenza drugs, including Remantadine, Tamiflu, Arbidol and Ingavirin, were used. The experiments were carried out on chick embryos. The resistance of influenza viruses to different concentrations of antiviral drugs was assessed by the level of reproductive suppression of 100 infectious doses for the virus. The dose of the drug that suppressed twofold the virus titer in the hemagglutination reaction compared to the control was considered as the inhibitory concentration (IC_{50}).

The Kazakhstan influenza B viruses showed a high degree of sensitivity to Tamiflu (IC_{50} values were 0.58 to 1.40 mg/mL). The strains studied were less sensitive to Remantadine since their reproduction was inhibited by the drug at concentrations of 13.13–37.50 mg/mL. The isolates exhibited absolute stability in relation to the drug Ingavirin, except for one virus, which showed a low degree of sensitivity, because it was suppressed to less than 50% when using the highest dose of the drug (50 mg/mL). The strains taken in the experiment turned out to be completely resistant to Arbidol.

The performed experiments thereby showed that the examined 2018 Kazakhstan isolates of influenza type B virus are sensitive to Tamiflu and Remantadine and resistant to Arbidol and Ingavirin.

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EPIDEMIOLOGICAL ANALYSIS OF MEASLES OUTBREAK IN GUINEA 2017–2018

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With the end of the largest ever known epidemic outbreak of Ebola in Guinea, since the beginning of 2016, comprehensive disease surveillance and response are under way. The five most common diseases, chosen according to their frequency in recent years, are monitored. These are viral hemorrhagic fevers, including Ebola and Yellow fever, cholera, meningitis, measles and poliomyelitis. Monitoring cases of measles shows that since the beginning of 2016 and despite the response organized in February 2016, confirmed cases are still being registered, and this occurs in several health districts.

The purpose of this paper was to describe the epidemiological profile of measles cases during the epidemic in 2016 and 2017. And also to study the results of the evaluation and the survey of immunization coverage in the framework of measles vaccination.

With the method of descriptive analysis, we developed a basic early warning system, a laboratory database and a national response plan

In 2016 (from 27 to 52 weeks of the year), 1304 suspected cases of measles were recorded, of which the blood was collected from 382 patients, which was 29.3%. Of these, 379 (99, 2%) were admitted to the laboratory, among them 193 (50.9%) were positive (IgM+ = 189, indeterminate = 4).

In 2017 (from 1 to 9 weeks), 2.133 suspected cases of measles were recorded, blood was withdrawn from 549 patients (25.73%). The laboratory received 443 samples (80.69) and 163 (36.8%) were positive. Confirmed cases of measles continue to be recorded, despite the ongoing vaccination and campaign to fight the disease in the outbreaks. This epidemic affected 17 prefectures in the country from 38. 73% of children is under the age of 5. There is no difference between the sexes (F = 50%, M = 50%). Coverage of vaccinations is 21 and 44%, respectively, in cases of confirmation and without measles, with a total vaccination coverage of 35%. 40% of cases of unconfirmed measles have IgM+ to rubella. Mortality is low due to lack of information. Determining the cause of the persistence of measles will help to stop the epidemic.

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ASSESSMENT OF THE RISK OF MEASLES INFECTION IN HOSPITAL

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There is no unambiguous assessment of the current epidemiological situation on measles. On the one hand, there is a decrease in morbidity, on the other — an increase in the number of seronegative women of childbearing age, thus affecting the measles incidence among the young children.

Assess the risk of measles infection in pregnant women, parturient women and newborns, as well as the need for serological examination of newborns aimed at the following anti-epidemic measures became possible with the introduction of infection in the hospital (maternity hospital). A total of 104 patients were examined in the laboratory. Blood serum was obtained from pregnant women (29), parturient women (46) and newborns (29) on the 2nd day when the measles case in the obstetric ward was revealed. The diagnosis of measles was confirmed by the ELISA (IgM) and PCR. The specific IgG antibodies were detected using the Anti-Measles Viruses ELISA (IgG) test system, Euroimmun (Germany).

In the study the seronegatives among the pregnant women consisted 31.0%, and among the parturient women 34.8%. In blood serum of the newborns IgG were detected in 21 patients (72.4%). Taking into account that among the examined persons were 22 mother–child couples, it was possible to confirm the presence of maternal immunity. Thus, in the sera of 6 infants IgG antibodies were not detected and their mothers were also seronegative. At the same time in the sera of the other 16 infants from the seropositive mothers IgG antibodies were detected. The IgG titers varied from 0.2 to 3.0 IU/ml the mean value consisted (0.70±0.45) IU/mL for infants and (0.60±0.35) IU/mL for their mothers.

The data obtained show that in case of the risk of spreading the measles infection and deciding whether to perform a procedure for determining the specific IgG in the newborn's sera, it is more appropriate to carry out a serological examination of the parturient women due to the identity of the content of measles antibodies in the sera of infants and their mothers.

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DEVELOPMENT OF PROTOTYPE OF UNIVERSAL INFLUENZA VACCINE BASED ON LIVE ATTENUATED INFLUENZA VACCINE VIRAL VECTOR

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Human influenza viruses are respiratory pathogens that cause annual epidemics and occasionally serious pandemic outbreaks. Seasonal influenza vaccination is the most effective way to control the spread of the disease; however it remains to be ineffective against pandemic influenza viruses due to the antigen mismatch. Therefore, the development of new universal vaccine with broad and durable effect is important issue for medical care. The extracellular domain of M2 protein (M2e) is highly conserved among all influenza A viruses and is widely used for generating broadly-reactive influenza vaccines. A new strategy of induction M2e-specific antibody is the expression of M2e tandem repeats in hemagglutinin (HA) molecule of live attenuated influenza vaccine (LAIV) used as viral vector. Recombinant LAIV viruses with chimeric HA proteins were generated by the means of reverse genetics. For that purpose BsmBI restriction site was inserted between signal peptide and HA1 subunit of HA genes of A/Switzerland/9715293/2013 (H3N2), A/Anhui/1/2013 (H7N9) or A/South Africa/3626/2013 (H1N1). Subsequently, four M2e tandem repeats were cloned into the inserted BsmBI cloning site. Recombinant LAIV viruses based on A/Leningrad/134/17/57 backbone were rescued by electroporation of Vero cells using Neon Transfection System (Invitrogen). All the LAIV-4M2e viruses actively replicated in eggs and preserved the temperature sensitive and cold-adapted phenotypes typical for LAIV viruses. Infectious virus titers were determined in eggs and MDCK cells incubated at different temperatures. Protective efficacy of new recombinant LAIVs against a panel of various influenza viruses was assessed in BALB/c mouse model. In addition, the recombinant

LAIVs were attenuated for mice. These data indicate that the 4M2e insertion did not affect LAIV virus replication characteristics. The expression of M2e epitopes by the recombinant viruses was confirmed by ELISA with M2e-specific antibody 14C2 (ab5416). The results of immunogenicity and cross-protective efficacy of the new LAIV-4M2e viruses will be presented.

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CIRCULATION OF COXSACKIEVIRUS A IN HAND-FOOT-MOUTH DISEASE IN SOUTHERN VIETNAM, 2015–2016

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Hand, food and mouth disease (HFMD), a common contagious disease that usually affects children, is normally mild but can have life-threatening manifestation. It can be caused by enteroviruses, particularly Coxsackieviruses (CA) and human enterovirus 71 (EV71) with highly variable clinical manifestation.

In 2011–2014, EV71 and CA16 were responsible for the HFMD outbreak in South Vietnam. However, CA6 and CA 10 were observed increased dramatically from 2015–2017. In 3 years, 1488 cases were detected positive for enterovirus from 3277 HFMD cases, the results are the more frequently presented serotypes as 908 EV-71 (61%) and 580 other EV none EV71 (39%).

The HFMD cases which were detected as other EV positive, had been sequenced and serotyped with results: CA6 (196.34%), CA10 (75.13%), CA16 (146.25%) and CA2.4, 5.8, 9; CB3.4, 5; ECHO6.9, 11.16, 25.30... (163, 28%).

Furthermore, serotype of CA 6, CA 10 replacement every year.

CA10 increased in 2016 and the presence of CA10 were 68% (69/102) in the group of Enterovirus non EV71. Our study demonstrates variety of enterovirus genotypes as viral pathogens in causing HFMD in Southern Vietnam. CA10 and CA6 were co-circulating together with EV-71 and CV-A16 in recent years.

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EPIDEMIOLOGY OF ADENOVIRAL INFECTION IN ST. PETERSBURG

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The modern methods of laboratory diagnostic for different virus significantly expanded etiological spectrum of acute gastroenteritis. Along with wide spread rota- and noroviral gastroenteritis, a large amount of cases with adenoviral etiology is registered. According to the published data, the adenoviral acute intestinal infections constitute from 1 till 15% of all diarrheal diseases and depends on the region.

The purpose of this study was to find the prevalence of adenoviral acute intestinal diseases in St. Petersburg, to estimate the significance of this problem, find the risk groups and other epidemiological features.

We used the official data from St. Petersburg center for registration of infectious and parasitic diseases in 2016–2017. Epidemiological investigation of 344 cases of adenoviral infection was performed by standard contact investigation. Molecular diagnostics was performed using PCR based tests.

The incidence level of adenoviral infection in St. Petersburg in 2016 was 4.1 per 100 000; in 2017 — 2.4 per 100 000. Adenoviral infection was registered in 90.0% in hospital patients, because of using high technology laboratory methods. Findings among outpatients were rare and were only in depth examination. From 2016–2017 adenoviral infection was found in 34 (9.8%) outpatients only. Adenoviral gastroenteritis was registered in all districts of our city, in 2016 more frequently in Viborgsky, Central and Primorsky districts of St. Petersburg; in 2017 more frequently in Kalininsky, Primorsky and Krasnogvardeisky districts. All the patients with this infection were recovered.

Monthly trend showed autumn-winter seasonality, in summer the incidence decreased. Analysis of the age structure of adenoviral infection showed that 60% cases were in the age group from 0 to 14 years. In children from 0 to 2 years old — 30% cases were registered, from 3 to 6 — 24.4%. The incidence level in the age group from 0 to 2 was 24.1 per 100 000 in 2017 (6 times increase from common level of this infection); in children from 3 to 6–18 per 100 000 (4 times increase from common level of this infection). We found the same tendency in 2016. Adenoviral infection is also registered among people of active age (in the age group 20–29 we found 12.9% cases; 30–39 — 7.1); in the elderly patients we found decreasing trend. It was only 3.2% cases of adenoviral infection in patients after 60. Diagnostic investigations on the etiology of acute intestinal infections were organized on different agents simultaneously. So 38% cases in this investigation were with associations of adenovirus with over etiologic viral and bacterial agents. Viral-viral associations were in 54% of all mixed cases. More frequently associative epidemic foci were forming with adenovirus and rotavirus (32%); adenovirus and norovirus (16%); with over viruses — 6% cases. The part of viral-bacterial associations in the adenoviral foci was 43% (33 cases). Among bacterial agents adenovirus more frequently associated with *Escherichia*, *Campylobacter*, opportunistic flora, rarely with *Klebsiella* and *Yersinia* associations with 3 etiologic agents was found in 4% cases. Two patients had adeno-, roto- and norovirus at the same time. We also found epidemic foci with mixed adenovirus, rotavirus and campylobacter infections. All mixed cases were found in hospital patients. Mixed infections had more serious clinic without specific clinical manifestation, and additional laboratory methods were required for identification.

This investigation showed significance of the problem of adenoviral infection in St. Petersburg; children from 0 to 2 years old were found to be a risk group for this disease. Autumn-winter seasonality was found. Epidemiological specific feature of adenoviral infection is forming mixed foci with other (viral and/or bacterial) etiologic agents in 38% cases.

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CHALLENGES FOR POLIO ERADICATION. RISK OF RE-EMERGENCY OF INFECTION IN POLIO FREE COUNTRIES

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The risk of importation of wild polioviruses (WPV) into polio-free countries remains till poliomyelitis is eradicated. Other risks of Polio Eradication Initiative are: circulating vaccine-derived polioviruses (VDPV) with nucleotide substitutions and recombinant profile; appearance of vaccine associated paralytic poliomyelitis (VAPP) and escape of polioviruses from polio vaccines.

Wild type 1 poliovirus, the causative agent of poliomyelitis outbreak in Tajikistan where vaccine coverage dramatically decreased, was imported into Russia and was isolated from poliomyelitis cases and healthy migrants' from Tajikistan. We isolated WPV1 from three children who arrived in Russia from Tajikistan. The percentage of migrants' children who were seronegative to three types of poliovirus was 30 times higher than it was among resident Russian children. We isolated twice as many polioviruses from healthy migrants' children as from patients with acute flaccid paralysis in Russia.

The transmission of pathogenic revertant type 2 poliovirus from the unvaccinated paralytic patient to four healthy contacts in a hospital illustrated the emergence of VDPV with increased transmissibility. The vaccine-derived poliovirus of type 3 which displayed 1.1% nucleotide substitutions in the genomic region VP1 was isolated from a patient with VAPP who received two doses of oral polio vaccine (OPV). Another VAPP patient excreted polioviruses of types 1 and 2 after vaccination with OPV. A month later he stopped to excrete poliovirus of type 2, but he continued to excrete poliovirus of type 1 for more than 4 months. We also revealed the excretion of vaccine poliovirus of type 2 from VAPP patient till 105th day after receiving four doses of oral polio vaccine. Vaccine poliovirus of type 3 was detected in the sample of unvaccinated 11-week-old patient with VAPP. We isolated the same poliovirus from the patient's sister who received 3 doses of inactivated polio vaccine and had high antibodies titers to polioviruses. She was the only possible source of poliovirus for the VAPP patient.

These data illustrate how poliovirus can persist in the population and confirm the possibility of limited spread of VDPV among well-immunized population. Reemergence of poliomyelitis can compromise Polio Eradication Initiative. It is indispensable to continue accurate surveillance and maintain polio free status of Russia as well as of other polio free countries.

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ROLE OF DIFFERENT TYPES OF ENTEROVIRUSES IN ETIOLOGY OF INFECTION ON CERTAIN TERRITORIES OF RUSSIA

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Epidemic peaks of enterovirus infection with the prevalence of different clinical forms of infection depend on different etiological factors. Outbreaks of hand, foot and mouth disease registered in the North-West of Russia in 2011–2012 were connected with Coxsackievirus A16 not detected previously in the region. The identification of two genetic variants closely related to strains isolated in France in 2010 and in Japan in 2011 suggested that Coxsackieviruses A16 implicated in these outbreaks had been brought to the North-West of Russia by two importation events. Echovirus 30 lineage which largely circulated in Russia in 2013 and caused outbreaks of meningitis in the North-West of Russia belonged to genotype H new for the region. Viruses implicated in outbreaks were closely related to the strains of genotype H detected in China in 2010–2013. Since earlier we detected in the country only Echovirus 30 of genotype Ec2 it is likely that Echovirus 30 of genotype H was imported into Russia from South-East Asia.

In 2016 Echovirus 30 of different variants of genotype H was implicated in epidemic peaks of enterovirus meningitis in Saratov and Kostroma regions. But a year later in Saratov region another type of enterovirus provoked a peak of enterovirus meningitis. It was Echovirus 18 which differed from viruses of the same type occasionally circulating in the North-West of Russia. In Murmansk and Leningrad regions in 2016 Coxsackieviruses A6 belonging to different genetic variants were the etiological factor of hand, foot and mouth disease. In Murmansk region and in the Komi Republic the cases of enterovirus infection with exanthema in 2017 were also connected mainly with Coxsackievirus A6. The strains of Coxsackievirus A6 identified in the North-West of Russia belonged to three sub-genotypes of pandemic genotype of Coxsackievirus A6.

Thus we detected Echoviruses 30 and 18 on territories where enterovirus meningitis was the leading form. On territories where enterovirus exanthema dominated the etiological factor of infection was Coxsackievirus A6. Our studies proved that surveillance of enterovirus infection aimed at acquiring new information about circulation of enteroviruses among population on different territories in different years is indispensable for prevention of propagation of enterovirus infection and for limitation of circulation of enteroviruses including the imported new serotypes/genotypes by means of using virological and molecular methods.

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MOLECULAR-GENETIC CHARACTERISTICS OF THE COXSAKIE A10 ENTEROVIRUS THAT WAS CIRCULATING IN THE CONSTITUENT ENTITIES OF THE RUSSIAN FAR EAST

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The 2017 epidemic season of enterovirus infection (EVI) was conditioned by circulation of Coxsackie A10 *(CA10)* in several constituent entities of the Far Eastern Federal District (FEFD) — the Khabarovsk, Primorsky Territories, Republic Sakha (Yakutia), Jewish Autonomous Region (JAR), Amursk and Magadan Territories. During the previous years the *CA10* was identified in individual cases and only in 2016 it was the cause of outbreaks in the Amursk city (the Khabarovsk Territory).

A total number of 90 strains of *CA10* were sequenced. A following phylogenetic analysis with the aid of BEAST program software and reference sequences obtained from the GenBank database was executed. A model of molecular clock was used to perform the evolutionary analysis.

Two genetic lines of *CA10* (A and B) were circulating in the observed constituent entities of the FEFD. The line A included enteroviruses (EV) isolated in the Republic Sakha (Yakutia) and Khabarovsk Region during 2016 as well as those that circulated in different regions of Russia in 2009–2013 and Europe in 2003–2010. The presented genetic variant was the source of the outbreaks in the Amursk city (the Khabarovsk Territory) registered in 2016. Divergence of the characteristics between Far Eastern and other Russian EV strains most likely took place in 2011 (CI: 2009–2012). The genetic line B was presented by *CA10* isolated in the FEFD in 2016–2017. The B-line strains isolated in the FEFD were divided into two clusters. First cluster was presented by the strains that circulated in the Khabarovsk, Primorsk, Magadan Territories and JAR in 2017 as well as those isolated in China in 2015. The most recent common ancestor (MRCA) for EV of the first cluster existed in 2013 (CI: 2012–2015). The second cluster included strains from the Republic Sakha (Yakutia) and Amur region isolated in 2016. However, this *CA10* variant did not circulate in the constituent entities of the FEFD in 2017.

During the last two years of observation the molecular-genetic research allowed to reveal circulation of the two Far Eastern *CA10* genetic lines of different origins and identify the time to their MRCA.

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DISTRIBUTION OF ROTAVIRUS G-, P-, I-, AND E-GENOTYPES IN NIZHNY NOVGOROD, RUSSIA

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Rotavirus infection is an important health problem all over the world. In Russia, under the conditions of the beginning of vaccination against this infection, knowledge about its pathogen is limited by the characteristic with the binary classification (G[P]-genotypes), based on the properties of the VP4 and VP7 genes encoding the rotavirus outer capsid proteins. Information about the other gene segments genotypes, as well as unusual and reassortant strains is not sufficient. The aim of this study was to determine the I (VP6) and E (NSP4) genotypes of rotaviruses detected in Nizhny Novgorod using the multiplex PCR method.

We used 55 rotavirus-positive fecal samples from children hospitalized with acute intestinal infection from January to May 2018. RNA of rotaviruses was extracted using "RIBO-prep" reagent kit (AmpliSens, Russia). RT-PCR was carried out with reagents manufactured by "Sileks" (Germany). G- and P-genotypes of rotaviruses were determined using previously published primers. To identify I- and E genotypes in multiplex PCR, fragments of 195 bp (I3), 273 bp (I1), 368 bp (I2) and 233 bp (E3), 305 bp (E2), 443 bp (E1), respectively, were amplified and detected by agarose gel electrophoresis.

I- and E-genotypes were determined in 51 samples (92.8%). In one sample only E-genotype (1.8%) was revealed, and in three — only I-genotype (5.4%). Mostly, the genotypes were detected in combination I1-E1 (52.7%). The set of I2-E2 was found in 30.9% of cases. In addition, the genotype I1-E2 (5.6%) was identified in three samples, I2-E1 and I3-E3 (3.6% together) were shown to be sporadic. The following combinations of G-, P-, I-, and E-genotypes were determined: G1-P[8]-I1-E1 (9.1%), G4-P[8]-I1-E1 (7.3%), G9-P[8]-I1-E1 (32.7%), G4-P[8]-I1-E2 (5.5%), G3-P[x]-I2-E2 (1.8%), G2-P[4]-I2-E2 (29.1%), G2-P[4]-I2-E1 (1.8%), G2-P[4]-I2-Ex (3.6%), G9-P[8]-I1-Ex (1.8%), Gx-P[8]-I1-E1 (5.5%), and Gx-P[x]-I3-E3 (1.8%).

Thus, the new method to identify the I- and E-genotypes was tested and their distribution was determined. Various combinations of G-, P-, I-, and E-genotypes of rotaviruses have been shown. The genotype G9-P[8]-I1-E1 was predominant (32.7%). The G4-P[8]-I1-E2, G3-P[x]-I2-E2, G2-P[4]-I2-E1 strains had probably a reassortant origin.

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DIAGNOSIS OF CYTOMEGALOVIRUS AND PARVOVIRUS B19 INFECTIONS IN SPECIAL GROUPS OF PATIENTS

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Some researchers described the reactivation of cytomegalovirus infection in immunocompetent patients with sepsis, burns, blood transfusions, massive surgical interventions, prolonged mechanical ventilation, use of steroids and vasopressors. In addition to herpesviruses, the reactivation of other latent viruses, in particular, parvovirus B19 (B19V), can also occur with developing immunodeficiency phenomena. With the existing concomitant pathology, these viruses significantly burdens the condition of patients.

For this reason the need for a qualitative and timely diagnosis of viral infections is increasing. PCR assay which capable of detecting even a few molecules of DNA is a progressive diagnostic method due to its high sensitivity. In this regard, quantitative detection of viral DNA can serve as a reliable criteria for significant activity of the pathogen, proving its etiological role in the development of a clinical syndromes.

The aim of the study was to create a test systems for quantitative DNA detection of CMV and B19V with hybridization-fluorescent detection of amplification products in the "real time" mode. It will help to establish the frequency of reactivation of latent viral DNA in critical condition and subsequently determine its effect on the course of the pathological process.

As a result of the studies for the first time in the Republic of Belarus a test systems for the qualitative and quantitative detection of CMV and B19V DNA by the real-time PCR method was created and registered by the Ministry of Health. The main characteristics of the developed test systems showed high values of analytical sensitivity (≥ 2 copies per run of 500 ME/ml), analytical and diagnostic specificity (100%), linear range (> 8 logarithms).

The created test systems, in addition to its use as a diagnostic tool, also can be used as a prognostic marker of infection, as a therapeutic marker for monitoring the success of antiviral therapy as well as for assessing the contagious nature of biological fluids. Thus, during the conducted studies using the test system, reactivation of CMV was detected in 28.6% (6 of 21) of patients in a critical condition with a viral load of 10 to 111 copies/ml. Also, a strong correlation between reactivation of CMV and established diagnosis of sepsis was found ($r = 0.73$). Reactivation of B19V was not detected in any of the 15 patients, which is inconsistent with the existing literature data and requires further researches.

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IMPROVEMENT OF TECHNOLOGY OF PRODUCTION OF HERPETIC VACCINE, CULTURAL, INACTIVE

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To improve methods of production and control of the vaccine, with the aim of developing a new innovative form of herpetic vaccine.

Vaccine strains of herpes simplex virus (HSV) type I (strain "US") and type II (strain "VN") are used as a seed material for preparing of herpesvirus vaccine. The monolayer cell culture (CC) of the primary fibroblasts of chick embryos (FECH) and the diploid cells of the human lung embryo (FLECH) were used for preparing of vaccine. Harvest virus of HSV-I and II types are collected in semi-finished products, which after freezing and thawing are inactivated with formalin. In a comparative plan, the semi-finished products accumulated on different cellular substrates are monitored, in accordance with the production schedule and the current regulatory documents. Semi-finished products are controlled for infectious activity, safety, toxicity, and absence of extraneous contamination. Control of specific activity is carried out in experiments on white rats.

It was necessary to use a high concentration of plant vaccine strains of HSV-I and II types when creating an innovative form of herpetic vaccine. We received a high yield of the virus when growing viruses on a cell culture of human origin, in particular, in the CC FLECH. It was found that the reproductive activity of vaccine strains on the FLECH was 6.0–6.5 lg TCD 50/ml. It was higher in 10–100 times than the activity of their reproduction on the FECH QC for HSV strains I and II type, respectively. The specific activity of semi-prepared foods prepared in different cell cultures was studied in animals. It was found that the specific activity of semifinished products manufactured on the CC FLECH exceeded by 10 times the activity of the semifinished products obtained at the FECH QC, which was determined by the index of neutralization of the sera of immune animals.

Conditions for increasing the reproductive activity of HSV seed strains have been developed, the concentration of viral antigens in the vaccine has been increased 10–100 times, using a substrate of diploid cell culture of human origin, compared with CC FECH. Methods for the production of herpetic vaccine have been improved, and the basis for the creation of a new innovative form of herpetic vaccine has been developed.

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IMMUNIZATION WITH UNIVERSAL INFLUENZA VACCINE ENHANCES IMMUNE RESPONSE TO SUBSEQUENT INFECTION

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This study evaluated the cellular and humoral responses, relative to conserved viral M2 and HA antigens, of previously immunized mice to sublethal influenza infection. We developed an experimental, recombinant protein universal vaccine Flg-HA2-4M2e features a hemagglutinin second subunit (aa76–130) consensus fragment of influenza A viruses belonging to phylogenic group 2 (HA2) joined with 4 tandem copies of M2e (viral M2 protein ectodomain); those fragments were sequentially linked to the C-terminus of flagellin. BALB/c mice were immunized intranasally 3 times (2 wk intervals, 10 μg/0.02ml); controls were administrated PBS, as above. Two weeks after final immunization, immunized and control mice were challenged with a sublethal dose (100MID) of influenza A/Aichi/2/68 (H3N2). Post-vaccination humoral immune response was characterized by high levels (serum, BAL) of anti-M2e IgG and IgA. One month post challenge, anti-M2e IgG levels in immunized mice were elevated 1.5 fold. In controls, infection did not lead to anti-M2e IgG formation in serum. Anti-M2e IgA in BAL was increased 3.5 fold in immunized mice and only 1.7 fold in controls. A significant rise in IgG titers against A/H3N2 virus in immunized mice (5.6 fold) compared to controls (2.5 fold) was noted. In lung, the post-vaccination response was characterized by the formation of M2e- and HA2 specific T-cells ($CD4^+$, single (TNF^+) and double (TNF^+IL2^+) producing effector memory cells — Tem). One month after challenge, TNF^+ and TNF^+IL2^+ M2e-specific T-em levels increased almost 10-fold. Double producers (IFN^+TNF^+) and triple producers ($IFN^+TNF^+IL2^+$) were also detected. The pool of HA2-specific double producing Tem ($TNF^+IL\text{-}2^+$) increased significantly (~4x), and TNF^+ mono and $IFN^+TNF^+IL2^+$ triple producers appeared. In control mice, infection resulted in the formation of fewer specific Tem cells. The results show that sublethal infection in mice pre-immunized with Flg-HA2–4M2e: enhanced Ag-specific local and systemic humoral responses; increased Ag-specific Tem lung populations; and led to the appearance of new cytokine secreting effector T memory cells.

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FEATURES OF POPULATION IMMUNITY AGAINST MEASLES AND RUBELLA VIRUSES. WHY DO ADULTS SUFFER?

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The main factor in the immunity of people to measles and rubella viruses is the presence in the blood of the protective level of specific antibodies. These antibodies appear both after disease, and after vaccination. The level of antibodies is maintained for many years by long-lived plasmacytes and memory B-cells. Repeated contact with the virus leads to the boost — an increase in the level of specific IgG. However, in the conditions of intensive vaccination of the population, the circulation of the wild virus is reduced and the probability of natural boosting vaccinated people with wild strains of viruses is reduced. According to the Russian vaccination calendar, vaccinations against measles and rubella viruses are given to children at 1 year and 6 years of age. At the same time, among the measles cases, a group of young adults 20–40 years old is singled out, which raises the question of the duration of postvaccinal immunity. Using "Vector Best" kits, the study of the anti-measles and anti-rubella immunity was conducted of age groups: up to 1 year, 1–2 years, 3–6 years, 7–14 years, 15–17 years, 18–30 years, 31–40 years, 41–50 years and 51–60 years on the territory of Moscow and the Moscow region for 2013 (the territory with an unfavorable epidemic situation). The serum from 654 randomly selected healthy individuals and 646 patients from the same region with a serologically confirmed measles infection were examined. A gradual increase in the percentage of people with protective levels of antibodies to rubella and measles viruses was found, reaching 81.3% for measles and more than 90% for rubella at the age of 7–14 years. At the same time, the percentage of those protected against rubella remained at an older age. While the most pronounced increase in the seronegative persons to measles virus (40% or more) in the 18 to 30-year-old age group was found, but in groups older than 40 years, the immunity reached 85–95%. A strong negative correlation was found between the incidence of measles and the level of tension of the population's anti-measles immunity ($r = -0.76$). Thus, an increase in the number of cases of sickness to 28% at the age of 18–30years and a decrease to 2.9% in 51–60 years was provided by a decrease (up to 55%) and an increase (up to 95%) of persons with protective immunity, respectively.

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RISK FACTORS FOR SARI AND INFLUENZA VACCINE EFFECTIVENESS IN SENTINEL SURVEILLANCE SYSTEM IN RUSSIA, THE SEASON 2017–2018

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The influenza sentinel surveillance system (ISS) in Russia includes 19 hospitals and 14 polyclinics located in Kaliningrad, St. Petersburg, Lipetsk, Moscow, Samara, Stavropol, Novosibirsk, Chita, Khabarovsk, Vladivostok. The standard definitions for SARI, ILI and ARI (according to WHO case definition) and case-based reporting were used in the study. The on-line data submission into the electronic database of the Smorodintsev Research Institute of Influenza from each Sentinel Site (SS) at the FBUZ Centers for Epidemiology and Hygiene was conducted on a weekly basis. Case-based form for each patient included the data on age, sex, date of disease onset, sampling for PCR, co-morbidity, ICU placement, the status of vaccination, treatment by antivirals, results the PCR detection of influenza viruses and other 7 respiratory viruses, outcome of the disease. Laboratory confirmed influenza (LCI) was registered in average in 20.8% of patients with SARI and in 16.3% of ILI/ARI patients. Severity of the clinical course of SARI determined as the proportion of SARI patients placed in the Intensive Care Unit of total SARI cases was estimated as 7.6%, LCI SARI cases as 2.3%. Influenza A(H3N2) virus caused 51.0% of SARI cases, influenza B (Yamagata lineage) and A(H1N1) pdm09 viruses were determined in 33.3 and 15.8%, respectively. The general trend of greater significance of LCI among SARI compared to the ILI/ARI was confirmed this season. Among 400 SARI patients with LCI only 17 (4.25%) patients were vaccinated. Among 320 LCI outpatients with ILI/ARI 22 (6.9%) patients were vaccinated. The effectiveness of vaccination assessed by the case-control method was estimated as 11% in prevention of admission with LCI SARI cases and 58% in prevention of LCI ILI/ARI cases. The concomitant somatic pathology and pregnancy were identified in 57.5% of SARI patients with LCI. This indicator was significantly higher (by 23.1%) in LCI SARI than in SARI caused by other respiratory pathogens. The leading role of pregnancy as a risk factor for hospitalization with LCI SARI (27.3% of SARI patients with influenza against 5.7% of SARI patients with other etiology) was confirmed in this season. In addition, significant increase ($p < 0.05$) of percentage of LCI SARI with cardiovascular diseases (16.5%) and diabetes (5.5%) compared to patients with SARI of other etiology (11.7 and 2.8% respectively) was established, and these patients' categories were assigned to the risk groups.

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RESULTS OF MOLECULAR DETECTION AND CHARACTERIZATION OF INFLUENZA AND OTHER RESPIRATORY VIRUSES IN RUSSIA, SEASON 2017–2018

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The influenza season 2017–2018 started significantly later than any of the seasons analyzed in the last five years. The epidemic lasted for 12 weeks (weeks 6–17), was of medium intensity and involved 10.4% of the population of the country with children aged 3–6 years being the most affected group. The average hospitalization rate of patients with ILI and ARI was 2.6% and was the highest in infants aged 0–2 years (5.4%). The number of influenza-associated deaths was two times higher this season compared to 2016–2017 which can be attributed to the circulation of A(H1N1) pdm09 viruses that still is the major cause of lethal influenza outcomes in the country. A total 72 759 patients were investigated by RT-PCR in 55 collaborating RBLs and 12 149 (20.7%) were positive for influenza, of which 39.3% were influenza A(H1N1)pdm09 viruses, 29.6% were A(H3N2) and 31.1% influenza B (Yamagata lineage) viruses. The first cases of influenza viruses were detected at the very beginning of the season (weeks 40–45.2017), however a distinct increase in the rate of detection was registered only from the week 2.2018 with the peak on the week 13–14.2018 and subsequent gradual decline. The certain differences in the etiology of morbidity between Federal Districts were registered. The impact of influenza and other ARI agents in different stage of, epidemic was determined. In the pre-epidemic period, the incidence growth was occurred mainly due to ARI (about 32.7%), especially due to rhinoviruses (RhV) and RSV(10.2 and 8.0% cases, respectively) while LCI were registered in 3.4% only. During the epidemic, the rate of laboratory confirmed influenza cases (LCI) detection increased up to 29.2% at simultaneous decrease in frequency of parainfluenza, adenovirus, bocavirus, coronavirus and, especially, RhV, to a lesser extent RSV infection. In the post-epidemic period, the role of influenza A(H1N1)pdm09, A(H3N2) and B viruses dropped up to 6.1; 6.9 and 3.6%, respectively, with increase of RhV (9.5% of diseases). Genetic analysis of influenza A(H1N1)pdm09 and A (H3N2) viruses circulating in 2017–2018 season showed that all analyzed viruses by the structure of surface genes encoding antigenic determinants corresponded to the vaccine strains recommended by WHO for the Northern Hemisphere for 2017–2018 epidemic season. However, significant changes in the internal genes of circulating viruses were revealed.

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PROSPECTS FOR MONOCLONAL ANTIBODIES USING IN DIFFERENTIAL DIAGNOSIS OF ADENOVIRUS INFECTION

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More than 50 types of adenoviruses (AV) cause human diseases. There are serological evidences that more than 80% of population suffer from AV infection. The variety of clinical manifestations of AV infection complicates its differential diagnosis. Currently polyclonal sera are widely used for these purposes in Russia. The development of highly sensitive and specific enzyme-linked immunosorbent (ELISA) and immunofluorescence (IF) tests with using monoclonal antibodies (MAbs) is an urgent task.

Highly sensitive and specific MAbs #1 and #2 were obtained at the laboratory of biotechnology. MAbs were conjugated with horseradish peroxidase (PX) and fluorescein isothiocyanate (FITC). Sensitivity and specificity of various combinations of MAb #1 or #2 and it's PX conjugates for detection of purified AB type 6 was examined by sandwich-ELISA. The ability of FITC conjugates to detect AV 3, 4, 6 types in infected A549 cells was examined by IF. Respiratory syncytial virus was used as negative control.

The specificity of examined sandwich-ELISA variants was high, the sensitivity widely varied. The best result (7 ng/ml of purified AV 6 type) was obtained with capture MAbs #1 and PX conjugate of these MAbs. For AV antigens detection in infected cell the most promising is MAbs #2 FITC conjugate that allows to detect AV of epidemic types in infected cells in form of nuclear localized clear fluorescence. Usage of MAbs for development of high sensitivity and specificity test-kits for differential diagnosis of AV infection provides new possibilities for medical practice. Diagnostic properties of developed sandwich-ELISA and MAbs #2 FITC conjugate will be investigated with clinical samples.

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THE ROLE OF MOLECULAR-GENETIC RESEARCH IN THE SYSTEM OF EPIDEMIOLOGICAL SURVEILLANCE OVER ENTEROVIRUS INFECTION IN THE RUSSIAN FAR EAST AND SIBERIA

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A seasonal peak of enterovirus (EV) incidence is typical for majority of the constituent entities of the Russian Far East and Siberia. The spectrum of identified EV is diverse. Genetic variability of EV leads to emergence of new sub-subtypes.

The goal of the research was to evaluate the role of genotyping and phylogenetic analysis in epidemiological surveillance over enterovirus infection (EVI).

Epidemiological analysis was performed based on the official reporting forms. A total number of 1474 environmental samples, biomaterial from patients with EVI and exposed persons were analyzed via molecular-genetic methods. Reconstruction of genetic affinity was performed using Bayesian modeling.

Circulation of Russian and foreign EV strains was registered in the Russian Far East and Siberia. The most epidemiologically significant strains were as follows — Coxsackie B-4, B-5, ECHO-6, 9, 30. During the last four years Coxsackie A, mostly Coxsackie A-6 was also identified. The breakouts of Coxsackie A-6-infection were registered in children's ensembles in the Amur, Sakhalin and Khabarovsk Regions. Most EV had a genetic relation to reference sequences obtained from the GenBank database. This indicates the possibility of importation of EVI from different countries. Epidemiological investigations confirmed that some cases were imported. That said, during the summer season of 2017 EVI was diagnosed in patients arrived from resorts located in Turkey, Vietnam and Tunisia. The diseases were caused by Coxsackie A-6, Coxsackie A-2, EV-A71C1 variants as well as EV-C104 that was never registered in Russia before.

Molecular-genetic research not only promotes the enhancement of diagnostic subsystem of epidemiological surveillance, but also improves evaluation of epidemiological situation in the constituent entities of the country, facilitates identification of territorial peculiarities of genetically isolated and epidemiologically significant EV variants circulation, helps to identify imported cases of EVI.

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CONTROL OF INFLUENZA VIA VACCINES: CHALLENGES AND PERSPECTIVES AS VIEWED BY VARIOUS STAKEHOLDERS

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Influenza remains one of the principal challenges of modern healthcare on a global scale. Despite vaccination efforts, morbidity and mortality — especially among high-risk groups during seasonal epidemics — are high. Each year more emerging and re-emerging strains of animal origin are designated as having pandemic potential. Vaccines are the cornerstone of influenza control, including mitigation of yearly epidemics and out-of-season outbreaks, as well as prepandemic preparedness. Challenges, however, still remain, and here we explore varying views of different stakeholders (international agencies, regulators and manufacturers) as one of the reasons why.

Influenza virus is constantly evolving, thus, recommended strains for seasonal vaccines are regularly updated. Current WHO position includes 3- and 4-valent vaccines; and a nominal 25% increase in manufacturing capacities is needed for the switch to the latter. Moreover, even a single change in strain recommendation would require manufacturers to develop a new process within 6 months at most, and strain yield and HA activity for the candidate virus may be lower than had been anticipated. Separate WHO recommendations for tropical countries (similarly to northern and southern hemispheres) are still highly debatable. Until then local authorities at the country level should make the decision; however, current-season vaccines may already (or yet) be unavailable.

Though effectiveness, safety and economical feasibility for influenza vaccines has been proved numerous times, manufacturing capacities worldwide are still lacking. Current technology is classic at best and utilizes chicken embryos, whereas promising approaches (e.g. cell cultures) would require overhauling of the whole monitoring (e.g. GISRS) and manufacturing system. Academia could generate a breakthrough (e.g. next generation vaccines), but the transition from a prototype even to a preclinical setting is a very high-risk and money-intense endeavor. Similarly, since there is no guaranteed market for prepandemic influenza vaccines, except periodic stockpiling by international or national bodies, R&D activities in this area for manufacturers are not a priority. Finally, we have lately seen a surge of support for the anti-vaccination movement.

Thus, combined efforts of all stakeholders are urgently needed to advance control of influenza via vaccines to the next stage and as part of the universal health coverage paradigm.

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CROSS-ANTIGENIC AND IMMUNOGENIC FEATURES OF CANONICAL AND NEW GENOGROUPS OF ENTEROVIRUS 71

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Enterovirus 71 (EV-A71) is a member of the *Enterovirus* A species. EV-A71 is a leading public health problem, because

it causes a range of illnesses from hand-foot-and-mouth disease (HFMD) to severe neurological manifestations. EV-A71 strains have been phylogenetically classified into genogroups: A to G. Whereas canonical genogroups B and C have been reported worldwide, new genogroups E and F were recently identified in Africa and Madagascar, respectively. The recent identification of the new Genogroups E and F raised the question of their cross-antigenicity and immunogenicity with the canonical ones.

We compared antigenic and immunogenic features of EV-A71 strains, which belong to the canonical (B-C) and the new (E-F) genogroups. The level of cross-protection induced by a given EV-A71 genogroup against viruses of other genogroups was estimated using a seroneutralization assay with human and rabbit *sera*, as well as a mouse monoclonal antibody.

Neutralization assays performed with diverse standardized human, rabbit, and mouse anti-EV-A71 sera or antibodies successfully neutralized all available isolates indicating a broad overall cross-antigenicity between the canonical genogroups B and C and the newly described genogroup E and F. By using collections of human sera from Cambodian patients with neutralizing antibodies against EV-A71 genogroup C, we evaluated the epidemiological risk of a population affected by a canonical EV-A71 genogroup from being protected against the new genogroups E and F. All human *sera* showed rather similar cross-neutralization activities between isolates of genogroups B, C, E and F.

Taken together, our results indicate that the antigenic features of all tested genogroups are quite similar among the serotype EV-A71. They also suggest that the neutralizing antibody response induced by strains of the canonical genogroups B and C is likely to be protective against the new genogroups E and F. Our findings provides valuable informations in terms of public health and EV-A71 vaccine development.

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CLINICAL-LABORATORY CHARACTERISTICS OF INFLUENZA INFECTION IN HOSPITALIZED ADULT PATIENTS IN THE EPIDEMIC SEASON 2017–2018

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Despite advances in the field of modern influenza vaccination and antiviral therapy, influenza and acute respiratory infections remain the most common diseases. The annual incidence is 19–20 thousand per 100 000 and the economic loss of about 90% of the losses from all infectious diseases. The death rate from influenza in the world is 0.01–0.2%, increasing in children under 2 years and those over 65 years of age, as well as in the development of pneumonia, as complications. We conducted a clinical and laboratory analysis of cases of influenza infection in the epidemic season 2017/18 in adult patients hospitalized in the Botkin Clinical Infectious Diseases Hospital. 423 medical charts were reviewed, with confirmed influenza infection by PCR. The analysis of the obtained results was carried out using the statistical package SPSS 17.0RU for Windows. The etiological composition was presented by influenza A viruses — 56%, 25% of them H1N1, H3N2 — 64%, undifferentiated influenza A viruses — 9%, influenza A+B — 2%, and influenza B viruses — 44%, 85% of them Yamagata, Victoria — 0.7% and 14.3% undifferentiated influenza B. At admission to the hospital, the condition of most patients was regarded as of moderate severity. More than 50% of patients were hospitalized before the 3rd day of illness. Among those admitted to the hospital 51.2% were men and 48.8% were women. The median age was 30.5 years. Comorbidity diseases were absent in most patients (65%). All patients received standard pathogenetic therapy. The clinical pattern was characterized by a marked intoxication syndrome, the median temperature of the body was 39.0 degrees. The duration of the intoxication syndrome was 5.6±0.4 days, and catarrhal syndrome was 8.1±0.5 days. 50% of the patients had complications: 12.5% of them — pneumonia, 12.5% — sinusitis and 18.3% — bronchitis. Duration of the hospitalization was 6.3±0.6 days. There were no lethal cases among the observed patients. In conclusion, it should be noted that influenza A viruses prevailed in the observed patients (56%), and among viruses influenza A-H3N2 (63%), among viruses of influenza B — Yamagata type viruses (85%). Hospitalization was in the early days. The clinical pattern was characterized by severe intoxication and catarrhal syndrome, frequent complications, including pneumonia (12.5%).

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VACCINE PROPHYLAXIS, DIAGNOSTICS AND GENOTYPES OF MUMPS (EPIDEMIC PAROTITIS) VIRUS

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Epidemic parotitis (EP, mumps) is an acute anthroponotic viral infection. Mumps virus is single-strand negative RNA genome virus. Its genome contains 7 genes encoding 5 internal proteins (P, L, M, V, I), the transmembrane protein SH and 2 surface proteins — hemagglutinin/neuraminidase (HN) and fusion protein F. It is important to emphasize that only antibodies to proteins F and HN have neutralizing activity.

Vaccination against mumps was introduced in the Russian Federation in 1981, that highly affected morbidity. Indeed, in 1970–1980 in Russia, 300 to 600 thousand cases of mumps were registered annually, while in 2015 as little as 127 cases were detected. The mass rejection of vaccinations in Western European countries affected the incidence of mumps in Russia. In 2017, 4443 people became ill. Among them, children under 14 were prevailed, although there were a lot of adults as well. Mumps is a serious viral disease; in 30–40% of cases it may be asymptomatic. It leads to the development of orchitis in 25% of diseased boys. The risk of miscarriage in mumps-infected is higher than even at rubella. For verification of mumps diagnosis in the Russian Federation mainly ELISA (domestic and foreign test systems) are used. However, a study of the blood of patients for the presence of specific antibodies of the IgG or IgM class is not enough either to establish the fact of active replication of EP, or to confirm both manifest and asymptomatic forms of the disease.

At present, there are 12 genotypes of the EP virus circulating in the world: A, B, C, D, E, F, G, H, I, J, K, L and Leningrad-3 (L-3), which has been assigned to a special group. The contagiousness of patients with mumps is not high, but the susceptibility is universal, it reaches 100% and lasts for a lifetime. Mumps outbreaks are recorded in populations with both high and low vaccine coverage.

Today in the world, more than 120 countries have introduced immunization schedules against mumps in their vaccination calendars, and in 72 countries they are absent. Advances in vaccine prevention are undoubtful. Over 37 years, 215 million people have been vaccinated in the

Russian Federation, 2500 lives have been saved, 2.5 million cases of serous meningitis have been prevented, tens of thousands of cases of orchitis (the probability of male infertility), pancreatitis, and diabetes have been prevented.

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INFLUENCE OF THE NEWCASTLE DISEASE VIRUS ON SOME INDICES OF CELL-MEDIATED IMMUNITY IN TUMOR-BEARING RATS

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The use of oncolytic viruses for biotherapy of tumors is a promising approach. It is assumed that the administration of some kinds of viruses into the tumor-bearing organism induces both direct and indirect antitumor effect. The potential use of Newcastle disease virus (NDV) in this field attracts attention of the researchers.

Our aim was to study the effect of the administration of NDV vaccine strain on some indices of cell-mediated immunity in rats after transplantation of carcinoma.

The experiment was performed on 19 white mongrel male rats with Guerin's carcinoma. The NDV vaccine strain La-Sota was inoculated once 5000 doses paratumorally 2 times a week, 4 times in total: in the first group of rats the course of NDV was started after tumor transplantation, in the second group — one week before tumor transplantation. Tumor growth was observed and lymphocytes` subsets were counted in peripheral blood samples collected from the femoral vein of animals in the dynamics of the course of NDV administration. The per cent of T- and B-lymphocytes were estimated by flow cytometer BD CantoII. The results showed stimulating effect of the NDV on the T-cell link of rats` immune system and made it possible to establish differences in the type of the immunological changes developing in tumor-bearing rats, depending on the time of administration of the virus relative to the time of tumor transplantation and their possible significance for obtaining a prophylactic effect on transplanted tumors in some animals. So the administration of NDV previous to tumor transplantation caused a marked increase of $CD3^+CD25^+$ (T cells expressing an early activation marker) and $CD3^+CD4^+$ lymphocytes` levels which persisted after tumor transplantation while the levels of $CD3^+CD8^+$ and $CD3^+$RT1b (T cells expressing a late activation marker) cells were decreased. This was the only group where in some rats tumor formation after transplantation was not observed or early regression was detected. On the contrary, in the control animals the highest $CD3^+CD8^+$ and the lowest $CD3^+CD25^+$ cells` levels were observed during the whole period. Administration of NDV to the rats of the first group after tumor transplantation produced effect neither on tumor growth nor on potentially antitumor factors of cell-mediated immunity. Thus we consider that if NDV is able to induce any antitumor effect it should be used before tumor transplantation; activation of Th cells should be achieved.

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THE EFFECT OF PARVOVIRUS B19 INFECTION ON RESULTS OF CHEMOTHERAPY IN PATIENTS WITH LYMPHOMA

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Parvovirus B19 infection (B19V) can cause severe complications in patients with hematological malignancies which explains the importance of understanding the influence of B19V on the results of chemotherapy (CT). The purpose of the study was to reveal the prevalence of B19V in patients with lymphomas and the influence of B19V on the CT results.

The study included 41 patients aged 48.9±2.3 years: 12 patients with Hodgkin's lymphoma (HL) and 29 with Non-Hodgkin's lymphoma (NHL) (21 aggressive, 8 indolent). Patients received CT according to the tumor immunophenotype. B19V DNA was determined in plasma and in bone marrow (BM) by qPCR, B19V IgM and IgG in the serum by ELISA.

78.0% of patients had B19V IgG, mean concentration was 158.1±12.9 U/mL. B19V DNA in plasma was detected in 7.3%, in BM in 48.8%. Viral load in plasma was 68.7±35.8 IU/mL, in BM — 438 240.0±281 316.8 IU/mL. Seroprevalence and the mean concentration of B19V IgG was higher in NHL than in HL (79.3% vs 75.0% and 161.4±16.3 vs 153.6±20.5, $p>0.05$). In NHL, the number of seropositive patients and the mean level of B19V IgG were higher in aggressive than in indolent tumors (81% vs 75% and 177.9±19.3 U/mL vs 114.4±22.8 U/mL, $p = 0.052$). B19V IgM were not found. B19V DNA in plasma was found only in NHL patients (10.3%). The frequency of B19V DNA detection in plasma was higher in indolent (12.5%) than in aggressive lymphomas (9.5%), while DNA concentration was higher in aggressive lymphomas (102.5±20.5 IU/mL vs 1.0±0.0 IU/mL, $p > 0.05$). B19V DNA detection frequency in BM was similar in HL (50.0%) and NHL (48.3%, $p>0.05$), but the mean B19V DNA concentration was higher in NHL than in HL: 624 496.9±395 398.3 IU/mL vs 3640.5±1649.2 IU/mL, $p > 0.05$. In NHL, B19V DNA in BM was more frequent in indolent than in aggressive lymphomas (50.0% vs 47.6%), and the average concentration was higher in aggressive lymphomas (865 689.2±541 738.6 IU/mL vs 21 516.3±19 352.8 IU/mL, $p > 0.05$). Complete remission was observed in 68.3% of patients, partial remission 17.0%, stabilization 4.8%, progression 9.9%. CT results depended neither on serostatus and B19V IgG concentration nor on B19V DNA presence in BM or plasma ($p > 0.05$).

All parameters of the viral infection (B19V IgG, DNA) were higher in NHL than in HL ($p > 0.05$). The mean concentration of B19V IgG was higher in aggressive NHLs than in indolent ones ($p = 0.052$). B19V infection did not influence results of antitumor CT ($p > 0.05$).

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ASSOCIATION BETWEEN HERPES VIRUS INFECTION AND INDICATORS OF OXIDATIVE STATUS OF TUMOR TISSUE IN GASTRIC CANCER

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Viral infection and oxidative stress are recognized as aggravating factors contributing to the neoplastic tissue transformation. Our purpose was to determine the influence of viral infections of tissues on the processes of the free radical oxidation in stomach cancer (SC).

We studied tumor tissues (TT) and intact tissues from the resection line (IT) obtained from 25 SC patients (mean age 62.8±2.1 years). DNA of CMV, EBV and HHV6 was determined by qPCR. Levels of malondialdehyde (MDA) were measured to assess the intensity of the oxidative stress; the function of the antioxidant component was evaluated by catalase, superoxide dismutase and glutathione peroxidase activities and levels of reduced glutathione.

The significance of differences was assessed by the Mann–Whitney U-test; correlation of the indices was calculated by the Spearman's method. Differences were considered significant at $p < 0.05$.

In total, herpes virus infections were observed in 96% of TT and IT. In TT, EBV DNA was the most frequent (88%); HHV6 (64%) and CMV (44%) DNA was less frequent ($p < 0.05$). The ranking and prevalence of the pathogens in IT were similar ($p > 0.05$). The mean viral load in TT was 2.3±0.4 lg DNA copies/10^5 cells for EBV and 1.4±0.3 lg/10^5 for HHV6, in IT — 2.2±0.3 lg/10^5 and 1.6±0.2 lg/10^5, respectively. No significant differences between TT and IT were found ($p > 0.05$). The mean CMV DNA amount in TT (1.6±0.2 lg/10^5) was 2.3 times higher than in IT ($p = 0.041$). The amount of EBV DNA in TT moderately correlated with the MDA level (304.3±50.1 nmol/mL) ($rs = 0.402$, $p < 0.05$); in IT, such correlation was not observed. Both in TT and IT, MDA levels in samples with high viral load (> 2 lg/10^5) were higher than in tissues with low viral load or with EBV absence (393.9 vs 324.6 nmol/mL, $p = 0.025$). MDA levels were 1.6 times higher in HHV6-positive TT than in negative ones (416.8 vs 262.8 nmol/mL, $p = 0.023$). For CMV, such correlation was not observed. We did not reveal any significant influence of viral infection on the antioxidant component.

Viral infections of the stomach tissues influenced the intensity of the lipid peroxidation processes: MDA levels in samples with high viral load were higher than in samples with low viral load or in EBV-negative in TT and IT, in HHV6-negative in TT; the amount of EBV DNA in TT correlated with MDA levels ($r_s = 0.402$, $p < 0.05$). The CMV influence on the oxidative stress development in SC was not observed.

4. ZOONOTIC AND PARASITIC INFECTIONS: CLINICAL, EPIDEMIOLOGICAL AND LABORATORY ASPECTS

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PCR ANALYSIS IN THE REAL TIME REGIMEN AS A LONG-TERM METHOD FOR LABORATORY DIAGNOSIS OF RICKETTSIOSIS

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The actuality of studying the natural foci of rickettsiosis and the expediency of researches for the revealing of rickettsia DNA in ticks of the Crimea are caused by peculiarities of the region that are favorable for the circulation and preservation of pathogens in the nature.

The purpose of the study was to define the contamination of ticks by rickettsia and determine their species belonging.

Tasks: the organization of ticks collection and carrying out of their specific identification; carrying out of the laboratory researches of ticks — PCR — analysis in the real time regimen (PCR-RV).

Materials and methods — epidemiological and literary data on the study of rickettsiosis in the Crimea; parasitological methods (collection of ticks for the standard flag and dragging, manual collection from animals), specific identification of the ticks, laboratory methods (revealing of rickettsia DNA by PCR-RV using reagents set "RealBest DNA Rickettsia species" ("Vector-Best", Novosibirsk).

1342 specimens of ticks are collected from August to October 2016 and analyzed in total. Specific composition is presented by: *Haemaphysalis punctata* — 65.3%, *Rhipicephalus sanguineus* — 21.8%, *Hyalomma marginatum* — 9.5% and *Dermacentor marginatus* — 3.4%.

Using the PCR test "RealBest DNA Rickettsia species" in 470 from 1342 nucleic acid samples isolated from individual ticks suspensions, DNA marker of rickettsia revealed, a site of the citrate synthase gene *(gltA)*, was detected. 114 positive samples of rickettsia DNA were selected for additional amplicons production and sequencing of their sequences by 3–4 genes (*gltA, ompA, ompB* and *sca4*). The received results of the sequencing were compared with the nucleotide sequences of the rickettsia DNA presented in the GeneBank database. The species of rickettsia was established for 3 to 4 genes.

Analysis of nucleotide sequences indicated about circulation in four analyzed species of ticks collected in the Crimea, in six species of rickettsia, five from them are pathogenic for humans: *R. conori, R. massiliae, R. aeschlimannii, R. mongolotimonae, R. slovaca*.

The PCR test "RealBest DNA Rickettsia species" allows detecting in the extracted nucleic acid samples the DNA-marker of rickettsia circulating on the peninsula and can be considered a long-term method for laboratory diagnosis of rickettsiosis.

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LESIONS OF THE GASTROINTESTINAL TRACT IN SCHOOLCHILDREN INVASED BY LAMBLIA

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In recent years, more and more often among residents of the Russian Federation, especially among children, cases of parasitic infestations have been recorded, among which a special place is occupied by lambliasis, which often occurs under the mask of the lesion of gastrointestinal tract and is not always recognized in time.

The purpose of the study was to analyze lesions of the gastrointestinal tract in schoolchildren invaded by lamblia.

Under supervision there were 55 children of school age of whom 60% were children with gastrointestinal lesions. The diagnosis was confirmed by a coprological examination of feces for lamblia cysts.

According to the results of ultrasound investigation, all children showed lesions of the gastrointestinal tract, manifested in the form of reactive changes in the pancreas — 2.1%, reactive changes in the liver — 15.2%, signs of biliary dyskinesia — 18.2%, combined liver and pancreatic lesions — 15.2%, combined liver and pancreas damage, and signs of biliary dyskinesia — 18.2%, liver damage and signs of biliary dyskinesia — 12.1%, as well as pancreatic lesions and signs of dyskinesia of bile ducts — 9.1%. In most cases — 75.8% of children received the drug Makmiror at the rate of 15–30 mg per 1 kg of body weight for 7 days. Albendazole was received by 24.2% of children at 12 mg/kg body weight.

Lambliasis was registered most often in children of primary school age, which may indicate an incomplete knowledge of the rules of personal hygiene. The main causes of the disease were non-compliance with personal hygiene and contact with domestic animals, more often with cat. The main complaints of children were abdominal pain, nausea, decreased appetite, loosening of the stool and allergic reactions to the skin.

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CLINICAL-EPIDEMIOLOGICAL AND LABORATORY-INSTRUMENTAL ASPECTS OF NON-ERYTHEMATOUS FORM IN PATIENTS WITH TICK-BORNE BORRELIOSIS

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Tick-borne borreliosis remains like the most common natural focal disease with a transmissive mechanism of the pathogen. Despite the introduction of advanced technologies of early diagnosis and modern methods of treatment doctors are faced with such a problem as medical examination and diagnosis of patients with tick-borne borreliosis.

The purpose of the work is to develop universal recommendations for clinicians for the management of patients with tick-borne borreliosis.

Tasks of the study was to compare the distinctive features of epidemiological anamnesis, clinical manifestations, indicators of laboratory and instrumental diagnostics and criteria of dispensary registration of patients with tick-borne borreliosis of erythema and non-erythema form.

We have analyzed about 34 patients from the maps of municipal institutions in Ulyanovsk. Forms of borreliosis were divided evenly into erythemic and non-erythemic forms in 17 patients (50%)

In the first 7 days 9 (26%) patients addressed, on 8–14 days — 5 (15%), on 15–30 days — 4 (12%) and 16 (47%) arrived at a later date. The complaint in 100% was the presence of itching and in 50% of erythema, which was accompanied by subjective sensations (burning sensation or compaction — 17 (50%), increase — 9 (26%) patients. In 3 (8%) patients complications with the defeat of the musculoskeletal system (rheumatoid arthritis) were revealed. Serological diagnosis (ELISA) was performed in 17 (50%) patients. Antibodies were found in 14 (41%), IgM levels ranged from 0.470 to 0.633. Terms of appearance were different (15–43 days). In 3 (9%) people the level was below normal. The remaining half of the patients were not examined for various reasons. Clinical and electrocardiographic manifestations of dysfunction of the circulatory system were noted in non-erythematous form (50%).

Serological diagnosis of tick-borne borreliosis by ELISA, due to the late appearance of antibodies in the early stages of little informative, which necessitates the introduction of modern rapid methods. Patients with non-erythematous form of tick-borne borreliosis require more attention and detailed laboratory and instrumental diagnosis, as there is a risk of complications from vital organs and systems.

4.4 doi: 10.15789/2220-7619-2018-4-4.4

DETECTION OF GENETIC MARKERS OF TICK-BORNE RICKETTSIOSIS WITH THE PCR

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There are seven species of pathogenic rickettsia belong to the spotted fever group were reported to be circulating in Russian: *R. conorii, R. sibirica, R. heilongjiangensis, R. slovaca, R. aeschlimannii, R. helvetica* and *R. raoultii*. But only first three of them were reported to cause the confirmed diseases in our country. The regions that are endemic for *R. sibirica* and *R. heilongjiangensis* are: coast of Primorsky Krai, the Amur River, coast of lake Baikal, the Reserve Krasnoyarsk Stolby, Altai, Khakassia. Crimean peninsula was reported to be endemic by *R. conorii*. All of these regions are the popular places for tourism. Real-time PCR test system "RealBest DNA Rickettsia species" (AO "Vector-Best", Novosibirsk) was developed to detect the DNA markers of the pathogenic rickettsia in clinical specimens. It also can be used for detection of rickettsia in ticks without defining the species. Using the developed test system, more than 7000 tick from 10 regions of Russia were tested. The percentage of rickettsia-infected ticks varied from 7 to 92% in dependence of region and tick's species. After the sequencing of DNA of positive samples in regions of genes *gltA, ompA, ompB* and *sca4* it was determined that there are 11 rickettsia species are circulating with 7 of them that are pathogenic: *R. sibirica, R. heilongjiangensis, R. conorii, R. slovaca, R. aeschlimannii, R. massilae* and *R. mongolotimonae*.

Also using the developed test system the DNA-markers of *R. sibirica* and *R. heilongjiangensis* were determined in the clinical samples (blood samples, urine, swabs of skin eschar, eschar biopsy) derived from patients that were hospitalized in the Far East, Western and Eastern Siberia with the diagnosis "tick-borne rickettsiosis". With the goal of the ability of determination of these two pathogens, the PCR test system "RealBest DNA Rickettsia sibirica/Rickettsia heilongjiangensis" was developed additionally. Using this test system it was found, that the frequency of presence of *R. heilongjiangensis* in tick varies from 0.7 for *I. persulcatus* to 29% for *H. oncinna*. The occurrence of the *R. sibirica* varies from 0.6 for *D. silvarum* up to 17% в *D. nuttalli*. It was proven that both of the developed PCR test systems can be successfully used for determination of circulating pathogenic rickettsia in natural foci, detection of their DNA markers in ticks for the diagnosis of the tick bitten people, as well as for analysis of clinical samples in the laboratory diagnostics tick-borne rickettsiosis.

4.5 doi: 10.15789/2220-7619-2018-4-4.5

DATABASE OF LEPTOSPIRA PROTEIN SPECTRA FOR MASS-SPECTROMETRY IDENTIFICATION

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Leptospirosis is found all over the world both in humans and in many species of agricultural, domestic and wild animals. The disease caused by individual serovars of the pathogen is characterized by a severe clinic and high mortality. *Leptospira* grow very slowly and only on special nutrient media. Together with the difficult pathogen isolation there is also the problem of its identification. According to the modern genosystematics several molecular biology methods were proposed to determine the *Leptospira* species. Mass-spectrometry direct profiling of proteins is easy to set up and widely used to diagnose most bacterial infections, while the available databases of *Leptospira* spectra are absent.

The aim of this study was the development of a protein spectra database for identification of the *Leptospira* species.

Our database contains information about 28 *Leptospira* reference strains of 28 serovars including eight most common species *L. interrogans*, *L. borgpetersenii, L. kirschneri, L. santarosai, L. noguchii, L. inadai, L. weilii, L. biflexa*, as well as the protein spectra of these strains in the format for the software "MALDI Biotyper 3.0". According to the serological classification the presented strains belong to 21 serogroups: *Icterohaemorrhagiae, Grippotyphosa, Canicola, Pomona, Tarassovi, Australis, Sejroe, Autumnalis, Bataviae, Ballum, Pyrogenes, Javanica, Hebdomadis, Louisiana, Panama, Lyme, Sarmin, Djasiman, Mini, Manhao, Sema-*

ranga. The database can be updated and edited. The protein spectra files can be entered into "MALDI Biotyper 3.0" and newer versions database by creating reference spectra and importing them into the Taxonomic Tree.

Protein spectra were obtained at 10–20-fold study of the samples extracted with acetonitrile/formic acid on a mass spectrometer "Microflex LT" (Bruker Daltonics, Germany) using a "Flex Control 3.3" program. The database was tested with eight strains isolated in Siberia and at the Far East in 2012–2016, and 18 *Leptospira* cultures from the collection of Gamaleya State Research Centre for Epidemiology and Microbiology. The results of identification of pathogenic *Leptospira* completely coincided with the data of multilocus sequencing. The created database is intended for specialists of microbiological and scientific laboratories engaged in diagnostics and study of leptospirosis.

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INFORMATION TECHNOLOGY APPLICATION FOR NATURAL FOCI INFECTIONS MONITORING AND PREDICTION

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Amur Region's climate-geographical features, flora and fauna specifics led to the emergence of persistent natural foci of several infections, including tick-borne encephalitis, hemorrhagic fever, tularemia, and listeriosis. Amur Region registers from 100 to 200 cases of natural foci infections every year. The largest share of these infections is tick-borne.

The Amur Region Rospotrebnadzor Service introduced in 2017 the Epidemiological Surveillance System (EpiS), creating one information space for the entire epidemiological service network in the region and ensuring connectivity with the primary healthcare organizations. EpiS enabled rapid collection and exchange of information, supports epidemiological investigations with accurate geo-location based on GLONASS/GPS coordinates and delivers outbreak early warning capabilities. The natural foci locations and Anthrax cases historical data for the past 180 years was converted into an electronic register of the territories and sites and embedded into the EpiS. It is planned to expand this registry for other natural foci infections.

Operational information on morbidity in conjunction with the historical information and other epidemiologically significant factors is displayed in the Emergency Operations Center (EOC) in near real-time on detailed regional maps. The EOC strengthens and supports current situation monitoring as well as in-depth epidemiological investigation and analysis of the situation.

In 2018 the Amur Region Rospotrebnadzor Service also introduced modern methods for predicting morbidity based on the deep neural networks technology. This forecasting method demonstrated its effectiveness for a number of infections, in particular, Influenza-like illness (ILI) and Acute Intestinal infections, due to the integrated consideration of historical data, socio-hygienic characteristics and environmental factors. The prognostic models are built on the entire available data archive and allow identifying stable patterns leading to a change in the morbidity dynamics. The prediction accuracy reached 85–90% for the ILI. The use of such forecasting methods allows strengthening preventive measures to combat infectious morbidity.

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ANALYSIS AND FORECASTING INCIDENCE OF BRUCELLOSIS IN THE REPUBLIC OF DAGESTAN

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From 300 to 500 cases of brucellosis among people are registered in Russian Federation annually. Sixty three percent of these cases occur in the North Caucasus Federal District (NCFD). Assessing the structure of the brucellosis incidences among the population of NCFD during 2005–2017 we found that 2122 reported cases (67%) occurred in the Republic of Dagestan.

The current situation calls for the need to improve methods of epidemiological diagnosis. To assess the epidemiological situation of brucellosis and to forecast the epidemic situation in the Republic of Dagestan for 2018 we analyzed the official statistics of brucellosis cases among people. The forecast for the number of cases was executed using two methods — the classical method of linear approximation and by the method developed by us, using the Wald's graph plot. The proposed method allows to determine the monthly minimum and maximum number of cases of brucellosis in the forthcoming period, therefore predicting the total minimum and maximum levels of morbidity.

According to the result of the statistical analysis, the threshold level of incidence of brucellosis disease (Mediterranean fever) in the Republic of Dagestan between 2005–2017 amounted to 14 cases. The average values of incidence of disease during long-term observations fluctuated between 12 and 20 cases during different seasons of the year, with the most cases occurring in June.

As a result of the conducted analysis, it was established that in the Republic of Dagestan in 2018, the monthly increase in the incidence level amounted to 1.6 new cases of brucellosis, the aggregate minimum prognosis of the incidence level is 10 new cases, while the maximum prognosis is 28 new cases. 123 cases are forecast in the Republic of Dagestan in 2018.

Therefore, according to the latest findings utilizing Wald's graph plot, it has been established that the brucellosis epidemiological situation in the Republic of Dagestan in 2018 is unstable. The exceedance of the threshold level points to the possible decline of the epidemiological situation. The suggested method of brucellosis epidemiological case forecast contributes to the optimization of the epidemiological process management, allows for the timely evaluation of the activity of the epidemic outbreak areas and prompt and swift decision making for the purposes of their localization and liquidation.

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THE DYNAMICS OF TICK VECTORS INFECTION RATE WITH IXODIC TICK BORRELIOSIS CAUSATIVE AGENTS IN KHABAROVSK REGION DURING THE EPIDEMIC SEASON OF 2017–2018

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Ixodic tick borreliosis are among the most prevalent illnesses in the group of tick-borne diseases in Russia.

The objective of the research was to perform a comparative evaluation of infestation rate of engorged Ixodic

ticks with *Borrelia burgdorferi* sensu lato *(B. burgdorferi* s.l.*)* and *Borrelia miyamotoi* in the Khabarovsk region during 2017–2018.

A total number of 1238 ixodic ticks were tested on the presence of the *B. burgdorferi* s.l. and 710 ticks were tested on the presence of *B. miyamotoi* DNA via Real-time PCR. Identification of the nucleic acids of the pathogens was performed using PCR kits "RealBest DNA B. miyamotoi", "RealBest DNA B. burgdorferi s.l." ("Vector-Best", Novosibirsk) according to the manufacturer's instructions.

The infestation rate of *Ixodes persulcatus* ticks (31.4±1.74%) with *B. burgdorferi* s.l. was statistically higher compared to *Dermacentor silvarum* (12.8±4.87%, $p < 0.05$) and *Haemaphysalis* spp. (16.6±2.86%, $p < 0.05$). No significant difference between infestation rates of different species of ticks with *B. miyamotoi* was found. During the start of the epidemic season was registered an elevation of vectors infestation rate with *B. burgdorferi* s.l. with a peak in July (33.1±4.33%, $p < 0.05$) followed by a consequent decline down to 11.8±5.53% ($p < 0.05$) in September. A decline in infestation rate of *B. miyamotoi* vectors from 10.4±2.63 to 5.8±1.46% ($p < 0.05$) was registered. From July to September the DNA of *B. miyamotoi* was not found. It is of importance that 14 ticks had a coinfection with *B. burgdorferi* and *B. miyamotoi* in 2017–2018. The Ct DNA value of *B. burgdorferi* s.l. was higher in most of the cases compared with Ct DNA value of *B. miyamotoi*.

The infestation rate of Ixodic ticks with *B. miyamotoi* was significantly lower compared to *B. burgdorferi* s.l. in Khabarovsk Region. The obtained results imply that during the start of the epidemic season (April-May) the risk of exposure of the population to *B. miyamotoi* is higher compared to summer-autumn period. Thus, it is important to study the competition between the pathogens in ticks and its value on the manifestation of the diseases in humans.

4.9 doi: 10.15789/2220-7619-2018-4-4.9

PATHOGENS, PESTICIDE RESISTANCE AND GENETIC DIVERSITY OF HUMAN HEAD LICE

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Pediculosis capitis or head louse infestation is the most prevalent parasitic infestation of humans. It is commonly perceived as an age-dependent rite of passage, and as an embarrassing social nuisance; however, head louse outbreaks do not raise any substantial public health concerns due to their assumed low capacity for transmitting the louse-borne pathogens associated with *Pediculus humanus humanus*, the human body louse. The purpose of this study was to screen head lice, *Pediculus humanus capitis* from Georgia, USA and Madagascar for *Bartonella quintana* and *Acinetobacter* sp. to determine the risk of exposure of rural populations with different levels of economic development to these pathogens. Other aims were to examine these lice for the occurrence of genetic markers for permethrin resistance using restriction fragment length polymorphism (RFLP/PCR) analysis and to evaluate the genetic structure of these head louse populations using microsatellite typing. The *kdr* permethrin resistance biomarker for the T917I mutation was detected by RFLP/PCR in 99.9 and 70% of lice from Georgia and Madagascar, respectively. *Bartonella* DNA was detected at similar levels in both set of samples (10.3 and 12.6%), while *Acinetobacter* sp. DNA was detected more frequently in Georgia lice (80.8%) than in Malagasy lice (42.1%). Microsatellite typing based on 3 sites revealed significant genetic heterogeneity among the lice tested, although head lice from Georgia were separated in 2 closely related clusters, while Malagasy lice exhibited more genetic diversity using Principal Component Analysis and Bayesian clustering. The results provide the first information regarding these combined characteristics of head louse infestations at these locations and can be used as a baseline for temporal surveillance of changes in circulating head louse populations, for monitoring louse susceptibility to permethrin-based pediculicides, and to track potential exposures and outbreaks due to louse-borne pathogens.

4.10 doi: 10.15789/2220-7619-2018-4-4.10

WHOLE GENOME-BASED CHARACTERIZATION OF *COXIELLA BURNETII* STRAINS ISOLATED IN RUSSIAN FEDERATION

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Information on the whole genome structure of the *Coxiella burnetii* strains circulating in Russian Federation (RF) is currently unavailable. The identification and study of differential-significant genetic markers of *C. burnetii* that are important from the epidemiological point of view remains a priority, including determining the hostal specificity.

The aim was whole genome-based characterization of *C. burnetii* strains isolated from different hosts.

We performed whole genome sequencing of four *C. burnetii* strains isolated from two host types (the human and the arthropods) in RF using the MiSeq technology (Illumina, USA). Genome assembling and alignement using Dugway 5J108-111 as the reference was performed with SPADes 3.9.0 genome assembler. Comparative analysis of the whole genomes of Russian strains was carried out between the investigated genomes and the whole genomes sequences of *C. burnetii* available in the NCBI database.

It was confirmed the conception of closed pangenome of species *C. burnetii* characterized by a low intraspecies genetic diversity, including the population circulating in the territory of RF. However, in Russian strains of *C. burnetii* unlike foreign strains the analysis of the variable part of the genome and the composition of unique genes revealed deletions of a part of them, which allows us to speak about their unique genomotype. Analysis showed pronounced clustering within a group of Russian strains by host type, the differences between genomes within clusters were minor. Comparing the number of deleted genome fragments it was found that surprisingly strains from arthropods had a significantly greater genome reduction compared with strains from human. These data are in contrast with the conclusions of a number of authors that the genomic reduction of *C. burnetii* strains isolated from arthropods is limited.

Thus, this corpus of data allow us to characterize the genomes of *C. burnetii* strains isolated in the territory of RF and to make assumptions about the hostal specificity of this pathogen, prompting further studies of its mechanisms.

4.11 doi: 10.15789/2220-7619-2018-4-4.11

INTERNATIONAL COLLABORATIVE PROJECT ON TICK-BORNE ENCEPHALITIS IN THE BARENTS REGION

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An international joint project on the surveillance of tick-borne encephalitis (TBE) in the Barents region was implemented in Norway and in NW Russia.

The project objective was to analyze hard ticks in endemic, non-endemic and borderline endemic areas within the Barents region, to verify the range of Ixodidae occurrence, and to define the northern limit of tick-borne encephalitis virus (TBEV) distribution.

Ticks were flagged in 2014–2015 (May-June) at several sites in Norway: from 58°N (Mandal) to 65°N (Brønnøysund), and in Russia: from 57°N (Pskov) to 64°N (Zachapino, the Arkhangelsk Oblast). TBEV was detected by real-time PCR.

Ticks collected in Russia were mostly *I. perculcatus*, while all those in Norway were *I. ricinus*. Each tick was studied individually. TBEV detected in Russian samples belonged to Siberian genotype, while in Norwegian samples it was only European genotype. TBEV prevalence in ticks collected in Russia was: 0.5% in St. Petersburg and in the Leningrad Oblast, 1.3% in the Pskov Oblast, 3.9% in the Arkhangelsk Region, 4.4% in Karelia.

In Russia fifty years ago scanty TBE cases were reported only in the south of the area under study, but now TBE is registered in most of districts, including the north of Arkhangelsk Oblast. In Norway TBE cases in humans are currently reported only in the south, however, TBEV is detected in questing ticks up to Brønnøy county. This northward shift of TBE in the northern Europe is a serious challenge to public health care.

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IS THERE A TRANSOVARIAL TRANSMISSION OF TAIGA TICK *(IXODES PERSULCATUS Sch.)* AND THE SHEEP TICK *(IXODES RICINUS (L.))* THE CAUSATIVE AGENT OF IXODID TICK-BORNE BORRELIOSIS *(BORRELIA BURGDORFERI* s.l.*)*?

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Ixodes ricinus (L.) and *Ixodes persulcatus Sch.* (Acari: Ixodidae) — the main vectors of pathogens of tick-borne borreliosis of humans. Transovarial transmission of the pathogen from the infected female to the eggs can serve as a mechanism for the vertical transmission of *Borrelia* to new generations of ticks in nature. At present, this issue has not been finally resolved, although it is generally believed that the transovarial transmission of *Borrelia* has no appreciable significance in maintaining their circulation and forming the level of infestation of adult ticks of the following generations (Korenberg et al., 2013).

Collected in May 2018 in natural biotopes of the Leningrad Region, adult females and males of taiga and sheep ticks were planted on rabbits for feeding. Of the 20 females of each species, 15 females of *I. persulcatus* and 17 females of *I. ricinus* were feed in June. In July, 15 females of *I. persulcatus* and 13 females of *I. ricinus* laid eggs. Determination of the presence of *B. burgdorferi* sensu lato complex DNA in females and samples of their clutches was carried out using the PCR method with hybridization-fluorescent detection in real time using a commercial set of AmpliSens (Interlabservis, Russia).The amplification was performed on a Quantcudio 3 thermocycler (Applied Biosystems, USA) A positive response to *B. burgdorferi* was found in 5 (38.5%) of *I. ricinus* females and 7 (46.7%) of *I. persulcatus* females. No laying eggs positive reaction did not. According to our results, transvarial transmission of *B. burgdorferi* sensu lato in *I. persulcatus* and *I. ricinus* is absent.

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FEATURES OF *BACILLUS ANTHRACIS* IDENTIFICATION BY MALDI-TOF MS

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Identification of *B. anthracis* is an important stage in laboratory diagnosis of anthrax, a dangerous infectious disease of humans and animals. The application of sensitive and rapid method of MALDI-TOF MS for this purpose is difficult owing to considerable homology of protein spectra of *B. anthracis* and closely related saprophytes of the genus *Bacillus*. It requires creation of databases of reference mass spectra of various representatives of the given genus and development of algorithms of their analysis.

The aim of the work was to develop a technical approach for reliable identification of *B. anthracis* with using MALDI-TOF MS.

We used 72 strains of saprophytes of the genus *Bacillus*, including strains belonging to the group *Bacillus cereus*, and 37 strains of the causative agent of anthrax, differing in their biological properties. To prevent spore formation and eliminate signals of spore proteins from spectra under study, cultures were reinoculated twice. Samples were prepared by lysis of 18-hour cultures and extraction of acid-soluble proteins by 80% TFA with the subsequent ultra-micro-centrifuge filtration. Collection of spectra

and their analysis were carried out using Microflex LT (Brucker) and its programs v. 3.3.64 and v. 3.3.65.

At the first stage we created 2 databases of mass spectra of reference strains: 1) saprophytes of the genus Bacillus and 2) strains of *B. anthracis*. When carrying out "blind" tests we revealed that fragments of peptide complexes over the range 2–12 000 Da in all representatives of both groups practically did not differ because of high degree of affinity. Thus, strains of closely related saprophytes were identified as *B. anthracis* and strains with high indicator SV on the contrary as saprophytes. When all spectra of cultures of both groups were pooled, identification became more correct, allowing to obtain the highest values of SV for strains of one species. The most optimum results of specific identification were obtained when identification of cultures was carried out using the program MALDI Biotyper RIC and construction of MsP-dendrogram was carried out using the program FlexAnalysis. In obtained dendrograms samples under study were clearly clustered with one of bacilli species represented in the base.

Thus, perfection of the scheme of reliable identification of *B. anthracis,* including accurate differentiation from closely related bacilli on the basis of MALDI TOF MS continues to remain urgent.

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SEARCH FOR SPECIES-SPECIFIC MARKERS FOR *BACILLUS ANTHRACIS* BY MALDI-TOF MASS SPECTROMETRY

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The use of the sensitive and rapid method of MALDI-TOF MS for identification of cultures of the causative agent of anthrax requires not only strict specificity, but also universality for all strains irrespective of their intraspecific variability of phenotypic properties.

The aim of the work was to reveal species-specific signals, common to all *B. anthracis* strains with various complexes of phenotypic properties.

We used 37 strains which included strains atypical in capsule formation, toxin production, nutritional requirements, activities of protease, lecithinase and hemolysins, ability to hydrolyze carbohydrates, as well as strains with different MLVA- and SNP-genotypes. Samples were prepared by lysis of 18-hour cultures in 80% TFA followed by ultra-micro-centrifuge filtration. The studies were carried out using Microflex LT instrument (Bruker). Collection of mass spectra and analysis of data were carried out using the programs v. 3.3.64 and v. 3.3.65. Analysis of spectra for frequency of signals was carried out using the program Microbe MS.

The occurrence of various combinations of phenotypic properties made it possible to discriminate 11 phenotypes. Individual spectra of each of these phenotypes (20 spectra of each strain) were analyzed and peak frequency was determined. For the further analysis we used peaks occurring at the frequency ≥ 95%, with their numbers in various groups varying from 2 to 32.

When comparing the peak frequency of all the 11 phenotypic groups we revealed the absence of common peaks with the frequency ≥ 95%. The distribution of signals which were identified in all the groups most often were as follows: 2601 Da — 82.2%; 4367 Da — 81.7%; 4666 Da — 76.4%; 6445 Da — 73.8%; 5206 Da — 72.8%. Earlier these peaks were not considered as specific markers of *B. anthracis*. The approach to choose markers we used when analyzing strains with a great number of phenotypic groups, including rare strains, may account for this. Markers of the system of ribosomal proteins, SASP and histone proteins, earlier described as species-specific markers, also occur in the spectra of strains from various groups, but at much lower frequency, and that may be connected with the production of various proteins or with various levels of their expression.

Thus, selection of species-specific peaks for identification of *B. anthracis* strains should be carried out taking into account the variability of their biological properties.

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ETIOLOGICAL CHARACTERISTICS OF MALARIA AND PREVALENCE OF HEMOGLOBINOPATHIES IN PATIENTS IN THE REPUBLIC OF GUINEA

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According to WHO, in 2016, malaria affected 216 million people in 91 countries, which is 5 million more than in 2015. The number of deaths from malaria in 2016 was 445 000 people. 90% of cases and 91% of deaths from malaria was from Africa.

There are more than 50 different types of hereditary hemoglobinopathies. They are most often found in regions with a tropical and subtropical climate, which correspond to geographic regions endemic for malaria.

The aim of our study was to determine the etiological structure of malaria and to assess the prevalence and variants of hemoglobinopathies in patients with malaria in the territory of the subrefecture Fria of the Republic of Guinea.

The study included 300 cases of malaria aged 0 to 70 years, from the hospital "RUSAL FRIGUIA" in town Fria from May to December 2017. Malaria was determine by a rapid test for the differentiated determination of antigen *P. falciparum* and pan-malarial antigen, with verification and validation of parasitemia by the method of thick drop and smear. The species belonging to the plasmodium was confirmed by the PCR method followed by sequencing. The type of hemoglobin was determined by method of electrophoresis.

The average age of patients was 15.8 years (from 1 month to 65 years), men — 53%. In 99% cases causative agent was *P. falciparum*, with parasitemia from 16 to 20 000 tr/μL. Hemoglobinopathy revealed in 20% of patients, first of all, sickle-cell anemia (85%). Lethal outcome was registered in 11 patients at the age from 2 to 14 years.

High parasitemia was associated with a more severe course of the disease. In patients with concomitant hemoglobinopathy revealed a less severe clinical course of malaria, characterized by relatively small parasitemia.

100% dominance of *P. falciparum* in patients with malaria in this region defines clinical vigilance regarding the severity of the course and the prognosis of the disease. Identifying concomitant hemoglobinopathies allow us to predict a favorable prognosis of malaria.

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PECULIARITIES OF MASS SPECTROMETRIC ANALYSIS OF BRUCELLA S- AND L-FORMS

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The causative agent of brucellosis, like many bacteria, is able to transform from S- and R- forms into L-form under the influence of various factors changing its biological

properties. Sometimes it is not diagnosed by the available certified diagnostic preparations and test systems. In this regard, it is urgent to develop an effective method for identifying the pathogen using MALDI-TOF MS.

The aim is to study peculiarities of protein profiles of *Brucella* S- and L-forms using mass spectrometric analysis.

The following *Brucella* strains of S- and L-forms were used in this study: *B. abortus* 544, *B. melitensis* 16 M, *B. suis* 1330, *B. abortus* I-206 of S- and L-forms, L-form of *Brucella* I-6, and L-form of *Brucella* I-7 from the collection of microorganisms of Irkutsk Antiplague Research Institute. Cultures were grown on Albimi agar at 37°C for 48 hours. Extraction was carried out with 70% formic acid followed by the addition of acetonitrile according to the "Instruction for Sample Preparation and Subsequent Mass Spectrometric Analysis of Pathogens of 1–3 Risk Groups" (Irkutsk, 2011). The spectra were collected using MicroFLEX mass spectrometer (Bruker Daltonics, Germany).

In the absence of *Brucella* spp. protein profiles in the database, identification of the pathogen did not provide reliable results. Therefore, during the first stage of the work the protein profiles of the following reference strains were added to the database: *B. abortus* 544, *B. melitensis* 16 M, and *B. suis* 1330. Thereafter the mass spectrometric study of the other representatives of these three species allowed achieving the reliable identification to the species level except L-forms of *B. abortus* I-206. After the introduction of *B. abortus* I-206 in L-form into the database, it became possible to identify L-forms of this species, in particular L-forms of *Brucella* I-6 and *Brucella* I-7.

Based on the results, it can be assumed that L- and S-forms of the same species differ significantly in protein profiles. Thus, we can recommend mass spectrometry with matrix-activated laser desorption/ionization for the accelerated identification of *Brucella*. For effective application of the method, it is necessary to create a representative electronic database of mass spectra of collection *Brucella* strains in both S- and L-forms.

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COXIELLA BURNETII PREVALENCE IN TICKS IN THE ULYANOVSK REGION

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Q fever is on record in the Ulyanovsk Region, and 3 cases were reported in 2013–2017. (average annual incidence rate over the period is only 0.08 per 100 000), but in fact the spread of Q fever is much higher judging by the results of seroprevalence survey in some districts where the antibodies to *Coxiella burnetii* were detected in 3.7% of healthy population. T*he role of ticks* in the direct transmission of *C. burnetii* to humans is small, however, being important participants of the pathogen circulation in natural and mixed foci of the infection they pose a real threat to animals, including agricultural, that contribute much to Q fever outbreaks in humans. Hence, monitoring of *C. burnetii* in ticks is essential for Q fever prevention.

The study objective was to assess the *C. burnetii* prevalence in ticks and to conduct subsequent genetic analysis of PCR products.

709 adult ticks (*Ixodes ricinus, Dermacentor marginatus*, *D. pictus*, *D. reticulatus*) were flagged from vegetation in forest and forest-meadow sites in some districts of the Ulyanovsk Region, and examined individually using standard PCR with the genus-specific primers flanking the 16S ribosomal RNA gene site. For PCR-positive results the amplicons were sequenced.

Genetic markers of *C. burnetii* DNA were detected in 5 ticks (*I. ricinus, D. marginatus, D. reticulatus)* from the Cilninsky, Ulyanovsky, Melekessky, Kuzovatovsky and Novospassky districts. The homology of the nucleotide sequence of the 16S rRNA gene of four PCR products was 99% as compared to the reference Nine Mile strain, while for one of them it was only 95%, that justifies the need to further study the heterogeneity of the microorganisms of the genus *Coxiella*. One *D. marginatus* (Novospassky district) was possibly infected with a less-investigated coxiella-like microorganism.

The existence of natural foci of Q fever was confirmed in 5 districts of the Ulyanovsk region. The genetic heterogeneity of *C. burnetii* circulating in the region was shown for the first time. The advisability of further study on the heterogeneity of microorganisms of the genus *Coxiella* is argued.

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WHOLE GENOME-BASED PHYLOGENETIC DIVERSITY AND GENOMIC EPIDEMIOLOGY OF LEPTOSPIRA

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Leptospirosis is an emerging zoonotic disease caused by pathogenic *Leptospira* strains. Each year, there is an estimated 1 million severe cases of leptospirosis and nearly 60 000 deaths worldwide. The genus *Leptospira* is highly heterogeneous and currently consists of 23 species and more than 300 serovars that can be isolated from diverse ecological niches and animal reservoirs. According to their phylogeny, *Leptospira* species are distributed in 3 groups: the pathogens, the intermediate species, which cause a milder disease, and the saprophytes, which do not cause disease in human nor animals.

Different serological and molecular typing methods have been used to study the epidemiology of *Leptospira*, but they are performed by few reference laboratories and usually designed for the most commonly found pathogens. Since the first complete *Leptospira* genome sequence was published in 2003, it is now possible to sequence bacterial genomes in a few hours at reduced cost. Whole-genome sequencing (WGS) has emerged today as an ultimate tool for both the identification of relevant genetic variations linked to virulence and for bacterial strain typing.

In this study, the taxonomic status of all species of the genus *Leptospira,* as well as 81 strains isolated from the natural environment across a wide geographic range, was evaluated by comparative genomics. Our results reveal that the genus *Leptospira* now contains 65 named species, including species from a new sub-lineage. Our findings show that the genus has a large and open pan-genome which further confirms the complexity of this genus. The availability of whole-genome sequences of *Leptospira* also allowed us to develop a core genome MLST (cgMLST) scheme targeting the entire genus of *Leptospira*. Our cgMLST represents a standardized, accurate, highly discriminatory, and reproducible method for differentiation among *Leptospira* isolates, allowing for comparison of and sharing typing results among laboratories worldwide.

This study will advance many aspects of the leptospirosis field including epidemiology, diagnostics, and basic knowledge including species diversity, evolution, ecology, and virulence.

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MONITORING VECTORS OF TICK-BORNE BORRELIOSIS, HUMAN GRANULOCYTIC ANAPLASMOSIS, HUMAN MONOCYTIC EHRLICHIOSIS IN STAVROPOL REGION IN 2017

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The epidemiological situation of tick-borne borreliosis in Stavropol region continues to be a unfavourable. Infection of humans occurs not only in natural centers of the infection, but also in outlying settlements, mostly in parks, squares and cemeteries.

The main vector of borreliosis are ticks belonging to the genus *Ixodes ricinus*.

The purpose of the study is research on ticks belonging to the genus *Ixodes ricinus* which transmit Ixodes tick-borne borreliosis, human granulocytic anaplasmosis (HGA) and human monocytic ehrlichiosis (HME).

Monitoring of infection of ticks infect with pathogens *(Borrelia burgdorferi, Ehrlichia chaffeensis* and *Anaplasma phagocytophilum*) in 2017 was carried out in 7 administrative territories of Stavropol region. The ticks were collected using a cloth drag-flag method (200 pools, 1037 specimens). PCR method has been used for detection of *Borrelia burgdorferi, Ehrlichia chaffeensis* and *Anaplasma phagocytophilum*.

Genetic material of the human pathogens such as *Borrelia burgdorferi* sensu lato has been found in 94% of the studied pools, *Anaplasma phagocytophilum* — 22.5%, *Ehrlichia chaffeensis* — 0.5%. Because the same genuses can transmit various infections, the mixed-infection method has been used. As a result of laboratory research, 20.5% of investigated pools the combination of pathogens tick-borne borreliosis and human granulocytic anaplasmosis (HGA), tick-borne borreliosis and human monocytic ehrlichiosis (HME) — 4.5%, tick-borne borreliosis, human monocytic ehrlichiosis (HME) and human granulocytic anaplasmosis (HGA) — 0.5%.

In Stavropol region the mixed-infected ticks *Ixodes ricinus* pathogens (tick-borne borreliosis, human monocytic ehrlichiosis and human granulocytic anaplasmosis) have been revealed. Considering the high level of infection caused by infected ticks, there is a probability for people to get infected in natural biotopes.

In order to prevent the emergence and spread of the infections, it's necessary to continue monitoring the infection and carry out measures for unspecified prevention.

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VARIABILITY OF THE LEVEL OF INFLUENCE OF RED VOLE POPULATIONS BY *HANTAVIRUS PUUMALA* IN THE REPUBLIC OF TATARSTAN IN 2015–2017

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One of the most common natural focal infectious diseases in Russia and the Republic of Tatarstan is hemorrhagic fever with renal syndrome (HFRS). During the period 2013–2017 in the Russian Federation, 39237 cases of HFRS were registered in 8 federal districts of the Russian Federation, in 59 regions. The Volga Federal District accounted for 82.9% of all reported cases of HFRS in the country. The average intensive incidence rate was 15.9‰ people, including 16.5‰ in the Republic of Tatarstan.

The viruses that cause HFRS belong to the genus *Hantavirus*, the family *Bunyaviridae*. On the territory of Russia, serotypes of hantaviruses pathogenic for humans are recorded: *Puumala, Seoul, Amur, Hantaan, Dobrava*. In the European part of Russia the serotype *Puumala* (PUUV) prevails, the main carrier of which is the red vole *Myodes glareolus*. Infections of people occur in the habitats of the red vole, so to take measures to reduce the incidence of it is important to know which of the rodent populations are most infected with the virus PUUV.

The objectives of the study were to determine the level of infection of populations of red vole with the virus PUUV in areas of the Republic of Tatarstan with high incidence of HFRS and assess the variability in the level of infection in three years. The objective of the study was to identify the PUUV virus in individuals of the red vole from the populations of several regions of the Republic of Tatarstan in 2015–2017.

Small mouse-like rodents were caught in the spring and summer-autumn periods 2015–2017 in the areas of habitat of red vole near the settlements. In 2015–2016, five were surveyed, and in 2017 — seven districts of the Republic of Tatarstan. Isolation of total RNA from rodent lung tissue was performed using a Trizol reagent (Invitrogen, USA). For the synthesis of the viral cDNA, the reverse transcriptase Thermo Scientific RevertAid Reverse Transcriptase (Thermo Fisher Scientific, USA) was used in a standard procedure and the resulting cDNA served as the template for PCR. PCR products were sequenced using 3730 DNA Analyzer (ABI, USA). The nucleotide sequences of the PCR products were compared to sequences from the GenBank database using the BLAST program.

In total, during the period 2015–2017 years 369 specimens of the red vole were examined by the PCR method, viral RNA was detected in 59 (16.2%) samples. However, in the years mentioned, the infection of rodents varied. So, in 2015, of the 111 samples tested, a positive result was obtained in 29 (26.1%) samples, in 2016 out of 102 samples — in 6 (5.9%), and in 2017 out of 156 — in 24 (15.4%). By PCR in all positive samples, only PUUV viruses were detected, other hantavirus serotypes were not detected.

High rates contamination of rodents in 2015 were observed in Laishevsky, Nizhnekamsky, Almetyevsky, Zelenodolsky districts; in 2016 — in Nizhnekamsky and Almetyevsky; in 2017 — in Tukaevsky, Laishevsky and Mamadyshsky districts of Tatarstan.

The conducted studies evidence to the circulation of hantaviruses of serotype PUUV in populations of red vole on the territory of natural foci located in seven surveyed regions of the Republic of Tatarstan. The level of infection of the voles for three years showed a significant variability — from 5.9 to 26.1%, though overall remained high and averaged 16.2%.

Thus, with a large number of moused rodents and a high level of infection of the red vole with hantavirus *Puumala* in the regions of the Republic of Tatarstan, the unfavorable epidemiological situation of HFRS remains.

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EPIDEMIOLOGICAL SURVEILLANCE OVER TICK-BORNE VIRAL ENCEPHALITIS IN ARKHANGELSK REGION

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In the areas with naturally occurring hot spots, the incidence of tick-borne infections (hereinafter, TBIs) represents one of the challenging issues of medical, social and economic nature. An infection among TBI which is most impactful in Arkhangelsk Region is tick-borne viral encephalitis (hereinafter, TBE).

The study sees its purpose as contributing to better epidemiological surveillance over TBE on the regional level, and is designed to analyze the contamination levels among ixodic mites using the PCR method.

The statistical data forms reported to the federal level, as well as of the studies into the contamination levels among ixodic mites were used for the analysis.

The study involved the analysis of TBI incidence in Arkhangelsk Region over the period from 2005 to 2017. It was found that the TBI incidence was exceeding the average score for Russia every year during the period analyzed (2.8-fold in 2017). The minimum level of TBI incidence was registered in 2017 — 3.6 per 100 000 residents, while the maximum one in 2009 — 9.9 per 100 000 residents. In 2017, the incidence rate of ixodic tick borreliosis (hereinafter, ITB) was 2.0 per 100 000 residents, which is 2.3 times lower than in Russia (4.6 per 100 000 residents). The contamination of mites with tick-borne encephalitis virus varied, during the period analyzed, between 3.4 and 16.8%, the average rate being 7.4%.

For the purposes of epidemiological surveillance over TBIs in Arkhangelsk Region, the molecular genetic method (real-time PCR) has been in use since 2012. The PCR-based studies conducted in 2016 to 2018, have found that the TBIs contamination rates among the ticks occurring in natural biotopes and removed from people, were as follows: TBE — 3.8%, ITB — 26.1%, HME — 2.7%, and HGA (not detected).

The application of molecular genetic methods in studying the contamination levels among ticks has enabled an increase in the range of microorganisms detectable in biological material, and has led to better awareness of TBE, ITB, and HME contamination levels among ticks, which, in turn, is essential to quality epidemiological surveillance, TBIs prevalence risk assessment and forecasting, and preventive interventions planning.

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IMMUNOLOGICAL SCREENING OF LEPTOSPIROSIS IN DOGS IN ST. PETERSBURG

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Leptospirosis is an acute infectious disease classified with zoonoses. It is a ubiquitous disease with severe clinical course, and with high lethality exceeding 20% for some etiologic forms. In urban areas domestic dogs proved to be one of the main source of this infection. The epidemiological well-being of the urban environment depends largely on epizootic processes taking place in the population of those animals.

The study objective was to conduct immunological screening of leptospirosis in dogs in St. Petersburg in order to identify their epidemiological danger for city residents.

In 2012–2017 we examined sera sampled from 720 domestic dogs in St. Petersburg. A standard technique was applied to detect the antibodies to leptospirae in the microagglutination reaction with a set of reference strains of living leptospira belonging to 12 serological groups.

In 165 samples (22.9%) we detected specific antibodies to leptospirae belonging to 3 serological groups: *Icterohaemorrhagiae* (49.7%), *Canicola* (43.0%), *Grippotyphosa* (7.3%). 60.0% of samples contained antibodies at a titer of 1:200–1:400, while 40.0% of our samples contained antibodies at a titer of 1:800 and higher. The seropositive animals were male (69.7%) and female (30.3%). The dogs got infected in some pet relief areas, often in a city park or square (67.0%), when swimming in urban and suburban water bodies (15.0%), or in unknown places (18.0%). The tendency towards the prevalence of *Icterohaemorrhagiae* serogroup in dogs' leptospirosis etiology, revealed at the turn of the century, is found to persist. The presence of antibodies to leptospira in the sera of domestic animals points to their infection with this pathogen and therefore to the existence of potentially dangerous source of this disease in St. Petersburg.

Active anthropurgic foci of leptospirosis exist within the limits of St. Petersburg city in the immediate environment of citizens, and domestic dogs are one of the sources of this infection. What gives rise to concerns is the fact that dogs are infected with *Icterohaemorrhagiae* and *Canicola* leptospira serogroups, that cause the most serious diseases in humans.

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A SURVEY ON CASES OF TICK BORNE ENCEPHALITIS IN ST. PETERSBURG

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The study objective was to bring to light the current environmental and epidemiological specificity of tick borne encephalitis (TBE) in St. Petersburg.

We analyzed the data on TBE incidence in St Petersburg published in "Data on infectious and parasitic diseases" (State Statistical Reporting, Form #2), FGBUZ "Federal Centre of Hygiene and Epidemiology of the Rospotrebnadzor", and those reported by Parasitology Department of FBUZ "Centre of hygiene and epidemiology in St. Petersburg" in 1996–2016.

It is found that people in St.Petersburg are at risk of exposure to tick bites, and of TBE infection acquire not only outside of the city, but also in the territory of their megapolis. Every year about 1000 humans are bitten by ticks in St. Petersburg.

I. persulcatus and *I. ricinus* are two main vectors of TBE virus, and the former dominates. The average TBE virus prevalence in flagged ticks is 0.61%.

There is a rise in number of medical care encounters related to tick bites in St. Petersburg. Thus, the tick-bite incidence rate (number of cases per 100 000 of inhabitants) increased from 141.9 in 1996–2002 to 288.9 in 2010–2016. Meanwhile, TBE incidence rate tends to go down both in St. Petersburg and countrywide. For instance, in St. Petersburg the average TBE incidence rate (number of cases per 100 000 of inhabitants) was 1.66 in 1996–2002, but dropped to 1.17 in 2010–2016. The maximal TBE incidence rate in St. Petersburg is reported in children (3–6 and 7–14 year old).

The absence of reported TBE cases in professionally-menaced groups of population testifies to the efficiency of preventive services among these contingents.

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COOPERATION OF ZOOLOGICAL GROUP AND THE PCR LABORATORY FOR EVALUATION OF EPIZOOTICS IN REPUBLIC OF BASHKORTOSTAN

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The aim of the study was to evaluate the work done to study the natural foci of tularemia in the Republic of Bashkortostan (RB).

The objectives of the study were to estimate the number of study district, the number of studies conducted, the nature of the samples studied, and the methods used.

Tularemia is a zooanthroponosis infection, characterized by the flood-marsh type of natural foci.

From 2014 to July 2018, 242 small mammals caught in the RB were examined for tularemia, of which two were infected. The first specimen was caught in the Krasnokamsky district in 2014, the second in the Gafuriysky district in 2018. These areas adjoin the natural focal point of tularemia in the city of Agidel, where in 2013, 5 cases of tularemia were reported.

Through the territory of the RB the Belaya River and its tributaries flows, therefore, in the years of active reproduction of small mammals, the dispersal of *F. tularensis* carriers along this watercourse is possible. In connection with this, the number of areas studied is also growing. In 2014 — 1 district, in 2015 — 5 districts, in 2016 — 14 districts, in 2017 — 5 districts.

Every year, the volume of conducted research, the types of investigated samples increased. Since 2016, were studied samples of water from open reservoirs, since 2017 — samples blood-sucking arthropods, and since 2018 samples of hydro fauna for research on tularemia. The total number of samples of environmental objects in 2014 was 45, in 2015 — 50, 2016 — 84, 2017 — 96, in 2018, 89 are planned.

Serologic methods (microreaction of agglutination, indirect haemagglutination reaction, inhibition of indirect haemagglutination) and PCR were used. It is planned to use the ELISA.

As the result of the study there was issued the order in the FBUZ "Center for Hygiene and Epidemiology in the Republic of Bashkortostan" about the ongoing monitoring of the epizootic condition of foci of tularemia in the area of Agidel city and the Krasnokamsky district.

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THE IMPACT OF GLOBAL CLIMATE CHANGE ON THE INCIDENCE OF TICK-BORNE ENCEPHALITIS IN THE EUROPEAN PART OF THE RUSSIAN ARCTIC

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The study objective was to estimate the impact of air temperature change on the incidence of tick-borne encephalitis (TBE) in the Arkhangelsk Region (AR) and in the Komi Republic (RK).

We analyzed TBE incidence rate (TBEIR) in RK in 1970–2017, and in AR in 1980–2017, its dependence both on the average annual air temperature and the local air temperature during the ixodid tick activity season, and satellite data on vegetation changes within the area under study.

In RK in 1970–1979, the average number of TBE cases per year was 1.4 (TBEIR was 0.1 per 100 000), while in 2008–2017 it was 15.2 (TBEIR was 1.8 per 100 000, i.e., 18 times higher than in 1970–1979). An even sharper rise in TBEIR was registered in AR. In 1980–1989 the average number of TBE cases per year was 1.6 (TBEIR was 0.1 per 100 000), while in 2008–2017 it was 64.4 (TBEIR was 5.4 per 100 000, i.e. 54 times higher than in 1980- 1989). A sharp rise in TBEIR in the Northern Europe is due both to the significant northward shift of TBE geographical distribution limits and to TBEIR significant growth in the southern districts. During the analyzed period both average annual temperature and the air temperature during the period of tick activity increased substantially. A strong correlation was revealed between the increase in the TBEIR and the rise in the air temperature. With the help of satellite technologies a pronounced growth of the vegetation index was detected.

The increase in TBEIR within the area under study was mostly due to the air temperature increment, especially during the period of tick activity. The increase in the local vegetation index bears witness to significant changes in the entire ecosystem under the influence of climate changes that provide more favorable conditions for increase in number of animal hosts of ixodid ticks, those being the main vectors of TBE virus.

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PATHOGENETIC TREATMENT OF SEVERE *P. FALCIPARUM* MALARIA: APPROACHES TO OPTIMIZATION

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Pathogenesis of malaria is associated with massive destruction of erythrocytes infected with plasmodium and a development of pathological reactions. One of the most severe clinical forms of malaria is the cerebral form, which is registered in almost 10% of all *P. falciparum* malaria cases. This is also the leading cause of death. The aim of this study was to optimize the pathogenetic treatment of severe *P. falciparum* malaria to prevent fatal outcomes.

During the years 2007–2016 44 patients (36 men and 8 women) with severe *P. falciparum* malaria aged 16 to 69 years old were treated in the intensive care unit of Moscow state Clinical Hospital No. 2. The verification of the diagnosis was based on clinical, epidemiological history and the results of blood smears. The severity of malaria in patients was mainly due to late hospitalization: between 5 to 10 days from the onset of the disease. On admission the level of blood parasites in patients was in the range from 2500 to 2 701 800 p/μl. The patients were treated in accordance with WHO recommendations (2006, 2010).

Ischemic damage of organs and hemorrhagic complications were prevented. In addition, a protocol of intensive care in patients with severe *P. falciparum* malaria was implemented: preventive extracorporeal hemocorrection methods were added without waiting for signs of uremia. This was carried out by a prolonged veno-venous hemodiafiltration procedure ("Prisma"), which resulted in the removal of a wide range of toxic and biologically active substances. This plasmapheresis procedure clears the plasma from fragments of dead parasites, toxic substances, and excessive amount of hemoglobin accumulated during hemolysis, thus reduces or prevents severe damage of the

kidneys. Suppression of malaria toxicity promotes a quicker restoring of an adequate immune response of the body.

This approach of intensive care with the preventive procedure of extracorporeal hemocorrection method led to a reduction in mortality from 84 to 6.8% in patients with severe forms of *P. falciparum* malaria.

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CURRENT PROBLEMS IN DIAGNOSTICS AND TREATMENT OF STRONGULOIDIASIS

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Long-term observations of patients with various helminthiases showed that strongyloidiasis remains one of the most problematic regarding diagnosis of the disease. It is difficult to make a differential diagnosis on the basis of clinical symptoms due to its polymorphism.

Diagnostic errors of strongyloidiasis were discovered in 38 enrolled patients. It should be noted that incorrect diagnosis led to a change in the nosological structure of the disease. Years ago strongyloidiasis was misdiagnosed with such pathological conditions as acute or chronic enteritis, pancreatitis, bile duct dyskinesia, eosinophilic pneumonia, food poisoning, food infection, typhoid paratyphoid and nontyphoid disease. In recent years, strongyloidiasis was taken for acute leukemia, malignant tumors, Whipple's syndrome and Crohn's disease. Other investigators (N.I. Tumolskaya et al., 2014) reported about "masks" of strongyloidiasis.

Despite a comprehensive approach to laboratory diagnostics of chronic strongyloidiasis according to guidelines, examination of stool specimens (baermann technique) and investigation of the duodenal contents are rarely implemented in clinical laboratories. Therefore parasitological diagnosis of helminthiasis often is established with a significant delay.

Ivermectin is currently the drug of choice in the treatment of strongyloidiasis. It is suitable for the treatment of acute, chronic and disseminated forms of the diseases. A Nobel Prize in 2015 was awarded for its discovery. In the Russian Federation, ivermectin is neither registered nor produced. An alternative drug albendazole is used with a daily dose of 400–800 mg 1–2 times for 3 days. Albendazol is a drug of foreign origin and is available all over the country. Its effectiveness is insufficient and in some cases repeated courses of treatment are required. With early diagnostics and treatment with effective anthelmintic drugs and adequate rehabilitation pathogenetic therapy, the prognosis is usually good, with the exception of immune compromised cases (HIV/AIDS, tuberculosis, non-specific inflammatory diseases, etc.).

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ANALYSIS OF POPULATIONS OF *BACILLUS ANTRACIS* STRAINS ON THE BASIS OF THEIR RESISTANCE TO SPECIFIC ANTHRAX BACTERIOPHAGES

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The specific anthrax bacteriophage lysis is a compulsory test in the scheme of identification of *B. anthracis* strains, however it does not enable us to estimate quantitatively the presence of phage resistant clones in the population of the strain.

The aim of the study was to investigate the population composition of two virulent strains of *B. anthracis* on the base of phage resistance to specific bacteriophages.

Spore suspensions of typical virulent *B. anthracis* strains 1 (SO) and 81/1 were used as suspensions in a 30% glycerin solution kept in sealed ampoules at 4–6°C for more than 20 years. Concentrations of phage corpuscles in experimental batches of bacteriophages Gamma A-26, BA-9, K-VIEV were, correspondingly, 8×10^9, 4×10^8 and 2×10^8 per 1 ml. Accurately 0.1 ml of a spore suspension in a concentration of 1×10^3 were applied to Hottinger's agar and spread over its surface. When the liquid was absorbed completely, one of the bacteriophage preparations was applied to test plates, moistening the whole surface of plates. Plates which were not treated with bacteriophage preparations served as controls.

Not a single colony grew on plates of both strains treated with bacteriophage Gamma A-26. Plates treated with bacteriophage K-VIEV showed a 2.9% growth of colonies of the control of the strain *B. anthracis* 1 (SO) and a 4.8% growth of colonies of the strain *B. anthracis* 81/1. Plates treated with phage BA-9 showed a 10.9% growth of colonies of the strain *B. anthracis* 1 (SO) and a 17.3% growth of colonies of the strain *B. anthracis* 81/1, correspondingly. For further determination of sensitivity to all the three bacteriophages we used 12 colonies of each strain, which showed resistance to phages K-VIEV and BA-9 at the first stage.

The retest showed that in both strains 16.7% of variants separated on the base of their resistance to bacteriophage BA-9 were sensitive to all the three bacteriophages. Among variants of the strain *B. anthracis* 81/1 which were selected from the plates treated with bacteriophage BA-9 such variants made up 20%, and in *B. anthracis* 1 (SO) — 10%. Among variants of both strains variants resistant to the action of bacteriophages Gamma A-26 and BA-9 and sensitive to bacteriophage K-VIEV were found. Among variants of *B. anthracis* 1 (SO) selected from cultures treated with bacteriophage BA-9, 80% were sensitive to bacteriophages BA-9 and Gamma A-26 and resistant to bacteriophage K-VIEV.

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COMPARATIVE CHARACTERIZATION OF SUBCULTURES ISOLATED FROM A POPULATION OF *BACILLUS ANTHRACIS* 1 (SO) STRAIN ON THE BASIS OF PHAGE RESISTANCE TO SOME SPECIFIC ANTHRAX BACTERIOPHAGES

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On the basis of many phenotypic properties the causative agent of the anthrax shows not only intraspecific variability between strains, but also intrapopulation variability in some strains.

The aim of the work was to study a complex of phenotypic properties and genetic characteristics of variants of the virulent strain *B. anthracis* 1 (SO) in the group of isolated on the basis of resistance to specific anthrax bacteriophages Gamma A-26, BA-9, K-VIEV and to carry out their comparative analysis.

We used the initial strain *B. anthracis* 1 (SO). Concentrations of phage corpuscles in experimental batches of bacteriophages Gamma A-26, BA-9, K-VIEV were, correspondingly, 8×10^9, 4×10^8 and 2×10^8 per 1 ml. The criterion for selection of cultural variants was their resistance to bacteriophages. Phenotypic properties and genetic characteristics of isolated subcultures were defined according to the Guidelines 4.2.2413-08.

After treatment of spore cultures on Hottinger's agar with each of the bacteriophages separately, incubation for 24 hours at 37°C, phage resistant cultural variants being distinguished from the initial typical strain by capsule formation and toxin production, hemolytic, proteolytic

and lecithinase activities, incapable of spore germination on the basal medium or on media with bicarbonate in conditions of increased CO_2 concentration were isolated.

In the group of subcultures resistant to bacteriophage K-VIEV 5 of the 8 subcultures were incapable of germination in the atmosphere of increased CO_2 concentration while among cultures of other groups there were no such strains. The group of 9 subcultures resistant to bacteriophage BA-9 included two cultures differing in their plasmid composition ($pXO1^-$, $pXO2^+$; $pXO1^-$, $pXO2^-$). All the 4 subcultures of the group resistant to bacteriophage Gamma A-26, had the genotypes differing from the initial strain and one of them also differed from the others of the group, exhibited low proteolytic activity, the absence of lysis of sheep erythrocytes and expressed ability to immunoprecipitation on a synthetic medium with anthrax γ-globulin.

Thus, in three groups of subcultures of the strain *B. anthracis* 1 (SO), which were isolated on the base of phage resistance to specific anthrax bacteriophages, we revealed not only variability of biological properties, but also peculiarities of phenotypic properties and genetic properties which are more characteristic of certain groups.

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MODERN DIRECTIONS IN OPTIMIZATION OF RABIES SURVEILLANCE

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Rabies is one of the oldest and most studied infections in both animals and humans. Despite the available opportunities for its specific prevention laid by L. Pasteur, it is still impossible to overcome rabies. Against the backdrop of a steady increase in the incidence of animal rabies, 191 cases of human infection have been reportedin Russiasince the beginning of the century. More than a thousand unfavorable rabies areashave been identified annually. At least 300 000 people on average seek for medical attention. Economic damage from animal bites amounts more than 3.5 billion rubles a year.

At the same time, recent scientific advances allow us to identify modern directions for the optimization of both epizootic and epidemiological surveillance of rabies. In view of the assessment of the current surveillance system in Russia, the main directions include the improvement of the information base, both epizootic and epidemiological diagnostics, as well as surveillance technology based on an integrated risk assessment and the introduction of molecular biological methods.

It is required to create one unifiedinformation resource that contains not only data on the incidence of both human and animal rabies, but also on the dynamics of epidemiologically significant risk factors. These factors include environmental, climatic and social conditions that contribute to the emergence and preservation of risks, as well as the biological characteristics of the pathogen. Thus, such modern resource allows to combine information collected by the participants of sanitary-epidemiological, medical, veterinary and other services. It will serve as a database to create a special geoinformation system in the future. The purpose of this system is the assessment of epizootic and epidemiological risks, as well as forecasting the situation of rabies on its basis. The importance of molecular diagnostics and monitoring also cannot be underestimated. The development ofnew diagnostic tests and scientifically based approach to monitoring organization contribute to enhancement of surveillance effectiveness.

5. YERSINIOSIS: TAXONOMY, PHYLOGEOGRAPHY, POLYMORPHISM OF PATHOGENICITY FACTORS AND SELECTIVE VIRULENCE

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YERSINIA PESTIS VOLE'S STRAINS: TAXONOMY, PHYLOGEOGRAPHY, POLYMORPHISMS OF PATHOGENICITY FACTORS AND SELECTIVE VIRULENCE

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Strains of *Yersinia pestis*, the causative agent of plague, can be divided into those possessing universal virulence and isolates conditionally pathogenic for guinea pigs and humans.

Purpose and objectives of our study were intraspecific classification, analysis of phylogeography, determination of polymorphism of pathogenicity factors and identification of selective virulence factors using postgenomic technologies.

More than 100 isolates from natural plague foci of the former USSR and Mongolia were studied with (i) DFR-, IS*100*-, CRISPR-, MLVA25-, and SNP-typing, (ii) sequencing of genes coding for several pathogenicity factors, (iii) 50 of the strains were underwent the whole genome sequencing, (iv) the whole genome sequences, mRNA or protein spectra synthesized at 37°C *in vitro* or in a dialysis chambers placed into guinea pig peritoneum cavities were compared in the bv. ulegeica strains subcultures differing in subcutaneous virulence for guinea pigs by more than five orders of magnitude, (v) knockout mutagenesis and subsequent complementation were used to assess the significance of identified potential selective virulence factors.

Y. pestis division into two subspecies differing in epidemiological significance is justified. DFR-, IS*100*-, CRISPR-, MLVA25-, and SNP-typing had very similar clustering ability. Polymorphism of the classic pathogenicity factors was not associated with selective virulence. One or several genotypes were isolated only in certain natural foci. Strain subcultures dramatically differing in their subcutaneous virulence for guinea pigs had identical nucleotide sequences in their genomes but fluctuated in mRNA and protein spectra. Most of the observed differences in the spectra of synthesized mRNA and proteins did not affect virulence for both mice and guinea pigs. According to our research, the main candidates for the role of selective virulence factors are methionine ABC transporter lipoprotein (WP_038931127.1) and class II fructose-bisphosphate aldolase (WP_002209962.1).

A rational variant of bringing the taxonomy of the plague microbe in accordance with the rules of the International Code of Bacterial Nomenclature and evolutionary taxonomy is proposed. Two new potential molecular targets for the prevention and treatment of plague are found.

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CREATING A SPECTRA LIBRARY FOR SPECIFIC MALDI-TOF MASS SPECTROMETRY IDENTIFICATION OF *YERSINIA ENTEROCOLITICA*-LIKE SPECIES

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Phenotypic identification of *Y. enterocolitica*-like species by biochemical tests is laborious and not always reliable. Among these species, identification of *Y. bercovieri*, *Y. mollaretii*, *Y. frederiksenii*, *Y. kristensenii*, *Y. intermedia* is a problem for clinical microbiologists, and usually misidentify them as *Y. enterocolitica*, an important food-borne pathogen. In last study, 1138 *Yersinia* strains isolated in Russia were analyzed by MALDI ToF mass spectrometry (MALDI ToF MS) using Microflex™ LT (Bruker Daltonics, Germany). We demonstrated the accurate MALDI ToF MS genus identification, and *Y. pseudotuberculosis* and *Y. enterocolitica* species identification (100% of strains).

However only 22.1% of strains 217 *Y. enterocolitica*-like species were further reliably identified to the species level (Score Value ≥ 2.3 correspond to highly probable species identification). Other *Y. enterocolitica*-like strains were misidentified as *Y. enterocolitica*.

The aim of this study was to create in-house spectra library for correct identifying *Y. enterocolitica*-like species.

MALDI ToF MS identification of the *Y. enterocolitica*-like species is limited by small number of their reference spectra available in the MALDI Biotyper database. The mass-spectra from 60 well-characterized *Y. enterocolitica*-like strains (53 were isolated in Russia, 7 are Institute Pasteur reference strains) were used to generate a project library: *Y. kristensenii* (n = 20), *Y. intermedia* (n = 15), *Y. frederiksenii* (n = 14), *Y. aleksiciae* (n = 5), *Y. bercovieri* (n = 4), *Y. mollaretii* (n = 2). To select strains, the obtained spectra of 263 *Y. enterocolitica*-like strains were used for cluster analysis. It was shown that *Y. intermedia*, *Y. frederiksenii*, *Y. kristensenii* spectra forms a few subclusters. So for library creation we selected the strains of spectra different subclusters.

For applying in-house spectra library additional verified strains *Y. intermedia* (n = 20), *Y. frederiksenii* (n = 15), *Y. kristensenii* (n = 20) and *Y. enterocolitica* (n = 20) were used. All strains were accurate identified to the species level with Score Values ≥ 2.3.

Thus the creating in-house spectra library allows to increase the specificity of *Y. enterocolitica*-like species MALDI ToF MS identification.

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EPIDEMIOLOGICAL AND CLINICAL PECULIARITIES OF PSEUDOTUBERCULOSIS OUTBREAKS

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Retrospective analysis of 29 pseudotuberculosis outbreaks in Siberia and at the Far East demonstrated the following patterns:

– outbreaks mainly belonged to short-term type I lasting no more than one incubation period;

– they were mostly registered in the children's organized groups (86,2%) including 12,0% cases in child-care facilities;
– they occurred more frequent in autumn-winter (46,2%) and spring (38,4%) periods of a year and were associated with consumption of vegetable salads in 72,4% cases;
– violation of sanitary-and-hygienic mode and technology of meal preparation from uncooked vegetables was noted in all outbreaks;
– laboratory confirmation of the diagnosis was set in 39,5% of the patients by PCR assay in the first days; bacteriological and serological diagnosis was confirmed in 14,1 and 64,6% after 2–3 weeks;
– *Yersinia pseudotuberculosis* was revealed in the population of synanthropic and wild small mammals in 52,9%, in transmission factor — in 46,7% from the total number of the studied outbreaks;
– all epidemic *Y. pseudotuberculosis* strains were O:1b serotype, possessed *ypm* gene of super-antigen, lacked of a high pathogenicity island (HPI), and belonged by plasmid content to single plasmid (pYV 47 MDa) and two-plasmid (pYV 47 MDa and pVM 82 MDa) strains with the identical frequencies;
– the peculiarity of clinical manifestation of pseudotuberculosis caused by *Y. pseudotuberculosis* with two-plasmid and chromosomal *tcpY1* gene (phagocytosis inhibitor) was the presence of the intoxication symptoms, fever, rash, damage of gastrointestinal tract, liver and joints with prevalence of medium-severe and severe course specific for Far Eastern scarlet-like fever (FESF);
– we discovered one more form of FESF clinical course caused by *Y. pseudotuberculosis* with *pYV* plasmid and lacking *tcpY1* gene. In this case all observed symptoms were poorly expressed, and pseudotuberculosis was developed in "minor" easier form mainly in children.

The revealed peculiarities of pseudotuberculosis outbreaks are necessary to take into consideration in epidemiological surveillance.

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INTRASPECIFIC DIVERSITY OF *YERSINIA PESTIS* CHAPERONE/USHER SECRETION APPARATUSES

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The Post-Antibiotic Era requires replacement of antibiotics with alternative antibacterials aimed at alternative molecular targets. One of such alternative approaches to treat infections are remedies targeting virulence. *Yersinia pestis* as many other Gram-negative bacterial pathogens use the chaperone/usher (CU) pathway to assemble virulence-associated surface fibers termed pili or fimbriae. *Y. pestis* has two well-characterized CU operons: the *caf* genes coding for the F1 capsule and the *psa* genes coding for the pH 6 antigen. There are eight additional CU secretion systems capable of assembling *Y. pestis* pilus fibers. When choosing new targets for effective treatment of infectious diseases, it is necessary to search for pathogenicity factors possessing structural conservatism, since polymorphism gives pathogens the opportunity to evade interaction with the drug.

Searches and comparisons of amino acid sequences of CU proteins from *Y. pestis* strains belonging to SNP-types 0.PE2, 0.PE3, 0.PE7, 0.PE4, 0.PE5, 1.ORI, 1.ANT, 2.ANT, and 2.MED were conducted using the databases of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) by MAUVE (http://darlinglab.org), BLAST (https://blast.ncbi.nlm.nih.gov), ProtParam tool (https://web.expasy.org/protparam), and protein sequence analysis (http://molbiol.ru/scripts/01_18.html). The usher genes for two of chaperone/usher pathways (*y1539-1544* and *y4060-4063*) were disrupted in all of the studied *Y. pestis* strains by an insertion sequence or premature stop codon, and thus these pathways are not expected to be functional. The phylogenetic-group-specific polymorphisms of amino acid sequences of the proteins from the *Y. pestis* CU secretion systems is inherent in five ushers (*y0562*, *y1858*, *y1871*, *y2390*, *y3480*), three molecular chaperone (*y2392, y3479, caf1M*) and three adhesin subunits (*caf1*, *y2388*, *y3478*). These polymorphic proteins are excluded from the list of potential *Y. pestis* molecular targets.

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THE OUTER MEMBRANE PROTEIN A (ompA) OF *YERSINIA PESTIS* IS NOT REQUIRED FOR VIRULENCE IN MICE AND RATS

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The plague bacterium *Yersinia pestis* has a number of well-described strategies to protect itself from the both cellular and humoral factors of the host's innate immunity. OmpA in several pathogens has been shown to mediate resistance to complement and antibacterial peptides, as well as play a role in invasion and intracellular survival. In this study, we sought to determine whether deletion of the *ompA* would render fully virulent *Y. pestis* strain attenuated in the mouse and rat models of plague.

Y. pestis Δ*ompA* mutant was constructed using the knockout mutagenesis. SDS-PAGE and Western blot analyses with anti-OmpA serum showed the absence of OmpA in *Y. pestis* Δ*ompA* cell lysates and outer membranes preparations. We could not detect any differences between *Y. pestis* wild type strains and their Δ*ompA* derivatives using a serum killing assay. The OmpA deficient mutants were 2 times less resistant to bactericidal action of polymyxin B as compared with the wild type strains. To assess the biological significance of OmpA in fully virulent *Y. pestis* strain *in vivo*, studies in a mouse and rat models of bubonic and pneumonic plague were performed. Inbred mice and rats were infected subcutaneously or intranasally to mimic bubonic or pneumonic plague and observed for 21 days. Comparative study of the virulence of *Y. pestis* mutant strains using subcutaneously and intranasally challenged mice and intranasally challenged rats did not reveal differences in their LD_{50}. The average survival time of mice and rats that succumbed to infection with the strain 231 or its isogenic derivative did not differ from each other. The estimated LD_{50} of the *ompA* mutant for subcutaneously challenged rats was approximately 10-fold higher than the LD_{50} of the wild type 231 strain.

The main outcome of our investigation is the finding that the loss of the ability to produce OmpA antigen did not influence virulence of Δ*ompA* mutant of *Y. pestis*. This argues against the usefulness of using OmpA as a molecular target for plague prophylaxis and therapy.

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GENETIC DETERMINANTS CHARACTERISTIC FOR *YERSINIA PSEUDOTUBERCULOSIS* STRAINS ISOLATED FROM PATIENTS WITH FAR-EAST SCARLETT-LIKE FEVER

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The illness caused by *Yersinia pseudotuberculosis*, which was firstly described in Russia (Vladivostok, 1959) and named Far East Scarlett-like Fever (FESLF), is manifested via fever, rash and injury of liver and joints. Our study was aimed to reveal phylogenetic relationships of the FESLF isolates with the *Y. pseudotuberculosis* population. Totally, 64 *Y. pseudotuberculosis* strains including 37 isolates from 6 FESLF outbreaks and 19 sporadic cases were used. A previously described MLST scheme was used to characterize clonal diversity. MLST analysis was extended by sequencing virulence genes *inv, yadA*, *yopE, cnf*. We found three MLST types among FESLF isolates: ST2 (n = 33), ST26 (n = 5), and ST32 (n = 3; specific for serotype O3). All but 1 vegetable isolate belonged to ST2, which was also found in 9 (60%) of 15 rodent isolates. ST2 prevailed among isolates from all sources. The ST2/ST26/ST32 sequence types formed a cluster at the eBURST scheme with ST2 and ST32 belonged to separating subclusters descended from ST26. Combining MLST with virulence gene sequence typing gave rise to 6 MVLST types. The concatenated sequences of 10 MVLST genes were used to build a maximum likelihood tree that divided into 2 subclades. One subclade united MVLSTs found in FESLF isolates and MVLST6, which was found in rodent isolates only. The second subclade united MVLSTs found in rodent and vegetable isolates. The analysis of virulence gene diversity revealed predominance of nonsynonymous substitutions among virulence genes, whereas basic parameters of nucleotide diversity were similar in virulence and housekeeping genes. Notably, unique *yopE* and *inv* alleles and a deletion of 946 bp in the *cnf* gene encoding cytonecrotizing toxin were found in all FESLF isolates independently on MLST type. The deletion in *cnf* resulted in a loss of the Rho-binding domain and toxin inactivation. The plasmid pYV was found in all strains. Additional plasmid pVM82 was found in all but 4 ST2 strains but not in other genotypes. The fact that full FESLF symptomatology is caused by several distinct genotypes supports the view that specific virulence traits are characteristic of FESLF-associated strains and suggests that the dominance of the ST2 genotype could be caused by its epidemiologic advantages rather than its pathogenic traits. This suggestion was supported by evolutionary analysis that rejected the hypothesis of equality of evolutionary rates for ST2 and other genotypes ($p < 0.05$).

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DYNAMICS OF MORBIDITY OF THE WEST NILE FEVER IN THE ASTRAKHAN REGION

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The variety and wide prevalence of arbovirus infections, the possibility of adverse outcomes determine the relevance of their study. In the territory of the Astrakhan region the epidemic focus of West Nile Fever is registered. The purpose of this study was to analyze the dynamics of the morbidity of the West Nile Fever in the Astrakhan region from 2014 to 2017. The analysis of "Data on infectious and parasitic diseases" (Form 1) in the Astrakhan region was carried out.

As our research has shown, West Nile Fever in the Astrakhan region is currently characterized by a low intensity of the epidemic process. 5 people in the Astrakhan region were affected by the West Nile Fever in 2014, the morbidity rate per 100 000 of the population was 0.5. The number of cases increased by 3.0 and 4.8 times respectively in 2015 and 2016. 15 people fell ill with West Nile Fever in 2015, and 24 people — in 2016. The mortality rate per 100 000 of the population was equal to 1.5 and 2.4 respectively. It should be noted that the number of people with West Nile Fever in Russian Federation as a whole increased by 1.5 and 4.9 times in 2015 and 2016, compared to 2014. The source of infection in West Nile Fever is mainly wild birds. The increase in the incidence rate in 2015 and 2016 in the Astrakhan region and in Russia may be associated with increased infection of migratory birds during their seasonal migration from the natural foci of West Nile Fever. Only one case of West Nile Fever was registered in the Astrakhan region in 2017, the mortality rate per 100 000 of the population decreased in 24 times compared to the previous year and amounted to 0.1. Children under the age of 14 years were 11.1% of all the patients with this arbovirus infection from 2014 to 2017.

Thus, the natural focus of the West Nile Fever remains in the Astrakhan region, which activity depends on both the sources of infection and its vectors influenced by the intensity of the epidemic process in endemic foci, seasonal migration of sources of infection and climatic conditions.

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POSSIBILITIES OF NON-INVASIVE METODS APPLICATION FOR DIAGNOSIS OF YERSINIOSIS IN CHILDREN

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The term "*Y. enterocolitica* and *Y. pseudotuberculosis* infections" or "yersiniosis" is applied for two infectious diseases caused by Yersinia. These are pseudotuberculosis and intestinal yersiniosis followed by intoxication, injuries to the gastrointestinal tract and multiple organ disorders in case of miscellaneous and multi-disorder disease types. According to course duration of the disease it is classified as an acute (lasts for one month), a protracted (no longer than 3 months) and a chronic form (longer than six months). Nowadays in the acute period and at relapse of the disease bacteriological and PCR methods are used for diagnosis. Sokolova and co-authors (2016) analysed data of the diagnosis procedure of infants with acute diarrhea treated in an infectious diseases unit and proved 3 times more of yersiniosis to be dected by the PCR technique than by using bacteriological tests. Thus, non-invasive PCR technique should be used more widely for acute form *Y. enterocolitica* and *Y. pseudotuberculosis* infections diagnosis.

During the winter rise in the incidence of yersiniosis we investigated the appendix tissue (n = 60) taken from a surgical unit in the acute period of the disease of the children and demonstrated 20% of positive results obtained by PCR (11 DNA of *Y. pseudotuberculosis* and 1 DNA of *Y. entero-*

colitica), only one *Y. pseudotuberculosis* strains (1.7%) was detected by the bacteriological method. The set of pathogenicity factors of this strain didn't prove to be usual for the strains isolated in the Russia which in most have the YPM genes (the superantigen *Y. pseudotuberculosis*-derived mitogen) and responsible for the typical pseudotuberculosis symptoms (rash, skin desquamation, red tongue). The obtained strain proved to have "high pathogenicity islands" (HPI) genes with ypm being absent. These "european" strains cause pseudotuberculosis with such symptoms as mesenteric lymphadenitis, acute appendicitis and gastrointestinal features. The traditional bacteriological technique proved to be effective both in theory and in practice.

Necessity of using both classical bacteriological and PCR methods thus proves to be important for understanding the symptoms yersiniosis.

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ONCE UPON A TIME IN THE FAR EAST: ON THE 50TH ANNIVERSARY OF THE DISCOVERY OF "REAL PSEUDOTUBERCULOSIS" AMONG HUMANS

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After the first description of pseudotuberculosis and its pathogen in 1884, the infection was for a long time considered a classic zoonosis. Single cases of human disease usually resulted in death. In the mid twentieth century W. Masshoff and W. Knapp (1953, 1954) described rare sporadic cases of the appendicitis caused by infection of mesenteric lymph nodes. However, the decisive turn in the discovery of the true face of human pseudotuberculosis occurred only after the unusual events in the Far East of the USSR. It all started with the fact that in Vladivostok in the spring of 1959 an infection affected more than 300 young sailors of one military unit. 200 people with a severe form of the disease were placed in the hospital. Epidemiologists believed that the cause of this outbreak could be massive foodborne infection.

The clinical findings of the disease were very polymorphic, which made diagnosis difficult. Most patients in the early days had mainly typical symptoms of scarlet fever, some — signs of hepatitis, others — appendicitis or arthritis. A new disease was called "Far Eastern scarlet fever — FESF" before the discovery of etiology (I. Grunin, G. Somov, I. Zalmover, 1960). Similar collective outbreaks began to be recorded annually in other regions of the Far East.

However, the nature of the infection remained a mystery for several years, until in 1966 the naval bacteriologist Vladimir Znamenskij did announce that he and his colleagues found in the feces of patients *Yersinia pseudotuberculosis*. However, authoritative scientists refused to believe a little-known provincial microbiologist. On the recommendation of reviewers, the scientific journal rejected the article sent. So, in order to prove his rightness, Dr. Znamenskij in January 1967 in Leningrad infected himself with the culture of *Y. pseudotuberculosis*, taken from a patient. After 6 days, he developed a severe septic form of FESF.

Doctors of the Military Medical Academy studied it in details and successfully cured. The results fully confirmed Znamenskij's hypothesis that FESF is a previously unknown form of human pseudotuberculosis. From then a new stage in the study of pseudotuberculosis began, which significantly changed the old ideas about this infection and its pathogen.

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MONITORING OF VACCINAL PROCESS IN HUMANS RESIDING IN THE ALTAI HIGH-MOUNTAINOUS NATURAL PLAGUE FOCUS

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The Altai high-mountainous natural plague focus has high epidemic potential; in 2014–2016 three cases of bubonic plague are registered in local residents.

The aim of this work — complex immunological examination of the humans residing at the territory of Gorno-Altai high-mountainous natural focus, vaccinated with a live plague vaccine.

Sixty volunteers earlier non-vaccinated against plague and living in the focus took part in the experiment. Blood sampling was performed before vaccination/revaccination after 1, 3 and 6 months.

Immunological efficiency of vaccination was estimated by: lymphocyte subpopulational composition; index of $CD4^+/CD8^+$ cells; IFNγ, TNFα, IL-4 production; NST-test; specific antibody titer to *Yersinia pestis* F1; typing of HLA class II genes. Processing of statistical data was performed using parametrical and nonparametric criteria.

No pathological alterations in lymphocyte subpopulational composition were revealed. Decrease of T-lymphocyte was registered in 3 months after revaccination due to increase of $CD3^-CD19^+$-cells. Increase of T-helper percentage, raising immunoregulatory index value and the general tendency to functional activity increase of immunocompetent cells in the NST-test in 1 month after revaccination indicates the presence of adaptive cellular immunity.

Positive seroconversion in the overwhelming majority of the vaccinated humans taking part in the testing indicates the adequate immune reconstruction of the body and development of specific antibody in reply to plague vaccine introduction. Increase of TNFα and IFNγ production, and also IFNγ/IL-4 ratio after vaccination indicate the increase of Th1-cell activity and development of the cellular immune response in humans. At the same time, decrease of IFNγ/IL-4 ratio and also TNFα associated with Th-1-cells occurs after revaccination indicating the shift to humoral immunity.

Commonly encountered HLA-DRB1 (*03, *04, *07, *08, *11, *13), HLA-DQB1 (*02, *03:01) and HLA-DQA1 (*05:01) gene alleles are defined. Possible associations of these alleles with TNFα and IL-4 production level and also with relative T-helper content and $CD3^-CD16^+$-cells are revealed.

Comparative analysis permitted to detect a number of the major parameters indicating to activation of cellular and humoral immunity in humans vaccinated against plague. Further immunological monitoring of the vaccinal process is necessary.

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HETEROGENEITY OF POPULATIONS OF THE FLEA *CITELLOPHILUS TESQUORUM ELBRUSENSIS* DETECTED ON THE BASIS OF ANALYSIS OF PROTEOMIC PROFILES BY THE METHOD OF MALDI-TOF MS

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The aim of the work was to analyze proteomic profiles of imago *C. t. elbrusensis*, the basic vector of the causative agent of plague in territory of the Central-Caucasian high-mountain natural focus of plague.

Proteomic profiles of 49 specimens of imago *C. t. elbrusensis* collected in populations of East and North Prielbrusye in June-August, 2017 were analyzed in the course of this work. All parasites were previously characterized by the following signs: sex, state of gastrointestinal tract, generative state of females. Each sample was studied individually by homogenization and extraction of proteins in 80% TFA. Spectra were collected and analyzed on MALDI-TOF mass spectrometer Microflex LT (Bruker, Germany) by using pre-established programs Flex Control V 3.3.5 and Flex Analysis v 3. (Bruker, Germany). The additional analysis of signal frequency and statistical processing were carried out using programs Microbe MS (Lash P., 2016).

The MSP analysis of the dendrogram constructed on the basis of super-spectra (generalized spectra of each sample) on the basis of differences in their protein composition showed clearly that *C. t. elbrusensis* was clustering into two basic geographical groups: a group of East Prielbrusye and a group of North Prielbrusye. At the same time the analysis of proteomic profiles of fleas of each of these groups revealed heterogeneity of protein composition of samples collected from points, most remote from each other in the region of 2–12 000 Da. It makes possible to differentiate some local proteotypes in populations of *C. t. elbrusensis* of basic geographical groups, each of which is characterized by certain frequency of both ribosomal and individual proteins denoting sufficiently long isolation of the given local populations of parasites — vectors of the causative agent of plague, owing to disconnection of settlements of their hosts — mountain sousliks in the conditions of mountain landscape of Prielbrusye.

5.12 doi: 10.15789/2220-7619-2018-4-5.12

THE GRANULOCYTES PHAGOCYTIC CAPACITY TO *YERSINIA PESTIS* IN BLOOD SAMPLES OF ANTI-PLAGUE VACCINATED PEOPLE ACCORDING TO FLOW CYTOMETRIC ANALYSIS DATA

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Phagocytosis is the basis of cellular immunity in bacterial infections, but there is currently no information on the phagocytic activity of human blood granulocytes for killed *Yersinia pestis* cells grown at 28°C and the effect of anti-plague vaccination on this indicator. In this work a problem of obtaining such information was solved for the first time with the help of flow-cytometric technology, which allows to evaluate objectively the indices of the phagocytic reaction in whole blood samples without allocation of phagocytes and serum from it. Heat-killed *Y. pestis* (EV NIIEG), *Escherichia coli* (25922ATS) and *Staphylococcus aureus* (209P) cells, stained with FITC, were used in the experiments. Individual phagocytic reaction indicators were determined in blood samples of people, living in the territory of the Caspian sandy natural plague focus (130 persons), before and one month after anti-plague vaccination with respect to three types of bacteria after 15 min of incubation *in vitro* by the method of Hasui M. et al. (1989), modified by us according torecommendations of White-Owen C. et al. (1992). The results were taken into account on the CyAn ADP™ Dako Cytomation flow cytometer using the Summit v.4.3 Built 2445 software. Against the background of high phagocytic indices for *E. coli* and *S. aureus*, respectively 97.3±0.24 and 98.5±0.13%, in relation to *Y. pestis* were recorded the reduced phagocytic activity of granulocytes 55.6±2.1% in blood samples before vaccination.The phagocytic numbers measured in the FITC fluorescence intensity units for blood granulocytes that absorbed *Y. pestis* cells were on average (Mean) twice lower than for *E. coli* and *S. aureus* at significantly higher coefficients of variation on this parameter (CV = 169±3.7%) in comparison with CV for *E. coli* (68.8±1.6%) and *S. aureus* (66.1±0.9%). A month after the anti-plague vaccination, the blood granulocyte phagocytic activity to *Y. pestis* increased to 82.4±2.8 ($p < 0.001$), indicating that a new cellular test for anti-plague immunity evaluation in humans may be developed on the basis of the rapid whole blood granulocyte phagocytic activity to *Y. pestis* cells determination *in vitro*.

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ANTI-PLAGUE VACCINATION STIMULATES THE NEUTROPHIL EXTRACELLULAR TRAPS FORMATION TO INCREASE THE YERSINIA PESTIS KILLING EFFICIENCY *IN VIVO*

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Neutrophil extracellular traps (NETs) formation is a recently described anti-microbial mechanism of neutrophils which involves the release of chromatin decorated with granular proteins in order to bind extracellularly and kill microorganisms. However, the role of NETs in anti-plague immunity is unknown. Our aim was to show that NETs participate in *Yersinia pestis* killing and significantly increase the bacterial clearance *in vivo*, when post-vaccination anti-plague immunity in mice is created. BALB/c mice were immunized subcutaneously by protective dose of live *Y. pestis EV* NIIEG cells (2.5×10^4) and results were recorded on the 21st day after vaccination. Contributionof NETs to bacterial killing was determined by intraperitoneal (i.p.) inoculation of 150 U/mouse micrococcal nuclease (MCN) or EDTA-inactivated MCN to vaccinated and control mice 10 min before i.p. challenge of 10^8 live *Y. pestis EV* cells, grown 48 h at 28°C. After 4 h, animals were killed and the collected peritoneal lavage (PL) were seeded on polylysine pretreated coverslides, where the percentage of NET-forming neutrophils (NFN) were determined by fluorescence microscopy using DNA staining with propidium iodide. Colony-forming units (CFU) in PL were evaluated using Hottinger agar after 72 h ofbacterial grown at 28°C. Phagocytic capacity of neutrophils to i.p. injected FITC-labeled *Y. pestis* cells was measured in PL samples byflow cytometry. Vaccinationstimulated NETs formationin response to live *Y. pestis* cells (from control NFN values 8.3±0.9 to 41.5±2.3%, $p < 0.001$ forn = 6) and this accompanied the increased bacterial killing, reflected in 10-fold decreasing of CFUin PLof vaccinated animals, against the background of the absence

of significant differences to 4 h in cell phagocytic capacity *in vivo*. MCN treatment decreased the NFN and increased the CFU values in vaccinated mice reaching control values, and this effect was reversed when MCN was inactivated. These results highlight the contribution of NETs is as an important cellular defense mechanism in anti-plague immunity.

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HLA GENE POLYMORPHISM IN PERSONS VACCINATED AGAINST PLAGUE

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The live-attenuated vaccine based on the *Yersinia pestis* strain EV NIIEG is still in use in the Russian Federation for the protection of people living in territories endemic for plague and provides a high degree of efficacy, but fluctuations in individual values of adaptive immunity in response to vaccination necessitate the establishment of genes that control the variability of the immune response. Human Leukocyte Antigen (HLA) genes play a decisive role in this process. In this study the distribution of HLA genes in people, vaccinated EV NIIEG live vaccine and living in the Caspian sand plague focus (Kalmykia and from Astrakhan), was investigated for their connection of HLA genes with indicators of immunity factors. The study involved 120 people. HLA gene typing was performed by multiplex PCR. Production of cytokines was determined by enzyme immunoassay. Statistical processing of the results was performed using the program "Statistica" 10.0. We determined that HLA-DRB1 alleles were more often in both regions *04(20–21%), *03(18%), *07(15–16%) and *01(10–15%). No significant difference was found, as well as in the reaction of cytokines in the inhabitants of both regions. The difference in the distribution of variants of the gene DRB1 and DQA1 was found in residents of the Lagan district of Kalmykia — the predominance of allele group DRB1*04 (40%) compared to DRB1*03(10%). The dynamics of cytokine production also varied by region of residence. 1 month after the vaccination, the levels of TNFα and IL-10 production increased in the residents of the Lagan district, and the inhabitants of the Black Soil district showed their decrease. The difference in cytokine production among residents of the Lagan district may be related to the special distribution of haplotypes of HLA.

The results show that the polymorphism of HLA genes has an effect on the level of cytokine secretion in response to the vaccinated EV NIIEG live vaccine. Further study of genes regulating the production of immune factors, will improve the understanding of the mechanisms of the immune response after vaccination, as well as contribute to the prediction of immunogenicity and effectiveness of vaccine products developed.

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WHOOPING COUGH — AN UNDERESTIMATED "ADULT" INFECTION

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Whooping cough is traditionally considered as a childhood infection. However, studies carried out in several countries, have shown that the actual incidence among adults is 10–100-fold higher than official statistics. Adults and older siblings become sources of pertussis for infants.

The aim of this study was to determine the true incidence of whooping cough in the adult population of St. Petersburg. The objective was to estimate the circulation of pertussis causative agent among the adult population of St. Petersburg (age ≥ 18 years), using antibody level to pertussis toxin as a marker of disease/natural booster in the last 12 months.

We examined 538 adults who applied to the medical center for blood tests for diagnosis of chronic nonpulmonary diseases, aged 18 to 82 years (mean age 41.2 years), 333 women, 205 men. Method: ELISA for the detection of antibodies to pertussis toxin (IgG, IgA). The IgG value ≥ 40 IU/ml was defined for categorization of whooping cough or contact with the patient during the last 12 months; including the IgG level ≥ 40 IU/ml in combination with a positive IgA level (≥ 12 IU/ml) or IgG ≥ 100 IU/ml with any IgA value for categorization of current or recent infection.

Anti-pertussis toxin IgG were detected in 87 patients (16.2% of those examined), including 27 patients (5.1%) with serological markers of recent infection. The proportion of seropositive persons was highest in the groups of 18–29 and 30–39 years (21.4 and 19.9%, respectively), followed by a decrease to 5.7% in the 50–59 age group; in the group of 60 years and older, the proportion increased to 13.9%. The proportion of patients with serological markers of recent infection was highest in the group of 18–29 years too (6.4%).

The wide involvement of adults in the epidemic process of whooping cough in St. Petersburg was revealed, particularly in the age group 18–39 years. Attention is drawn to the increase in the proportion of seropositive patients older than 60 years due to the increasing risk of a more severe and complicated course of the disease in this age group. It is necessary to include pertussis as a cause of prolonged cough in the training cycles of the post-graduated medical education for the "adult" physicians.

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CHARACTERISTICS OF A MOBILE LABORATORY FOR MONITORING AND DIAGNOSTICS DURING EPIZOOTOLOGICAL INVESTIGATION IN THE MONGOLIAN PART OF THE TRANSBOUNDARY SAILUGEM PLAGUE FOCUS

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Spread of *Yersinia pestis* of the basic subspecies in the Russian part of the transboundary Sailugem natural plague focus and the followed epidemiological complications required the assessment of the situation in the Mongolian part of the focus. So, since 2017 joint Russian-Mongolian epizootological examinations are performed at its frontier sites. Peculiarities of the investigations in 2018 were connected with using of a mobile laboratory for monitoring and diagnostics (MLMD) on the basis of "KAMAZ" lorry that appeared in the Altai Antiplague Station in 2017.

MLMD autonomy permitted to conduct researches in immediate proximity from the examined sites with daily delivery of the material. Combing, dissection, sampling were performed in a specially equipped jurt. Two samples were taken from the whole mammal carcasses: liver and spleen pieces were placed in a plastic test tube for homoge-

nizing, chest cavity lavage — in a usual microtest tube 1.5 ml. The same samples and one more probe of parenchymatous bodies in a test tube were taken from fresh carcasses for homogenizing with addition 2%-formalin in 1000 µl volume for detection of *Yersinia pestis* capsule antigen (F1). Spinal or bone marrow from birds-of-prey food debris, mummified carcasses, bones were taken in two test tubes for homogenizing (one tube with formalin).

In MLMD ectoparisite taxonomic identification was performed. After the necessary sample preparation the agent express-diagnostics was conducted in all received probes with the subsequent bacteriological examination only the positive samples. 100-µl liquid phase samples from agonizing, dead and birds' pecked animals, ectoparasites found out on them, bone remains were used for ICH-tests (FBUN GNTS PMB, Obolensk). All samples were examined by real-time PCR on a Rotor Gene Q instrument (Qiagen, Germany) and RNGA-RNAt. Specific fragments of *Y. pestis* DNA were amplified from all 39 ICH-positive samples at early cycles (since the 5th) and *Y. pestis* cultures were isolated. In total 60 positive responses were received in PCR including 50 replies that were confirmed by F1 detection in RNGA-RNAt, 47 *Y. pestis* subsp. *pestis* cultures were isolated.

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CLONING OF THE YERSINIA PESTIS TRANSALDOLASE GENE

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One of the antigenic complexes of the plague pathogen is fraction V (FV) (Bozhko et al., 2006). Investigation of the components of FV using two-dimensional protein electrophoresis, Western blott and mass-spectrometric analysis allowed determining that the composition of FV includes transaldolase, which has immunochemical activity with the monoclonal antibodies against FV (Arsenieva et al., 2017; Trukhachev et al., 2017). Transaldolase is an enzyme of the pentose phosphate cycle. The characteristics of the *Y. pestis* transaldolase and the *Y. pseudotuberculosis* transaldolase are similar. Perhaps, transaldolase of pathogenic *Yersinia* is the "moonlighting protein" (González-Rodríguez et al., 2012, He Y. et al., 2015).

The aim of the study was the cloning of the transaldolase gene of *Y. pestis* (*talB*) into a high-copium vector. After amplification of DNA with talF- and talR-primers, the 1059-bp PCR fragment including the *talB* gene of *Y. pestis* was cloned into the corresponding sites of pGEM-T using the set of reagents pGEM®-T Easy Vector Systems (Promega), resulting in pGEM-T-tal. Transformation of *E. coli* strains was performed using standard protocols (Sambrook J., 2001). The recombinant clones was selected on LB agar containing 100 µg/ml ampicillin, 0.5 mM IPTG and 80 µg/ml X-Gal. The few selected recombinant clones were detected insert of about 1000 nucleotide pairs in the plasmid vector with help the method extraction plasmid DNA (Kado et al., 1981). Analysis of the recombinant DNA of these strains using talF-, talR- and pT7-primers in PCR showed that there was an embedding of a fragment containing a *talB* under the control of the T7 promoter. The resulting recombinant *E. coli* pGEM-T-tal strain which carried the plasmid containing the *Y. pestis talB* gene reacted with the horse hyper immune antiplague serum and serum from rabbits immunized against FV in the gel precipitation reaction. The control strain containing only the vector plasmid pGEM-T didn't react with the serum. The *Y. pestis EV* strain served as a positive control.

Thus, recombinant strain of *E. coli* pGEM-T-tal containing gene of immunologically active *Y. pestis* transaldolase in plasmid was obtained. A tool for further study of immunogenic and protective properties of one of the components of FV *Y. pestis* was created.

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THE PRESENT EPIDEMIOLOGICAL CHARACTERISTICS OF YERSINIOSIS IN THE RUSSIAN FEDERATION

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The objective of this study was to carry out a retrospective analysis of pseudotuberculosis and intestinal yersiniosis surveillance data in the Russian Federation during the period 2013–2015.

Federal statistical observation data and federal subjects of Russia data were analyzed.

Presently the intestinal yersiniosis prevails in the etiological structure of yersiniosis, with proportion of 60%. A statistically significant decreasing trend in incidence is observed, the annual average incidence of pseudotuberculosis and intestinal yersiniosis per 100 000 population is 0.82±0.05 and 1.90±0.11 correspondingly.

The intensity of the epidemic process of yersiniosis varies greatly across different regions of the country. The registration of pseudotuberculosis is noted in approximately 49% of the entities of the Russian Federation, intestinal yersiniosis is registered more evenly — in 77% of the entities. The maximum incidence of yersiniosis is noted in a number of the entities of the North-West Federal District, the Siberian Federal District, the Far Eastern Federal District, where morbidity rates exceeded the federal average rate by 2–15 times.

The proportion of outbreak morbidity of pseudotuberculosis decreases, sporadic cases prevail. For the time period 2013–2015 10 outbreaks with a total of 110 diseased persons were reported. The incidence of intestinal yersiniosis is sporadic.

In the age structure of patients with pseudotuberculosis children predominate (65%) mainly in the age group 3–6 years (32%). In 2015 incidence in this age group was 5.2 per 100 000 persons, this is 17 times higher compared with adults and 2 times higher compared with children in the age group 1–2, 7–14 years. The ratio of children and adults with intestinal yersiniosis is practically 1:1 — 45 and 55%. The maximum incidence is noted among children in the age group 1–2, 3–6 and 7–14 years (3–32%) — 3.5, 3.4, 2.9 per 100 000 persons respectively. The incidence among adults was lowest (0.8 per 100 000 persons).

During this epidemiological study it was shown what the pathogens are principally transmitted to humans through fresh vegetables (11–61%) and fruits (3–32%). Thus meat and meat products, milk and dairy products are often not investigated as the possible sources of intestinal yersiniosis infection. The diagnosis is confirmed mainly by the serological methods — 49–91% of cases, by the PCR — only 1–15%.

5.19 doi: 10.15789/2220-7619-2018-4-5.19

MALDI-TOF MASS SPECTROMETRY ANALYSIS FOR DETECTION OF THE RIBOSOMAL MARKERS TO IDENTIFY OF *YERSINIA KRISTENSENII* STRAINS

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Y. kristensenii belongs to the group of nonpathogenic *Y. enterocolitica*-like species. Method of the biochemical profiling is not always allowed for adequate phenotypic identification of *Y. enterocolitica*-like species. MALDI-TOF mass spectrometry is an effective tool for bacterial identification, which results are based on simple mass spectral peak-matching with the reference spectra from the taxonomic database, with no peak assignment, so *Y. kristensenii* strains are usually identified incorrectly as *Y. enterocolitica*.

The aim of this study was to analyze at the statistical level the capacity of MALDI-TOF MS to distinguish between *Y. kritensenii* and *Y. enterocolitica* by searching of ribosomal proteins as discriminate markers.

Soluble proteins were extracted from intact cells of five *Y. kristensenii* and five *Y. enterocolitica* well-characterized strains by an EtOH-FA method. From each strain, no less than 20 mass spectra were obtained, which were used to create the main spectra. Digital format (.CSV) data of 10 main spectra were exported to the free statistical software "Mass-Up" for detection of discriminante peaks by use the exact Fisher test. Designation of the potential biomarkers was performed by comparing their molecular weights with the data of *Y. enterocolitica* ribosomal proteins in the database UniProtKB/Swiss-Prot-Expasy with using the TagIdent tool.

The 26 genus-specific peaks was detected in all strains of both species could not be assigned to a protein mass using the available datebases. Among the common peaks, were designated only two: the peak m/z 7265 corresponding to the ribosomal protein L29 (theoretical m/z 7261) and the peak m/z 9998 identified as the 30S ribosomal protein S15. The biomarker peak, differentiating the *Y. enterocolitica* species, m/z 5429 (theoretical mass at m/z 5426) corresponded to the 50S ribosomal protein L34, but all spectra of *Y. kristensenii* strains had a molecular weight shift of this peak to m/z 5443, presumably because of the amino acid exchange in this protein. As well as the unidentified *Y. enterocolitica* differentiating peak m/z 3884, had a shift of molecular mass to m/z 3909 in all spectra of *Y. kristensenii*.

It is suggest that the spectral peaks of a non identify protein m/z 3884 and of the ribosomal protein L34 m/z 5429 are biomarkers of the *Y. kristensenii* species. This results evidence the possibility of using a mass spectrometric method, coupled with the bioinformatic approach to detection of discriminante markers, for differentiation between of strains of *Y. enterocolitica*-like species.

6. TUBERCULOSIS AND MYCOBACTERIA: MOLECULAR APPROACH*

6.1

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PERFORMANCE OF GENEXPERT MTB/RIF IN THE DIAGNOSIS OF EXTRAPULMONARY TUBERCULOSIS IN MOROCCO

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Tuberculosis is commonly associated with lung diseases, but can also affect other parts of the body (extrapulmonary tuberculosis [EPT]). A rapid diagnosis is essential to initiate a specific and effective treatment. The diagnosis of EPT is a real challenge because of the paucibacillary nature of samples. GeneXpert MTB/RIF is a rapid automated diagnostic test that allows the detection of the presence of *M. tuberculosis* as well as mutations in the hot-spot region of the *rpoB* gene associated with rifampicine resistance. The objective of this study was to evaluate the performance of the GeneXpert MTB/RIF test for the diagnosis of EPT.

We analyzed 304 clinical samples collected in the Laboratory of Mycobacteria and Tuberculosis of Pasteur Institute of Morocco, between 2016 and 2017. Of these samples, 113 were pleural fluids decontaminated using the Petroff method and 191 biopsies (78 lymph nodes and 113 pleural biopsy), decontaminated using the Loeweinstein method. All samples underwent smear microscopy, culture on Loeweinstein–Jensen medium and tested with Xpert MTB/RIF.

The study population included 192 patients, 54.2% were men and 45.8% women. The age of the patients ranged from 2–78 years with the majority of the patients in the age group 25–45 years. The sensitivity of GeneXpert was 51.47% for all samples and 83.3% for lymph nodes.

Our study clearly shows that GeneXpert MTB/RIF test presents limitations in the diagnosis of EPT. In view of these results, it would not be appropriate to use only this technique for the diagnosing of EPT.

6.2

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ANALYSIS OF GENE MUTATIONS ASSOCIATED WITH *MDR AMONG MYCOBACTERIUM T*UBERCULOSIS STRAINS ISOLATED IN MOSCOW REGION

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The aim of this study was to determine the prevalence and variants of mutations in *M. tuberculosis* genes associated with the development of multidrug-resistance (MDR) as well as their correlation with genotypes in the study of clinical isolates obtained from patients with tuberculosis (TB) in hospitals from the Moscow region.

179 randomly selected *M. tuberculosis* clinical isolates from TB patients collected from 2008 to 2016 years were included in this study. One isolate from each patient was used. The molecular characteristics of *rpoB*, *katG* genes and *inhA* promoter, resulting in rifampin and isoniazid resistance (MDR), were obtained by Sanger sequencing. All specimens were subjected to spoligotyping; spoligotypes were compared to SITVIT_WEB database. Pearson χ^2 test was used to check pairwise differences.

All clinical isolates were divided into 2 groups of genotypes according to the results of spoligotyping: Beijing (72.6%, n = 130) and other genotypes collectively named "non-Beijing" genotypes (27.4%, n = 49). Beijing genotype had *rpoB* Ser 531> Leu mutation in 62.9% of cases whereas non-Beijing genotypes in only 15.8% of cases. Other variants of *rpoB* mutations were detected in only 6.3% of Beijing strains versus 28.1% of non-Beijing strains. The wild-type *rpoB* gene was observed in Beijing genotype in 30.8% of cases whereas in non-Beijing genotype in 56.1%. Statistically significant differences were obtained for all comparisons between two groups ($\chi^2 = 9.21$, $p < 0.01$).

We also obtained statistically significant differences in the analysis of combinations of *katG* gene and *inhA* promoter for Beijing and non-Beijing genotypes, respectively: in the case of simultaneous presence of mutations in them, in 13.9% and in 34.9% of cases ($\chi^2 = 8.59$, $P = 0.0036$) or wild-type in 20.4 and 39.5% of cases ($\chi^2 = 20.64$, $p < 0.0001$), as well as in the presence of genetic changes in only *katG* gene, 62.0 and 20.9% ($\chi^2 = 20.64$, $p < 0.0001$). However, no statistically significant differences were noted when comparing *inhA* promoter mutations occurred alone without *katG* mutations which was observed in a small proportions in both genotypes — 3.7 and 4.7%.

We established some specific features in clinical isolates of *M. tuberculosis* in Moscow region.

6.3

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COULD THE NEW INSIGHTS INTO PZA RESISTANCE PROVIDE ROUTE TO SHORTER MORE EFFECTIVE TB THERAPY?

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Pyrazinamide (PZA) is a pro-drug that is transformed into pyrazinoic acid (POA) by mycobacterial PncA enzyme. A very wide range of mutations in *pncA* result in clinical and *in vitro* resistance. In the last two years multiple groups have demonstrated that a low pH is not required for the activity of POA against tuberculosis as was previously widely assumed. Furthermore, laboratory mutants against POA have been generated in multiple laboratories under different conditions. Mutations in a range of genes have been observed but always including *clpC1* and/or *panD*. A direct activity of POA against mycobacterial PanD has been demonstrated but evidence of activity against other genes associated with *in vitro* resistance is disputed or lacking. It has been suggested that PZA is a dirty drug with multiple targets but we recently

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proposed an alternative explanation: POA is primarily active against PanD but PanD is only essential if the bacteria are expressing a stringent response, the other genes associated with resistance in some way disrupt the stringent response and eliminate the sensitive phenotype. This suggests a critical role for the stringent response in the life cycle of *M. tuberculosis* as compounds are being developed that target this pathway we suggest these compounds are particularly promising compounds for the treatment of tuberculosis.

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IDENTIFICATION OF MUTATIONS OF RESISTANCE TO FLUOROQUINOLONES, AMINOGLYCOSIDES AND ETHAMBUTOL IN RIFAMPICIN-RESISTANT *MYCOBACTERIUM TUBERCULOSIS*

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The aim of the study was to identify resistance mutations to second-line anti-tuberculosis drugs in patients with *Mycobacterium tuberculosis* clinical samples resistant to rifampicin. Samples of biomaterial from 35 adult residents of the Tyumen region in West Siberia with established by GeneXpert system presence of rifampicin-resistant mycobacteria tuberculosis (MBT) were examined in our study using the MTBDRsl kit (Hain Lifescience, Nehren, Germany) according to the manufacturer's instructions. The mutations were identified in genes encoding DNA gyrase *(gryA)*, 16S RNA *(rrs)*, and arabinosyltrasferase *(embB)* associated with resistance to fluoroquinolones (FLQ), injectable aminoglycosides/cyclic peptides (AG/CH) and ethambutol (EMB) respectively.

In 9 of the examined samples (25.7%) the MBT resistance to all three groups of drugs was revealed. In the remaining 26 samples, the MBT sensitivity to one or two groups of drugs can be assumed. Samples from 24 patients (92%) were genetically susceptible to AG/CH (in 2/3 cases solo, in 1/3 — in combination with sensitivity to ethambutol (5 samples) and fluoroquinolones (1)). Most samples demonstrated genetic resistance to fluoroquinolones (97%) and ethambutol (80%), and 30% of samples are resistant to aminoglycosides/cyclic peptides.

Among the *gyrA* mutations, 11 were in codon 90 (A90V), 43 — in codon 94 (of which 4 — D94A, 6 — D94N, D94Y, 30 — D94G and 3 — D94H). No mutation in codon 91 (S91P) was detected. In 22 samples 1 mutation was detected, in 4 — 2 mutations, in 6 — 3 mutations, and in 1 — 5 mutations. Among the mutations found in the *rrs* gene, 8 are in codons 1401–1402 (A1401G, C1402T) and 10 — in codon 1484 (G1484T), all mutations in codons 1401–1402 are combined with the presence of a mutation in codon 1484. Among mutations in *embB* (codon 306) in three cases replacement of M306I = 306 ATG/ATA, M306V, in 26 — replacement of M306I = 306 ATG/ATC/ATT was revealed. In 24 cases only one variant of the mutation is found, in 2 — both, and in 18 samples in the presence of a mutation there is no marker of wild type.

In conclusion, preliminary data on the genetic structure of MBT strains resistant to rifampicin and second-line anti-tuberculosis drugs were obtained in tuberculosis patients from the Tyumen Region in Siberia.

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MOLECULAR TYPING OF *MYCOBACTERIUM KANSASII* — A GLOBAL PERSPECTIVE

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To date, over 180 nontuberculous mycobacteria (NTM) species have been identified and almost 30 of these species have been reported as the causative agents of pulmonary and extrapulmonary diseases. *Mycobacterium kansasii* is the sixth most frequently isolated NTM species across the world. The isolation rate of this pathogen, among other NTM, has been calculated at 5% in Europe and 4% globally. In Poland and Slovakia, the recovery of *M. kansasii* from respiratory samples is particularly high, being 36% and 35%, respectively.

The genetic heterogeneity of *M. kansasii* is defined by the presence of seven molecular subtypes. Most of the disease-related strains belong to subtype I and II, while the others (III-VII) have usually been linked to environmental sources. Therefore, subtyping of *M. kansasii* isolates from human samples may be helpful for clinical diagnosis.

The aim of this study was to determine the distribution of *M. kansasii* subtypes among clinical isolates from 19 countries on 4 continents.

A total of 475 isolates recovered between 2000 and 2017 from as many patients with suspected *M. kansasii* disease were analyzed. The isolates were collected from 19 coun-

tries across 4 continents. For PCR restriction-enzyme analysis (PCR-REA) subtyping, protocols described by Telenti et al. (2003) (*hsp65* gene) and Bakuła et. al. (2016) (*tuf* gene) were used. The patients were categorized as having *M. kansasii* disease following the American Thoracic Society 2007 diagnostic criteria.

The vast majority of isolates (392; 82.5%) presented patterns characteristic for subtype I. Forty-three (9%) isolates exhibited subtype II pattern. There were 19 (4%), 2 (0.4%), 2 (0.4%) and 13 (2.7%) isolates representing subtypes III, IV, V, and VI, respectively. Four (0.8%) isolates gave inconsistent results (mixed subtype — I/II). The subtype I–VI isolates were obtained from both disease-associated and non-disease associated cases. Of two subtype IV isolates, one was obtained from a non-disease associated case, and the other one from a patient with unknown status. For two subtype V isolates, data concerning *M. kansasii* disease were unavailable.

The highest frequency of *M. kansasii* subtype I isolations was observed for Poland (140/142; 98.6%), and the lowest for Estonia (2/7; 28.6%).

This study demonstrated that subtype I represented the vast majority of *M. kansasii* clinical isolates worldwide. Since all *M. kansasii* subtypes detected (I-VI) were isolated from both disease-related and non-related cases, subtyping of the species does not permit differentiation between disease and non-disease states that did and did not cause definite disease. Furthermore, the genetic diversity of the *M. kansasii* population showed important regional variations.

The study was performed within the framework of the Fight Against Tuberculosis in Central & Eastern Europe consortium (https://fate-consortium.org).

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GENETIC DIVERSITY OF MULTIDRUG-RESISTANT *MYCOBACTERIUM TUBERCULOSIS* ISOLATES IN PAKISTAN

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Tuberculosis (TB) remains an inglorious leader among infectious diseases in mortality, with its annual toll of 1.7 million lives worldwide. Pakistan ranks 5th among the world's highest TB burden countries and the 6th among countries with the highest burden of drug-resistant TB, including multi-drug resistant (MDR)-TB. However, very limited data are available on the genetic structure of *M. tuberculosis* strains circulating in this country.

The objective of this study was to explore the genetic diversity of multidrug-resistant *M. tuberculosis* isolates from Pakistan with two different methodologies, i.e. spoligotyping and 24-loci MIRU-VNTR typing.

The study included 130 MDR-TB isolates, recovered from as many patients from Pakistan, between January 2013 and June 2015. Conventional drug susceptibility testing was performed using the standard 1% proportion method on the Löwenstein-Jensen medium, as described elsewhere. Spoligotyping was performed with a commercially available kit (Mapmygenome India Ltd., Madhapur, India) according to the manufacturer's protocol. MIRU-VNTR analysis was carried out at 24 loci, as described earlier. Phylogenetic clades of *M. tuberculosis* were assigned according to signatures provided in the SITVIT database (http://www.pasteur-guadeloupe.fr:8081/SITVIT_ONLINE).

Spoligotypes were obtained for 127 (97.8%) isolates. Based on a SIT number in the SITVIT database, all isolates presented 53 different profiles split into 14 clusters (n = 88, 69.3%, 2–30 isolates per cluster) and 39 (30.7%) unique patterns. MIRU-VNTR typing identified 128 unique types (98.5%) and one cluster (n = 2, 1.5%). When spoligotyping and MIRU-VNTR typing was used in combination, only two, out of 130 isolates, clustered both in both methods, resulting in a clustering rate of 1.5%.

Upon phylogenetic analysis, 101 (77.7%) isolates were classified into 12 clades, with the most prevalent being CAS1_DELHI (n = 53, 41.7%) followed by T1 (n = 14, 11%) and BEIJING (n = 10, 7.8%). The remaining 9 families (CAS, MANU2, EAI5, T2, LAM10_CAM, H1, X1, H4 and CAS2) involved 24 (18.9%) isolates. Twenty-six (20.5%) isolates could not be assigned to any specific lineage.

This study provides a snapshot of the genetic diversity of *M. tuberculosis* strains circulating in Pakistan. The compactness of the drug resistant *M. tuberculosis* population structure was apparent, as three major lineages, i.e. CAS1_DELHI, T1, and BEIJING comprised more than half (60.6%) of the isolates studied. Furthermore, the exceptionally low clustering rate

Suggest that recent transmission does not play an important role in the incidence of MDR-TB in Pakistan.

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UPDATE ON VIRULENCE FACTORS IN MYCOBACTERIA

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Although the majority of mycobacteria represent harmless environmental bacteria, a few mycobacterial species have evolved into major human pathogens. *Mycobacterium tuberculosis*, the etiological agent of human tuberculosis, is the most dominant mycobacterial pathogen in terms of global patient numbers and gravity of disease.

The molecular mechanisms by which *M. tuberculosis* induces disease are complex and result from a long-lasting host-pathogen co-evolution that might have started already by its *Mycobacterium canettii*-like progenitors. Recent research has revealed numerous factors implicated in the pathogenesis of *M. tuberculosis*, although the pathogen still holds many secrets of its successful strategy to circumvent host defences and persist in the host. As many pathogenicity factors relate to the exchange and secretion of biomolecules by *M. tuberculosis*, special emphasis is given to secretion pathways that enable *M. tuberculosis* to circumvent immune defence mechanisms mounted by the host. These factors might represent new, alternative targets for development of combination therapies that would enhance the efficacy of the immune system in controlling *M. tuberculosis* infections. Similarly, selected secretion systems may also represent important virulence factors in selected non-tuberculous mycobacteria. Here, recent insights into evolution of selected factors of *M. tuberculosis* and selected other mycobacteria that are involved in host-pathogen interaction will be discussed.

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GENOTYPING OF MULTIDRUG AND PRE-EXTENSIVELY DRUG-RESISTANT *MYCOBACTERIUM TUBERCULOSIS* ISOLATES FROM A HIGH INCIDENCE TB AREA IN MOROCCO

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The emergence of extensively drug-resistant tuberculosis (XDR-TB) has raised public Health concern for global control of TB. Although drug resistance-associated mutations in multidrug-resistant *Mycobacterium tuberculosis* complex (MTBC) isolates in Morocco were characterized, mutations in Pre-XDR/XDR isolates and their genotypes have not been reported previously. Resistance to second line antituberculosis drugs (SLDs) is mainly due to mutations in specific genes: *gyrA* and *gyrB* for resistance to fluoroquinolones (FQs), *rrs*, *eis* and *tlyA* for resistance to injectable drugs (kanamycin (KAN), amikacin (AMK), and capreomycin (CAP).

A collection of 70 MTB isolates already characterized as MDR and 100 pan-susceptible isolates randomly selected from a high incidence TB area in Morocco were enrolled in this retrospective study. The mutation profiles associated with resistance to SLDs: FQs and injectable drugs were assessed by DNA sequencing. Target sequences for four genes were examined: *gyrA* and *gyrB* (FQs), and *rrs* (KAN, AMK, and CAP) and *tlyA* (CAP). The fingerprint of each isolates was established by spoligotyping and 15-loci MIRU-VNTR typing methods.

Molecular analysis showed that 26.7% of MDR isolates are pre-XDR strains harboring mutations in *gyrA* gene. The most prevalent mutation involved in FQ resistance was Asp94Gly (50%). None of the isolates harbored mutations neither in *gyrB* nor in *rrs and tlyA* genes. Likewise, none of the pan-susceptible isolates displayed mutations in targeted genes. Spoligotyping of MDR MTB isolates resulted in 4 and 5 orphan and unique patterns respectively, and 61 strains in 9 clusters (2–26 strains per cluster) with a resulting clustering rate and recent transmission rate of 87.1% and 74.3% respectively. The most prevalent spoligotype was SIT42 (LAM; 37% of isolates). The repartition of strains according to major MTBC clades was as follows LAM (46.1%) > Haarlem (59%) > ill-defined T superfamily (17%) Haarlem (7%) > clade S (6%) > Beijing (3%) > T2-T3 and Beijing-like (1%). Of note, CAS (Central Asian) and EAI (East-African Indian) strains were absent in this setting. Subsequent 15-Loci MIRU typing failed to find any cluster of SIT/MIT, all clusters established by spoligotyping were splited, 70 unique MLVA-MtbC15 profiles were generated with a resulting clustering and recent transmission rate equal to zero meaning that all MDR strains are not a part of an established transmission chain and that the developpement of drug resistance in this setting is likely a result of inadequate treatment rather than primary resistance. HGDI analysis of the 15 MIRU loci showed that loci 10, 40 and Mtub04 were highly discriminative in our setting. All pre-XDR isolates harboring mutations in *gyrA* gene had not specific/paticular pattern generated by any of the two methods.

This study provides a first global snapshot of MDR MTBC population structure in Morocco. The results obtained (i) highlight the need for rapid detection of mutations associated with resistance to SLDs in order to adjust timely the treatment and to interrupt the propagation of virtually untreatable form of the disease, (ii) confirm that TB in Morocco is almost exclusively transmitted through evolutionary-modern MTBC lineages belonging to principal genetic groups 2 and 3 (Haarlem, LAM, T), with extremely high level of biodiversity seen by 15-MIRU typing, (iii) validate the use of spoligotyping in conjunction with 15-MIRU-VNTR scheme for future investigations in Morocco that should ideally use modified 15-loci MIRU-VNTRs (to include MIRU 23 instead of MIRU16), (iv) confirm the use of both the two typing methods to understand the transmission dynamic of tuberculosis in this setting.

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THE CORRELATION BETWEEN LEVELS OF PHENOTYPIC RESISTANCE AND GENOTYPIC MUTATIONS OF *M. TUBERCULOSIS*

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Rapid identification of drug resistance of *Mycobacterium tuberculosis* allows an earlier initiation of an adequate treatment regimen that potentially can reduce TB morbidity, mortality and transmission. New diagnostic methods have provided a promising solution for rapid and reliable detection of drug resistant TB. Despite the fact that rapid molecular assays are less accurate than the culture-based methods and raise the possibility of false negative results, the molecular characterization of the resistance spectrum of *M. tuberculosis* isolates offers the opportunity for overcoming the phenotypically detected resistance. So far, mutations within the *rpoB* gene confers a different level of phenotypic resistance for rifampicin (RIF) as well as mutation in *inhA* is link with low resistance to isoniazid (INH).

Objective was to study the correlation between phenotypic and genotypic resistance of *M. tuberculosis* on different levels of drug concentrations.

The genotypic resistance profiles for isoniazid and rifampicin of *M. tuberculosis* sputum isolates were assess by MTBDR*plus* and where correlated with culture based (MGIT-960) drug sensitivity test results. The different level of inhibitory concentrations of rifampicin and isoniazid of individual strains, assessed by MGIT-960 equipped with EpiCenter TB eXiST, were correlate correspondingly with the mutation types in the *rpoB* gene, and the presence of *inhA* mutation in the same *M. tuberculosis* isolates.

The *M. tuberculosis* isolates from 4568 patients with pulmonary tuberculosis were assess. 64.2% of them were INH resistant, and in 1,9% (n = 86) of these isolates, resistance was conferred by *inhA* mutation only. RIF resistance was detected in 61.9% of subjects, and in 27.2% (n = 762) of these the mutation *rpoB531* was missing. 30.6% of INH resistant *M. tuberculosis* strains, conferred by inhA mutation only and 28.6% of RIF resistant *M. tuberculosis* strains without S531 mutation, were sensitive to high concentrations of drugs by phenotypic DST.

The correlation of genotypic tests results with phenotypic resistance levels can be crucial forward a personalized approach in TB patient treatment, stopping the spread of drug resistance and promotion of the optimum use of the few drugs available for resistant TB treatment.

6.10 doi: 10.15789/2220-7619-2018-4-6.10

GENOMIC EPIDEMIOLOGY OF TUBERCULOSIS: FROM WITHIN HOST EVOLUTION TO GLOBAL MIGRATION PATTERNS

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The availability of thousands of *Mycobacterium tuberculosis* genomes allows not only to infer how the pathogen emerged and spread but also to identify specific loci associated. I will present our work using evolutionary analyses to generate a high-resolution picture of the emergence, global spread and local transmission of the pathogen as well as genomic determinants associated. I will show how non-selective processes like genetic drift contribute to the genomic diversity of the pathogen with downstream consequence at the transcription and methylation levels. In the second part of the talk I will also discuss different approaches to identify and track new drug resistance determinants using a combination of functional genomics and genomic diversity analyses from different countries and from different patients through time and space. Overall our analyses reveal new bacterial factors associated to virulence and drug resistance. I will show however how the frequency of genetic variants associated to different traits, even if advantageous, depend on the conditions of local TB control more than on the high fitness of the bacterial genotype.

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EMERGENCE OF BEDAQUILINE RESISTANCE AFTER COMPLETION OF BEDAQUILINE-BASED DRUG-RESISTANT TB TREATMENT: A CASE STUDY FROM SOUTH AFRICA

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Treatment outcomes for drug-resistant tuberculosis (DR-TB) are poor with only 52% of MDR-TB and 24% of XDR-TB patients successfully treated. To address the global DR-TB epidemic WHO has released guidelines for the use of bedaquiline (BDQ) for the treatment of rifampicin-resistant or MDR-TB for specific indications. However, standardised methods to perform drug susceptibility testing (DST) have not been defined and BDQ resistance mechanisms remain poorly characterised.

Illumina NextSeq whole genome sequencing (WGS) was used to characterise serial *Mycobacterium tuberculosis* (Mtb) isolates from a patient receiving BDQ in Khayelitsha, South Africa. Phenotypic drug susceptibility testing (DST) for BDQ was performed in MGIT-960 media (concentration 1 μg/ml).

WGS showed an initial infection with a strain resistant to 7 drugs (rifampicin, isoniazid (low-level), ethambutol, ethionamide, fluoroquinolones, pyrazinamide and streptomycin). Following initial treatment failure with a standardised MDR-TB regimen, the patient was placed on a regimen containing 6 effective drugs (including BDQ, based on WGS). Isolates taken prior to BDQ initiation were BDQ-susceptible (phenotypically). WGS of subsequent serial isolates revealed the acquisition of a variant in *Rv0678* (conferring BDQ-resistance) one month after stopping BDQ treatment. Subsequent isolates showed the loss and gain of several other *Rv0678* variants, with only one variant (138 G insertion) fixed in the last available isolate. All isolates with *Rv0678* variants were BDQ-resistant.

The systematic gain and loss of *Rv0678* variants in isolates taken after completion of BDQ-based treatment illustrates the complex ongoing evolution patterns of *M. tuberculosis* as the concentration of BDQ decreases in the patient (long half-life). An alternative explanation is the emergence of existing BDQ-resistant Mtb from lesions which rupture following continuation of treatment without BDQ and after stopping all TB treatment. The emergence of BDQ resistant *M. tuberculosis* following stopping of treatment poses a risk of transmission of BDQ resistant clones to close contacts. Monitoring of pre-existing and emerging BDQ resistance should be a priority for all routine use and should continue post BDQ cessation.

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WHOLE GENOME SEQUENCING SHEDS LIGHT ON THE TRANSMISSION DYNAMICS OF A MULTI-DRUG RESISTANT *MYCOBACTERIUM TUBERCULOSIS* OUTBREAK OVER 23 YEARS IN A HIGH INCIDENCE SETTING

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Whole genome sequencing (WGS) has shown that *Mycobacterium tuberculosis* strains are more genetically diverse than previously assumed and that traditional genotyping methods cannot discriminate strain heterogeneity with high resolution, which may mask their ability to accurately define the directionality of an outbreak, particularly in high tuberculosis (TB) incidence settings.

The objective of this study was to examine the evolution of a single *M. tuberculosis* cluster defined by a particular IS*6110* RFLP pattern to understand transmission and strain diversity over time.

Clinical *M. tuberculosis* isolates (n = 97) with identical IS*6110* RFLP fingerprint patterns were selected from a longitudinal sample bank of *M. tuberculosis* isolates collected from a high TB incidence suburb in the Western Cape, South Africa from 1993–2015. DNA was extracted from *M. tuberculosis* cultures for WGS and subsequent analysis. Available WGS of *M. tuberculosis* isolates from surrounding suburbs were screened and additional isolates

from the same time period with the same genotype were included in the phylogenomic analysis. The genomic variants identified by WGS were used for phylogenomic inference, drug resistance prediction and to determine genomic distances between isolates.

WGS analysis revealed unexpected genomic diversity within the seemingly homogenous IS*6110* cluster of *M. tuberculosis* isolates. Despite the IS*6110* RFLP based uniformity, at least six non-time dependent sub-clusters and several orphan-isolates were evident from the WGS-based phylogeny and genomic comparisons. Sub-clusters gained drug resistance conferring mutations (beyond MDR) on multiple occasions and *M. tuberculosis* isolates from surrounding suburbs were observed throughout the phylogeny.

IS*6110* RFLP typing underestimated the complexity of this 23-year outbreak. This study suggests that there is continuous circulation and reintroduction of this *M. tuberculosis* cluster in the community setting. Even with the advent of the WGS-era, confirming direct epidemiological links or outbreak directionality remains a challenge in high TB burden, low-income settings.

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A 15-YEAR SPATIOTEMPORAL ANALYSIS OF *MYCOBACTERIUM TUBERCULOSIS* LINEAGES 1 AND 2 IN CHIANG RAI, THAILAND

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Chiang Rai is the northernmost province of Thailand with high burden of tuberculosis (TB) and high TB death rate. Chiang Rai consists of various ethnic groups in three different geographic areas including (1) the bordering area in the northern and northeastern, (2) the central area, and (3) the outlying districts in the southeastern, southern and southwestern.

We aimed to assess the spatial and temporal distribution of *Mycobacterium tuberculosis* (MTB) lineages in the three different areas over 15 years.

Whole genome sequence (WGS) data was used to classify the genotypes of 1497 MTB using lineage-specific single nucleotide polymorphisms (SNPs). The spatiotemporal distribution of MTB lineage was analyzed in the three different areas during early 2000s (2002–2006), late 2000s (2007–2011) and 2010s (2014–2018). Stoddart and Taylor's index (G) was calculated to determine genotypic diversity of MTB lineage in the different settings.

In 2000s, lineage 2 (East Asian) was a highly predominant genotype (45%) in Chiang Rai followed by lineage 1 (Indo-oceanic) (41%). In 2010s, lineage 1 became the most dominant genotype (51%) replacing lineage 2 (35%). The overall change in predominant lineage from lineage 2 to lineage 1 was caused by a dramatic increase proportion of lineage 1 in the central area (from 44 to 53%) and in the outlying districts (from 39 to 59%). In the bordering area, a combined impact of increasing distribution of lineage 1 (from 32 to 40%) and other lineages (from 18 to 24%) was an additional cause of changing predominant lineage. The Stoddart and Tayloy's analysis of genotypic diversity showed that the central area had the highest diversity of MTB lineage ($G = 4.14 \pm 0.46$) followed by the bordering area ($G = 3.67 \pm 0.76$) and the outlying districts ($G = 3.63 \pm 0.62$).

Our study combining genotypic and space-time analysis has revealed a dynamic population changes in MTB lineage over 15-year period in Chiang Rai. Further studies on social determinants and patient's demographic data in the different geographic areas have the potential to provide effective TB control in the different setting.

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MOLECULAR-GENETIC METHODS OF DETECTION OF TUBERCULOSIS AND ITS DRUG RESISTANCE IN ARKHANGELSK REGION IN 2017

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Burden of tuberculosis (TB) is decreasing in Arkhangelsk region in northwestern Russia with incidence declining from 45.9/100 000 in 2012 to 21.6/100 000 in 2017 in civil society (excluding penitentiary society). Introduction of molecular-genetic tests of detection of TB and its drug resistance (DR), including multidrug-resistant (MDR) and extensively drug resistant (XDR) TB, is one of the key components of regional TB program. It plays an important role in improvement of diagnostics and management of TB patients in the region.

The objective was to evaluate performance of molecular-genetic tests used for detection of TB and its DR among patients with TB registered in civil society in Arkhangelsk region in 2017.

Line probe assay (LPA) (Hain Lifescience, Germany) and GeneXpert MTB/RIF (Cepheid, USA) were used for detection of *M. tuberculosis* (MTB) and its DR alongside conventional sputum smear microscopy (SM) and culture. All patients were initially tested with SM followed by LPA (Genotype MTBDR*plus*) if result of SM was positive or GeneXpert if result was negative using the same sample. In case of DR to isoniazid and/or rifampicin, Genotype MTBDR*sl* was performed to detect additional DR to fluoroquinolones and injectables, including XDR. In cases suggestive of nontuberculous mycobacteria (NTM), SM or culture positive with negative results of LPA or GeneXpert for MBT, identification was performed using Genotype Mycobacterium CM/AS.

Total of 214 "new cases" and 28 "relapses" of TB were registered in Arkhangelsk region in 2017. MTB was detected in 160 (74.8%) out of 214 "new cases" and all of them were tested for DR using molecular-genetic tests. 53 patients (31.1%) had MDR-TB, among them 3 patients had additional DR to injectables and 2 patients had XDR-TB. Among 28 "relapses" MTB was detected in 24 (85.7%) patients. 13 patients (54.2%) had MDR-TB, among them in 1 patient additional DR to injectables and in 2 patients to fluoroquinolones was detected. NTM associated disease was diagnosed in 4 patients (2 — *M. avium*, 1 — *M. gordonae*, 1 — *M. interjectum*).

All TB patients in Arkhangelsk region were tested with molecular-genetic tests before the treatment enabling quicker diagnostics and earlier treatment initiation. Early diagnosis ensures proper treatment regimen for patients with TB and NTM diseases. As a result management of TB patients is improved leading to better treatment outcomes and subsequently reduced TB transmission in the region.

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DETECTION OF EXTRACELLULAR *MYCOBACTERIUM TUBERCULOSIS* SMALL RNAs

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According to WHO, tuberculosis infection is one of the top ten deadly infections in the world, and about one-fourth of the human population is a carrier of latent tuberculosis infection (LTBI) without manifestation of disease symptoms. The LTBI associated with the conversion of *M. tuberculosis* bacilli to a dormant, metabolically inactive state; however, the molecular mechanisms of this change is not well studied. In many researches the small RNAs (sRNAs) was proposed as regulators of these processes. The aim of the study was detection of sRNA transcripts in cultural supernatant of *M. tuberculosis* strain H37Rv and into the blood serum of mice (C57Bl) infected with LTBI.

Mycobacterial cells were grown in Middlebrook 7H9 containing 10% ADC supplement at 37°C and harvested at different growth phase for use. The culture of *M. tuberculosis* was centrifuged at 6000g for 20 min at 4°C. The supernatant was filtered through 0.22 μm filters to remove the remaining bacteria. Bacterial total RNA was extracted from *M. tuberculosis* cultural supernatant by ExtractRNA reagent (Evrogen, Moscow, Russia), followed by digestion with Turbo DNase-free kit (Ambion, Austin, TX, USA) to remove contaminating DNA. cDNA was synthesized with 1.5 μg of total RNA by M-MLV (Evrogen, Moscow, Russia) transcriptase and random hexadeoxynucleotides according to the manufacture's instruction. The quantitative RT-PCR (qPCR) was carried out with the qPCRmix-HS SYBR (Evrogen, Moscow, Russia) and the CFX-96 real-time PCR detection system (BioRad, USA). All primers used in this study were designed using VectorNTI 11 (Invirogen, USA) and GeneRunner software (http://www.generunner.net) and synthesized at Evrogen (Moscow, Russia). LTBI C57Bl mouse model was designed as previously described (Shramko et al., 2010). The certain sRNA transcripts have been detected in *M. tuberculosis* culture supernatant at different growth phases (exponential phase, stationary phase and late-stationary phase) and into the blood serum of mice infected with LTBI. Obtained data allow us to propose *M. tuberculosis* sRNAs as new markers for LTBI diagnostic in the future.

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TB PORTALS PROGRAM: DATA-DRIVEN MULTINATIONAL CONSORTIUM AGAINST DRUG-RESISTANT TUBERCULOSIS

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TB Portals Program (TBPP) is an international initiative that is founded, developed and steered by doctors, researchers, IT specialists and radiologists to combat drug-resistant tuberculosis (DR-TB). The efforts of hospitals and biomedical research institutes from ten countries on three continents are organized and supported by TBPP to actively establish and grow an integrated open-access network of innovative tools and data from real patient cases of TB (tbportals.nid.nih.gov). The TB Portals collect, analyze, standardize, and present anonymized clinical, laboratory, and socioeconomic data, full bacterial genomes, and radiological data (CXR and CT).

The TBPP database currently has more than 1300 published (22 250 total cases), 75% of which are MDR or XDR-TB. Clinicians supply and validate all patient data. Once validated, the data become published with open access status in accordance with NIH FAIR principles. Importantly, both original and derived (expert-based and computational annotations) data remain patient-centric, i.e. linked to a unique patient identifier. This cornerstone principle enables users to define and analyze cohorts of patients, augmenting OMICS studies with diverse clinical information.

To date the database contains 730 published (1300 total) fully sequenced and annotated *Mycobacterium tuberculosis* genomes associated with the patient case record. We will highlight several TBPP projects studying 1) genomic signatures for TB relapse and reinfection, and 2) comparative analysis of *M. tuberculosis* strains isolated from sputum vs. surgically removed parts of lungs.

TBPP assists doctors and researchers in testing and refining their hypotheses with friendly and powerful tools. Starting from genomics and the molecular basis of drug resistance, we will demonstrate how our online tools enable anyone to simultaneously look at clinical, microbiological and radiological evidences in order to (1) search for genomic clues for inconsistencies in existing DR-TB diagnostics and to (2) study common and countries-specific evolutionary patterns of *M. tuberculosis*.

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SIMPLIFYING NGS APPROACHES TO OPTIMIZE TRACING OF TRANSBORDER SPREAD OF *MYCOBACTERIUM TUBERCULOSIS*

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Molecular epidemiology, and more recently genomic epidemiology, based on whole genome sequencing (WGS), improve our understanding about the transmission dynamics of *Mycobacterium tuberculosis* in a population. However, in many countries, including many of those with a high burden of TB, systematic genomic epidemiology cannot be implemented. Trying to find a solution to this situation, we propose an alternative line of progression, which tries to conciliate the discriminatory power of WGS with the speed, low cost and simplicity of PCR-based approaches. The cost of this shortcut is that it sacrifices the complete knowledge of all the transmission clusters in a population, because it needs to focus on surveying the strains that deserve special attention because they are more actively transmitted, or correspond to high-risk MDR or XDR strains. This short-cut strategy has proved to be efficient to survey actively transmitted strains, to fast track outbreak-strains, to update the presence of high-risk strains in a population or to give an urgent answer to public-health alerts, such as to rule out secondary cases due to the importation of XDR-TB cases. More recently, we are integrating this strategy to optimize the characteriza-

tion and tracking of trans-national transmission events. We have activated a decentralized multinational network of surveillance nodes. This network simultaneously analyze the cross-border distribution of relevant strains by means of sharing the same set of strain-specific PCRs. Once new cases infected by the surveyed strains are captured by the strain-specific PCRs the isolates are characterized by WGS. From these data we to define in detail the network of relationships between the involved cases. It allows us to differentiate transmissions after arrival of migrants to the host countries from independent importations from their countries of origin. Integrative multinational efforts supported on novel simplified strategies can transform the way in which we survey TB transmission in a new global scenario.

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GENETIC DIVERSITY AND DRUG RESISTANCE OF *MYCOBACTERIUM AVIUM* IN ITALY

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Mycobacterium avium complex is responsible for most of the human-associated nontuberculous mycobacteria infections. *M. avium* is classified into 4 subspecies, each endowed with specific pathogenetic and host range characteristics; among these, *M. avium* subsp. *hominissuis* (MAH), that is usually isolated from human and swine sources, is an important pathogen that causes infections in the respiratory tract, lymph node, and, occasionally, soft tissue of immunocompetent patients; moreover, it causes disseminated diseases in patients with human immunodeficiency virus infection. In Italy, as in many other countries worldwide, MAH is the most common cause of nontuberculous mycobacteria infection and the incidence of MAH infections is increasing. In the present study, we determined the VNTR-based genetic diversity of a collection of 71 MAH human strains isolated from 2010 to 2016 in order to estimate the genetic relationships among MAH isolates in our setting. Moreover, we performed the clarithromycin susceptibility test in order to investigate whether there was any association between the VNTR pattern and the minimal inhibitory concentration (MIC) of clarithromycin. The VNTR analysis revealed 24 distinct VNTR patterns; of these, 16 patterns were unique, while 8 patterns were shared by 2 or more isolates, thus yielding 8 lusters including a total of 55 isolates. Our results showed that most MAH isolates displayed a close genetic relationship, indicating that the MAH genotypes are quite homogeneous in our geographical area. Such genotypic stability of the MAH strain population circulating in our region supports the hypothesis of the presence of possible local sources of infection and transmission pathways at the local level.

Clarithromycin showed strong antimicrobial activity against MAH isolates, as indicated by the high proportion (94.4%) of susceptible strains. No significant association between VNTR genotype and MIC of clarithromycin was found; moreover, due to the small number of resistant isolates, it was not easy to evaluate the correlation between VNTR genotypes and clarithromycin susceptibility.

Further investigations on larger collections of MAH strains of human, animal and environmental origin, are needed both to define the correlation between genotypes and clinical features or drug resistance and to clarify the sources of infection and the specific transmission pathways of our region, in order to achieve a better control of MAH infection.

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GENOTYPES OF *MYCOBACTERIUM TUBERCULOSIS* ISOLATES FROM DIFFERENT ORGANS OF PATIENTS WITH GENERALIZED TB AND HIV-COINFECTION

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The purpose of the study was to genotype *Mycobacterium tuberculosis* isolates from internal organs of patients from St. Petersburg, Russia, with generalized tuberculosis (TB) and HIV coinfection, to assess possible association between the *M. tuberculosis* genotype and localization of TB disease.

A total of 128 strains of *M. tuberculosis* were recovered from 55 patients with HIV infection of stages 4 or 5 and generalized TB with multiple lesions of internal organs (from 2 to 10), were studied. Most of the patients had affected lungs (50 cases), intrathoracic and intra-abdominal lymph nodes (46 and 34 cases respectively), spleen (40 cases), kidneys (32 cases), brain and meninges (32 cases). *M. tuberculosis* isolates were cultured from lungs (48), intrathoracic and intra-abdominal lymph nodes (40), spleen (20), kidneys (14), meninges and brain (7). *M. tuberculosis* were isolated from one affected organ in 11 patients, and in the remaining cases isolates from 2 or 3 affected organs (16 and 28 persons, respectively) were obtained. Genotyping was performed by spoligotyping (all strains) and IS6110-RFLP typing (Beijing genotype), the obtained profiles were compared with the international database SITVIT_WEB and a proprietary database (Narvskaya, 2003), respectively. The data were subjected to statistical analysis.

67.3% (37 of 55) patients were infected by *M. tuberculosis* Beijing genotype. Of the 37 patients infected with Beijing strains, strains of cluster A0 were isolated from 14 patients, B0 from 5 patients, and 10 other RFLP types were obtained in the remaining 18 patients. The non-Beijing genotypes were represented by LAM (4), Ural (5), T (5) and 4 others (1 strain of each genotype), all of which belong to the Euro-American lineage of *M. tuberculosis* (lineage 4). No difference was observed for isolates from different organs of the same patient. Of the 37 patients infected with Beijing strains, the lungs were affected in 33 patients, intrathoracic lymph nodes in 28, spleen in 28, intra-abdominal lymph nodes in 28, kidneys in 24, and brain and meninges in 24 patients. Of 18 patients infected with non-Beijing strains, lungs were affected in 17 patients, intrathoracic lymph nodes in 18, spleen in 12, intraabdominal lymph nodes in 11, kidneys in 8, brain and meninges in 10. Comparison of different organs for association with infection by Beijing and non-Beijing strains did not reveal statistically significant differences: lungs ($P = 0.890$); intrathoracic lymph nodes ($P = 0.503$); spleen ($P = 0.777$); intra-abdominal lymph nodes ($P = 0.970$); kidneys ($P = 0.448$); brain and meninges ($P = 0.886$).

Beijing genotype was predominant among *M. tuberculosis* isolates studied. Among Beijing family strains, cluster A0 (corresponding to the Central Asian Russian strain) prevailed. There were no statistically significant differences between Beijing and any of non-Beijing genotypes with regard to frequency of isolation from particular organs.

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PHYSIOLOGICAL IMPACT OF THE EVOLUTION OF THE *rpoB* MUTATION

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Bacilli within an infected lung cavitary lesion spontaneously evolve mutations that confer resistance and are subsequently selected following antibiotic treatment. During this evolutionary process both drug susceptible and drug resistant bacilli may be present. This mix state of susceptive and resistant bacilli captured at a distinct point in time may change during the course of infection and drug selection. The complexity of the population structure in each sputum sample may thus define the outcome of molecular and phenotypic drug resistance testing which in turn may determine how the patient will be treated. We hypothesize that the *rpoB* mutation will influence the transcriptome of the rifampicin mono-resistant isolate compared to the progenitor rifampicin susceptible isolate.

A sputum sample from an individual patient containing a heterogeneous population of both a rifampicin mono-resistant Beijing Ser531Leu clone and its susceptible progenitor was selected. DNA was extracted and sequenced using the Illumina HiSeq platform and analyzed using an in-house bioinformatic pipeline. RNA was extracted and sequenced using the Illumina platform and analyzed using Chipster, an open source bioinformatic platform.

The small number of variants between the two isolates suggests that the resistant isolate evolved from the susceptible progenitor. Our comparative transcriptomic analysis showed that microevolutionary events within the *rpoB* gene had a considerable influence on transcription. Consequently, the expression of bacilli's stress response, sigma factors, and regulatory genes were down regulated. This in turn led to a down-regulation of expression of a large number of genes, suggesting that the rifampicin resistant mutant has an altered physiology.

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MYCOBACTERIUM TUBERCULOSIS DRUG RESISTANCE MUTATIONS AND UNDERSTANDING OF PK/PD: TREATMENT AND CARE IMPLICATIONS

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Russian Federation has the third-highest burden of multidrug-resistant tuberculosis (MDR-TB) in the world and complicated by high rates of human immunodeficiency virus (HIV) co-infection which leads to mortality and risk for acquired *Mycobacterium tuberculosis* drug resistance. Treatment outcomes may be a consequence of pharmacokinetic/pharmacodynamics (PK/PD) variability.

In Irkutsk, we aimed to describe pharmacokinetic variability, minimum inhibitory concentrations (MICs) for key anti-TB drugs and their molecular correlates of resistance, and to determine if PK/PD variability associates with treatment response.

Consecutive people living with HIV initiating TB treatment at Irkutsk Regional TB Referral Hospital were recruited. After 2 weeks of treatment, medications were directly administered and plasma samples collected at 2 and 6 hours after administration. Drug concentrations were measured using validated liquid chromatography-mass spectrometry assays for peak concentration (C_{max}), the highest value in the dosing interval, and area under the concentration curve from time 0 to 6 hours (AUC_{0-6}). *M. tuberculosis* MIC testing was performed using the MYCOTB Sensititre plate. A drug was classified as active when C_{max} was greater than MIC. PK/PD variability as a predictor of treatment outcome was determined by classification and regression tree (CART).

69 patients with HIV had PK/PD testing. Mean age was 34 years (SD±6.2), 45 (65.2%) were male. Mean CD4 count was 180 (±202) cells/mL. Thirty-six (52.2%) had drug susceptible TB, 10 (14.5%) MDR-TB, 17 (24.6%) pre-extensively drug-resistant (XDR)-TB and 6 (8.7%) with XDR-TB. Based on PK/PD testing, patients were treated with a lower number of active drugs (3.25±1.40) compared to the number presumed to be active when initially prescribed (4.81±0.94), $p \leq 0.001$. Fifty patients had treatment outcomes and 16 (32.0%) had treatment failure. In CART analysis, regardless of molecular mutation for drug resistance, having less than 4.5 active drugs as redefined by PK/PD testing, correctly identified 15 of 16 (93%) of patients with treatment failure.

In Irkutsk, PK/PD testing predicted treatment outcome for patients with HIV/TB. Screening for mutations in *M. tuberculosis* resistance determining regions is an important method for constructing initial regimens, but should be followed by PK/PD testing to attain the highest likelihood of drug activity.

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GENOMICS AND LOCAL ADAPTATION OF *MYCOBACTERIUM AVIUM*

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Mycobacterium avium subsp. *hominissuis* (MAH) is a human pathogen that causes *M. avium* complex (MAC) lung disease, which is difficult to cure by current antibiotics treatment. It has been suggested that MAH circulates between the human body and the environment. Despite its clinical significance, the genetic mechanisms underlying local adaptation of this pathogen are unknown due to a lack of population-wide genomic data. To overcome this issue, we evaluated the genetic population structure of MAH using genome-scale data from 36 global strains (including 12 Japanese strains sequenced in this study), and then sought to identify alleles unique to Asian populations by comparative genomic analysis. The population structure analysis was extended to include 652 global strains using the multiple-locus variable-number tandem repeats data set, which revealed that two genetic population groups dominated the Asian isolates.

By analyzing mutual homologous recombination and gene content, we revealed that MAH reproduces sexually and has an unlimited gene repertoire. The results of these analyses predict the presence of a chromosome

exchange mechanism called "distributive conjugative transfer (DCT)," recently discovered in other mycobacterial species (Gray et al., Science. 2016. 354: 347–350). We also identified chromosomal loci where allelic variation was correlated with geographic origin. One noteworthy discovery was that the origin of genes responsible for trehalose biosynthesis differed between Asian and other MAH populations. Furthermore, we observed transmission of alleles encoding "mammalian cell entry proteins" between East Asian populations. Therefore, sexual reproduction, likely via DCT, plays a critical role in local adaptation of MAH.

As a new concept, we present a model for the life cycle of *M. avium*, in which *M. avium* generates progeny with diverse genomes via "mating" in a common environmental pool, prior to infection of human hosts. After infection, the progeny is subjected to natural selection within the host, followed by the re-release of clones with adaptive alleles into the environment. This concept may also be relevant for other mycobacterial species.

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UTILITY OF WHOLE GENOME SEQUENCING (WGS) OF *MYCOBACTERIUM TUBERCULOSIS* COMPLEX ISOLATES IN PRACTISE

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Since 2016, all culture positive *M. tuberculosis* complex isolates have been subjected to WGS to allow comparison with the current laboratory diagnosis in the Netherlands. The utility of WGS was investigated for 1) identification of (sub) species and genotypes; 2) drug susceptibility testing; and 3) investigation on tuberculosis transmission.

The new SNP-IT (SNPs to identify TB) method, developed at the RIVM/the Netherlands and Oxford University/the UK, traces SNPs shared exclusively by members of each (sub) species, lineage, and sub-lineage. The total number of unique SNPs identified ranged from 23 for *M. bovis* to 6.837 for *M. canettii*. The SNP-IT method was applied to 1.157 routine samples from 2016/2017 in the Netherlands and compared with identification by Reverse Line Blot, PhyResSe, and Coll SNP-barcode. This comparison showed that SNP-IT more accurately identifies all animal (sub) species and is more specific in identifying sub-lineages of lineage 4. A small proportion (n = 176) of lineage 4 isolates could not be identified by SNP-IT due to high similarity to the H37Rv reference genome; these are in the Coll SNP-barcode system identified as lineage 4.5/4.7/4.8.

Phenotypic drug susceptibility testing (MGIT) was compared with the detection of resistance-associated mutations by WGS for first-line antibiotics rifampicin, isoniazid, ethambutol, and pyrazinamide. In total, 1.134 isolates from 2016/2017 in the Netherlands were included. For all drugs, the negative predictive value (NPV) was > 99%. In general, rifampicin and isoniazid had most optimal scores. For rifampicin, the sensitivity was 100%, specificity 99.8%, the positive predictive value 95%, and the NPV 100%. This was 98%, 99.2%, 92.5%, and 99.8%, respectively, for isoniazid. WGS was also able to predict intermediate/low level resistance for rifampicin, isoniazid, and pyrazinamide. A minority of isolates showed discrepancy between MGIT and WGS results; these isolates are re-tested to explain discrepancy results.

Both VNTR typing and WGS were applied to all isolates from 2016. In total, 535 isolates were genotyped, of which 25% (134/535) were clustered by VNTR and 15% (82/535) by WGS. The proportion of identified epi-links among WGS clustered cases (50%) was much higher than among VNTR clustered cases (31%). This study was repeated with isolates from 2016 and 2017 to analyse transmission over two years.

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MINOR GENETIC DETERMINANTS OF SECOND-LINE INJECTION DRUGS RESISTANCE IN *MYCOBACTERIUM TUBERCULOSIS*

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Second-line injection drugs group, which include kanamycin (KAN), capreomycin (CAP), and amikacin (AM), is one of the cornerstones used in the treatment of MDR tuberculosis. Though the main resistance mechanism leading to cross-resistance to all three drugs described in *Mycobacterium tuberculosis* is the alteration of 16S rRNA, other mechanisms also could be found in clinical strains, such as promoter mutations of the *eis* and *whiB7* genes leading to KAN resistance, and TlyA inactivating mutations leading to CAP resistance. In consequence, a noticeable number of resistant strains do not carry any known mutations.

We performed the next-generation sequence analysis of the 5 Beijing and one Haarlem lineage clinical *M. tuberculosis* strains with discordant results of phenotypic resistance to injection drugs, and genetic analysis of *rrs*, *eis*, *tlyA*, and *whiB7* loci. The sequencing data were analyzed using the Galaxy web platform (http://usegalaxy.org). Further bioinformatic analysis of the obtained SNPs was performed with custom Python scripts and public databases ReSeqTB and PolyTB.

We found 2126–2229 SNPs for Beijing lineage and 1606 SNPs for Haarlem lineage isolates compared to the referent H37Rv strain. Upon the exclusion of known mutations associated with resistance, fitness compensation and deep bioinformatic analysis, the list of candidate SNPs, potentially associated with resistance, was shortened to 10–100 for each strain. The novel putative mechanisms of resistance included mutations in elongation factor EF-G, phosphotransferase Aph, hypothetical protein Rv0147, secretion protein EspG2, and aspartate aminotransferase AspC.

The diversity of drug resistance mechanisms reflects the complexity of microevolution of *M. tuberculosis* and impacts the sensitivity of molecular tests. Improvement of our knowledge of drug-resistance mechanisms would facilitate the discovery of new drugs together with the prediction of drugs interactions and promote the development of molecular assays.

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PECULIARITIES OF THE TUBERCULOSIS, HIV AND HIV-ASSOCIATED TUBERCULOSIS INCIDENCE IN THE FAR EASTERN FEDERAL DISTRICT OF THE RUSSIAN FEDERATION

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Objective of the research was to analyze the dynamics of the tuberculosis (TB), HIV and HIV-associated TB incidence in the Far Eastern Federal District (FEFD) of the Russian Federation.

The data of the official statistical forms No. 8, No. 33, No. 61 was used. Modern statistical methods were applied.

The TB incidence declined by 43.2% (from 85.1 to 48.3‰) during 2008–2017 in the Russian Federation when prevalence declined by 42.2% (from 190.7 to 109.8‰). The most pressing tuberculosis epidemiological situation persisted in the FEFD. During a decline of TB-incidence in the FEFD in the years of 2009–2017 by 32.9% the regional index was still higher compared to the Russian-wide rate by 1.8 — 1.8 times. HIV-infection in 2017 reached 36.0‰ versus 14.0‰ registered in 2008 in the FEFD. During the analyzed period HIV-incidence accession rate in the FEFD constituent entities changed repeatedly. In 2017 the HIV prevalence rate in FEFD was lower than the Russian-wide index by 2.2 times. The highest HIV prevalence in the FEFD was registered in the Primorsky, Sakhalin Territories and the Chukotka Autonomous Region (57.3; 50.9 и 50.0‰ respectively). The HIV-TB associated incidence in the Russian Federation reached 8.3‰ in 2017 versus 4.3‰ in 2009. During the analyzed period of time percent of the HIV-positive patients with associated TB increased from 6.4 to 17.2% in the Russian Federation. In the FEFD the same index raised from 2.0 to 4.8%. This said the Russian-wide index consistently exceeded the FEFD rate by 3.1–4.6 times. The highest accession rates of the present index in the FEFD were registered in the Khabarovsk Territory — 9.9%, the Amur region — 9.4%, the Republic Sakha (Yakutia) — 6.6%.

The FEFD is a leading territory of TB-incidence for several years. An increase in the number of HIV-positive people as well as HIV-TB co-infected patients was registered in the region. Substantial differences of the analyzed indices in different constituent entities of the FEFD were registered.

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GLOBAL WHO POLICIES ON MOLECULAR METHODS FOR TB DIAGNOSIS

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The World Health Organization's (WHO) global strategy for TB prevention, care and control for 2015–2035 (known as the End TB strategy) calls for the early diagnosis of TB and universal drug susceptibility testing (DST), highlighting the critical role of laboratories for rapidly and accurately detecting TB and drug resistance. This requires ensuring access to WHO-recommended rapid diagnostics and universal access to drug-susceptibility testing (DST) for all patients with signs and symptoms of TB. WHO defines universal access to DST as rapid DST for at least rifampicin among all patients with bacteriologically confirmed TB, and further DST for at least fluoroquinolones among all TB patients with rifampicin resistance. The WHO estimates that 10.4 million persons developed tuberculosis (TB) worldwide in 2016, including 490 000 cases of multidrug-resistant TB (MDR-TB) and 110 000 cases of rifampicin-resistant TB (RR-TB), needing the same second-line treatment regimen.

The objective of the study was to present WHO policies on molecular methods for TB diagnosis.

Xpert MTB/RIF and/or Ultra are recommended as initial test for diagnosis of all persons with signs and symptoms of TB. Line probe assays are recommended as rapid diagnostic tests for detection of resistance to Isoniazid, Rifampicin, Fluoroquinolones and Amikacin. DNA Sequencing is becoming increasingly important as a reference method for detecting mutations associated with resistance to first and second line anti-TB drugs.

According to WHO policies, molecular methods play critical role in global fight with TB.

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PREVALENCE OF NONTUBERCULOUS MYCOBACTERIUM spp. STRAINS ISOLATED FROM CLINICAL SPECIMENS AT NORTH ESTONIA MEDICAL CENTRE IN 2001–2017

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Mycobacteria are widespread microbes in nature. Mycobacteria that are not causative agents of human and animal tuberculosis are designated as non-tuberculous mycobacteria (NTM). NTM caused infections start to become more frequent in the recent years. This report summarizes data of the NTM isolates reported at North Estonia Medical Centre in the period 2001–2017. In Estonia, physicians are not requested to report infections involving NTM species. Reports of the prevalence of MTBC are available in the Estonian Tuberculosis Registry. In contrast, reporting of NTM suspected to be involved in a disease, does not represent the absolute occurence and distribution of the NTM species and disease. These reports are not verified and any association with clinical data should be interpreted with caution.

The incidence of tuberculosis in Estonia shows a trend of decrease. The aim of the study was to analyse the prevalence of NTM strains on the basis of clinical specimens.

The NTM species were isolated from clinical material that was sent to the mycobacteriology laboratory for diagnostic purposes during 2001–2015. The pathological material was decontaminated by NaOH+NALC and cultured on Löwenstein–Jensen and Middlebrook media. The NTM species were identified by the GenoType Mycobacterium CM/AS test (Hain Lifescience GmbH).

In 2001–2017, of 114 907 investigated specimens, 12 790 (10.9%) MTBC and 1020 (1.04%) NTM strains were identified. The leading NTM species isolated were *Mycobacterium avium* (41.7%), *M. gordonae* (17.5%), *M. fortuitum* (9.3%) and *M. intracellulare* (6.8%). The rarely isolated NTM were represented by *M. kansasii*, *M. xenopi*, *M. szulgai* and *M. abscessus*. Of the NTM, 72% were cultured from sputum, 5% from blood and 23% from other materials. In total, 85 patients were infected with NTM in 2001–2005, 210 in 2006–2010 and 331 in 2011–2017. Of the NTM infected patients, 35% were > 65 years old and 9% were < 30 years old. A TB and HIV coinfection was found in 317 (8.3%) and NTM-HIV coinfection was found in 56 (10.5%) patients.

During the last 17 years the prevalence of NTM in patients' material has increased approximately three times. The most prevalent species is *M. avium*.

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THE INFLUENCE OF THE H2 COMPLEX ON *MYCOBACTERIUM AVIUM* INFECTION IN MICE

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Mycobacterium avium is the opportunistic pathogen in humans, animals and birds and the most common cause of non-tuberculous mycobacterial lung infections worldwide. Analogously to other mycobacterial infections, its antigens are presented predominantly in the context of the Class II *MHC* molecules resulting in activation of $CD4^+$ T cells producing IFNγ, the key cytokine in antimycobacterial response and infection control.

Addressing genetic control of *M. avium*-triggered disease, we compared two congenic strains of mice on the B6 genetic background established in our lab — B6.I-100 ($H2\text{-}A^jE^j$) and B6.I-139 ($H2\text{-}A^{jb}E^j$) — that carry different alleles encoding the β-chain of the *H2-A* gene. After aerosol *M. avium* challenge, B6.I-139 mice died earlier and displayed more severe cachexia compared to B6.I-100 mice. Measurement of the CFU counts in lungs and spleens at weeks 8, 12 and 18 post infection, revealed significant differences in the lung phenotype at the early phase (more CFUs in the lungs of B6.I-100 mice). Assessment of lung pathology demonstrated diffuse inflammation in the lung tissue of B6.I-139 mice at week 8 post infection and granulomata containing foamy macrophages and necrotic zones during the chronic phase. Flow cytometry and immunohistochemical staining revealed higher neutrophil inflammation in the lungs of B6.I-100 mice, accompanied by an increased expression of genes involved in neutrophil attraction. The level of proinflammatory TNFα, but not IL-6 and anti-inflammatory IL-10 and TGF-β, was higher in the lungs of B6.I-100 at an early stage of infection. Importantly, lung $CD4^+$ T cells from more resistant B6.I-100 mice were more activated ($CD44^{hi}CD62L^{lo}$ phenotype) and produced significantly more IFNγ in response to mycobacterial antigens during chronic stage of infection. Higher numbers of lung $CD4^+$ T cells in B6.I-139 mice in 8 weeks after challenge may reflect an attempt of the host to control infection early after challenge, apparently not successful. Of note, it is not clear whether interstrain differences in disease progression reflect differences in the efficacy of antigen presentation between $H2\text{-}A^b$ alleles and subsequent T cell activation, or T cell exhaustion during chronic stage of the immune response. Overall, our data suggest that the allelic differences in the H2-A molecule are involved, albeit moderately, in control to *M. avium* infection.

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MYCOBACTERIUM AVIUM-TRIGGERED DISEASE: HOST GENETICS AND IMMUNITY IN MOUSE MODELS

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Mice of the I/St strain are extremely susceptible to *Mycobacterium tuberculosis* but resistant to *M. avium* infection, whereas B6 mice show a reversed pattern of susceptibility. By directly comparing: (i) characteristics of susceptibility to two infections *in vivo* (ii) architecture of lung granulomata and (iii) expression of genes encoding regulatory factors of neutrophil influx in the lung tissue, we demonstrate that genetic susceptibility of the host determines the pattern of lung pathology. *M. avium*-infected B6 mice and *M. tuberculosis*-infected I/St mice are prone to develop necrotizing granuloma surrounded by hypoxic zones, massive neutrophil influx and B-cell follicles in the lung tissue. These mirror-type lung tissue responses demonstrate that the level of genetic susceptibility of the host to a given mycobacterial species largely determines characteristics of pathology, and emphasize the importance of host genetics in pathogenesis. Segregation genetic analysis and development of novel H2-recombinant congenic strains allowed dissection of genetic control of two infections. Regarding susceptibility to and severity of *M. avium*-triggered disease, involvement of two distinct genes was clearly demonstrated: the *Slc11a1* (former *Nramp1*) gene, acting as a major genetic factor, and the classic Class II *MHC* gene *H2-Ab*, a minor modifier of susceptibility pattern.

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EVOLUTION AND TRANSMISSION OF *MYCOBACTERIUM TUBERCULOSIS* RESISTANCE TO FLUOROQUINOLONES

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Fluoroquinolones (FQs) have been widely used for the tuberculosis (TB) treatment for decades and *Mycobacterium tuberculosis* strains resistant to FQs have been reported globally. In the past few years, we had gained some insights into the evolution and transmission of *M. tuberculosis* FQ-resistance. Firstly, we found that FQ-resistance mostly appeared in multi-drug resistant (MDR) *M. tuberculosis* strains. We observed the emergence and transmission of FQ-resistance in clinical clustered (as defined by whole-genome sequencing) MDR cases and we speculated that the general inclusion of FQs in the first-line treatment regimen in western China may contribute to the high resistance rate among MDR cases. By studying the within-host heterogeneity of *M. tuberculosis*, we proved that the evolution of FQ-resistance is associated with the emergence and competition of several resistance related mutations in DNA gyrase genes, a process for selecting highly resistant and low-cost strains. Lastly, we studied the mechanisms of primary ofloxacin-resistant strains to acquire resistance to moxifloxacin (new generation FQ) and we found a secondary mutation in DNA gyrase associated with this process.

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EPIDEMIOLOGY OF EXTRAPULMONARY TUBERCULOSIS IN ALBANIA, 2010–2016

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Extrapulmonary tuberculosis (EPTB) is a therapeutic challenge. Possible reasons include ist under- and overdiagnosis/reporting. Here, we report results of the cross-sectional retrospective review of the epidemiology of EPTB in Albania from 2010 to 2016.

The objectives of the study were to find out epidemiological characteristics of EPTB and to explore risk factors, and challenges in the diagnosis and management of EPTB in Albania.

We used data from National TB Program and included all cases of TB diagnosed in the Albania from 2010 to 2016. Information on age, sex, year of diagnosis, and anatomic location of the site of disease was retrieved from central database of National TB Program.

In Albania during 2010–2016, 925 cases of extrapulmonary TB were reported, males were 581 (63%) and females 344 (37%). The number of cases diagnosed per year was as follows: 170 (38.2%) in 2010, 129 (30%) in 2011, 108 (25.7%) in 2012, 141 (29.7%) in 2013, 147 (36%) in 2014, 117(28.2%) in 2015 and 113 (27.2%) in 2016.

Sputum smear examination, X-ray and culture examination and tissue biopsy were carried out in 58; 42.3; 18 and 15% of patients respectively for EPTB diagnosis. The most affected age group was < 65 years (23%). Pleural effusion (35%) and lymph node (15.7%) were the most common types of extrapulmonary TB.

Patients live in urban areas (60%) rather than rural (40%). The mean age of EPTB patients is 44.5 and pulmonary TB patients is 41.2. Incidence of EPTB decreased from 5.5/100 000 in 2010 to 5.1/100 000 in 2016.

In Albania, extrapulmonary TB in 2010–2016 showed a slight decrease in incidence, although the rates are still very high. Diagnosis of extrapulmonary TB was made according to national guidelines, however long delay has been reported in most cases before the final diagnosis. Microbiological proof is the key to diagnosis and treatment, and tissue biopsy that should be required regularly.

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DETERMINANTS OF TB RELATED DEATH FROM TUBERCULOSIS PATIENTS IN THE NORTHERN THAILAND

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Tuberculosis is the most common cause of deaths from respiratory infection in Thailand. Understanding the risk of death could provide useful information to provide better clinical care for TB patients. *N-Acetyltransferase 2 (NAT2)* gene is the main determinant of isoniazid (INH) metabolism. *NAT2* rapid acetylator contributes to lower anti-TB drug (INH) serum concentration and increased risk of treatment failure and relapse from INH based TB regimens.

The aim of this study was to determine the effect of *NAT2* acetylator status on TB related death.

TB patients were recruited from the TB registry during 2002–2011 in Chiang Rai province, Thailand. The *NAT2* acetylators (rapid, intermediate and slow) were determined by haplotype specific polymerase chain reactions, HS-PCR. These groups of patients were excluded from further analysis: 1) patients who did not receive the INH based regimens for TB treatment; 2) patients who did not receive the INH based regimens longer than 2 weeks and 3) patients who died within the first 2 weeks of TB treatment. Mortality-associated risk factors within 1 year of treatment were analyzed using Cox-regression model.

Of 1.076 TB patients who met study criteria, 213 (19.8%), 495 (46.0%) and 368 (34.2%) belonged to *NAT2* rapid, intermediate and slow acetylate group respectively. In total, 115 patients died within 1-year follow-up. In the multivariate analysis, rapid *NAT2* acetylator status increased the risk of death when compared against *NAT2* intermediate acetylator (adjusted hazard ratio [aHR]: 1.83, 95%CI: 1.15–2.91). The effect of *NAT2* rapid acetylator on deaths is more significant in HIV positive TB patients (aHR 2.68, 95%CI:1.14–6.26). The risk factors associated with death was different among the *NAT2* acetylators. In *NAT2* rapid acetylator group, elderly people, HIV positive, past TB history and smoking status was increased the risk of death.

The *NAT2* rapid acetylator is related to the mortality during TB treatment. The inadequate treatment with the doses of standard regimens caused by *NAT2* rapid acetylator might increase the risk of death in TB patients, these NAT2 pharmacogenetic risks are interacting with other clinical risk factors, which is depended on the acetylator status.

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CLICHES AND DOGMAS IN MOLECULAR TUBERCULOSIS RESEARCH

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I will present a personal critical view on some hot issues of the molecular epidemiology of tuberculosis, in particular, regarding an uncritical use of some well known online tools and resources. Invaluable for establishing terminology and classification in molecular epidemiological studies of *Mycobacterium tuberculosis*, they are limited by our insufficient knowledge of genome evolution and uncritical perception of their indications. This is exemplified by partly inadequate (sub)clade assignment due to imperfect decision rules, and misleading methodological approach when scientifically unsound phylogenies are built from spoligotyping data.

To begin with, I propose the following definitions. First, "molecular mythology" that relies on minimal array of references that conveniently support long-lasting clichés. Second, "molecular iconography" that relies on dogmatic perception of the current knowledge when online databases are uncritically regarded as ideal icons. Finally, I introduce the term "click science". In contrast to the fascinating and sophisticated click languages, "click science" relies on uncritical and simplified perception of knowledge and a dogmatic, iconographic view of indications provided by increasingly convenient online tools and databases. For example, spolTools is an example of the click phylogenetics when a plethora of statistics is generated in few clicks but their exploration is minimal. In its turn, click systematics is exemplified by SITVIT's (i) reader-unfriendly huge tables with different possible percentages and (ii) easy to read but partly inadequate (sub)clade labels.

Labels are convenient for classification, but should be revisited in the context of modern knowledge. The "we have been taught this way" approach reflects the mentality of a conservative teacher rather than a creative researcher. As Heidegger once said, "knowledge does not think"; indeed why think when it already knows? As far as science is concerned, this quotation from Henry Gee's "The accidental species" is much more appropriate: "Science is about neither Facts nor Truth, but the quantification of doubt".

Below are examples of some clichés pertaining in molecular epidemiology of tuberculosis.

Firstly, pathogenic properties of the Beijing genotype are traditionally listed as increased virulence, association

with drug-resistance, high transmissibility and clustering. But they do vary among endemic and sporadic strains, in different settings and hosts, even at within country level.

Secondly, Russian epidemic clone Beijing B0/W148 was commonly regarded as widespread across all Former Soviet Union. In reality, its geographic distribution shows a peculiar clinal gradient with highest frequency in Siberia and sharp decrease in the Asian part of the former Soviet Union.

Thirdly, a global spread of LAM RD-Rio sublineage has been claimed and was attributed to its particular pathogenic properties. A comprehensive analysis of available data shows that RD-Rio strains are rarely present in Russia and East Asia. It appears that there is no global dissemination of RD-Rio due to specific virulence properties of these strains but rather their spread due to human migration (if such migration did take place).

A confusing terminology, misclassification and false clustering are not abstract issues but make a scientific discussion meaningless, and I will propose some courses for improvement of the situation.

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ANALYSIS OF SECONDARY RESISTANCE OF *MYCOBACTERIUM TUBERCULOSIS* TO SECOND-LINE ANTI-TUBERCULOSIS DRUGS IN CASABLANCA

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Tuberculosis (TB) is a major public health problem in Morocco, despite multiple strategies and funds allocated. According to epidemiological data, with about 31.452 TB cases detected in 2016, and a national incidence of about 91 per 100 000 inhabitants per year, TB pose a serious threat to TB management in Morocco, with Casablanca being one of the most affected region.

The objective of this retrospective study was to evaluate secondary resistance of *Mycobacterium tuberculosis* to 2nd line anti-tuberculosis drug in Casablanca.

In this retrospective study, 1300 patient samples from different CDTMRs and hospitals across Casablanca and regions over a 2-year period from January 1st, 2015 to December 31st, 2016 were analysed. Conventional techniques, such as BKD microscopic examination, BKC culture and antibiotic sensitivity were used for the diagnosis of TB.

Our results show that among the 1300 samples analyzed, 600 (46%) were found positive for MTB, of which 58.33% were male and 41.66% were female. Patients aged between 20 and 40 years was the most affected group, representing 78% of patients. Data using the conventional Petroff decontamination and homogenization technique for isolation, identification as well as titration of the "BK" strains were as follows: Negative culture (54%), Positive culture (46%). The antibiogram used for this study gave the following results: 53% were wild strains, 47% were mutants, among which 18% were "MDR" strains and 1% were "XDR" strains.

The results of the present study reflect the importance of a good management of TB cases in order to succeed the treatment regime adopted at the national level and the success of the fight against this scourge in Morocco.

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DEVELOPMENT OF THE EXTERNAL QUALITY ASSESSMENT SCHEME FOR NON-TUBERCULOUS MYCOBACTERIA DRUG SUSCEPTIBILITY TESTING IN EUROPEAN UNION

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Non-tuberculosis mycobacteria (NTM) are increasingly associated with pulmonary and extrapulmonary disease in humans. Although identification and drug susceptibility testing (DST) of NTMs comprises a significant part of the tuberculosis (TB) reference laboratory activities in the European Union (EU), currently no internationally recognised external quality assurance (EQA) schemes exist for NTM DST. It lacks standardization and evidence on how to interpret DST results for specific drugs is limited.

Recognising the need for harmonization of methodologies in EU/EEA, European Reference Laboratory Network for Tuberculosis (ERLTB-Net) in 2017 conducted a pilot study among National Reference Laboratories (NRL) aimed at understanding methods employed for identification and DST and developing an EQA scheme for NTM DST. Pilot study comprised a survey followed by an EQA round using identical panels comprising 10 well characterised rapid (*M. abscessus*) and slow (*M. avium*) isolates.

Completed questionnaires were received from a total of 32 NRLs (97.0% response rate). Thirty NRLs routinely perform identification of NTMs with a majority (77.4%; N = 24) using line probe assays as a primary means of NTM speciation. Reports containing minimum inhibitory concentrations (MICs) and result interpretation for five key drugs (Clarithromycin (CLA) Amikacin (AMK) Moxifloxacin (MOX); Linezolid (LIN), and Doxycycline (DOX) for *M. abscessus* only) were received from 21 NRLs (95.5% response rate).

Interlaboratory agreement rates were higher for *M. abscessus* isolates; all five strains were found to be resistant to DOX by all NRLs (MIC 8.0–16.0 μg/ml). Relatively minor variations were seen in MICs and their interpretations for MOX and AMK while for LIN MIC ranges for individual isolates varied greatly (2.0–32.0 μg/ml) across NRLs resulting in a lower agreement with regards to results interpretation. For slow growers AMK and MOX appeared to be the most problematic drugs both in terms of MIC determination (ranging 4.0–64.0 and 0.5–8 μg/ml in individual strains, respectively) and interpretation.

The results show that inter-laboratory reproducibility is insufficient, highlighting the need for expanding EQA schemes for NTM DST. As EQAs for *M. tuberculosis* complex DST have led to more reliable and reproducible phenotypic DST, we propose to follow a similar approach for clinically relevant NTM.

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POLYMICROBIAL BIOFILM FORMATION AS A POSSIBLE CAUSE OF UNEXPECTED DEFAULTED TREATMENT OF PULMONARY TUBERCULOSIS

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Microbes rarely exist as single species planktonic forms as they have been commonly studied in the laboratory. Instead, the vast majority exists as part of complex polymicrobial biofilm communities attached to host and environmental surfaces. *Mycobacterial tuberculosis* (MBT) is no exception. A number of researchers have shown that in the experiment *in vivo* model, MBT can form biofilm-like structures in the lungs.

The aim of the study is to demonstrate the role of tuberculomas satellite microbiota as example of polymicrobial biofilm existent in a lung of TB patients.

Our study of clinical MBT strains shown less 5% of them were able to produce mature biofilms (pellicle) on a liquid medium. Although we might expect that pathogenic MBT could gain obvious advantage in case of growth in necrotic foci in lungs and it should keep this ability in the first passage *in vitro*. It was found feature of MBT strains produced pellicle on liquid medium to grow on Levinshtein–Jensen by specific R colonies. It looks as disk with a convex center, "UFO-colonies". It was shown on *in vitro* model that about of 50% clinical MBT strains can coexist together with *Bacillus licheniformis*, also isolated from sputum of TB patient. Moreover, after pellicle formation by bacilli in the first 3 days, the growth of MBT was continued for next 30 days under the bacillary pellicle. It is very important that investigated bacilli had a high tolerance to streptomycin, ethionamide, isoniazid and ethambutol, e.i. to four of the 12 basic anti-TB drugs.

The study on 16S rRNA metagenomic and massively parallel sequencing (NGS) DNA of several tuberculomas was conducted. It was shown that quantity of MBT genomes were less 3% in all cases. The vast majority species belonged to Gram-positive *Firmicutes* like *Staphylococcaceae* and also a small amount of Gram-negative taxons was found.

We can assume that anti-tuberculosis therapy is confronted with not only MBT, but with polymicrobial biofilm communities, which formed by the etiological agents of tuberculosis and also by a large number of other satellite microorganisms in lungs. It is very important that this microbial community in TB-patient lungs of should form a cumulative resistance to anti-tuberculosis therapy during long-term treatment. We can expect that the cumulative resistance of a polymicrobial biofilm in the TB-patient lungs may be significantly differing from the resistance of detected in the clinical laboratory TB strains.

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BACTERIAL WGS AND HOST GENOME-WIDE SNP ANALYSIS OF TUBERCULOSIS PATIENTS IN THAILAND

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Mycobacterium tuberculosis has been a human pathogen for a long time, providing ample opportunities for genomic interactions between the two organisms. Evidences of co-evolution has been reported. We have performed genomic studies in a cohort of tuberculosis patients in Chiangrai, northern Thailand. The genomes of *M. tuberculosis* isolated from 1170 patients during 2003–2010 were sequenced. The genomes of the same patients were also evaluated using high-density SNP arrays. The bacteria were genetically heterogeneous, with majority belonging to various sublineages of lineages 1 and 2. Refinement of classification of lineage 1 were proposed and a few novel sublineages of the others were identified especially in remote populations. The patients mostly belonged to three genetic groups, identified by principal component analysis, and three self-identified ethnicity groups. The profiles of patients infected by sublineages varied especially among sublineages of lineage 2. There were strong correlations between the bacterial genotypes and human ethnicity. GWAS identified a few genes associated with particular genotypes of the bacteria. Together with historical records, this study indicated that both the founder effects and co-evolution may explain the associations. This study provided some insights to the bacterial host interactions and useful information for the development of vaccines and other control measures for tuberculosis and is being replicated in a cohort of 600 patients in 2016–2018 with some patients studied by WGS.

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POPULATION STRUCTURE OF *MYCOBACTERIUM TUBERCULOSIS* ISOLATES FROM TB-HIV COINFECTED PATIENTS IN OMSK REGION, WEST SIBERIA, RUSSIA

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A clear trend of the increasing incidence of tuberculosis (TB) associated with HIV infection is observed in the Omsk region in West Siberia. The TB-HIV incidence increased from 0.3 in 2006 to 15.2 in 2017 per 100 000 population. The aim of this study was to analyze the population structure of *Mycobacterium tuberculosis* isolated from TB-HIV coinfected patients.

A total of 150 *M. tuberculosis* isolates were recovered from 150 patients with pulmonary tuberculosis in 2013–2017 were included in this study. They included 110 men (74.8%) and 40 women (25.2%), the average age was 35.2 years (from 22 to 58 years). *M. tuberculosis* culture and drug susceptibility testing were performed according to standard protocols. DNA was extracted from *M. tuberculosis* isolates using the recommended method. Beijing genotype was detected by PCR analysis of the *dnaA-dnaN*::IS*6110* insertion. Beijing B0/W148 cluster was identified by PCR analysis of the *Rv2664-Rv2665*::IS*6110* insertion. Spoligotyping was performed according to standard protocol (Kamerbeek et al., 1997) and the profiles were compared to SITVIT_WEB (http://www.pasteur-guadeloupe.fr:8081/SITVIT_ONLINE) for family assignment which was corrected by expert assessment. A chi-square test was used to detect any significant difference between the two groups.

Almost 3/4 of the studied *M. tuberculosis* isolates belonged to the Beijing genotype (109/150, 72.6%). Beijing B0/W148-cluster (Russian MDR Beijing clone) included 29 isolates (26.6% of Beijing population). Majority of the Beijing isolates (62/109; 56.8%) belonged to the Beijing 94-32-cluster (Central Asian/Russian strain).

Forty-three non-Beijing isolates were subdivided into 17 spoligotypes shared by 1 to 5 isolates. They represented the following genetic families: LAM (n = 19), T (n = 10), Ural (n = 6), Haarlem (n = 3), X (n = 1); for two isolates the family status was "unknown".

Population structure of *M. tuberculosis* isolates from TB-HIV coinfected patients in Omsk region is dominated by the Beijing genotype (72.6%) while the other, non-Beijing families belong to the Euro-American superlineage. Beijing genotype is dominated by the isolates of the epidemiologically important Beijing 94–32 cluster (56.8%).

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LOOKING INSIDE THE FOREST: FROM CLASSICAL GENOTYPING OF *MYCOBACTERIUM TUBERCULOSIS* TO WHOLE GENOME SEQUENCING IN HIGH MULTIDRUG RESISTANCE SETTINGS

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Molecular typing of *Mycobacterium tuberculosis* is an increasingly important public health tool that can provide a framework to investigate the dissemination and emergence of specific strains. Classical typing methods have relied upon the genetic analysis of repetitive loci, whose presence, number and layout on the *M. tuberculosis* genome have enabled the distinction between clinical isolates of different genotypes.

Over the last decade, the massive development of Next Generation Sequencing and ability to carry out Whole Genome Sequencing (WGS), which provides the ultimate resolution power, has revolutionized bacterial typing by enabling one to infer on the directionality of tuberculosis (TB) transmission. Herein, the importance of seeing deeper in the genome of *M. tuberculosis* will be analysed in two distinct epidemiological scenarios: the emergence of strains associated with drug resistance due to migratory movements and, the discrimination and study of the transmission dynamics of endemic multidrug and extensively drug resistant strains.

Regarding the emergence of drug resistant strains, WGS does provide sufficient evidence to delineate and discriminate within cross-border clusters that were otherwise impossible to discriminate. In Portugal, this has been of special relevance for multidrug resistant (MDR) superclusters of the Beijing family in Europe (such as the 94-32 and 100-32 types) that are spreading through vast geographical areas. This can be of great importance to inform concerted efforts aimed at screening migrant populations arriving from high-incidence settings and new epidemiological links can be uncovered even within the country. The same inability to discriminate using classical typing methods can be generated by outbreak strains whose circulation is occurring for decades. In such a scenario multiple transmission sub-clusters are usually present and WGS can effectively resolve these transmission networks. Good examples are the KZN, Lisboa or Q1 strains, all of which associated with extensively drug resistant (XDR) TB. Furthermore, recent evidence obtained by WGS shows that MDR-TB and XDR-TB within Lisboa and Q1 clades has emerged multiple times instead of more conservative predictions based on classical typing. Some roadblocks still lie ahead, but, the latter also highlights the advantage of genome-wide based phylogenetic analysis of *M. tuberculosis* clinical isolates in TB surveillance and, the need for a switch from classical typing to WGS-based typing.

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ADVANCES IN THE STUDY OF MOLECULAR BASIS OF RESISTANCE TO NEW ANTI-TB DRUGS

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Bedaquiline is an effective drug for the treatment of MDR and XDR tuberculosis allowing up to 85% cure rate in complex therapy. Unsuccessful treatment is accompanied with elevation of bedaquiline MIC and acquisition of mutations in *mmpR* and *atpE* genes. However, the clinical significance of mutations detection is still obscure due to an insufficient number of clinical isolates, characterized by phenotypic and molecular methods.

Bedaquiline MIC of clinical MTB isolates from patients, who obtain complex therapy including bedaquiline, were tested using both the agar proportion method on 7H11 plates and Bactec MGIT system. Genes *mmpR* and *atpE*, associated with an elevated MIC of bedaquiline, were sequenced.

191 clinical isolates were divided into several groups based on the genetic analysis: strains with wild-type sequences of all analyzed genes; heteroresistant strains, where both wild-type and mutant sequences could be identified; isolates where only mutant, or mix of different mutant sequences was found; and a group of isolates with the mutated *atpE* sequence. Most of the strains, isolated prior the bedaquiline treatment, had wild-type sequences and liquid media MICs ranged from 0.06 to 0.50 mkg/ml with the mode at 0.12 mkg/ml. Isolates with mutated *mmpR* gene possessed MIC range of 0.12–4.00 mkg/ml with mode at 0.25 mkg/ml. Heteroresistant isolates had an intermediate MICs from 0.12 to 2.00 mkg/ml. Four isolates with AtpE substitutions (D28N, A63P, A63V) had bedaquline MICs of 4.00 and 8.00 mkg/ml. The MICs distributions of wild-type and mutated isolates on 7H11 media had the distinct border between 0.06 mkg/ml and 0.12 mkg/ml: most of the strains with a MIC of ≥ 0.12 mkg/ml bore mutations.

During the treatment with bedaquiline, intermediate resistance emerged by selection of *mmpR* mutations, and high-level resistance caused by substitutions in AtpE. Our results also raise the question of reliability of currently used critical bedaquiline concentrations for 7H11 agar (0.25 mkg/ml) and Bactec MGIT 960 (1 mkg/ml) tests.

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THE IMPLEMENTATION OF NEXT-GENERATION SEQUENCING FOR EPIDEMIOLOGICAL STUDIES AND DRUG RESISTANCE INVESTIGATIONS IN MICRO-EPIDEMICS INVOLVING PEDIATRIC TUBERCULOSIS PATIENTS

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In Latvia, the childhood TB epidemiology trends very clearly reflected the increase of TB transmission from the year 1992 and the decrease of transmission rate since 2001. There was also a small increase of TB notification rate in children in 2011 which clearly predicted an increas-

ing incidence of adult TB cases in 2012, and was related to the economic crisis in Europe. The best strategy for TB case detection in children is contact investigation allowing early diagnosis, which, in turn, allows the implementation of the prophylactic treatment of TB infection, provides successful treatment outcomes, and prevents death. Current molecular diagnostic methods of *Mycobacterium tuberculosis* (Mbt) usually provide limited information that is often not sufficient for the local outbreak and transmission investigations. Implementation of the modern approaches such as Next generation sequencing technologies in the epidemiological studies of childhood TB has a potential to combine TB diagnosis, drug resistance profiling and epidemiological analysis into one test helping to initiate personalized treatment for every patient timely and correctly.

Case report. A patient, 29 years old, was diagnosed with the 3rd TB episode in her life in 2017. Mbt cultures were obtained and genotyped. Molecular genotyping results showed different spoligo and IS6110 RFLP patterns for all three TB episodes in years 2001, 2011, and 2017.

Epidemiological anamnesis revealed that the first TB episode at the 14 years of age in patient was identified in 2001 during household contact investigation — patient`s uncle was diagnosed with TB in 2001. Uncle had TB relapse in 2006. Genotyping results of the uncle`s both Mbt cultures obtained in 2001 and 2006, and patient`s Mbt culture obtained in 2011 revealed the identical spoligotype (SIT1) and IS*6110* pattern with 17 bands for both patients. These results indicated the high possibility of the transmission in the household contact. However, genotyping results from patient's Mbt culture obtained in 2017 showed different genotype.

Whole genome sequencing (WGS) was used for in-depth characterisation of *M. tuberculosis* isolates associated with matched pairs of TB cases. The obtained results were in accordance to the genotyping and drug resistance. In addition, the obtained data provided additional resolution of the microevolution of Mbt subpopulations.

The addition of WGS to the epidemiological data and social network analysis could improve the confirmation of the epidemiological links and evaluation of the transmission dynamics of TB. Additionally, rapid WGS data can be used to identify molecular evidence for strain-specific phenotypic variability including anti-mycobacterial drug resistance, further providing rapid onset of appropriate treatment.

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MOLECULAR EPIDEMIOLOGY OF TUBERCULOSIS IN LATVIA

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Tuberculosis is still one of the major infectious diseases in Latvia, causing serious health problems. While the incidence of the disease has steadily declined in the country since year 2001, the rates of drug-resistant tuberculosis are among the highest within the European Union. Molecular genotyping of *M. tuberculosis* plays an important role both in clinical studies and in the epidemiological investigations, allowing to describe and characterize pathogen`s population structure. Our previous studies have shown that in Riga and Riga region the majority of *M. tuberculosis* isolates belonged to lineage 4 (Euro-American) and lineage 2 (East-Asia). The family distribution of the isolates comprised 25% Beijing, 27% T, and 25% LAM (Latin-American Mediterranean) isolates, while Haarlem, Ural, and X families were detected in 11, 6, and 3%, respectively. A high proportion of Beijing and LAM isolates is alarming, as these *M. tuberculosis* genotypes have been often associated with remarkable pathogenic features such as drug resistance and increased transmissibility. TB incidence in the Latvian region Latgale seems to be higher than the average, and in-depth studies of *M. tuberculosis* isolates in this region could provide additional resolution for the characterization of the lineages circulating in the country. The Latgale region borders with Lithuania in the South, Belarus in Southeast, and Russian Federation in the East. *M. tuberculosis* isolates in this region were studied by the Spoligotyping and IS*6110* RFLP genotyping methods. In total, 56 (73.7%) samples of 76 bacteriologically confirmed TB cases in the year 2017 were available for molecular analysis. The results showed that 52% of isolates could be classified as common genotypes in Latvia (SIT1, SIT42, SIT50, SIT53, SIT254, SIT262, SIT283, SIT1292), while 48% of isolates belonged to SITs which are rarely found in the country or were unique (SIT45, SIT47, SIT52, SIT65, SIT118, SIT150, SIT278, SIT1175, SIT1451). The most common spoligotype belonged to the T1 lineage (SIT53, 16%) followed by SIT1, SIT47 and SIT254 (9% each). Within all samples studied, 14 isolates (25%) formed 4 different clusters with 3–5 members in each. The epidemiological links were confirmed for nine patients in 3 clusters (SIT47, SIT65, and SIT1292). When the prevalence of different spoligotypes was analysed between different countries, a similarity between particular genotypes in Latvia and neighbouring countries was observed. In-depth analysis of these isolates on the international scale could be very useful in order to investigate the possible transmission dynamics of *M. tuberculosis* strains.

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FUNCTIONAL RELEVANCE OF *MYCOBACTERIUM TUBERCULOSIS* DIVERSITY: FROM GENOTYPES TO IMMUNE RESPONSES AND DISEASE SEVERITY

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The genetic diversity of tuberculosis (TB)-causing bacteria has surprised us over recent years. A growing body of evidence attributes a functional relevance to this diversity, both at the clinical and immune response levels. Investigating the full diversity of *Mycobacterium tuberculosis* in nature is however impossible. We recently moved from the study of limited collections of *M. tuberculosis* to an oriented approach, aimed at covering a representation of *M. tuberculosis* heterogeneity. For this, we studied over 600 TB patients in Porto and over 300 matching *M. tuberculosis* isolates. We show a highly homogeneous phylogenetic structure of *M. tuberculosis*, with nearly all cases belonging to Lineage 4 (L4). Within the L4 clade, the most represented sublineage was LAM. This host-pathogen sympatric distribution was however shak-

en by the presence of HIV or diabetes co-morbidities, which oriented the selection of 2 LAM *M. tuberculosis* isolates from TB patients with no comorbidities, but with different TB severities. Despite their close genetic structure, we are finding a distinctive pattern of cytokine production by human and mouse macrophages infected with either isolate. Interestingly, the high TB severity-associated isolate is a poor inducer of cytokine responses. Mechanistically, we relate this poor induction of cytokine production with a differential capacity of the bacteria in activating the host transcriptional machinery, as well as the inflammasome. Furthermore, a different *in vivo* progression of infection by the selected *M. tuberculosis* isolates was also observed. Most notably, the isolate associated with high TB severity showed higher dissemination patterns from the lung to the liver and spleen than that associated with mild TB. To investigate if the link TB severity-cytokine response was broader, we tested the cytokine response induced in human peripheral blood mononuclear cells by a series of other *M. tuberculosis* isolated from patients with different TB severities. A segregation of isolates was observed, with poor inducers of cytokine responses being generally associated with high TB severity. Collectively, our findings suggest that bacterial-intrinsic properties modulate the intensity of the initial immune response with likely consequences for the severity of TB. Our studies open interesting avenues for TB interventions, namely by raising the importance of considering the pathogen diversity when designing host-directed therapies.

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THE ROLE OF THE IS*6110* IN MICRO- AND MACROEVOLUTION OF *MYCOBACTERIUM TUBERCULOSIS* LINEAGE 2

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Genomes of *Mycobacterium tuberculosis* complex members contain the insertion sequence (IS) *6110* which, due to its high quantitative and positional variability, has become a widely used marker in epidemiological studies. The element plays an important role in microorganism genome plasticity, but still many consequences and causes of transposition have not been fully described. This work studies the transposition mechanism of IS*6110* and its impact on the evolution of *M. tuberculosis* (*Mtb*).

Whole-genome sequencing data of 902 *Mtb* lineage 2 isolates was obtained from NCBI and ENA databases. Phylogenetic sublineages were determined based on SNP analysis (120 samples belonged to the ancient Beijing (17 proto-Beijing, 28 Asia Ancestral 1, 13 Asia Ancestral 2, 38 Asia Ancestral 3), 782 samples belonged to the modern Beijing (10 Asian African 1, 29 Asian African 3, 65 Asian African 2, 43 Pacific RD150, 140 Europe/Russia W148 outbreak, 361 Central Asia) (E. Shitikov et al., SciRep, 2017). ISMapper was used to determine the sites of integration of the IS*6110* (Hawkey et al., BMC Genomics, 2015).

We obtained 17 972 points of insertion, which belonged to 865 independent positions in the H37Rv genome. The mean copy number per genome was 19.92 (from 9 to 25). To describe the evolution of an element in the genome, we arranged our samples in the order corresponding to a phylogenetic tree constructed on the basis of SNPs. We determined the stepwise mechanism of transposition, in which the transition to a new subpopulation is accompanied by a change in the localization of several copies of IS. It is important to note that the localization of the element in the ancestral population does not change, which implies a transposition only by "copy-paste" mechanism. In addition, we defined genes (537 sites (256 genes)) and intergenic regions (328 sites), where the element was integrated. Sixteen genes previously identified as being essential under different experimental conditions were found to contain IS. Further we carried out identification of IS*6110* mediated LSPs which showed the presence of recombination events (deletion) between inversely oriented elements.

In conclusion, we determined the evolution and role of IS*6110* for *Mtb* lineage 2 strains. We identified evolutionary and subpopulation-specific sites of integration which can be used for typing and subsequent research.

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NGS DETERMINATION OF MYCOBACTERIAL TRANSRENAL DNA AS POTENTIAL TOOL OF CLINICAL DIAGNOSTIC

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Sputum is a major object for monitoring TB treatment and diagnostics it is strictly depended from bacterial load. There is a substantial need for less variable and more reliable specimen for the diagnosis of tuberculosis and for treatment monitoring. The objective of this study is to estimation diagnostic power of full genome sequencing (NGS) of soluble mycobacterial transrenal DNA (mtr-DNA) in urine of TB patients and TaqMan tests designed after analysis of metagenomic data.

DNA patient with pulmonary tuberculosis (TB) isolated from 4 ml. of urine by QIAamp Circulating Nucleic Acid Kit. Detection of TB positive samples made by previously developed PCR targeted to 45 base pairs fragment of mycobacterial genome. It was chosen 2 positive PCR samples. It were mapped on the reference genome *M. tuberculosis* (NC_000962.3) by BWA. In total were mapped 16 579 paired reads of the one sample (0.83%) and 1 783 754 (64%) of the second sample respectively. There were also analyzed mapped DNA sequences with more than 4x coverage. The median length of mtr-DNA found as 20 bp.

It was found 156 mtr-DNA fragments repeated in both samples. The median length of DNA fragment was found was 20 bp. Five fragments including part of 16S rRNA gene were chosen for design primer and TaqMan probes for targets from 43 to 60 bp. Length of primers and probes were reduced by Locked Nucleic Acid (LNA) bases. The sensitivity and specificity of the developed tests was determined by known DNA samples from urine. The result obtained did not reveal a significant improvement in the sensitivity of the new tests. PCR-RT cutoff remained approximately 40 cycles, like in previously developed tests.

High specificity and sensitivity of NGS and low of PCR suggest that diagnosis and monitoring of tuberculosis by mtr-DNA should be based on NGS, rather than on PCR.

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MOLECULAR EPIDEMIOLOGY OF TUBERCULOSIS IN KAZAKHSTAN, 2006–2018

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The implementation of various projects aiming to develop basis of molecular-epidemiological monitoring of tuberculosis infectious agent by combining scientific and technical potential of specialists from several research and medical centers resulted in extensive experience and contribution to understanding epidemic process of tuberculosis in Kazakhstan. In particular, we established an updated information database of genetic profiles using 24-MIRU-VNTR and spoligotyping, a set of reagents designed to carry out molecular profiling of mycobacterial isolates, as well as protocols based on reduced and expanded panels for stream high-throughput screening in 96- and 384-well format.

The studies were conducted on clinical isolates of *M. tuberculosis*, collected from 2006 to 2018 in hospitals of Kazakhstan. The samples were characterized by the resistance to first and second line antimicrobials using cultivation on Lowenstein–Jenssen media and BACTEC MGIT 960. Genotyping: manual 24MIRUVNTR-typing (Supply et al., 2006) and spolygotyping (Kamerbeek et al., 1994), compared with MIRUVNTR*plus* and SITVIT_WEB databases. Additional typing of hypervariable loci QUB-18, QUB3232, VNTR-3820, VNTR-4120 was used when insufficient genotypes' differentiation was observed (Iwamoto et al., 2007; AllixBeguec et al., 2014). Genetic polymorphism of drug resistance was determined by TB-TEST system (BIOCHIP-IMB, Russia), and GenoType MTBDRplus kits (Hain Lifescience, Germany).

Our pilot study of 2007–2008 established that Beijing strains present a special epidemic threat for Kazakhstan as they are the main reason for the majority of MDR tuberculosis cases and are widely distributed in different regions of Kazakhstan (Skiba et al., 2015). Further study (2012–2014, with 576 genotyped samples of drug-resistant strains) allowed not only to confirm the previous conclusions, but also clarified prevalence and impact of other genetic families in Kazakhstan. Both of these studies led to identification and confirmation of a separate genetic group, which we named KAZ-1.

These studies have shown some cases of tuberculosis caused by several mycobacterial strains simultaneously. We have also encountered genotype changes in patients during their hospital treatment. This finding formed the basis for the next project on the study of nosocomial transmission of drug-resistant tuberculosis (2015–2017). The result of this project was registration of several cases of genotype change in hospitalized patients at different treatment stages. This targeted monitoring also helped to record the first case of LAM RD-Rio strain in Kazakhstan.

The overall results inspired us to use the developed research algorithm that combines reduced VNTR panel with TB-TEST biochip system in the ongoing research to reveal the connection between drug-resistance mutations and strain genotype, and to better understand the epidemic process of tuberculosis in Kazakhstan.

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NEXT-GENERATION SEQUENCING OF DRUG RESISTANT *MYCOBACTERIUM TUBERCULOSIS* STRAINS — FIRST SLOVENIAN EXPERIENCE

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Slovenia is low-incidence country with incidence rates of tuberculosis (TB) cases around 6.0 in last several years. In our country, the percentage of resistant TB is very low with sporadic cases of multidrug resistant (MDR) TB. The aim of our study was to examine the feasibility of a full-length gene analysis for the drug resistance related genes (*inhA, katG, rpoB, embB*) using Next-generation sequencing Ion Torrent technology and compare the results with those obtained from conventional phenotypic drug susceptibility testing (DST) in 61 TB isolates from our National mycobacterial culture collection. TB strains included were either susceptible or mono-, poly-, or multidrug resistant by phenotypic DST. High concordance between genetic (Ion Torrent technology) and standard phenotypic DST testing for isoniazid, rifampicin and ethambutol was observed with sensitivities of 68.2; 100 and 100%, and specificities of 100; 80 and 88.2%, respectively. In conclusion, the next-generation sequencing analysis successfully predicted drug resistance with significant shortening of time needed to obtain the resistance profiles from several weeks to just a few days.

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WGS IN ROUTINE DIAGNOSTICS OF TUBERCULOSIS — PREDICTION OF DRUG RESISTANCE AND GENOTYPING

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The aim of the study was to compare the performance of whole genome sequencing (WGS) and conventional assays for drug susceptibility testing and genotyping of *Mycobacterium tuberculosis* in a routine laboratory.

All *M. tuberculosis* cultures sent to the Finnish mycobacterial reference laboratory in 2014 were tested by Mycobacteria Growth Indicator Tube (MGIT) for first-line drug susceptibilities. Genotyping was performed by 24 loci MIRU-VNTR typing and spoligotyping. WGS was performed with the Illumina MiSeq system. For prediction of drug susceptibility, the data were analyzed using five software tools (PhyResSE, Mykrobe Predictor, TB Profiler, TGS-TB and KvarQ). Clustering analysis was performed using Ridom SeqSphere+ (Ridom GmbH, Germany) cgMLST v2 (2891 targets) for genomes assembled by Burrows-Wheeler Aligner (bwa). Isolates with allelic distance ≤ 12 formed a cgMLST cluster. In addition, SNP analysis using the GATK tools (Broad institute, Cambridge, MA, USA) was performed and clusters based on distance of ≤ 12 and ≤ 1 SNPs were formed.

The sensitivity of the five software tools to predict any resistance among strains was almost identical, ranging from 74% to 80%, and specificity was more than 95% for all software tools except for TGS-TB. The sensitivity and specificity to predict resistance to individual drugs varied considerably among the software tools.

Among the 211 isolates, 15 clusters comprising 36 isolates (19.9%) were found by conventional genotyping. Of these, 15 isolates (36.1%) were clustered similarly to six clusters also by cgMLST analysis. Furthermore, four strains that did not cluster by conventional genotyp-

ing clustered by cgMLST analysis resulting in 19 (9.0%) clustered isolates by cgMLST. By clustering analysis with the distance ≤ 12 SNPs, 18 isolates clustered into 7 clusters. With the 1 SNP cut-off, three clusters with a total of seven strains were found and these were similarly clustered also by cgMLST and conventional genotyping analysis.

A reliable prediction of drug susceptibility can be obtained with WGS combined with data analysis with software tools. In routine practice, *M. tuberculosis* isolates can be screened with WGS for mutations associated with drug resistance, and only resistant strains confirmed with the MGIT system. Compared to conventional genotyping methods, WGS analysis is more discriminatory, reducing the risk of false clustering and unnecessary contact tracing.

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SINGLE NUCLEOTIDE POLYMORPHISMS IN *hsp65* AND MACPPE12 GENES OF *MYCOBACTERIUM AVIUM* subsp. *HOMINISSUIS*

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Mycobacterium avium subsp. *hominissuis* (*MAH*) represents a group of environmental bacteria known as opportunistic pathogens of animals and humans, especially HIV positive. Polymorphisms in *hsp65* and MACPPE12 genes are used for identification, intra-subspecies differentiation and phylogenetic studies of *MAH* populations.

The aim of our study was to identify single-nucleotide polymorphisms (SNPs) in *hsp65* and MACPPE12 genes of clinical isolates from Russian patients with pulmonary and disseminated mycobacteriosis and to assess phylogenetic relationships of geographically distant *MAH* populations.

The sequence analysis of the 3'-portion of the *hsp65* gene and MACPPE12gene was applied for 40 MAH strains isolated from humans with mycobacteriosis (including 19HIV-positive) in St. Petersburg, Russia (2008–2011). The nucleotide sequences were aligned to the reference genome of *M. avium* subsp. *hominissuis* 104 (NC_008595.1.).

In total, the 40 *MAH* strains were classified into three different *hsp65* sequevars: code 1, code 2 and code 3. The majority of *MAH* strains (72.5%) belonged to code 1, the same sequevar as for *MAH* strain 104. The code 2 and code 3 included 3 (7.5%) and 8 (20%) strains, respectively. The largest *hsp65* sequevar code 1 has observed only in 4.7% of isolates from Japan and absent in Korean human isolates. The sequevars code 1 and code 2 predominated among *MAH* strains in the USA, Canada, Belgium.

The sequence analysis of the MACPPE12 gene revealed 20 SNPs grouped into nine sequevars at the nucleic acid level: NA01, NA02, NA03, NA06, NA10, NA13, NA14, NA19, and NA_Rus01. Among 20 SNPs eight were nonsynonymous resulting in seven sequevars at the amino acid level: AA01, AA02, AA04, AA07, AA08, AA13, and AA_Rus01. The sequevar AA02 consisted of three different NA variants with synonymous SNPs profiles: NA02, NA03, and NA06. Half of the *MAH* strains belonged to the sequevar AA02 (type NA02). The predominant cluster AA02 (type NA02)/code 1 and the unique variant AA_Rus01 (NA_Rus01) were identified among *MAH* strains from Russia. The present study demonstrated the prevalence of the sequevar AA02 in MAH strains isolated from humans in Russia, Japan, and Korea.

Thus, we confirmed the relative conservativeness of the nucleotide sequence of the *hsp65* gene but the polymorphism of the MACPPE12 gene. A comparative analysis of the SNPs profiles of the *hsp65* and MACPPE12 genes allowed to identify differences and similarities between geographically distant populations of *MAH*, which highlighted the variability of the global population of *M. avium* species.

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MOLECULAR EPIDEMIOLOGY OF TUBERCULOSIS IN ALBANIA (2006–2011)

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Tuberculosis (TB) epidemics in Albania has been stable over the past years with a gradual decreasing incidence (from 18.7 to 14.8 per 100 000 inhabitants, in the period 2001–2016) with a slight deterioration in 2013 (16.8 per 100 000 inhabitants). First insight data (2008) on TB molecular epidemiology showed a moderate Recent Transmission Index (RTI) (28%) and a high level of genetic diversity.

We aimed with this study to better understand the correlation of ubiquitous and autochthonous *Mycobacterium tuberculosis* complex (MTBC) genotypes with available demographic and epidemiologic data over a six-year period, in Albania.

MTB strains isolated in Albania (n = 745, 1 isolate per patient) between 2006 and 2011 were analyzed by spoligotyping and MIRU-VNTR typing by 24 loci scheme. The data obtained were compared with MIRU-VNTRplus database. Using molecular typing 486 (65.23%) isolates (patients) were distributed into 113 clusters and the remaining 259 (34.77%) isolates had a unique pattern. The cluster sizes ranged from 2 to 21 isolates per cluster. RTI ((nc — c)/n) resulted 50.07%. The most predominant lineages were Ghana (28.59%), Haarlem (19.73%) UgandaI (18.79%), LAM (7.11%), Ural (5.64%), TUR (3.89%) and Caprae (3.49%). Other lineages identified were Cameroon (1.74%), X (1.48%), S (1.21%), Bovis (0.54%), Delhi/CAS (0.54%, Beijing (0.4%) and West African 2 (0.13%). This study highlighted the predominance of five shared spoligotypes: ST 53 (T1) (n = 166, 22.28%), ST 4 (LAM3 and S/convergent) (n = 39, 5.23%) and ST 42 (LAM9) (n = 38, 5.10%) ST 613(T1) (n = 37, 4.97%) and ST 47 (H1) (n = 35, 4.70%). Of the unknown spoligotype signatures three were more frequent than others (4.70%, 3.22%, 3.22%), their origin and historical link to other genotypes is yet unknown. Among the MLVA MtbC15-9 types, MLVA 15411-85 and MLVA 15419-69 (both of unknown spoligotype signatures) resulted the predominant types involved in recent transmission in Albania (two biggest clusters identified with 21 and 19 identical isolates respectively).

In conclusion, MTBC genetic population in Albania is highly heterogenous. TB epidemics in Albania is fueled mostly by evolutionary-recent lineages. It is largely dedicated to recent transmission (50.07%). Autochthonous genotypes result linked to the 2 biggest clusters identified. One of them is found exclusively in Tirana (MLVA 15411–85). The new MTBC genotypes will require further molecular characterization.

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THE IMPACT OF THE DELETION OF THE MMP-1 GENE ON THE EXPRESSION OF SYMPTOMS AND THE EFFECTIVENESS OF TREATMENT IN PATIENTS WITH PULMONARY TUBERCULOSIS

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The objective of the study is to examine the impact of the deletion of the MMP-1 gene on the development, expression, dynamics of clinical syndromes and the effectiveness of treatment in patients with pulmonary tuberculosis.

73 patients with pulmonary tuberculosis, receiving treatment in the hospital of the state health care facility "Regional TB Dispensary" in the city of Astrakhan. Depending on the genotype of MMP-1, three groups were formed: the first group — with G1/G1 — 15 people (20.5%), the second — with G2/G1 — 27 people (50.7%) and the third one — with G2/G2 — 21 people (28.8%).

During the treatment, the disappearance of symptoms of intoxication and bronchopulmonary disorders in patients of the 1st and 2nd groups occurred earlier than in the 3rd group ($\chi^2 = 11.5$, $p = 0.02$). Dissolving of infiltration in the lung tissue also started earlier in the first and second groups ($\chi^2 = 9.2$, $p < 0.05$). The discharge of *Mycobacterium tuberculosis* (MBT) stopped earlier in patients of the 1st group. After four months of treatment, MBT were not determined by any method in 94.6% of the patients in the 2nd group and in 90.4% in the 3rd group ($\chi^2 = 9.9$ $p = 0.07$). Closure of decay cavities after 2 months of treatment was observed in 70% of cases in patients of the 1st group, in 53.6% of cases — of the second and in 15.6% of cases — of the third. In 47.4% of patients with G2/G2, destructive changes persisted for more than 4 months of treatment ($\chi^2 = 10.3$, $p = 0.03$).

Patients with genotype G2/G2 have a tendency to a protracted tuberculosis. The study of the polymorphism of the MMP-1 gene in patients with pulmonary tuberculosis can be used as a prognostic criterion of the clinical course of tuberculosis, correction of specific therapy, and justification for prescribing drugs affecting collagen metabolism.

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IN VITRO ACTIVITY OF BEDAQUILINE AGAINST NON-TUBERCULOUS MYCOBACTERIA

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Infections caused by non-tuberculous mycobacteria (NTM) have become more frequent in the last years since they have emerged as important pathogens in immune-compromised patients. Unfortunately, their treatment is long, toxic and often with poor results. Bedaquiline (BDQ) (trade name Sirturo; Janssen Therapeutics, Inc.) has been approved for the treatment of multidrug-resistant tuberculosis and there is increasing interest in its potential use for treating NTM infections.

The aim of this study was to determine the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) of BDQ against clinical isolates of NTM obtained from different hospitals. We investigated the possible role of gene mutations on the activity of BDQ against NTM by sequencing the *atpE* gene.

The MIC was determined by the broth dilution method in 7H9 medium supplemented with OADC and glycerol using the resazurin microtiter assay (REMA). The MBC was determined by conventional 7H10 plate agar dilution method.

Range concentration of BDQ in REMA was assessed from 2 to 0.0035 μg/ml. Each experiment was performed in triplicate. The MIC of BDQ was found at ≤ 0.015 μg/ml. The MBC of all NTM tested was found to be higher than 4 times the MIC. No nonsynonymous mutations in the *atpE* gene that conferred BDQ resistance in all NTM tested were identified.

BDQ exhibited a strong inhibitory effect against all the NTM clinical isolates tested. These promising results indicate that BDQ could be potentially useful for the treatment of NTM. However, according to the MBC data it lacks bactericidal activity. Nevertheless, BDQ could still have excellent potential for use in patients with NTM infections and further investigation is needed.

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EMERGING OPPORUNISTIC PATHOGEN *MYCOBACTERIUM ABSCESSUS* IN SLOVENIA: MOLECULAR ANALYSIS OF RESISTANCE GENES COMPARED TO MIC METHOD

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Mycobacterium abscessus complex (MABSC) is a group of three closely related subspecies: *M. abscessus* subsp. *abscessus* (MAA), *M. abscessus* subsp. *bolletii* (MAB) and *M. abscessus* subsp. *massiliense* (MAM). Correct species identification is important, especially for patients with cystric fibrosis (CF), due to distinct molecular resistance profiles among subspecies. Gene *erm(41)* with T/C polymorphism at nucleotide 28 is responsible for inducible macrolide resistance (only in MAA and MAB, in MAM gene is not functional), meanwhile mutation in *rrl* gene causes high-level macrolide resistance. Amninoglycoside resistance occurs with mutation in *rrs* gene.

We aimed to diferentiate Slovenian MABSC isolates to subspecies level and to assess their molecular resistance profile. Selected isolates were further tested with microdilution method to to obtain full phenotypic resistance profile.

Molecular analysis was performed on 37 clinical isolates recovered from 37 patients in the period 2000–2017 from Slovenian National Mycobacterial Collection. GenoType NTM-DR (Hain Lifesciences, Nehren, Germany) was used to identify subspecies and resistance mutations. Antimicrobial susceptibility testing (AST) was performed on selected isolates with reference microdilution method using Sensititre RAPMYCO microplates (TREK Diagnostic Systems, Cleveland, Ohio, USA). Susceptibility and resistance were assessed according to CLSI guidelines.

GenoType NTM-DR showed the highest prevalence rate for MAA 72.9% (27/37), followed by MAB 16.2% (6/37) and MAM 10.8% (4/37). Resistance profile showed that T28 polymorphism in *erm(41)* gene is present in all MAB and MAM isolates and in 88.8% (24/27) MAA iso-

lates. Mutations in *rrl* and *rrs* genes were not detected. AST for 4 MAM isolates confirmed that inducible resistance is not present even with *erm(41)* T28 mutation. AST for 3 MAA with *erm(41)* C28 polymorphism showed MIC values below 2 mg/L which is interpreted by CLSI guidelines as sensitive strain. AST showed that MIC values for amikacin are between 8–16 mg/L interpreted as sensitive and concordant with molecular analysis.

In Slovenia, for macrolide and aminoglycoside resistance, phenotypic and genotypic results of *Mycobacterium abscessus* complex are concordant. Prevalent subspecies is MAA where high percentage of strains have inducible macrolide resistance. No other unknown genetic mutation was present in our isolates that can cause inducible macrolide resistance which is important for treatment patients with CF, where clarithromycin is first drug of choice.

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MOLECULAR FEATURES OF *MYCOBACTERIUM TUBERCULOSIS* ISOLATES FROM PATIENTS LIVING IN CLOSED CITY IN THE URAL REGION, RUSSIA

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Novouralsk is a closed town in the Sverdlovsk region, Middle Ural area in Russia, with a total population of 81 500 and travel and residency restrictions. We aimed to identify the molecular-epidemiological features of *M. tuberculosis* circulating in Novouralsk under these specific conditions.

A total of 87 *M. tuberculosis* clinical isolates obtained between 2013 and 2016 from TB patients living in Novouralsk town were analyzed. According to clinical data, 34 (39.1%) of TB patients were HIV-infected. 53 (60.9%) of TB cases were newly diagnosed. Using real-time PCR we divided *M. tuberculosis* clinical isolates into Beijing/non-Beijing genetic groups. Beijing genotype variant B0/W148 was detected by multiplex PCR assay. VNTR loci MIRU26 and QUB26 were used for subtyping Beijing strains. Spoligotyping was used for further subtyping non-Beijing isolates. Drug susceptibility testing for first and second line drugs was performed by absolute consentration method.

Genotyping identified the predominance of the Beijing genotype isolates (75.5%), among new TB cases, that is almost 20% higher than the average for the Ural region ($p < 0.05$). 52.8% isolates belonged to variant Beijing B0/W148. The majority of Beijing isolates — 35 (40.2%) had seven copies in MIRU26 and QUB-26 loci. Nine (10.3%) Beijing B0/W148 isolates had 2 copies in QUB26 locus that was unusual for this genetic cluster; six patients from this group had TB/HIV co-infection. Seven (8.0%) of non-Beijing isolates belonged to SIT35 spoligotype (Ural family). 20.7% of patient had prison history and 72.2% of them were infected with B0/W148 genotype. The MDR prevalence rate was higher than in Sverdlovsk region (66% vs 43.9%, $p < 0.05$) and MDR status was associated with the Beijing B0/W148 genotype (94% and 6% of its isolates were MDR and polyresistant, respectively).

Epidemiological situation with TB in Novouralsk is characterized by high level of TB/HIV co-infection, predominance of Beijing B0/W148 isolates, which is an underlying reason of high level of MDR-TB.

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PREVALENCE AND DIVERSITY OF NONTUBERCULOUS MYCOBACTERIA IN DIFFERENT REGIONS OF THE RUSSIAN FEDERATION

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Today, more attention is being paid worldwide to the nontuberculous mycobacteria (NTM) infections due to their increase in various regions of the world. The prevalence of different NTM species depends on the geographical location. The prevalence and distribution of the NTM species in Russian Federation have not been sufficiently studied to date.

The objective of this study was to demonstrate the diversity of NTM species isolated from patients in different regions of the Russian Federation. NTM were isolated from solid and liquid media from patients with suspected tuberculosis/mycobacteriosis in the period from July, 2013 to June, 2017 and identified using GenoType Mycobacterium CM/AS assay (Hain Lifescience, Germany) and real-time PCR assay co-developed with Syntol LLC (Moscow, Russia). Seventeen NTM species were identified in 1400 cultures from 876 patients. Exact species was identified for isolates from 840/876 (95.89%) patients. The prevalence of slowly growing NTM was 76.5% (643/840). The most common species was *M. avium* (223/846, 25.5%). Also the high incidence rate (in descending order) has been shown for *M.gordonae* (115/846, 13.12%), *M. lentiflavum* (113/846, 12.9%), *M. intracellulare* (80, 9.13%), *M. fortuitum* (83/846, 9.47%), *M. kansasii* (62/846, 7.1%), *M. abscessus* (52/846, 5.9%) and *M. xenopi* (31/846, 3.53%). 16 of 17 identified species were detected in Moscow region. This may be due to a large number of cases analyzed in this region in comparison with other regions. It was shown that NTM species distribution in Central Federal District, European part of Privolzhsky Federal District and Kaliningrad (Northwest Russia) was similar to European countries: MAC 33–39%, *M. gordonae* — 10–20% and *M. fortuitum* — 5–13%. The species distribution of NTM for neighboring Syktyvkar and Perm (North of European Russia) was similar and was characterized by high rates of *M. fortuitum* infection and low rates of *M. avium* infection. NTM, isolated from the border city of Khanty-Mansiysk were also characterized by low occurrence of *M. avium* and prevalence of *M. gordonae.*

To conclude, during the study period, 1400 samples collected from 876 patients in 6 federal districts were analyzed. The greatest species diversity — 16 NTM species — was shown for Moscow region. In most of the regions anayzed, slow-growing NTMs prevailed and the most abundant were species belonging to MAC complex. However, even on a relatively small number of observations, some regional features can be observed.

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MOLECULAR CHARACTERIZATION OF *MYCOBACTERIUM BOVIS* ISOLATES FROM CATTLE IN BULGARIA

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The observation of the population dynamics, diversity of genotypes and dissemination of *Mycobacterium bovis* strains in Bulgaria and neighboring countries play

an important role in the modern epidemiological investigations of tuberculosis in animals at the regional and international level. Bovine tuberculosis represents a significant economic burden to the agriculture of the affected countries. From 2000 to 2015 the disease shows cyclicity in private farms in different regions of Bulgaria. This study is a first molecular investigation of animal tuberculosis in the veterinary medicine in country. The macroscopic and microscopic observation of 35 diagnostic materials from slaughtered cattle, received in the National Reference Laboratory of animal tuberculosis were studied with the three molecular methods: RD4-PCR, spoligotyping and MIRU-VNTR. In 27 of the examined lymph nodes we found specific lesions for bovine tuberculosis. The findings were confirmed bacteriologically and by conventional PCR. To differentiate *M. bovis* from other *M. tuberculosis* complex subtypes, we used primers flanking specific deletion (RD4) in the genome of *M. bovis* and obtained the 446 bp DNA product. The spoligotyping subdivided the strains into 3 spoligotypes shared by two to 20 strains. Further molecular investigations of *M. bovis* strains are needed to characterize the genetic diversity and population structure of *M. bovis* strains isolated from cattle in Bulgaria. New information will be added to the global database in the field of molecular epidemiology of the prevalence of *M. bovis* strains in the cattle population in Bulgaria, which will allow comparative analysis with data from the Balkan region and Europe.

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INTERNATIONAL VALIDATION OF ANALYSIS PIPELINES FOR WHOLE GENOME SEQUENCING DATA OF *MYCOBACTERIUM TUBERCULOSIS* ISOLATES

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The aim of this multicenter study was to validate and compare different pipelines used for analysis of the Whole Genome Sequencing (WGS) data of *Mycobacterium tuberculosis* isolates.

All 535 *M. tuberculosis* isolates of culture positive cases in the Netherlands in 2016, were subjected to WGS, in addition to the routine application of VNTR typing. Transmission suggested on basis of identical VNTR profiles of cases in 2016 was further investigated by municipal health services and 41 epi-links were traced. Fastq.gz files of all 535 samples were analysed in four different WGS pipelines to facilitate international comparison: 1) SNP-based method at the RIVM/Bilthoven/The Netherlands; 2) SNP-based method at Oxford University/UK; 3) SNP-based method and 4) cgMLST at Borstel/Germany.

In all pipelines, shorter than 12 SNP distances between the 41 epi-linked cases was observed. One epi-linked pair revealed a higher genetic distance of 27 SNPs in the Bilthoven pipeline, due to poor sequence quality resulting in low coverage. In general, the genetic distances between isolates of the epi-linked cases were smaller in the Oxford and Borstel pipelines (0–3 SNPs), than in the Bilthoven pipeline (1–11 SNPs). All pipelines clustered roughly the same cases, more isolates without identified epi-links were clustered in the Oxford (n = 34) and both Borstel pipelines (n = 32 in the SNP pipeline and n = 39 in the cgMLST) than the Bilthoven pipeline (n = 29).

Also, some cases not clustered by VNTR were clustered by WGS. Patient characteristics revealed that in some of these pairs of cases an epi-link, missed by VNTR typing, was likely.

Several differences were observed among the pipelines with regard to the version of reference genome used, software used for mapping and SNP calling, (repetitive) regions excluded in the analysis, the minimum number of reads to support SNPs, and the minimum allele frequencies. The RIVM pipeline was adapted in the light of these results to function more in line with other international laboratories pipelines, facilitating the comparability of results.

International standardization on all these variables is necessary, and subsequently on the SNP cut-off to be applied to WGS clustering, to allow international-laboratory comparison of WGS data and reliable investigation of cross-border transmission.

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RNA-BASED DRUG SUSCEPTIBILITY TESTING OF *MYCOBACTERIUM TUBERCULOSIS*

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Multidrug resistant tuberculosis (MDR-TB) is one of the major WHO health concerns. One of the challenges that hampers the effective response to MDR-TB is the long turnaround time of phenotypic Drug Susceptibility Testing (DST) of *Mycobacterium tuberculosis*. To counter this, new fast and sensitive DNA-based methods were successfully introduced over the last years. However, these (a) are based on the knowledge on resistance mutations, (b) do not distinguish living from dead cells, (c) ignore all intrinsic resistance mechanisms, and (d) ignore the influence of compensatory mutations.

We introduce a next-generation diagnostic test based on quantification of drug-specific RNA biomarkers. The basic principle is that a brief antibiotic exposure triggers specific transcriptional responses in susceptible, but not in resistant, microbes within a few hours. This has the advantage that long culture-dependent steps are avoided, yet the resistance phenotype is detected independent of the specific cause of resistance.

First, the global transcriptional response of two *M. tuberculosis* strains to 10 anti-TB drugs was determined using RNAtaq-Seq. A set of highly responsive genes was selected for each drug and RNA-targeting probes were designed.

Next, the RNA-based DST was developed in 96 well format. In short, 200 µl of a positively flagged MGIT™ (BD) culture is spiked with a drug, while a replicate is incubated in absence of the drug. Multiplex mRNA quantification is performed directly on crude cell lysates using a combination of the bead-based MagPix™ (Luminex) and Quantigene™ Plex (Thermo Fisher) technology.

The normalized expression levels are combined to one numeric value which determines the drug susceptibility of the investigated strain.

We successfully developed 8 primary sets of RNA biomarkers for ten 1st-line, 2nd-line and new drugs. Taking isoniazid as proof of principle, we present a biomarker set of 5 responsive genes and 3 normalizing genes, which enables to distinguish susceptible, low- and high resistant TB strains after 6 hours incubation. Next, preliminary results demonstrate that the biomarker sets can successfully discriminate between susceptible and resistance strains for the selected drugs.

We present a robust, RNA-based DST without the need for RNA extraction. The assay was proven to be efficient for isoniazid. With a total of 8 biomarker sets under optimization, the drug resistance profile of up to 14 drugs can be determined.

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POPULATION STRUCTURE OF *MYCOBACTERIUM TUBERCULOSIS* IN RUSSIAN REGIONS BORDERING EU COUNTRIES

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Population structure of *Mycobacterium tuberculosis* in Russia is characterized by predominance of the Beijing genotype strains. A high burden of multidrug-resistant (MDR) tuberculosis in the northwestern Russia may have a negative impact on TB control programs in the neighboring countries of the European Union.

We aimed to assess the population structure of *M. tuberculosis* in northwestern Russian regions (Karelia, Kaliningrad, Murmansk, Pskov) bordering countries of the European Union (Finland, Norway, Estonia, Latvia, Lithuania and Poland).

A total of 304 *M. tuberculosis* isolates (2013–2017) from newly diagnosed TB patients in geographically distant regions of northwestern Russia were studied: Republic of Karelia (n = 78), Pskov (n = 89), Kaliningrad (n = 73) and Murmansk provinces (n = 66). The Beijing genotype family and its particular clusters B0/W148 and 94–32 were detected by analysis of specific markers in *dnaA-dnaN*::IS*6110*, *Rv2664-Rv2665*::IS*6110* and *sigE98*, respectively. Non-Beijing isolates were subjected to spoligotyping followed by comparison to the SITVIT_WEB.

These 4 Russian regions differed to some extent in the prevalence of the Beijing genotype: from to 55.1% in Karelia, 44.9% in Pskov, 63.0% in Kaliningard and 51.5% in Murmansk. Beijing B0/W148-cluster was identified in 17.9; 6.7; 19.2 and 10.6% isolates, respectively. The prevalence of the Beijing 94–32-cluster did not differ: 28.2; 29.2; 28.8 and 30.3%, respectively. In Pskov and Murmansk, most strains were drug susceptible (62.9 and 62.1%), while 17.9% and 25.8% were MDR, respectively. In contrast, MDR strains prevailed in Karelia and Kaliningard — 51.3 and 43.8%, while 41.0 and 36.9% strains were susceptible. In total, for four regions together, 90.2% (37/41) strains of the Beijing B0/W148-cluster were MDR. Beijing 94–32-cluster strains showed much lower rate of MDR — 34.8%, (31/89) ($P < 0.0001$), while 41.6% (37/89) were susceptible. Among non-Beijing *M. tuberculosis* the largest families were T (14.1 and 15.1%) in Karelia and Kaliningard, LAM (23.6%) in Pskov, and Ural (19.7%) in Murmansk.

M. tuberculosis population in the northwestern Russian-EU border provinces is marked by predominance of the major clonal complexes of the Beijing genotype (B0/W148 и 94–32). However, these clones significantly differed in the proportion of MDR. A circulation of the MDR-associated isolates of the Beijing B0/W148-cluster presents the major concern for local and national TB control programs in the Russian regions and likely adverse impact on the neighboring EU countries.

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DRUG RESISTANCE IN *MYCOBACTERIUM TUBERCULOSIS*: FROM PHENOTYPIC MIC-ANALYSIS TO WGS FOR ROUTINE DRUG SUSCEPTIBILITY TESTING

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The phenotypic methods used for drug susceptibility testing (DST) of *Mycobacterium tuberculosis* generally take weeks or months and require culture and manipulation of large numbers of highly infectious bacilli. Drug resistance in *M. tuberculosis* is almost exclusively a consequence of genomic mutations and so molecular determination of resistance offers a rapid, potentially cost effective, and safer alternative.

In 2016, the SRL in Stockholm introduced whole genome sequencing (WGS) for the molecular typing of clinical isolates of M. tuberculosis. At the same time, the WGS analysis made information of various putative drug resistance-related mutations available, which was utilized to complement the routine phenotypic DST. Since 2016, the WGS information on resistance to all clinically relevant drugs is analysed and routinely reported together with the extended phenotypic DST results for all the Swedish multidrug resistant (MDR) isolates.

Retrospectively, we have performed an observational study to compare the WGS data to the phenotypic DST to predict the resistance phenotype from the genetic sequence. The study included 877 clinical TB isolates (only one sample per patient was included) received by the Public Health Agency of Sweden between 01.01.2016 and 31.12.2017. The analysis of the isolates' resistance profiles for the first and second line drugs, obtained from the WGS and Bactec MGIT 960, is ongoing.

Simultaneously, we are evaluating the microbroth dilution technique used for the MIC determination of 150 consecutive (MDR) *M. tuberculosis* together with the corresponding WGS analysis as a part of a collaboration with the CRyPTIC (Comprehensive Resistance Prediction for Tuberculosis: an International Consortium) network. The MIC determinations of drug susceptible and resistant *M. tuberculosis* isolates are necessary in the work to establish correct critical test concentrations for phenotypic DST as well as to identify the minimal, moderate and high confidence mutations. Such coupled analysis will more accurately define clinical drug resistance and may possibly constitute the DST algorithm of choice in an increasing number of laboratories.

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MOLECULAR EPIDEMIOLOGY OF TUBERCULOSIS IN MONGOLIA: SOURCES AND PATHWAYS OF MDR *MYCOBACTERIUM TUBERCULOSIS* STRAINS

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Mongolia is a country with a high burden of tuberculosis (TB). The emergence and spread of multidrug resistance (MDR) TB in Mongolia is associated with problems of early diagnosis and the possibility of cross-border spread of MDR-TB along the Trans-Siberian Railway line from Russia or China. The objective of this study was to reveal sources and pathways of *Mycobacterium tuberculosis* (MTB) strains in Mongolia.

A total of DNAs of MTB from Mongolia (309 strains) were studied. RD 105/207 and 24 loci MIRU-VNTR typing were applied for genotyping and the results were analyzed by MIRU-VNTRplus application. PCR-real time typing was used to identify subtype CC2/W148 by the specific deletion in the *kdpD* gene. Mongolian MIRU-VNTR patterns of MTB were compared with evaluable published Chinese and Russian profiles.

All tested 309 MTB isolates distributed to four lineages: Beijing (228/309 — 73.8%), LAM (33/309 — 10.7%), T (30/309 — 9.7%), H (9/309 — 2.9%), and orphan (9/309 — 2.9%). 21 clusters uniting 187 strains were identified. Out of 228 Beijing strains 165 were clustered and significantly associated with MDR-TB cases from Ulaanbaatar and big railway stations settlements ($p < 0.001$), but MIRU-VNTR profiles from all Mongolian provinces were common.

24-MIRU-VNTR profiles of Mongolian and Russian strains were differed. The three largest clusters consisted of Beijing strains with MLVA MtbC15-9 profiles of 342-32 (58 isolates), 3819-32 (33 isolates) and 1773-32 (33 isolates). Strains with such profiles were found among Russian isolates in single cases in the Irkutsk, Buryat and Zabaikal regions. Subtype CC2/W148, which was primary source of MDR strains in Russia, not found among Beijing strains in Mongolian cohort. Phylogenetic analysis established high genetic close between Mongolian and Chinese profiles of Beijing strains, but Mongolian isolates were grouped in specific cluster.

We determined that there were no significantly transmission of MDR strains from Russia to Mongolia. Our study confirms possibility of cross-border spread of Chinese strains in the past, but now Mongolia has own MDR strains source.

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MOLECULAR EPIDEMIOLOGY OF TUBERCULOSIS IN EASTERN SIBERIA AND FAR EAST

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The highest burdens of tuberculosis in Russia have Asian regions. Beside of social and health care problems this area gained specific geographic spread of *Mycobacterium tuberculosis* (MBT) genotypes and high level of multi drug resistance (MDR). The aim was to carry out comparative evaluation of distribution epidemic MBT strains in Eastern Siberia and Far East of Russia.

We studied 1419 clinical MTB strains collected in Irkutsk region (598), Buryatia (306), Sakha (Yakutya) (351), Zabaykalsky Krai (65) and Primorsky Krai (99). RD 207, 105 181 analysis and 24 loci MIRU-VNTR typing were applied for genotyping and the results were analyzed by MIRU-VNTRplus application. PCR-real time typing was used to identify subtype CC2/W148 by the specific deletion in the *kdpD* gene.

Beijing strains were dominant and varied from 73.6% in the Irkutsk region (Primorsky Krai (72.7%), Zabaykalsky Krai (64.6%), Buryatia (64.4%)) to 43.3% in Sakha (Yakutia). Our study showed that minor Russian genotypes T (13.3%), Haarlem (7.4%) and especially S (13.5%) presented in Sakha (Yakutia) may reflect the MBT population spectrum existing in Russia before Beijing expansion in the 20th century. Yakut's strains of genotype S is unique epidemic group with high level of MDR (77.4% of all S isolates).

As there are in all Russian regions the subtypes CC1 and CC2/W148 were prevailing among Beijing strains. We identified that fifty percent of the Beijing isolates belonged to CC1 group in Irkutsk region, Primorsky Krai and Sakha (Yakutia). Buryatia and Zabaykalsky Krai had unexpectedly low level of CC1, as there were endemic subtype Beijing BL7 (MIT 642) in a quarter of strains from the Beijing collection. The RD 181 deletion absence, the genetic variation of profiles and the presence common clusters of strains of different ethnic groups patients confirm the occurrence of stable circulation of endemic and epidemic BL7 (MIT 642) subtype among the domestic population. The size of subtype CC2/W148 cluster was various from 26.3% in Irkutsk region cohort [Zabaykalsky Krai (24.6%), Primorsky Krai (22.2%), Buryatia (14.0%)] to 13.7% in Sakha (Yakutia). The significant frequency excess of primary MDR was detected only in CC2/W148 subtype vs other epidemic strains of Beijing and nonBeijing genotypes in all areas except Buryatia, where endemic subtype BL7 (MIT 642) shared MDR burden with CC2/W148.

The genotypic structure of the MBT population in the Asian Russia mainly reflects the epidemic expansion features of the Beijing subtypes.

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MOLECULAR DIAGNOSTICS OF TUBERCULOSIS: CLINICAL ASPECTS AND CHALLENGES OF IMPLEMENTATION

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The modern standard of bacteriological diagnostics includes the following set of diagnostic procedures for simultaneous testing of a clinical specimen from a patient suspected of tuberculosis: luminescent microscopy, cultivation on solid media (Finn and Levenstein–Jensen), use of the liquid medium of the BAKTEK 320/960 analyzer, molecular genetic methods in various formats: real-time PCR, GenXpert TB-Rif, Hain test, biochips. The emergence of new technologies does not lead to the rejection of ineffective classical methods. So, in my opinion, the presence of molecular genetic methods in the algorithm of a clinical doctors allows to abandon the microscopy of a clinical material due to low sensitivity and specificity and thereby

optimize costs. However, all the possibilities of molecular genetic methods are not taken into account. So, if the use of GenXpert TB-Rif is absolutely indicated in the case of acute progressive lesions with impaired vital functions in the patient, this technology is less effective with limited diagnostic processes without destruction and inferior to the effectiveness of real-time PCR. The spectrum of detected mutations associated with the development of drug resistance of the causative agent is not taken into account. If more sensitive and cost-effective PCR-based real-time test systems allow detecting only 8 mutations in three genes (*katG*, *inhA*, *rpoB*) that determine the drug resistance of the MBT in 90% of cases, the increase in migration will lead to an increase in cases of tuberculosis with other genetic markers of resistance. In clinical practice, the high risk of falsely positive results from molecular genetic studies in the analysis of material obtained from instrumental or surgical interventions is almost not considered. There is no discussion of the ineffectiveness of the use of molecular tests in the control of chemotherapy at early stages of treatment with a pronounced activity of the process. A clear algorithm is needed for each clinical task.

7. HIV, HEPATITIS AND OTHER SOCIALLY SIGNIFICANT INFECTIONS

7.1 doi: 10.15789/2220-7619-2018-4-7.1

THE ANALYSIS OF TRANSMITTED HIV-1 VARIANTS AMONG ACUTELY INFECTED PEOPLE WHO INJECT DRUGS USING NGS APPROACH

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The phenomenon of a genetic bottleneck, i.e. transmission of one or a few variants of the virus, has been widely studied for sexual transmission, but for people who inject drugs (PWID) the available data are not concludive.

The objectives of the study were real-time detection and follow-up of individual cases of acute HIV-1 infection (AHI) and analysis of the genetic variability with SGA and NGA approaches.

We analyzed full-length *env* genes of transmitted strains using single genome amplification (SGA) and Bayesian Evolutionary Analysis Sampling Trees (BEAST) approach. We also implemented the PrimerID Illumina MiSeq approach for ultra-deep sampling of a fragment of the *env* gene to look for the presence of minor transmitted variants.

Among PWID screened for the study 25% were seropositive. The calculated AHI incidence was 9.3 per 100 person-years. We report 7 cases of acute HIV-1 infection among active PWIDs and 8 potential sexual partners of PWID. Among all the cases studied by SGA and Primer ID approaches we detected a homogeneous viral population likely produced from a single viral variant.

We also detected one case of a secondary infection from a different donor. Adding to previously published data we have analyzed 19 cases of AHI subtype A in St. Petersburg, and at least 74% had a homogeneous viral population confirming a strong genetic bottleneck during parenteral transmission.

The data confirm our original discovery of the genetic bottleneck in HIV transmission among PWID.

7.2 doi: 10.15789/2220-7619-2018-4-7.2

VIRAL HEPATITIS B AND C IN THE ARKHANGELSK REGION: LONG-TERM DYNAMICS OF INCIDENCE AND CROSS-SECTIONAL STUDY OF MARKERS AMONG ADULT POPULATION

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Viral hepatitis B and C (VHB and VHC) represent a serious problem for national health care, affecting the young working population of the country.

The purpose and objectives were to analyze the long-term incidence of VHB and VHC in the Arkhangelsk region and to study the prevalence of VHB and VHC markers among adult population in Arkhangelsk city.

The statistical data forms reported to the federal level were used and a population-based study was carried out as a part of the Norwegian-Russian project. A quota sampling method was used to recruit 1243 adults aged 18–39 years. All participants were tested on VHB Antigen (HBsAg), VHB core antibodies (anti-HBc), VHB surface antibodies (anti-HBs) and VHC (sum antibodies) using an enzyme-linked immunosorbent assay.

Over the past 30 years, the incidence of acute VHB (AVHB) in the Arkhangelsk region decreased in 40 times, the incidence of chronic VHB (CVHB) — in 2.8 times. Nowadays, the incidence of CVHB is in 15 times higher compared with the incidence of AVHB; the incidence of CVHC is in 87 times higher compared with the incidence of AVHC. The prevalence of VHB markers (HBsAg and/or anti-HBc) was 11.8% in men and 10.2% in women in a population-based study. Among men, 1.1 and 1.3% of women were positive on HBsAg; 41.8% of men and 50.9% of women were positive only on anti-HBs. All three tests were negative in 46.4% of men and 38.9% of women. Among men, the percentage of positive for VHC markers was 6.4%, among women — 4.3%. Co-infection of VHB and HCV was found in 1.5% of men and 0.3% women.

Despite the progress made in the control of VHB and VHC, a pool of sources of infections remains in the population. Therefore, preventive work should be continued.

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IMMUNOPATHOGENESIS HIV AND MATHEMATICAL MODELING

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The study of HIV immunopathogenesis is the most important prerequisite for the search of new and improving the existing antiviral and immunomodulating medicines and vaccines used for the treatment and prevention of HIV infection.

The accumulated data of the HIV infection and the functional human physiological system reactions on it indicate that multifactorial mechanisms, which determine the development, course and outcomes of HIV infection, are mediated by a great number of physiological and pathological processes with various positive and negative feedbacks.

Due to the complexity of HIV interactions with the human body, the completely new interdisciplinary and interdisciplinary approaches are in urgent need. These approaches should include various bioinformatics and system analysis methods for the identification of immunobiological protection factors in HIV infection and comprehensive understanding of its pathogenesis. Thus, the advances in genome screening for the cellular proteins with anti-HIV activity identification may serve as the base for the promising approach for the HIV treatment and prevention. In turn, the methods of multiscale mathematical modeling

should be used to solve the tasks of characterization and conceptual analysis of meta-data on the "HIV-host" interaction system. The multiscale mathematical modeling methods may help in the studies focused on the sensitivity of viral infection "stabilization points" towards virus replication and immune reactions in the acute phases of infection. Moreover, the these methods are necessary for the estimation of prolonged ongoing immune stimulation effects, the degree of damage to microenvironment and tissue structures of lymph nodes, and the decrease in proliferative potential and the pool of central $CD4^+$ T-cells.

Lastly, the special emphasis isgiven to the theoretical analysis of HIV relationships with macroorganism, taking into account the virus evolution in the conditions of lymphocytes and macrophages phenotype changes during the adaptive reconstruction of the immune system.

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CHALLENGES AND PERSPECTIVES IN HEPATITIS C VIRUS (HCV) RESEARCH IN AN ERA OF DIRECT ACTING ANTIVIRAL (DAA) THERAPY

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Elaboration of *in vitro* models enabled studies of the HCV life cycle and a search of inhibitors of viral replication, leading to the development of effective DAA targeting non-structural proteins involved in virus replication (NS3/NS4A protease, NS5B polymerase and NS5 inhibitors) with cure rates of more than 95%. Despite this outstanding success of modern medicine and substantial progress in our knowledge of the virus, infection control might be only effective when antiviral therapy and vaccination are combined, since individuals that have been cured with DAAs remain susceptible to reinfection. Another limitation of DAA is their low genetic barrier, resulting in the emergence of drug escape-variants. Alternative or complementary approaches have been thus considered to target host factors required for accomplishment the virus life cycle: cell entry, assembly or release, related to lipoprotein metabolic pathways. Such drugs would have high genetic barrier and pan-genotypic activity.

HCV represents a difficult target for vaccination due to its considerable variability (7 genotypes, 67 subtypes and genetically diverse "quasispecies"). Continuous mutations result in changes in E1E2 envelope glycoproteins targeted by neutralising antibodies and help HCV to evade humoral immunity. The structure of HCV particles circulating in the blood of infected patients remains elusive due to their association with very low-density lipoproteins (lipo-viro-particles). Moreover their size and composition evolve during infection. Shielding of the envelope epitopes by lipoproteins and glycoproteins, cell-to-cell virus spread, and its dissemination by exosomes represent important escape mechanisms that contribute to propensity of HCV to establish chronic infection.

The development of HCV vaccine requires better understanding how antibodies interfere with the virus and of the mechanisms of CD4 T helper cell failure during infection, a predictor of progression to chronicity. An HCV vaccine eliciting T cell responses rather than neutralising antibodies is considered and is currently in clinical testing. Notably, the goal of vaccination is a partially protective vaccine, able to prevent development of persistence, not necessarily infection. A vaccine might be equally needed to restore immune dysfunction of cured patients to prevent re-infection. The development of permissive and immunocompetent animal model(s) is required for further studies of HCV vaccines and HCV-related pathogenesis.

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FREQUENCY AND THE CLINICAL SIGNIFICANCE OF OCCULT HEPATITIS B VIRUS INFECTION

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The detection of hepatitis B virus surface antigen (HBsAg) in serum remains the mainstay in the diagnosis of chronic hepatitis B viral infection and screening for hepatitis B virus in most developing countries, include Russia. Symptoms in chronic hepatitis B viral infection may range from mild nonspecific symptoms in patients with minimal liver damage to ascites, peripheral edema, and encephalopathy in patients with advanced liver disease. Anti-HBc may be the sole marker of resolved hepatitis B viral infection, as anti-HBs, which is neutralizing and so appears after the clearance of HBsAg, may disappear from serum many years after the resolution of hepatitis B viral infection. Occult hepatitis B viral infection is characterized by the absence of detectable HBsAg and present of HBcAb. The objective of study was to identify cases of occult hepatitis B viral infection from patients diagnosed with chronic viral hepatitis and determine it clinical significance.

2236 adult patients with chronic B virus infection were enrolled in study. Serological markers for hepatitis B virus were determined with immunoenzymatic assay and viral DNA — by polymerase chain reaction. For assessment of liver fibrosis was used transient elastography.

Out of all, 42.2% patients had tested negative for the HBsAg and positive HBcAb serologic marker. HBsAb (more 10IU/l) were detected in 28.1% occult hepatitis B. DNA of virus in blood was detected by polymerase chain reaction (threshold 100 IU/l) in 4.3%. In case of using the sensitive test system (threshold 10 IU/l) DNA was detected in 100%. ALT levels were different: N — in 21.8% patients, 1–2N — 41.7%, 2–5N — 27.0%, more 5N — 9.5%. The severe staging of liver fibrosis (F3-F4) is established in 55.5% (F3 — in 3, 6%, F4 — in 51.8%). The moderate staging of liver fibrosis (F1-F2) was 44.5% (F1 — 15.5%, F2 — 29.1%). The severity of chronic liver disease in terms of Child–Pugh score was: class A — 6.3%, class B — 15.5%, class C — 78.2% ($p < 0.001$). Mortality in the cohort of patients with occult hepatitis B virus infection was 13.2% and among patients with cirrhosis — 25.5%. Hepatocellular carcinoma was diagnosed among patients with liver cirrhosis in 1.8%.

The long-term persistence of the virus in the liver may induce a very mild but continuing necroinflammation that — if other causes of liver injury co-exist — may favor the progression of the chronic liver disease toward cirrhosis.

7.6 doi: 10.15789/2220-7619-2018-4-7.6

HEPATITIS A PREVALENCE AMONG CHILDREN IN BOKE AND KINDIA PROVINCES (REPUBLIC OF GUINEA)

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There are no reliable statistical data on the hepatitis A reported cases number among the Republic of Guinea population, included children. One of the morbidity estimation method is the antibodies to hepatitis A virus prevalence estimation in different age groups. Aim of study: to estimate

the seroprevalence of hepatitis A antibodies among children in Guinea by analyzing the detection of antibodies to hepatitis A virus in the local population. Materials and methods. The study was carried out in 2017 year in the Russian-Guinea Research Center for Epidemiology and Prevention of Infectious Diseases of Rospotrebnadzor (Kindia, Republic of Guinea) laboratory by St. Petersburg Pasteur Institute researches (St. Petersburg, Russia) with the assistance of the Republic of Guinea specialists. Serum samples were obtained from 71 conditionally healthy children living in the provinces of Boke (39 samples) and Kindia (32 samples) at the age 0–18 years (mean — 7.4±5.1 year), both sexes (male — 46.5%, female — 53.5%). There are no data about hepatitis A vaccination or case of hepatitis A in the past. Antibodies of the IgG class to hepatitis a virus were determined by enzyme immunoassay with the use of the test systems Vektohep A-IgG (manufactured by Vector-Best, Russia).

Antibodies of the IgG class to the hepatitis a virus were detected in 84.5% of samples. Seropositive persons at the age 0–5 years was 72.9% (95% CI: 55.9–86.2%), at the age 0–10 years — 77.6% (95% CI: 63.38–88.23%), at the 0–15 year — 83.0% (95% CI: 71.73–91.24%). The study was conducted in 1987–1988 years by A.P. Ivanov et al. showed the presence of antibodies IgG class to hepatitis a virus in children 0–10 years in 82.0% of cases, 0–15 years in 74.0%. There is no gender difference in antibody identification at the children 0–15 years (males and females 82.1% and 85.7% respectively, p = 0.5110), and among children 0–10 years (male and female — 76.2% and 84.0% respectively, p = 0.2167). In accordance with the WHO criteria, if antibodies detected in more than 50% of cases among children 0–15 years and less than 90% among children 0–10 years, this indicates the medium seroprevalence of hepatitis A in the population.

The prevalence of hepatitis A in accordance with our data, is at the middle level and has not significantly changed over the last 30 years.

7.7 doi: 10.15789/2220-7619-2018-4-7.7

HIGH BURDEN OF HEPATITIS B IN VIETNAM: IMPACT OF A HIGHLY HETEROGENEOUS VIRAL POPULATION

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South-East Asia is highly endemic area for hepatitis B. In Viet Nam, 8.4 million individuals were estimated to live with HBV infection that resulted in 23 300 deaths in 2005. Here, we investigated naturally occurring genetic variants of hepatitis B virus circulating in general population in Viet Nam.

A total of 3080 adults of 18–79 years old from 16 regions (An Giang, Binh Duong, Dong Nai, Ha Giang, Hoa Binh, Hue, Kien Giang, Lam Dong, Kontum, Nghe An, Ninh Binh, Quang Tri, Thai Nguyen, Hi Phon, Kanh Hoa and Thanh Hoa) were enrolled in this study in 2012–2014. All serum samples were analyzed for the presence of HBsAg with Monolisa® HBsAg detection kit (Bio-Rad, USA) or rapid test (Alere Determine™ HBsAg, USA). As a result, 309 (10.03%, 95% CI, 8.99–11.15) out of 3080 adults were positive for HBsAg. HBV DNA was extracted from HBsAg positive serum samples. HBV genotypes were determined by phylogenetic analysis based on S or P genes.

A total of 117 HBV isolates were genotyped. Six HBV subgenotypes (B2, B4, B6, C1, C5; I) and two recombinant forms (B/C; C/B) were identified. Subgenotype B2 was found in 4 (3.42%, 95% CI 1.34–8.46) isolates; B4 — in 82 (70.09%, 95% C I 61.26–77.64); B6 — in 2 (1.71%, 95% CI 0.47–6.02); C1 — in 20 (17.09%, 95% CI 11.35–24.93); C5 — in 1 (0.85%, 95% CI 0.15–4.68); I — in 3 (2.56%, 95% CI 0.88–7.27); recombinant forms B/C — in 3 (2.56%, 95% CI 0.88–7.27) and C/B — in 2 (1.71%, 95% CI 0.47–6.02). The phylogenetic analysis revealed that Vietnamese HBV strains of subgenotypes B4, B2 and C1 formed the several distinct clusters that separated from other strains isolated in Asia. HBV strains belonged to other subgenotypes were scattered among Asian variants. Subgenotype I was found only in the northern mountain region. Based on "a" determinant in S protein the HBV strains were classified into four subtypes: adr, adw2, ayw1, ayw3. No amino acid substitutions, which may alter HBsAg antigenicity or be responsible for vaccine escape were detected in preS region as well as in major hydrophilic region of the S region.

The predominance of HBV subgenotype B4 in all studied regions indicates crucial impact of this HBV variant on the persistence infection in Viet Nam. The high genetic diversity of viral population highlights the multiple sources of infection, successful spreading of a variety of viral variants and provides insight into the driving force of the HBV epidemic process in Viet Nam.

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MOLECULAR-GENETIC CHARACTERISTICS OF THE HEPATITIS B IN THE NANAYSKY DISTRICT OF THE KHABAROVSK TERRITORY

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Hepatitis B continues to stay a pressing issue due to frequent development of chronic cases of the disease.

Aim of the research was to analyze the genetic diversity of the hepatitis B virus (HBV) circulating among the indigenous population of the Nanaysky District of the Khabarovsk Territory.

A total number of 82 samples (59 women, 23 men) of blood plasma were obtained from the Nanaysky District patients with the diagnosis of chronic hepatitis B (CHB). According to the ethnic composition, there were 62.3% of Nanai people, 32.9% of Russians, Udege and Evenks totaled by 2.4% each. The HBV DNA was detected using the PCR kits "AmpliSens®HBV-FL" and "AmpliSens®Monitor-FL" (Central research institute of epidemiology of the Rospotrebnadzor, Russia). The PCR was followed by genotyping using a two-step PCR with primers to a conservative region of overlapping S and P genes. Phylogenetic analysis was performed with the MEGA6.0 software. Neighbor-Joining method was used to build the phylogenetic trees. Nucleotide distance was estimated via Kimura method.

HBV DNA was found in 46 (56.1%) samples of the blood serum. The viral load levels in 13 (28.3%) patients was low (< 10^3 ME/ml), in 26 patients it was intermediate (10^3–10^6 ME/ml) and in 7 cases it was high (> 10^6 ME/ml). The phylogenetic analysis was performed for 43 nucleotide sequences. Genotype D was dominant and was found in 34

samples (79.1%). The phylogram showed that the strains of one genotype divided into three subgenotypes: D1 — 1 (2.9%), D2 — 15 (44.2%) and D3 — 18 (52.9%). The genotype C was detected in 7 (16.3%) patients and four of them formed a cluster with Chinese samples that were registered in the GenBank database. Genotype A was isolated in 2 (4.6%) samples and formed a cluster with strains isolated in Poland and Belgium.

HBV genotype D comprised out of subgenotypes D1, D2, D3 and prevailed among the CHB patients living in the Nanaysky District of the Khabarovsk Territory. The second prevalent strain was genotype C. Genotype A was detected in individual cases.

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THE OCCURENCE OF HEPATITIS C MARKERS AMONG RESIDENTS OF THE KINDIA PREFECTURE OF THE REPUBLIC OF GUINEA AND THE KHANH HOA PROVINCE OF VIET NAM

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Hepatitis C virus (HCV) infection plays an important role in liver diseases. The burden of HCV infection continues to be significant in low- and middle-income countries, especially in Asia and Africa. The global elimination of HCV by 2030 is possible with the advent of effective diagnostic methods available to the majority of the population. The aim of the study was to estimate the prevalence of serological and molecular HCV markers among the apparently healthy people in Kindia region of the Republic of Guinea and Khanh Hoa region of Viet Nam.

Serum blood samples were obtained from apparently healthy adults of the Kindia prefecture (n = 248, the average age was 41.59±9.89) of Republic of Guinea and Khanh Hoa province (n = 256, the average age was 41.98±11.73) of Viet Nam. The presence of total anti-HCV and the specific antibodies to the core, NS3, NS4, NS5 HCV proteins were determined using ELISA-kits (Diagnostic Test Systems LLC, Russia). RNA HCV in the serum samples was detected by real-time PCR using the "AmpliSens HCV-FL" kit (FBIS "CRIE", Russia). The confidence interval (95% CI) was calculated by the Wilson method.

Totally, anti-HCV was detected in 9 (3.63%; 95%, CI 1.92–6.75) of 248 adults from Kindia; in 3 (1.17%; 95%, CI 0.40–3.39) of 256 adults from Khanh Hoa. The uncertain results of the anti-HCV were obtained in 6 (2.42%; 95%, CI 1.11–5.18) of 248 residents of Kindia; one (0.39%; 95%, CI 0.07–2.18) of 256 residents of Khanh Hoa. RNA HCV was detected only in one (0.39%; 95%, CI 0.07–2.18) of 256 adults from Khanh Hoa, while RNA HCV was not detected in serum blood samples from Kindia.

The results of the occurrence of HCV markers in apparently healthy residents of both Kindia Prefecture and Kanh Hoa province do not differ from the available estimated metaanalisys data on the HCV prevalence in West Africa and South-East Asia. In order to assess the dynamics of the epidemic process, it is necessary to study HCV infection in different ethnic groups throughout the territory of both countries.

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POLYMORPHISM THE *CCR2* GENE IN THE ST. PETERSBURG POPULATION

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HIV infection is one of the main socially significant diseases of the world population. Resistance/susceptibility to HIV-1 infection is different. Chemokine receptors such as *CCR2* play an important role during infection with HIV-1. The gene for the chemokine receptor *CCR2* locates in the short arm of chromosome 3. The replacement of nucleotide *G* by nucleotide *A* at position 190 in the *CCR2* gene results in the replacement of the amino acid valine by isoleucine at position 64 (*CCR2-V64I*) in the primary sequence of the protein. This replacement slows the development of AIDS in HIV-infected. In Europeans the allele frequency of *CCR2-V64I* is 8–10%, blacks — 15–17%, Mongols — 20–25%. Knowing the frequency of polymorphic allele distributions can help predict the epidemic situation in the region. The aim of the work was to study the frequency of alleles of the *CCR2* gene in St. Petersburg.

The study examined a group of 411 conditionally healthy donors aged 0 to 95 years living in St. Petersburg. Genomic DNA was isolated from biological samples using commercial kits (Interlabservice, Russia). *CCR2* genotype polymorphism was detected by pyrosequencing on the PyroMark Q24 instrument (Qiagen) using primers of our own design.

Factors "sex" and "age" had no a significant influence on the frequency of distribution of the studied alleles. The distribution of genotype frequencies in the studied population does not differ from the Hardy–Weinberg Equilibrium. Wild-type genotype (*GG*) was detected in 320 people. 6 people were carriers of the genotype *AA*, and 85 people were heterozygotes (*GA*). Frequency genotype of the *CCR2* wild-type (*GG*) was 77.9%. Heterozygotes (*GA*) were 20.7%, homozygotes *AA* were 1.4%. The frequency of allele *G* was 0.88, allele *A* was 0.12. Thus, more than 20% people of the population in St. Petersburg have a protective allele of the *CCR2* gene.

The high incidence of allele *CCR2* makes it reasonable to screen HIV-infected people and groups at risk for HIV infection. The obtained data can be used to predict the development of the AIDS epidemic in St. Petersburg.

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RECONSTRUCTION OF RECOMBINATION SITES IN GENOMES OF GENOTYPE 2 HEPATITIS C VIRUS STRAINS USING BIOINFORMATICS METHODS

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Hepatitis C virus (HCV) is an important human pathogen, causing an estimated 180 million chronic infections and annually 3–4 million new infections worldwide. Due to its genetic heterogeneity, HCV has been classified into seven major genotypes and about 80 subtypes. Although the different genotypes and subtypes share basic biological and pathogenic features they differ in clinical outcomes, response to treatment and epidemiology. HCV recombination raises many questions concerning its mechanisms and effects on the epidemiological and physiopathological features of the virus. The first natural recombinant strain of HCV was identified as recently as 2002. Since then,

there have been only a few more than a dozen reports including descriptions of HCV recombinants. However, the frequency of recombination may have been underestimated because not all known HCV recombinants are screened for in routine practice. However, the development of bioinformatics technologies allows for effective screening of genomic sequences for the presence of recombination signals.

Recombination analysis was performed with the Recombinant Detection Program (RDP) version 4.61. This tool provides statistical evidence for the breakpoint site by using six methods (RDP, Geneconv, maximum chi-square, Chimaera, SiScan and 3-seq). A recombination analysis was conducted on 237 complete genome sequences of genotype 2 HCV strains, extracted from the ViPR database. The analysis by the RDP was phylogenetically corroborated by NeighborNet method using the SplitsTree 4.14.1 program and statistically confirmed by the PhiTest method.

Using the RDP method, 116 unique recombination events with a high degree of reliability ($p < 0.01$) were found in the strains studied. The presence of recombination was confirmed by phylogenetic reconstructions. Some of these events occur in dozens of strains, including those belonging to several different subtypes, which may indicate their ancient origin. Futhermore, many strains contain more than one recombination site, and in some cases these areas can overlap. Analysis of parental strains showed that recombinations occur both within and between subtypes, and parental strains often come from different geographic regions. These results may indicate that genotype 2 hepatitis C virus strains have a higher potential for recombination variability than it was thought before.

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LOW HEPATITIS B VIRUS DNA BURDEN DOES NOT ALWAYS PROTECT FROM LIVER CANCER DEVELOPMENT

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Due to the considerable number of patients chronically infected with hepatitis B virus (HBV) worldwide (260 million), persistent infection with this agent still represents the principal etiology of the main form of primary liver cancer, hepatocellular carcinoma (HCC). Two decades ago, it has been shown in Far-Eastern countries, that the risk of HCC development increases considerably when high loads (> 2.0E+04 IU/mL) of circulating HBV DNA are measured in the plasma of the patients. This observation prompted the release of therapeutic guidelines recommending to treat the patients when HBV DNA exceeds 2.0E+03 IU/mL. We recently conducted a molecular analysis on Peruvian patients with HCC infected with HBV genotype F (n = 53). Half of these patients were remarkably young (< 40 years) and their livers unscathed with cirrhosis. Two-third of them were carrying HBV surface antigen, while the remaining were occultly infected. Remarkably, a single patient was displaying HBV DNA loads above the threshold for mandatory treatment. In tumor and non-tumor liver tissues, HBV DNA copy number per cell was usually very low (0.1–10% of infected cells) and no clonal integration was detectable. HBV genome mutations usually observed at this stage of the liver disease (Stop precore, basal core promoter double mutations, pre-S deletions) were infrequent (< 25% of cases) in this series. In tumor cells, innate immune response was inactive while most DNA repair systems were strongly activated. Our data suggest that for some populations such as American Indians, or in case of infection with specific HBV strains such as genotype F, the standard procedures of surveillance of patients at risk for HCC are ineffective. Our observations imply that the local patho-physiological context should prevail above guidelines generated from analyses conducted on populations with different geo-anthropological backgrounds. In addition, our observation is somehow reminiscent of the HCC presentation described in Yupik people from Alaska who are also infected with HBV genotype F, the endemic genotype of the Americas. It suggests that similar adverse situations might affect populations with American Indian ancestry. In that respect, research on populations living in Siberia, on the other side of Bering strait, might provide some interesting comparison points. Larger surveys should now be conducted to confirm and refine these preliminary observations.

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THE PREVALENCE OF HIV-1 DRUG RESISTANCE MUTATIONS IN PATIENTS WITH LOW ADHERENCE TO ANTIRETROVIRAL THERAPY IN THE LENINGRAD REGION

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Against the backdrop of an increase in the number of people embarking on antiretroviral therapy (ARVT), the manifestation of drug resistance of HIV is becoming an increasing obstacle to the fight against the epidemic.

Variants of the virus that have drug resistance are usually able to accumulate in the body in the event of interruptions in the intake of antiretroviral drugs. Therefore adherence to therapy is one of the most important factors in the formation of HIV resistance.

The aim of this work is to study the structure of mutations in the HIV genome associated with drug resistance in patients with low adherence.

Blood plasma samples from 269 patients were examined for the detection of mutations in drug resistance. Previously, the history of patients was studied: sex, viral load during therapy, adherence to the latter.

Among patients there are 139 (51.67%) people with low adherence to therapy. In the aggregate of patients with low adherence, men predominate (57.55%). This may be due to the peculiarity of the psyche, more typical for male patients. It is also important to note that majority of patients with low adherence belong to disadvantaged groups of people: people who use alcohol and drugs that do not have a permanent place of residence, etc. As a result of an unstable lifestyle, such patients most often interrupt therapy. This, in turn, leads to changes in ARVT regimens, which together with a high viral load (74% of patients with viral load exceeding 10 000 copies/ml) is a factor contributing to the formation of drug resistance.

One of the reasons for the drug resistance of HIV is the appearance of mutations in parts of the virus genome associated with the synthesis of viral enzymes, which are the main targets of therapy. Analysis of the results of studies on the presence of HIV drug resistance revealed several common mutations: M184V (51.08%), K103N (18.71%), L74V (12.95%), K101E (11.51%), A62V and G190S (10.79%), the remaining mutations occur in less than 10% of cases.

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PARENTERAL VIRAL HEPATITIS IN CHILDREN IN RUSSIA, PARTICULARLY IN THE NORTHWESTERN FEDERAL DISTRICT

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Among acute viral hepatitis in children aged 0 to 14 years nosological entities with enteric transmission prevail over the parenteral ones which account for not more than 3–5%. Thus, in 2017 out of 1851 cases of acute viral hepatitis reported in the Russian Federation among the children under 15 years of age 10 cases were of acute hepatitis B and 40 cases — of acute hepatitis C. 417 Cases of chronic viral hepatitis in children aged 0–14 years were reported in Russia in 2017. As well as in adult population, chronic hepatitis C predominates, accounting for 83.7% of all reported cases. Relative share of chronic hepatitis B is 14.9% and that of an unspecified chronic hepatitis — 1.4%. Preventive measures against infection with hepatitis B and hepatitis C contributed to significant decrease in the parenteral hepatitis incidence rate. Timely implementation of a wide hepatitis B immunization program for 1-year old children in the Northwestern federal district resulted in the fact that since 2013 the number of cases of acute hepatitis B has not exceeded 1–2 and in some years, there were no such cases at all. Within the Northwestern federal district in 2009–2010 acute hepatitis C was reported both in infants and older children. Decrease in incidence rate in infants from 5.1 per 100 000 people in 2009 down to 1.78 per 100 000 people in 2017, as well as lack of reported cases in older age groups, shows the improvement of epidemiological situation. The incidence rate of chronic hepatitis B and hepatitis C in children is more than 40 times lower than the incidence rate in adults. In 2017 in the Northwestern federal district the incidence rate of chronic hepatitis C in children was 6.5 times higher than that of chronic hepatitis B (1.5 and 0.2 per 100 000 people, respectively). Despite of positive dynamics in the parenteral hepatitis incidence rate, the total number of pediatric patients in the Northwestern federal district over the last years has remained constant (250–300 children), and the attack rate in 2017 was 3.7 per 100 000 people in case of chronic hepatitis B and 11.7 per 100 000 people in case of chronic hepatitis C. Thus, in spite of decrease in its incidence rate in children, the problem of post-transfusion hepatitis infection remains of high concern.

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MONITORING OF LONG-TERM ANTIVIRAL TREATMENT OF CHRONIC HEPATITIS B

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Rapidly progressing and hard to treat HBeAg-negative chronic hepatitis B is for the prevalent type of the disease in the world, including Russia. Antiviral therapy by nucleot(z)ide analogues is aimed at the permanent suppression of hepatitis B virus replication. Undefined duration of the nucleot(z)ide analogues treatment in HBeAg-negative patients is a serious problem taking into account absence of adequate predictors of the disease course after treatment discontinuation. Thus, our task is to analyze the results of the long-term antiviral therapy of HBeAg-negative chronic hepatitis B patients. Analysis was performed in 79 HBeAg-negative patients with confirmed chronic hepatitis B who had not previously treated. The administration medicine was telbivudine in a daily dose of 600 mg (n = 49) or entecavir in a daily dose of 0.5 mg (n = 30). The therapy course, which lasted from 5 months to 7 years, also included patients with a viral load less than 2.0×10^4 IU/ml in presence of severe hepatic fibrosis (F3 or F4 stages by METAVIR). Efficacy of the treatment was evaluated based on the activity of ALT and the level of HBV DNA, monitoring also included the biochemical and serological parameter measurements. The study had shown that viral load reached undetectable levels in 92.3% of cases after 52 weeks of therapy ($p < 0.05$). Five patients out of six non-responders, received telbivudine. A significant decrease in the proportion of patients with ALT levels above upper line normal (16.8% vs. 44.3% at the beginning of the treatment), as well as severity of cytolytic activity (ALT levels 109.8±102.4 IU/L vs. 68.8±39.2 IU/l at the beginning of the treatment) ($p < 0.05$) were noted. The remarkable fact was the decrease in the proportion of patients responding to treatment at 104–156 weeks of antiviral therapy. In most cases, failure of the therapy was associated with the telbivudine administration, and telbivudine replacement with entecavir was associated with increase of the virological response rates. Thus, it can be concluded that treatment with telbivudine is currently impractical due to the high level of virological breakthroughs. Entecavir has demonstrated higher efficacy during the treatment, lasting for up five or more years. However, there is a number of issues related to the prediction of the relapse risks after discontinuation of the nucleot(z)ide analogues therapy, which remains unsolved and requires further studying.

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DEVELOPMENT AND APPROBATION METHOD OF IDENTIFICATION MUTATIONS THE RESISTANCE OF THE HEPATITIS C VIRUS TO DIRECT-ACTING ANTIVIRAL AGENTS (DAAs)

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Hepatitis C Virus (HCV) treatment has been improved dramatically thanks to the introduction of direct-acting antiviral agents (DAAs). These antivirals have significantly increased response rates (up to 98%) and greatly reduced treatment duration. Currently available DAAs are classified into four categories given their molecular targets in the HCV replication cycle: NS3/4A protease inhibitors bind to the active site of the NS3/4A protease; NS5A inhibitors interact with domain 1 of the NS5A dimer; nucleotide analog NS5B polymerase inhibitors are incorporated into the nascent RNA chain resulting in chain termination by compromising the binding of the incoming nucleotide; nonnucleoside NS5B polymerase inhibitors. However, the high replication rates of HCV, can lead to the extreme mutations in the virus.

The objectives were development and approbation the method of identification mutations the resistance of the hepatitis C virus to direct-acting antiviral agents (DAAs).

The subtype-specific oligonucleotides were designed based on HCV sequence alignments from NCBI HCV database. The sequences were retrieved with the inclusion criteria of belonging to full genome sequences (confirmed non-recombinant genomes), being devoid of large insertions/deletions, and corresponding to 1a, 1b, 2a. It is these subtypes that are most common in Russia. Specific oligonucleotides were designed to therapeutically relevant regions: NS3/4A, NS5A, NS5B.

The methodological pipeline described here is adequate to characterize in-depth mutant spectra of HCV populations, and it provides a tool to understand HCV diversification and treatment failures. The pipeline can be periodically extended in the event of HCV diversification into new genotypes or subtypes, and provides a framework applicable to other RNA viral pathogens, with potential to couple detection of drug-resistant mutations with treatment planning.

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THE SIGNIFICANCE OF THE HIV RESISTANCE ANALYSIS IN ANTIRETROVIRAL THERAPY

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The widespread use of antiretroviral therapy, the development of new drugs and treatment regimens are inherently associated with the emergence of HIV resistance. Systematic laboratory monitoring of patients with ineffectiveness of antiretroviral therapy is necessary.

Objective was to assess the significance of the resistance analysis in the current algorithms for control HIV infection in individuals.

Blood plasma samples of 66 patients from different regions of the North West Federal District were used in the work. All patients were referred for testing the drug resistance of the virus due to the virological ineffectiveness of antiretroviral therapy. The nucleotide sequences obtained with the use of a set of reagents for the detection of mutations of drug resistance to antiretroviral drugs AmpliSens® HIV-Resist-Seq.

For this group of patients two main scheme of therapies were used: the first one included two drugs from the Nucleoside Reverse Transcriptase Inhibitors group in combination with one drug from the Non-Nucleoside Reverse Transcriptase Inhibitors group and the second one from the Protease Inhibitors group in combination with two drugs from the Nucleoside Reverse Transcriptase Inhibitors group. The analysis of nucleotide sequences in the protease gene showed that most commonly there are mutations that cause drug resistance to groups of nucleoside and non-nucleoside reverse transcriptase inhibitors. In 45 patients (60%) a mutation in the position of M184V was detected, which is responsible for the presence of a high level of resistance to lamivudine. In 34 (52%) cases, a mutation was found in the K65R position, responsible for the cause of resistance to abacavir and didanosine. In 12 (18%) patients, a combination of mutations M184V + L74V, also resulting in resistance to didanosine and abacavir, was detected. This indicates that when the therapy is changed, the percentage of the most frequent mutations increases, along with the appearance of new ones, which indicates the need for an analysis of the resistance of HIV to antiretroviral therapy not only in cases of virological inefficiency of therapy, but also during the change of therapy and similarly, naive patients should be analyzed for existing mutations to optimize treatment.

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FORECASTING OF INCIDENCE OF HAV WITH USE OF THE SCHEDULED PLAN OF WALD

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The incidence of viral hepatitis A (HAV) has high epidemiological significance. According to The Federal service for supervision of consumer rights protection and human welfare in Russian Federation during 2010–2016. 44 666 cases of HAV among adult population, and the children's population 21 308 respectively are registered.

For the purpose of assessment of an epidemiological situation on HAV the analysis of official statistical data on incidence of HAV is carried out to the Moscow in 2016 and forecasting of an epidemic situation for 2017. The forecast of number of cases was carried out with use of linear approximation and the developed method of forecasting of incidence of HAV with use the plan — Wald's graphics.

It is established by the results of the analysis of the dynamics of the incidence of HAV in Moscow, using the method of linear approximation in 2017, the incidence of HAV in adults and children was 240 and 115 cases These statistical indicators characterize the epidemiological situation as unfavorable.

According to the results of the statistical analysis, the threshold level of the incidence of HAV in Moscow among children for the period 2010–2016 analyzed. 11 cases were made, which is defined as an incremental total, with a minimum monthly prognostic level of 7 cases, a maximum of 17. The monthly increase in the number of diseases in the dynamics of the analyzed year was 0.9 cases. The forecast of the maximum number of diseases of the HAV in 2017 exceeds the threshold level of morbidity and indicates a possible worsening of the epidemiological situation in Moscow. The forecast of the total minimum and maximum incidence rate in 2017 will be 84 and 204 cases respectively.

Based on the results of the analysis of the incidence of HAV for the period from 2010 to 2016, using the Wald schedule it was shown that the epidemic situation in Moscow among the adult population and among the children under 17 is estimated as unstable. The method of analysis and forecasting using the Wald schedule allows to predict the total minimum and maximum levels of the incidence of HAV, which is of great importance for practical public health and makes it possible to adjust planned preventive and antiepidemic measures.

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RISK FACTORS OF PERINATAL TRANSMISSION OF HEPATITIS C VIRUS

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Worldwide, the virus of hepatitis C (HCV) infected 2–3% of the population (more than 185 million people). The proportion of pregnant women with hepatitis C (HC) is 1–2.4%. In previous studies, it was reported that perinatal transmission of HCV is due to the level of viral load of HCV, HIV co-infection, increased activity of alanine aminotransferase, duration of anhydrous period. The use of amniocentesis and internal monitoring of the fetus may increase the risk of perinatal transmission of HCV. There is no evidence of a reduction in the risk of HCV transmission from the mother to the baby in cesarean section and the rejection of breastfeeding.

The aim of the study was to determine the risk factors for perinatal transmission of the hepatitis C virus.

To diagnose perinatal transmission of HCV, 140 children born to women with HS were examined. The diagnosis of congenital HS was established when HCV RNA was detected in the blood plasma of a child older than 2 months twice with an interval of at least 3 months or anti-HCV in a child over 18 months old using commercial reagent kits. The ELISA method was used to detect antibodies to HCV ("ELISA-HCV-Ab", The Republican Scientific and Practical Center for Epidemiology and Microbiology, Belarus, Monolisa HCV

Ag-Ab ULTRA, Bio-Rad, France). Real-Time-PCR was quantified and measured quantitatively by HCV RNA (Real-Best HCV RNA Quantitative, Vector-Best, Russian Federation). The statistical processing of the data was carried out with the help of the program "Statistica 10.0" ("StatSoft", USA) and "MedCalc 10.2.0.0" (MedCalc, Mariakerke, Belgium). In order to describe the qualitative features, the share (P) of the trait was calculated, the ratio of the odds of the event in one group to the chances of the same event in another (OR) and its 95% confidence interval (95% CI) was determined. The clinical significance of the level of quantitative indicators was assessed using ROC analysis. Data were presented as AUC (area under the curve), its 95% confidence interval (CI), Se (sensitivity, %), Sp (specificity, %). Differences were considered statistically significant at $p < 0.05$.

Perinatal transmission was 5.1% and occurred in 7 of 140 children. The probability of perinatal transmission of HCV increased with a level of direct bilirubin > 5.4 μmol/L in the mother's blood at the gestational age of 27–37 weeks (AUC = 0.86; 95% CI 0.72–0.94; Se = 100%; Sp = 78.6%, $p < 0.001$) and a viral load of HCV > 1 200 000 IU/ml in 37 weeks or more (AUC = 0.72, 95% CI 0.60–0.82, Se = 100%, Sp = 54.8%, $p = 0.033$).

The risk factors for perinatal transmission of HCV were the first pregnancy (OR = 7.7, 95% CI 1.4–42.5, $p = 0.018$), drug use during pregnancy (OR = 14.5, 95% CI 1.5 -137.4, $p = 0.018$), two or more episodes of acute respiratory infection during pregnancy (OR = 12.2, 95% CI 1.2–119.5, $p = 0.029$), cervical injury in labor (OR = 10.7, 95% CI 1.8–65.0, $p = 0.009$), compensatory-adaptive placental reactions (OR = 8.5, 95% CI 1.5–46.6, $p = 0.013$).

Perinatal transmission of HCV is caused by behavioral factors of the mother, biochemical and virological characteristics of HCV infection, concomitant pregnancy diseases, maternal traumatism of the mother, pathomorphological features of the ulcers.

8. INFECTIOUS IMMUNOLOGY AT THE PRESENT STAGE

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CREATION OF THE IMMUNOFERMENTIC TEST SYSTEM FOR DETECTING C3 COMPLEX COMPONENT WITH THE USE OF PEPTIDOGLYCAN

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Any inflammatory process is accompanied by activation of the complement system, as well as increased production of the acute phase protein — C3. C3 binds to the surface membrane of the bacterial cell and enhances the formation of new C3b. It is known that corynebacteria colonize all the mucous open cavities of a person, and their metabolites play a role in the system of immunity.

The aim of the study was creation of an ELISA system for participation in C3 activities due to its ability to bind to peptidoglycan corynebacteria.

The ELISA method offers sorption in the wells of a micro-panel of peptidoglycan. Then, a solution containing a human complement component C3 with unknown activity is introduced into the wells. The incubation is carried out in the presence of EDTA to block all pathways of complement activation. C3 binds to the sorbed peptidoglycan, followed by removal of the onion content and introduction of the enzyme conjugate with antibodies against the human C3 component, washing out the unbound conjugate, introducing the substrate of the conjugated enzyme, and calculating the components of C3 by the amount of the product of the enzymatic reaction.

The kit contains a flat-bottomed micro-panel with sorbed peptidoglycan, a conjugate combined with antibodies to C3 components as a standard. The incubation takes place in the presence of EDTA.

Use of the proposed test system to identify the identified deficiencies in the blood serum of patients with ENT pathology (bronchitis, tonsillitis, sinusitis, otitis) is determined by the increased content of C3 complement components in comparison with its content in the sera of healthy individuals by 2 times.

Obtained data that with ENT — pathology in blood serum of sick people there is activation of C3, responsible for the subsequent launch of the entire complement system.

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FEATURES OF THE SUBPOPULATION COMPOSITION CYTOTOXIC T-LYMPHOCYTES IN CHILDREN WITH CONGENITAL CHRONIC HEPATITIS B

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Hepatitis B is an infectious disease caused by the hepatitis B virus, which has a high tropicity to hepatocytes and is capable of transitioning to a chronic form (HBV). The highest frequency of chronization is in children, especially with perinatal infection (up to 90%). An effective mechanism for protecting the body from viral infections is the development of a cytotoxic immune response, during which naive cytotoxic T lymphocytes proliferate and undergo several stages of differentiation, acquiring the ability to kill infected cells. In congenital CHB, the development of an ineffective variant of such a response in children can be a consequence of both immaturity of their immune system and disorders during its formation in the presence of the virus. The determination of the ratio of groups of cells at different stages of this differentiation can contribute to the study of the mechanisms of this pathology.

Purpose of the study was to evaluate the effectiveness of the formation of effector cytotoxic T-lymphocytes in children with chronic hepatitis B acquired due to perinatal infection.

The material of the study was peripheral blood of 9 children diagnosed with CHB, after perinatal infection and without severe accompanying pathologies, as well as 6 conditionally healthy children, aged 7 to 14 years. The following populations of cytotoxic T-lymphocytes ($CD45^+/CD3^+/CD8^+$) were analyzed by multicolor analysis on a BD FACS Canto II device: naive ($CD45RA^+/CD62L^+$), central memory cells (CM, $CD45RA^-/CD62L^+$), effector memory cells (EM, $CD45RA^-/CD62L^-$) and "terminally differentiated" EM (TEMRA, $CD45RA^+/CD62L^-$).

In the blood of the patients studied, a significant decrease in the absolute amount of cytotoxic T lymphocytes was found: 0.41 (0.33–0.60) 10^9/L versus 0.64 (0.48–0.76) 10^9/L in healthy subjects, $p = 0.0496$; the tendency to decrease the absolute number of TEMRA cells is 0.06 (0.05–0.09) 10^9/L versus 0.16 (0.08–0.27) 10^9/L in healthy and relative number of cells — 3.5 (2.6–4.5)% versus 6.6 (3.6–11.2)%. Also, there were no differences in the number of naive cytotoxic cells — 0.22 (0.16–0.29) 10^9/L and 10.2 (8.5–13.9%) versus 0.25 (0.23–0.28) 10^9/L and 10.6 (9.6–12.2)%, the central memory cells — 0.03 (0.02–0.05) 10^9/L and 1.6 (1.2–2, 4)% versus 0.04 (0.04–0.06) 10^9/L and 1.9 (1.5–2.5)% in healthy and effector memory cells — 0.10 (0.05–0.0, 17) 10^9/L and 4.2 (2.7–8.8)% vs. 0.14 (0.08–0.21) 10^9/L and 5.6 (3.9–9.0)% in healthy.

As a result of the study, it was shown: in the peripheral blood of children with congenital CHB, absolute amounts of cytotoxic T-lymphocytes were reduced; there is a tendency to decrease absolute and relative amounts of "terminally differentiated" cytotoxic T-lymphocytes, which may indicate the depletion of this pool of cells due to migration to the injured organ, or a violation of their formation. At the same time, there are no pathologies in the content of naive cytotoxic cells and memory cells.

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INFLUENCE OF MONOSTRAIN AND MULTISTRAIN AUTOPROBIOTICS ON MICROBIOTA AND IMMUNITY OF RATS WITH INTESTINAL DYSBIOSIS

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The aim of the study was to find the differences in autoprobiotics effects on intestinal microbiocenosis and immune system of rats with antibiotic associated dysbiosis.

Intestinal dysbiosis of male Wistar rats was induced by ampicillin and metronidazole. Indigenous enterococci (group E), lactobacilli (group L), bifididobacteria (group B) were isolated from feces before the antibiotic usage and then separately or as mixture of three strains (group M) were given to the animals for 4 days. Rats from control group 1 (C1) didn`t receive autoprobiotics. Animals from control group 2 (C2) didn`t receive antibiotics and autoprobiotics. The study of fecal samples, collected on 4th and 9th days of experiment, was performed by RT-PCR and by metagenome 16S rRNA analysis. The cluster differentiation of lymphocytes was analysed using flow cytometry.

Dysbiosis is characterized (on 4th day) by excessive abundance of filum *Gammaproteobacteria*, *Proteus* spp. (Pr) and, *Klebsiella* spp. (K), and decrease of *Faecalibacterium* sp. (F), *Prevotella* spp. (Pv), *Bacteroides* spp., *Lactobacillus* spp. populations. The decrease abundance of opportunistic enterobacteria was minimal in groups C1 and M. Low efficacy against Pr and K, main decrease of *Lactobacillus* spp. and *Paraprevotella* spp. content in M group coincided with maximum shifts in cluster differentiation of lymphocytes: increase of B-cells, NK-cells, decrease of T-cells and $CD3^+CD8^+$ T-lymphocyte. Surprisingly no significant changes in the immunogram of rats from the group C1 could be detected.

Indigenous enterococci stimulated growth of bacteroides and inhibited growth of lactobacilli and Pv. This autoprobiotic demonstrated low antagonistic activity against Pr. Indigenous lactobacilli inhibited the growth of the Pr and restored the number of Pv. and F. Significant decrease of both K and Pr percentage abundance and the increase of F were observed in group B.

Changes in the composition of microbiota correlated with changes of immunity in different experimental groups. The increase in the abundance of F correlated with the increase of $ThCD3^+$.

$CD25^-FoxP3^+$ content in the spleen in groups L and B. It was found that content of Th $CD44^+62L^+$ lymphocytes in the blood, which was reduced in group B and E inversely correlated with the abundance of *Gammaproteobacteria*.

It should be noted that implementation of autoprobiotics besides the influence on microbiota have a significant effect on immunity, which varies depending of the type of autoprobiotic. The mechanisms of immunomodulatory effects of autoprobiotcs are not yet clear and require further studies.

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POTENTIAL INFLUENCE OF IMMUNOMODULATORS ON THE PRODUCTION OF INTERFERON-GAMMA AND INTERLEUKIN-10 IN LABORATORY ANIMALS VACCINATED AGAINST PLAGUE

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The improvement of specific prophylaxis of infectious diseases involves the search for new highly effective immunomodulators to influence the activation of the factors of active and adaptive immunity. As potential components that increase the effectiveness of live plague vaccine, polyoxidonium and Ingaron (Interferon gamma human recombinant), preparations with a stimulating effect on immune system are of some interest.

The comparatives analysis of polyoxidonium and Ingaron effect on interferon-gamma and interleukin-10 production in BALB/c mice when immunized with culture of *Yersinia pestis* vaccine strain EV line NIIEG were studied.

200 BALB/c mice were subcutaneously immunized with a *Y. pestis* EV NIIEG culture at dose of 2.5×10^4 (group 1), in combination with polyoxidonium at dose of 4 μg (group 2), or with Ingaron in dose of 150 IU (group 3), intact mice (group 4) served as controls. The content of cytokines in blood was determined by enzyme immunoassay on the 3rd, 7th, 21st and 90th days after injection of preparation using commercial test systems (eBioscience, Austria).

On the 3rd day of the experiment, significant increase in the level of both interferon-gamma and interleukin-10 was established in all animals of experimental groups (1 — 58.3 and 29.0; 2 — 57.2 and 65.9; 3 — 83.2 and 45.6 pg/ml, respectively), compared with intact mice (26.2 and 11.1 pg/ml). An increase in the level of cytokines by 3–4 times was noted in the experimental group at 7 and 21 days after immunization. A significant decrease in the amount of interferon-gamma (1 — 48.4; 2 — 35.6 and 33.2 pg/ml in 3 group) was showed after 3 months, but it remained high compared to control (16.3 pg/ml). Content of interleukin-10 in blood of group 1 and group 3 mice decreased sharply by 90th day of observation (to 16.5 and 12.7 pg/ml), while in group 2 remained at high level (34.6 pg/ml), which indicates a possible prolonged action of polyoxidonium on production of cytokines — biomarker for anti-plague immunity.

Thus, potentiating effect of immunomodulators in laboratory animals vaccinated against plague has been established.

8.5 doi: 10.15789/2220-7619-2018-4-8.5

NEUTROPHIL/LYMPHOCYTE DISBALANCE AS A PREDICTOR OF VAGINITIS

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Infectious vaginosis or cervicitis is a frequent cause of ginecologist's appointment. Vaginitis is vaginosys of bacterial, viral or fungal etiology. During this inflammation vagina or cervix surface leucocytes count increases significantly. We were interested how white blood cells (WBC) count changes in peripheral blood during vaginitis and/or cervicitis.

The aim of the study was to estimate absolute and relative WBC count in peripheral blood from patients with and without vaginitis.

A total of 26 (mean age 40±19) women visited IDC ginecologist between 28 April to 12 June 2018. Informed agreement was received from all patients. Blood samples were collected in 5 ml K3 EDTA Vacuette tubes. Alifax Roller was used to erythrocyte sedimentation rate (ESR) determination, Sysmex XN was used to complete blood cell count (CBC) determination. The ginecologic slides were heat-fixed and stained with azur-eosine.

Microscopic exam of normal cervix slides shows not more 15 leucocytes in field-of-view (fov) at 1000 total magnification. Normal vagina slides contains 0–10 leucocytes in fov. Peripheral WBC rates was graded according to age. So we divided patients into 4 groups: with normal leukocyte count in the blood and urogenital tract (n = 10), with normal WBC in blood and elevated WBC in cervical and/or vaginal slides (n = 8), with normal blood and increased WBC values in the urogenital tract (n = 4) and abnormal WBC in hole blood and normal urogenital parameters (n = 4).

Parameters of peripheral blood in this group were compared with the group with normal values. The most numerous group had abnormal parameters both in blood and in cervix or vagina.

Nonparametric comparison in the groups with the Mann–Whitney test showed a statistically significant ($p = 0.0062$) increase in the absolute number of peripheral blood lymphocytes in patients with inflammation in the urogenital tract.

The imbalance of peripheral blood lymphocytes/neutrophils as a possible immunity disorder allows infection to cause vaginitis. Therefore, periodic monitoring of the complete blood cell count and measures leading to the normalization of the CBC can help prevent the inflammation of the urogenital tract.

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THE TRECs AND KRECs FREQUENCY IN THE BLOOD IN A POPULATION OF ST. PETERSBURG

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TRECs (T-cell receptor excision circles) and *KRECs* (kappa-deleting recombination excision circles) are surrogate markers of maturation of T-cells and B-cells. *TRECs* and *KRECs* quantification can be used for detection of primary or acquired immunodeficiency. However, to detect immunodeficiency, it is required to know the population values of the excision rings concentration.

The aim of this work was to determine the values of *TRECs* and *KRECs* in the blood of healthy donors in St. Petersburg.

Blood of healthy volunteers aged from 0 to 95 years (total 160 people) was used in the research. *TREC/KREC* copies were assessed by quantitative PCR. Calibrators for PCR are kindly provided by the Institute of chemical biology and fundamental medicine (Novosibirsk, Russia).

There was no significant correlation between the concentration of *TRECs* or *KRECs* from sex. At the same time there was a significant negative correlation between the number of copies of *TREC*/10^5 lymphocytes (Spearman correlation coefficient $r = -0.836$; $p < 0.0001$) or the number of copies of *KREC*/10^5 lymphocytes ($r = -0.641$; $p < 0.0001$) from age.

All group was divided into 7 age groups: newborns, 3 months – 9 years, 10–19 years, 20–29 years, 30–39 years, 40–49 years, older than 50 years. There was statistically significant reduction of the content of *TREC*s in the blood after 10 years and after 30 years. The number of *KREC*s was significantly decreased after 10 years. Then there are no significant differences in the number of *KREC*s between groups of 20–29 years and groups older than 30 years. At the same time the number of *KREC*s in the group of 10–19 years is significantly higher compared to adults over 30 years. Further experiments are needed to clarify whether the number of excision rings in human blood stabilizes after a certain age.

Thus, for first population values of excision rings concentration in blood of healthy donors of St. Petersburg were determined in this work. These data can be used to detect various immunodeficiency states.

8.7 doi: 10.15789/2220-7619-2018-4-8.7

COMPARATIVE ESTIMATION OF SENSITIVITY OF SEROLOGICAL REACTIONS FOR ESTIMATION OF IMMUNITY AGAINST THE CAUSATIVE AGENT OF TULAREMIA

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Tularemia is an anthropozoonotic natural focal acute infection. According to Russian biological safety regulations compulsive immunization and specific immunity estimation is carried out in accordance with established regulations for all the employees, who work with the causative agent of tularemia. Immunological efficacy of vaccination as well as specific diagnosis of tularemia is carried out using serological reactions (ELISA, MAT, IHAT) and/or skin allergic test, which causes extra body burden of antigens. Accordind to methodological guidelines 4.2.2939-11 (RU) for estimating of post-vaccination immunity it is allowed to apply one of the serological methods. It is widely recognized that ELISA is the most sensitive serological assay, including for tularemia. Serological reactions are carried out *in vitro*.

The purpose of the work was to compare sensitivity and specificity of ELISA and IHAT designed for detection of antibodies to *F. tularensis* antigens.

Blood serum samples were obtained from people, who had been immunized with live tularemia vaccine 1 month and 5 years before the assay. As a negative control the blood sera of donors with no anamnesis of a natural infection or vaccination against tularemia were used.

Detection of specific antibodies was carried out using tularemia serodiagnostic test produced by the Stavropolsky Antiplague Scientific Research Institute, an experimental ELISA test system, and "ELISA classic Francisella tularensis IgG" (SERION, Germany) to be considered for reference, following the manufacturers' guidance. To obtain the experimental ELISA test system, LPS extracted by Westphal method [1965] was used.

Of the 16 donors' samples in the ELISA, 7 turned out significant titres that exceeded the dilution of 1: 400, and 9 — negative. The data obtained in the ELISA were completely correlated with the results of "ELISA classic Francisella tularensis IgG", which was used as a verification test. In IHAT, positive reactions were found in 15 donors, negative in one. False positive reactions of IHAT can be associated with the immobilization of whole *F. tularensis* cells on the erythrocytes with antigens capable of cross reactivity The use of IHAT is justified for the diagnosis of tularemia if it is the case of antibody titres increase in dynamics. To estimate the effectiveness of immunologic adjustment after vaccination, ELISA seems to be preferable.

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THERAPEUTIC EFFICACY OF MONOCLONAL ANTIBODIES AGAINST LETHAL TOXIN OF *BACILLUS ANTHRACIS* IN A MOUSE MODEL

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Despite insignificant number of anthrax cases in the Russian Federation, the antitoxic drug development is going on. That's connected with the threats of terrorist acts and the presence of a large number of cattle burial grounds in the Russian Federation. The inhalational and intestinal form of the disease is enhanced by complexity of diagnosis, thus anthrax may be particularly dangerous. At the late stages of anthrax infection antibiotic therapy turns out to be ineffective and the patient has a risk of quick death due to a large amount of the lethal toxin accumulated in the patient's blood. At this stage antibodies capable of neutralizing, primarily, the lethal toxin (LT)

could be effective. Previously we obtained mouse monoclonal antibodies (mAbs) to the III and IV domain of the protective antigen (PA) and the I domain of the lethal factor (LF) of *Bacillus anthracis*, which promise to be effective as anti-LT drugs as was shown in the toxin neutralization experiment *in vitro*.

The aim of the study was to determine the ability of mAbs to Id LF (6G9), IIId PA (1D6) and IVd PA (1E10) to neutralize the LT of *B. anthracis* in a mouse model.

To determine the LD50 of LT in BALb/c mice, we injected the toxin into the retroorbital sinus from 50 μg/mouse to 6.25 μg per mouse using the double dilution. To determine the therapeutic effect of the mAbs in mice (9 animals per group) we injected mAbs to Id LF (6G9), IIId PA (1D6) or IVd PA (1E10) intraperitoneally at the following doses: 10, 25, 50, 75, 100 and 200 μg/mouse. After 24 hours of mAbs injection, the mice were immunized with LT retroorbitally at a dose of 4-fold increasing LD_{50}, and the animals were monitored for 7 days.

LD_{50} of LT for BALb/c mice was identified at 12.5 μg/mouse. The analysis of the mAbs against PA and LF with different domain specificity showed that the preliminary injection of all the analyzed mAbs protected the animals from LT. The most effective toxin-neutralizing effect was shown by mAbs against Id LF (6G9) and against IIId PA (1D6), which in dose 25 μg/mouse protected mice against death from LT. The mAb against IVd PA (1E10) also protected mice from the action of LT, but this one required a larger dose: of 100 μg/mouse.

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8.9 doi: 10.15789/2220-7619-2018-4-8.9

THE CLINICAL, IMMUNOLOGICAL AND LABORATORY PARAMETERS IN PATIENTS WITH LEPTOSPIROSIS IN ST. PETERSBURG

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Leptospirosis — infection, characterized by multiorgan failure. Despite the long-term study of leptospirosis, the immunopathogenesis of this disease remains insufficiently covered. It is assumed that the severity and outcome of leptospirosis infection depend on the type and concentration of cytokines produced.

The aim was to Study and generalization of data on the course, clinical and immunological parameters in leptospirosis.

The study included 102 patients with a confirmed diagnosis of leptospirosis. The control group consisted of 39 practically healthy people. Static data processing was performed using software package STATISTICA.

With the diagnosis of "leptospirosis" the hospital received only 11.8% of patients. Late treatment of patients for medical care was noted — 6.5±1.2 days from the onset of clinical manifestations, with the period of stay in hospital treatment averaged 20.6±2.8 days.

During the study period, kidney damage was characteristic of leptospirosis (78.4% of cases), with a decrease in diuresis in the early stages of development of the disease was noted 38.2% of cases, acute renal failure in 19.6% of cases.

Liver lesions were observed in 94.1% of cases. The activity of enzymes ALT and AST exceeded the norm by 2–3.5 times. The level of bilirubin was exceeded by 8.9–12.3 times, which was clinically manifested by jaundice of the skin and icteric sclera.

The levels of cytokines IL-8, MCP-1, TNFα, IL-10 in patients with leptospirosis was significantly higher than in the control group ($p < 0.05$). In dynamics, attention is drawn to the increase in the level of proinflammatory cytokines MCP-1, TNFα on the background of a decrease in IL-10, which may indicate the incompleteness of the inflammatory process. During the study, we noted that high levels of MSR-1 were found in individuals with icterohemorrhagic leptospirosis during the severe course of the infectious process.

Due to the complexity of the diagnosis of leptospirosis, there is a need to create mathematical models for predicting the course of the disease, based on objective laboratory data and clinical manifestations. The prognostic value of such models can be increased due to the knowledge of immunopathogenesis of the disease, and the inclusion of such an important factor as the production of pro- and anti-inflammatory cytokines in these patients.

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CYTOPROTECTIVE POTENTIAL OF MONOCLONAL ANTIBODIES AGAINST *BURKHOLDERIA PSEUDOMALLEI*

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Melioidosis is a disease caused by *B. pseudomallei*, belongs to the group of particularly dangerous bacterial infections. No specific preventive treatment of melioidosis has been developed. The aim of the study was evaluate the effectiveness of Mabs as cytoprotectors from the *B. pseudomallei* toxic effects. We used the panel of Mabs against melioidosis (PpmI, 3C6, 6A11, 6E7, PpmII, 2A6, 2H7, 2F11) and some melioidosis antigens with confirmed toxicity (*B. pseudomallei* 100, 57576, 51274, 59361). The experiments were carried out in L-929, CHO-K1 cell cultures lines, obtained from Institute of Cytology, St. Petersburg, Russia. We injected into the well with the formed monolayer a mixture of the antigen (40 μg/μl) and monoclonal antibody at three different doses (1 μg/μl, 0.5 μg/μl, 0.25 μg/μl). A comparative study of *three different doses* were performed during 3 days. The results of the study were identical on both cell lines. We established that each monoclonal antibody has different cytoprotective properties. It was shown that 2F11 (0.5 μg/μl) neutralized the *B. pseudomallei* 100 toxic effect throughout the all period of observation. Mabs PpmI, 6A11, 2A6, 2F11 at a dose of 0.5 μg/μl provided a cytoprotective effect to *B. pseudomallei* 57576 after 3 days and we observed an increase in the number of cells. Mabs PpmI, PpmII, 3C6, 2F11 gave protection even at a dose of 0.25 by day 3. We indicate that Mab PpmI protected cell lines at a dose of 1 μg/ml during exposure *B. pseudomallei* 59361 during the all period of observation. While Mabs 3C6, 6A11 provided protective properties at a dosage of 0.5 μg/ml only by day 2. The toxic effect of *B. pseudomallei* 51274 antigen on cell lines was neutralized by Mabs 3C6, 2H7, 2F11 at a dose of 1 μg/ml during the all period of observation. Thus, the protective properties of melioidosis Mabs prove the possibility of their use as components of experimental vaccines.

8.11 doi: 10.15789/2220-7619-2018-4-8.11

INNATE AND ADAPTIVE IMMUNITY CYTOKINES IN NASAL MUCOSA AND BLOOD SERUM OF ALLERGIC RHINITIS PATIENTS

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The study was aimed to evaluate the cytokine profile in nasal secretion and blood serum in patients with seasonal and perennial allergic rhinitis (AR) with a potential for additional sensitization with microbial allergens.

The inclusion criteria for AR were as follows: a diagnosis of AR for more than 2 years, the absence of nonallergic disorders of the nasopharynx, age of patients from 4 years to 60 years. Control group: healthy volunteers at the age of 3–43 years without any allergic disorders at examination. In order to evaluate the innate and adaptive immunity, the cytokine profile of blood serum (IL-4, IL-10, and TGF-β) and nasal secretion (TSLP, IL-1β, TNFα, and GM-CSF) was determined. To determine TSLP, TGF-β, IL-10, and GM-CSF concentrations, enzyme-linked immunosorbent assay kits were used (eBioscience, Bender MedSystems, R&D Systems, MN, USA).

The epithelial cells of the upper airways are capable of synthesizing TSLP where this cytokine affects DCs causing their maturation and activation. We have noticed a significant correlation (r = 0 46, p = 0 014) between the TSLP concentration in nasal secretion and allergen-specific antibodies (IgE) level to *S. aureus* enterotoxin (allergen component m80) in patients with PAR. There was a significant correlation (r = 0 56, p = 0 008) between TSLP and GM-CSF cytokine concentrations in nasal secretion of these patients. There was a significant correlation between TSLP cytokine concentrations in nasal secretion and those in allergen-specific antibodies (IgE) to the allergen component m3 of Aspergillus fumigatus in the patients with SAR (r = 0 43, p = 0 023). GM-CSF cytokine is produced by upper airway epithelial cells in an allergic inflammation. There was a significant correlation (r = 0 58, p = 0 007) between GM-CSF concentrations in nasal secretion and those in allergen-specific IgE antibodies to the allergen component d1 of *D. pteronyssinus* in patients with PAR.

Staphylococcal superantigens might be one of the stimuli of local TSLP hyperproduction by the epithelium. There was a significant correlation between GM-CSF concentrations in nasal secretion and the intensity of sensitization to a staphylococcal enterotoxin (SEB) in the patients with perennial allergic rhinitis. The patients with perennial allergic rhinitis and additional high sensitization to SEs demonstrated a higher TNFα production profile due to macrophage and T cell activation by these toxins.

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ANALYSIS OF TOXIN-NEUTRALIZING ACTIVITY OF MOUSE MONOCLONAL ANTIBODIES AGAINST PROTECTIVE ANTIGEN OF *BACILLUS ANTHRACIS*

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Anthrax exotoxin is the main virulence factor of *B. anthracis*. It consists of 3 proteins: protective antigen (PA), edema factor (EF) and lethal factor (LF). Acting in binary combinations, heptamers of PA with LF or EF forming the lethal or edema toxins (LT or ET), correspondently. Highly specific monoclonal antibodies (mAbs) can block or inhibit the action of the toxin and might be used as therapeutical agents against the threat of anthrax infection.

The aim of the study was to determine the ability of mouse mAbs against 3 (1D6F10, 4F5C7, 4F6F8, 7E5B7, 9D5G9) and 4 domain (1E10A1) PA of *B. anthracis* to neutralize the destructive influence of LT on mouse macrophage cell line J774A.1.

The toxin-neutralizing activity of mAbs was assessed by MTT assay. Four different concentrations of rPA and rLF were chosen to show the partial and complete lethal effects of this complex — from LD_{50} (0.5 μg/ml rPA and 0.1 μg/ml rLF) to LD_{100} (4 μg/ml rPA and 0.8 μg/ml rLF). Two different concentrations of mAbs were chosen to show ability to resist toxic action of LT — 4 and 40 μg/ml, that demonstrate the amount of antibodies, which can presumably bind a half of the PA molecules and its 5 times bigger quantity. As controls were used cells without treatment, dead cells and cells with the same concentrations of LT.

The strongest toxin-neutralizing effect was demonstrated by 3 mAbs of 6, which even at a low concentration in LD_{100} of LT contributed to the survival of cells above 50% (4F5C7 — 63%, 4F6F8 — 56%, 1E10A1 — 80%). In addition, excess concentration of mAb 1E10A1 completely prevented toxic damage on cells. The rest showed low activity (7E5B7 and 9D5G9) or presented a potentiating influence against LT (1D6F10).

MAb 1E10A1 can be recommended for further *in vivo* studies in animal models to confirm toxin-neutralizing activity.

The work was supported by the Sectoral Scientific Program of the Russian Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing.

9. ANTIBIOTIC RESISTANCE OF MICROORGANISMS: CURRENT ISSUES OF DIAGNOSIS AND WAYS TO OVERCOME

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THE EFFECT OF SUB-BACTERICIDAL DOSES OF ANTISEPTICS ON DNA AND PHENOTYPIC MARKERS OF VIRULENCE OF MICROORGANISMS

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Local antiseptics in sub-bactericidal concentrations are active against antibiotic-resistant microorganisms, including Gram-negative bacteria. The studies were performed *in vitro* and *in vivo*, on the culture of fibroblast cells of human embryonic skin. Our study shows antiadhesive activity of QATs against *S. aureus*, their ability to suppress hyaluronidase and staphylococcal plasmocoagulase. Poviargol (silver nanoclusters) is able to suppress protein A of staphylococcus, prevents the formation of microbial biofilms on biotic and abiotic surfaces. Polyhexanide shows antiadhesive properties against Gram-positive and Gram-negative bacteria, enhances the effect of antibiotics against resistant microbes due to increased permeability of the cell wall, affects plasmacoagulase, collagenase. The effect of sodium hypochlorite on microbial DNA was assessed by UV spectroscopy and electrophoresis. For the first time, a dose-dependent effect of sodium hypochlorite on individual nucleotides and polynucleotides was obtained, and complete destruction of the plasmid DNA of *Escherichia coli* DH5-Alpha strain was demonstrated. It has been established that the interaction with sodium hypochlorite involves the destruction of the secondary structure of DNA (denaturation) and the chemical modification of nitrogenous bases, presumably chlorination. The presence of a secondary structure slows down the chemical reaction of sodium hypochlorite with nitrogenous DNA bases. The ability of sodium hypochlorite to destroy formed (48 h) microbial biofilms of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* has been studied. Various antiseptics in non-bactericide concentrations complexly affect the antibiotic-resistant microbial cell: increase the permeability of the cell membrane, inhibit the enzyme-inactivators of antibiotics, suppress the epidemic factors of the transfer of antibiotic resistance markers by transduction and conjugation.

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METABOLIC ACTIVITY OF PLANKTON IN COMPARISON WITH BIOFILM PHENOTYPE SOME MICROORGANISMS OF HUMAN MICROBIOTA

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An important interest for the detection of serious metabolic disorders of macroorganism, and its microbiota, are tricarboxylic acids common to bacteria and mitochondria of eukaryotic cells, and phenylcarboxylic acids. They are called "sepsis-associated metabolites", since the imbalance of the profile of phenylcarbonic acids in the blood is most noticeable in septic states.

The purpose of our study is to carry out a comparative assessment of production sepsis-associated exometabolites by clinically-significant bacteria in biofilms and planktonic form.

Strains were used: *S. aureus* ATCC 25923, *S. epidermidis* ATCC 14990, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603, and clinical isolates of these species, isolated from the blood of ICU patients. Biofilms of these microorganisms were grown according to the method developed by us. The determination of exometabolites was carried out using gas chromatography-mass spectrometry (GC-MS).

S. aureus and *S. epidermidis*. Lactate was produced by the planktonic form in a substantially greater concentration than by biofilms. As for the biodegradation products of aromatic amino acids (phenyllactic and p-hydroxyphenyllactic acid), a more intense production by the *S. aureus* biofilm was demonstrated as compared to plankton.

K. pneumoniae and *E. coli*. The metabolism of the investigated *Klebsiella* strains to a large extent coincides with the metabolism of *E. coli*. The *K. pneumoniae* biofilm produced phenyllactic and para-hydroxyphenyllactic acids significantly more actively than the planktonic form.

Conclusions: 1. processes associated with the hydrolysis of crbohydrates in biofilms are less intense than in the planktonic form of the same microorganisms. This is indicated by a significant difference in the concentrations of lactate between plankton and biofilm; 2. the processes associated with hydrolysis of proteins take place in biofilms much more intensively.

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STUDY OF ANTIMICROBIAL RESISTANCE IN MEDICAL INSTITUTIONS IN CONAKRY (REPUBLIC OF GUINEA)

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Extensive apparition of resistance of pathogenic microbes to many antibiotics causes a serious preoccupation of public health agencies in developing countries. WHO considers the surveillance on this resistance development as one of the important task of public health system of every country.

The aim was to study of problem of antibiotics resistance in Republic of Guinea.

We studied 875 bacterial strains having medical importance, from some medical institutions of Conakry city. The identification was accomplished on commercial mediums or on nutritive mediums prepared in IRBAG. For each strain one made an antibiogram with agar-agar precipitation method on Muller–Hinton medium. The antibiotic discs of more than 20 antibiotics were test-

ed. The interpretation of results we accomplished according to criterions of Committee on antibiograms of French Microbiological Society.

There were identified the following pathogen bacteria: *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudamonas aeruginosa, Salmonella* sp., g. *Shigella* sp., *Staphylococcus aureus, Streptococcus* sp., *Streptococcus β-hemoliticus*. It was established that resistance to different antibiotics isn't the same for different bacteria. Occurring everywhere resistance to antibiotics in medical institutions of Conakry is conditioned by following causes: anarchy use of these drugs by population and by unreasonable physician recommendations. One uses antibiotics often without physician prescriptions, its sale is not controlled, the storage conditions are not kept, one use expired medicines. It increases the bacteria resistance. As an example the sharp down of susceptibility to tetracycline is conditioned by its wide distribution on the Guinean medicine market on obtainable prices. One uses this drug for self treatment.

Our study showed the wide development of resistance of pathogenic bacteria to antibiotics in Guinea by reason of its easy accessibility on medicine market. It's necessary the monitoring of antibiotics use with permanent control of the susceptibility of pathogenic bacteria to them. One must control the quality of antibiotics incoming on the market. These drugs should be used only by physician prescription or of other medical personnel having rights to do it. It is necessary the wide national, regional and international collaboration for this problem resolution.

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MICROBIOLOGICAL ASPECTS OF PURULENT SEPTIC INFECTIONS BREAKOUT INVESTIGATION IN KHABAROVSK CITY

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The aim of the study — to conduct an etiological interpretation of purulent-septic infection cases registered in August-September of 2017 among newborns in the maternity hospital of Khabarovsk city. A total of 8 cases of different diseases were registered (nosocomial pneumonia, urinary tract infection, omphalitis, subcutaneous whitlow, conjunctivitis).

A microbiological examination of newborns and medical staff of the maternity hospital followed by phenotypical and molecular-biologic evaluation of the isolated strains was performed. Cultures of *Staphylococcus aureus* and *Klebsiella pneumoniae* were isolated from different sites of infection from children and personnel. Vitek-2 Compact and MALDI-TOF Biotyper analyzers were used to identify the bacterial strains. The antibiotic sensitivity of the isolated strains was evaluated by disc diffusion method to 22 drugs.

S. aureus isolated from 3 children (oral pharynx, whitlow) and 13 employees did not belong to the multiresistant MRSA strains. Two groups of *K. pneumoniae* isolated from 3 newborns (oral pharynx, sputum, omphalitis) and from 7 staff members (urine, oral pharynx) differed from each other by antibiotic sensitivity. The strains isolated from newborns were producers of β-lactamase (typical feature for nosocomial strains) and were susceptible to 4 antibacterial drugs out of 22 evaluated. All strains isolated from the personnel were susceptible to the most of the antibiotic drugs. The RAPD-PCR confirmed the difference between two groups of *K. pneumoniae* strains. All strains isolated from the newborns were typed as genovariant A when those isolated from personnel belonged to different types (B, C, D, E).

The phenotypical and molecular-biological survey of *K. pneumoniae* strains isolated during the period of purulent-septic infections breakout in the maternity hospital of the Khabarovsk city indicates on the difference between two identified bacterial strains. It highlights the fact that during the breakout there were two independent epidemic foci of *K. pneumoniae* infection among newborns and medical staff.

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SEROVAR SPECIFIC ANTIMICROBIAL SUSCEPTIBILITY OF SALMONELLA

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Antimicrobial susceptibility of 564 *Salmonella* strains isolated in 2014–2017 in St. Petersburg from the patients with gastroenteritis was studied. Three leading serovars — *S.* Enteritidis, *S.* Typhimurium and *S.* Infantis accounted for 89.4% of strains (78.9; 5.9 and 4.6%, respectively). Other serovars were represented by single strains. 79.4% of *Salmonella* were resistant to at least 1 antimicrobial group: *S.* Enteritidis — 86.3; *S.* Typhimurium — 63.6; *S.* Infantis — 88.5%.

S. Enteritidis is characterized by high level of resistance to fluoroquinolones (75.0%) and nitrofurans (70.0%). Beta-lactam resistance was noted to ampicillin (2.7%) and extended spectrum cephalosporins (1.8%). Resistance to other antimicrobial groups ranged from 0.4 (aminoglycosides) to 8.8% (tetracyclines). Multidrug resistance (3 and more groups) was unusual for this serovar and was found in 10.8%.

In *S.* Typhimurium resistance to many antimicrobial groups was noted: tetracycline (45.5%), ampicillin (39.4), aminoglycosides (21.2), trimethoprim/sulfamethoxazole (21.2), fluoroquinolones (18.2). This serovar is characterized by the highest proportion of beta-lactam resistance: ampicillin (39.4) and extended spectrum cephalosporins (9.1). Multidrug resistance was found in every fourth strain of this serovar.

S. Infantis were characterized by very high proportion of the resistance to fluoroquinolones, nitrofurans, tetracyclines and trimethoprim/sulfametoxazole (88.5; 80.8; 80.8 and 61.5%, respectively). This serovar was characterized by multidrug resistance: almost 8 out of 10 strains. At the same time, resistance to extended spectrum cephalosporins and chloramphenicol has not been noted.

Rarely isolated serovars were characterized by lower proportion of antimicrobial resistance (31.7%): resistance did not exceed 20.0% (quinolones) and ranged from 1.7 (extended spectrum cephalosporins and aminoglycosides) to 15.0 (nitrofurans). Multidrug resistance was observed in 10.0%.

So, in *Salmonella* isolated in St. Petersburg in 2014–2017, the resistance to drugs of choice for the treatment of light and medium-severe salmonellosis (nitrofurans), as well as complicated and severe forms of salmonellosis (fluoroquinolones) is very high. This is typical for the leading serovar *S.* Enteritidis, causing up to 80% of cases of salmonellosis. The use of these antimicrobials should be accompanied by a mandatory antimicrobial susceptibility testing for timely correction of the treatment.

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PERINATAL LISTERIOSIS: THE MOUSE MODEL

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The Gram-positive bacterium *Listeria monocytogenes* is typical sapronontic pathogen. *L. monocytogenes* causes listeriosis, a severe disease with multiple manifestations including stillbirths and meningitis of newborns, in humans and a wide range of domestic and wild animals. The invasion factor of the internalin family InlB is involved in crossing the maternal-fetal barrier (Disson et al., 2008). Previously, we compared human and wild animal *L. monocytogenes* strains and described several naturally occurring InlB variants. We demonstrated that InlB variants differed in the ability to support intragastric infection in mice (Sobyanin et al., 2017). The aim of this work was to compare effects of InlB variants on perinatal infection. The mouse model was used. The InlB variants differing in 10 amino acid substitutions were expressed under the same promoter in the *L. monocytogenes* strain EGDeΔinlB. Work with animals was performed with approval of local bioethical committee. Mice were intragastrically infected on the 14th day of pregnancy, euthanized 1 and 3 dpi, bacterial loads were determined by plating. One of two InlB variants provided infection of both placentas and fetuses while another did not. Bacteria carrying InlB variant 14 but not the variant 9 were revealed in placentas 24 and 72 hpi. 65% of placentas and only 20% of fetuses were infected. Fetus infections was correlated with placenta infection. Infection was unequal for different fetuses in the same animal with bacterial loads ranges from individual bacteria to 10^5 CFU per fetus. Obtained results suggested that some but not all InlB variants might promote perinatal infection upon intragastric infection and that the infection of each placenta happens individually.

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PHENOTYPES AND GENOTYPES OF CLASSICAL AND HYPERVIRULENT *KLEBSIELLA PNEUMONIAE* CLINICAL STRAINS ISOLATED IN MOSCOW IN 2013–2018

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Klebsiella pneumoniae is causative agent of community-acquired and healthcare-associated infections including pneumonia, bloodstream infection, surgical site infections, liver abscess and meningitis. Multidrug resistant (MDR) *Klebsiella* belonged to classical branch of *K. pneumoniae* (cKp) have been included recently into the ESKAPE group of pathogens. On the other hand, in the last two decades hypervirulent *Klebsiella* phylogenetic branch (hvKp) emerged and spread around the world. In this study, we aimed to investigate phenotypes and genotypes of virulence and antibacterial resistance for 500 *K. pneumoniae* clinical strains collected in 2013–2018 from the patients of several Moscow hospitals.

Virulence factors and antibiotic resistance profiles between classical and hypervirulent *K. pneumoniae* isolates were compared. It was shown that hvKp strains were attributed to international sequence types ST23, ST86, ST65, and to novel clones ST2174 and ST2280, while strains of cKp — to international clones ST218, ST395, ST11, ST39, ST48, ST147, ST833, ST20, ST13, and ST3346. All hvKp strains had hypermucoviscosity phenotype, capsule types of K1, K2 and K57; carried 5–7 pathogenic genes (regulator of mucoid phenotype gene *rmpA*, aerobactin gene *aer*, iron uptake system gene *kfu*, allantoin metabolism gene *allR*, lipopolysaccharide synthesis genes *uge2* and *wabG*, and fimbrial gene *fimH*). On the contrary, cKP strains had no hypermucoviscosity phenotype, identified capsule types were K57, K62, K47, K14, K27, K28, K60, and K420. Such strains carried four or less pathogenic genes (they did not have *rmpA*, *aer*, and *allR* genes).

Major cKP strains in this study expressed the MDR phenotype, resistance to three or more classes of antibacterials; while more than 50% of them were resistant to six or more classes (beta-lactams, aminoglycosides, fluoroquinolones, sulfonamides, nitrofurans, phenicols). Molecular mechanisms of MDR include beta-lactamase genes (bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{OXA}, and bla_{NDM}), class 1 integrons carrying gene cassettes (*dfr, aac, aad,, aph,* etc.), and efflux pumps (*oqxAB, mph, cml,* etc.). Among hvKp strains two groups were described: (1) most of them were mainly susceptible to antibacterials carrying few resistance genes, (2) some strains accepted MDR plasmids carrying resistance genes, and expressed MDR phenotype.

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RELATIONSHIP BETWEEN MICROORGANISMS IN THE VAGINAL BIOTOPE OF SUBFERTIL WOMEN

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The aim of investigation was to evaluate the features of microecology of the lower genital tract of women with infertility.

A retrospective analysis of microbiological data of the vaginal discharge of 345 subfertil women was carried out. To assess the share of different types of microorganisms in the structure of the microbiota the coefficient of species constancy was used. To quantify the interaction between members of the microbiocenosis, the Jacquard similarity coefficient was used.

The nature of the relationship between the main members of the microbial community in the vaginal biotope of women with infertility should be considered antagonistic. The phenomenon of mutualism was characteristic only between *Lactobacillus* spp. and *Peptostreptococcus* spp. Typical *E. coli* have a significant ecological community with these bacteria, the relationships of their can be characterized as synergistic. Similar ecological synergism was revealed for *S. epidermidis* and lactobacilli. It was shown that in subfertil women *E. coli* acquires the functions of stabilizing strain and its activity often associated with both a change in the species composition of lactobacilli and their functional characteristics. In a similar situation despite the prevalence of microbial antagonism in the vaginal microbiota, *Lactobacillus* spp. "admit" the existence of *E. coli*, *Enterobacter* spp., *S. aureus*, *S. epidermidis*, *S. haemolyticus*, *S. agalactiae*, *Enterococcus* spp. and *C. albicans.* Under such conditions, the negative influence of commensal microorganisms on lactobacilli is enhanced and its have manifestation by a marked decrease in their numbers and functional activity, as well as a decrease in the sensitivity of the associates to the biocidal factors of lactobacilli when coexisting.

Thus, the microecological approach to assessing the state of the microbiota provides the necessary information on the relationship between individual microorganisms in its composition and can be a valuable tool in deciphering the mechanisms for reducing fertility associated with nonspecific infections.

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IMPROVEMENT OF BACTERIAL BIOFILM'S INVESTIGATION

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Existing approaches to the investigation of biofilms haven't a unified methodology. Researchers use different solvents, allow deviations from the original technique. Each of them distorts the results and doesn't allow them to be compared.

The aim of investigation was to evaluate the possibility of using alcohol and acetic acid for dissolution of crystal violet, as well as additional dye Lugol's solution for the coloring biofilms.

The studies were carried out on *S. aureus*, *S. epidermidis*, *E. coli*. To form biofilms, the strains were cultured in flat-bottomed plates for 48 h. Biofilms were stained with a 0.1% solution of crystal violet (CV). In part of the studies after the coloration of biofilm with CV, Lugol's solution (LS) was used for 2 min. Extraction of dyes was performed with 70 and 95% alcohol and 33% acetic acid. Results were taken into account by measuring the optical density of solutions at a wavelength of 590 nm. Statistical analysis of the data was carried out using the paired version of the Student t-test. The threshold value was taken as $p < 0.05$. The data are given as the arithmetic mean (M) and its error (m).

It was shown that the use of 70% alcohol qualitatively elutes CV from biofilms than 95% one. This may be due to the fact that 95% alcohol narrows the pores of the cell wall and the dye is less efficiently released into the solution. The acetic acid more efficiently elutes the CV from biofilms formed by grampositive microorganisms. The use of LS after staining by CV showed the best results, but only if the extraction was carried out with a solution of acetic acid prepared with alcohol. This approach seems most optimal. LS fixes the dye in the cell and dissolution with a mixture of alcohol and acetic acid makes it more possible.

It was shown that extraction of CV is best performed with 70% alcohol. When fixing CV by LS, it is more appropriate to use a solution of acetic acid in alcohol.

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EFFECT OF METAL OXIDE NANOPARTICLES ON THE EXCHANGE OF GENETIC MATERIAL BETWEEN BACTERIA

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The development of resistance to antibiotics in bacteria is one of the most serious threats to global public health. The spread of resistance genes to antibiotics is caused by the increase and misuse of antibiotics in medicine and in animal feed. Nanomaterialscan increase the efficiency of horizontal transmission of multiresistance genes localized in plasmids between bacteria. Some studies have indicated that nanomaterials can cause damage to bacterial membranes, possibly by forming reactive oxygen species and can deliver DNA or RNA molecules to animal or plant cells. It was shown earlier the increase in efficiency of horizontal transmission of multidrug-resistant genes localized in plasmids by alumina nanoparticles (NPs) [1]. In this study, we observed the effect of different metal oxide NPs on the horizontal transfer of antibiotic-resistance genes between different *Escherichia coli* strains.

For the analysis of plasmid horizontal junctions, NPs of metal oxides Ta_2O_5, HfO_2, Fe_3O_4, ZrO_2, TiO_2, Al_2O_3 were used at a concentration of 5 mmol/L. The studies were conducted between *E. coli* strains containing different plasmids with different resistant cassettes. The transconjugants growth after incubation with metal oxides NPs was fixed on medium with appropriate antibiotics. The effect of metal oxide NPs was compared with control samples without addition of NPs.

In this study, we showed that the addition of NPs metal oxides can enhance the efficiency of horizontal gene transfer between different bacteria.

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SONOCHEMICAL NANOSTRUCTURING OF ANTIBIOTICS IS A NEW APPROACH TO INCREASING THEIR EFFECTIVENESS AGAINST RESISTANT STRAINS

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One of the most urgent problems of modern medicine is bacterial resistance to antibiotics. Development of new treatment approaches is a laborious and expensive process. One of the strategies for developing new antimicrobial agents is a modification of existing antibiotics. Sonochemical nanostructuring of antibiotics can become a cheap alternative to modern complex methods. In this regard, the aim of this research was to analyse the antimicrobial activity of sonochemically-modified tetracycline against sensitive and resistant strains.

Escherichia coli Nova Blue TcR (with antibiotic resistance) and *E. coli* 292–116 (without drug resistance) were used in this study. Tetracycline, a broad-spectrum antibiotic, was modified using industrial sonicator UIP1000hdT (Hielscher, Germany). The effectiveness of antibacterial properties was estimated using the disc-diffusion method and spectrophotometry analysis of liquid cultures. The results were confirmed by flow cytometry after staining with propidium iodide and Syto-9 dyes. The antimicrobial action of the modified antibiotic solution during long-term storage has also been studied.

The ultrasound processing time determines the change in antimicrobial properties against both sensitive and resistant cells. As a result of sonochemical treatment, the effectiveness of antibacterial properties increases up to 25% against the resistant strain and up to 100% against the sensitive strain. The long-term storage at +4°C does not reduce the antimicrobial properties.

The obtained data shows that sonochemical modification of antibiotics can be a new promising and cheap approach to the development of new drugs effective for antibiotic therapy against drug resistance strains.

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DEVELOPMENT OF FUNCTIONAL NANOSTRUCTURES EFFECTIVE AGAINST BACTERIA BIOFILMS INCLUDING MULTIDRUG RESISTANT BACTERIA

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The emergence and distribution of antibiotic resistant strains of bacteria is one of the most world's pressing public health problems. Multidrug resistant (MDR) bacteria exhibit resistances to most antibiotics and, sometimes, to nearly all commercially available antibiotics. Besides, the persistence of bacteria in the body mainly in the state of biofilm reduces significantly the effectiveness of antibacterial therapy. Even antibiotic-sensitive strains in the state of the biofilm are weakly responsive towards antibiotics. There is an urgent need for development of new antibiotic treatment strategies against MDR bacteria and bacteria biofilms. The most potentially successful strategy may be the transition from classical therapy to the use of high-tech tools based on nanomaterials.

The purpose of this study was the development of new antimicrobial agents based on nanostructured materials of organic and inorganic origin. Antibacterial properties of nanostructured classical antibiotics (tetracycline), metal particles and their oxides, magnetically controlled nanocomposites with encapsulated antibiotic (ciprofloxacin) were investigated.

The preliminary results demonstrate the high antimicrobial activity of the developed materials. Nanostructuring of tetracycline increased its effectiveness up to 40% against a resistant strain, compared with the original antibiotic form. Particles of metals and their oxides showed excellent antimicrobial properties, including against antibiotic-resistant strains, but many of them can cause some cytotoxic effect in the macroorganism. Magnetically controlled nanocomposites with encapsulated ciprofloxacin demonstrated an increase in efficiency of up to 76% compared to the initial form of the antibiotic due to magnetically controlled effects of mechanical disintegration of the biofilm, accumulation and release of the composite inside the biofilm. Also, synergism in antibacterial action may be due to local alkalization due to recrystallization of calcium carbonate.

Thus, the results of this study can create a scientific basis for the development of new antimicrobial agents based on nanostructured materials effective against biofilms and antibiotic-resistant strains of bacteria.

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KLEBSIELLA PNEUMONIAE AND ITS GENES OF RESISTANCE TO BETA-LACTAMAMS IN PSYCHIATRIC HOSPITAL

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The aim of the study was to characterise of phenotype and genotype of antibiotic resistance in *Klebsiella pneumoniae*, isolated in psychiatric hospital.

215 strains of *K. pneumoniae*, isolated from sputum, urine, wounds and blood of the patients in 2016 were studied. Bacteria were identified using classical methods, antibiotic resistance was studied according to MUK 2004, Clinical guidelines for antimicrobial sensitivity determination, 2015. Beta-lactamases gene detection was performed by PCR.

Klebsiella showed high resistance levels to inhibitor-protected penicillins (86.5%) and cephalosporins (77.7%). Resistant to fluorochinolones were 51.6% strains, to carbapenems (meropenem) — 32.6% of isolated strains. The lowest resistance level was observed in amikacin and fosfomycin — 17.2% and 4.6% resistant strains respectively. Multidrug resistant were 59.3% of isolated *K. pneumoniae* strains. Almost a quarter — 24.7%, of strains showed associated resistance to inhibitor-protected penicillins, cephalosporins, fluoroxhinolones and carbapenems. Extreme antibiotic resistance was observed in 9.8% isolates — they were sensitive to colistin. Detection of beta-lactamases genes was performed in 30 cultures, bla_{CTX} was found in 80.0% of strains, bla_{TEM} — in 70.0%, bla_{OXA-48} and bla_{NDM-1} were found in 6.7% and 86.7% of strains respectively; bla_{OXA-48} gene was combined with bla_{NDM-1} in 6.7% isolates. The most frequently encountered beta lactamases were combination of bla_{CTX}, bla_{TEM} and bla_{NDM-1} (70.0%). Other genes combinations were revealed less frequently: bla_{CTX}, bla_{TEM} and bla_{NDM-1} were present in 6.7% strains, bla_{CTX} and bla_{NDM-1} — in 3.3%. Bla_{NDM-1} only was detected in 6.7% *K. pneumoniae*,

Resistant to antibiotics strains of *K. pneumoniae* prevailed in psychiatric hospital, third of isolates were resistant to carbapenems. The most common combination of resistance determinants for carbapenem-resistant strains was bla_{CTX}, bla_{TEM} and bla_{NDM-1} — 70%. Spread of carbapenemases, mostly NDM-1 producing *K. pneumoniae* strains, is a dangerous sign of the significant decrease in carbapenems efficacy towards infections, caused by *K. pneumoniae* and confirm the necessity of antimicrobial resistance monitoring in hospitals strains.

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RESISTANCE TO ANTIBIOTICS OF DIARRHEAGENIC *ESCHERICHIA COLI* IN PSYCHIATRIC HOSPITAL

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The aim of the study was estimation of antibiotic resistance in diarrheagenic *Escherichia coli*, isolated in psychiatric hospital in St. Petersburg.

Study of susceptibility to antibiotics in 123 strains of *E. coli* was carried out according to guidelines MUK 4.2.1890-04, 2004. Cultures were isolated from feces of patients of a psychiatric hospital in 2016–2017.

Diarrheagenic *E. coli* in psychiatric hospital were represented by enteroinvasive (48.0%) and enterotoxigenic *E. coli* (47.2%) mainly. Ratio of enterohemorrhagic *E. coli* (EHEC) was small (4.9%). Enteroinvasive *E. coli* (EIEC) included representatives of 3 serogroups, with prevalence (34.1%) of O144. Other serogroups were rare (8.1% for O151 and 5.7% for O124 strains). Enterotoxigenic *E. coli* (ETEC) included representatives of 3 serogroups, most common were O6 (26.1%) and O25 (20.3%). Only one strain of O85 serogroup (0.8%) was isolated in 2017. EHEC was presented with one serogroup O1 (4.9%). Number of strains of diarrheagenic *E. coli* increased two-fold in 2017 compared with 2016. The proportion of the EIEC O144 increased eight-fold. There was more than two-fold increase of ETEC O25. Proportion of *E. coli* belonging to other serogroups changed slightly with the exception of EHEC O1, the number of strains decreased from 5 in 2016 to 1

in 2017. Study of susceptibility of *E. coli* showed that resistant to antibiotics strains prevailed (61.8%) and the ratio of such strains increased almost two-fold in 2017 (72.1%) from 2016 (43.2%). Most isolates were resistant to ampicillin and inhibitor-protected penicillins such as amoxicillin/clavulanate (61.0%). Proportion of such strains increased almost two-fold in 2017 (72.2%) from 2016 (40.9%). Resistant to fluoroquinolones, cephalosporins were 3.2% and 2.4% strains, more than 20 times less. All of them were isolated in 2016. Isolates resistant to two preparations at the same time were most frequent (58.5%). The proportion of multidrug resistant cultures was low (2.4%). There were only 3 isolates resistant to ampicillin, inhibitor-protected penicillins, fluoroquinolones and cephalosporins.

Number of strains of diarrheagenic *E. coli* increased two-fold in 2017 compared with 2016, most of them were EIEC O144 and ETEC O25. Antibiotic-resistant cultures prevailed. Multidrug resistant strains were rare. All strains were susceptible to carbapenems, most of them — to fluoroquinolones and cephalosporins.

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MOLECULAR ANALYSIS OF PATHOGENS OF PARTICULARLY DANGEROUS BACTERIAL INFECTIONS: FROM THEORY TO PRACTICE

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The modern period of development of medical and biological science is characterized by significant successes in the field of structural analysis of microorganisms and wide technological possibilities of obtaining living objects with given properties. A new scientific trend has emerged — synthetic biology. One of the urgent goals is the application of molecular methods in the practice of epidemiological analysis, to determine the source of infection and the pathways of the spread of the microbial pathogen, to assess its virulence and other properties.

At the present time, considerable material has been accumulated on the genetics of pathogens of anthrax, plague, cholera, brucellosis and other extremely dangerous microorganisms. The algorithms of PCR analysis have been developed in determining the epidemiological significance of isolate strains and their taxonomic features. There is experience of genotyping using MLVA, MLST, SNP and other methods, as well as the analysis of the complete genomic sequence (WGS).

Taking into account the levels of strain analysis (diagnosis of infection or epidemiological analysis), the following current research areas can be identified:

– Detection and identification: application of nucleic acid amplification methods for the differentiation of living and dead cells; introduction of multiplex (multifactor) PCR analysis technologies; creation (completion) of databases of mass spectra of microorganisms; introduction of methods of direct mass-spectrometric analysis of clinical material.

– Molecular typing: the creation of sequential (optimal) genotyping algorithms for each species; application of protein profiling methods for typing pathogens.

– Application of information systems, epidemiological analysis: creation of own databases of full-genomic sequencing; genomic profiling of pathogens in specific areas; creation of complex software products using the data of geographical information systems and predictive modeling.

As a result, an algorithm for bioinformational analysis should be developed for the epidemiological investigation of outbreaks (cases) of infectious diseases, including those caused by new (atypical) genetic variants of pathogens of especially dangerous infections.

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DIFFERENTIATION OF *KLEBSIELLA* spp. STRAINS FOR SENSITIVITY TO ANTIBIOTICS USING MASS SPECTROMETRY ANALYSIS MALDI-TOF

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Klebsiella spp. strains are frequent causative agents of health care-related infections. Those strains especially dangerous if they circulate in hospitals. They are usually antibiotic-resistant. Therefore, information about the sensitivity of the isolated strain is necessary in the shortest time for proper etiological treatment.

The aim of our study was to assess the possibility of using mass spectrometry analysis (MALDI-TOF) for the rapid prediction of a selected *Klebsiella* spp. strain resistance.

The study used 195 strains of *Klebsiella* spp. isolated in various medical centers of St. Petersburg. All strains were identified by MALDI-TOF. Antibiotic sensitivity was studied by the disco-diffusion method in accordance with the recommendations of EUCAST 8.0. We used 19 antibiotics from 5 classes for testing strains: aminoglycosides, beta-lactams, beta-lactams of extended spectrum, quinolones and carbapenems.

A hierarchical clustering of spectra was made using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) to determine the relationship between clusters. We used Pearson correlation coefficient between variable values of peak intensity in spectral profiles as a measure of the distance between individual mass spectra. We identified 2 significant difference in the spectrum of the cluster. One cluster included spectra of strains resistant to all studied classes of antibiotics, second cluster — strains sensitive to them (Distance Level — 0.65). It was also found that all strains included in the clusters, which differ from others by Distance Level more than 0.2, have the same profile of antibiotic resistance.

As a result of this work, we have formed profiles of phenotypic resistance of *Klebsiella* spp. strains to 19 antibiotics and 5 classes. The prospect of using the results of the study is a significant reduction in the study of biological material from the patient. Thus, simultaneously with the identification of *Klebsiella* spp. strains by MALDI-TOF it is possible to predict the sensitivity of the isolated strain to different classes of antibiotics or even to one of them. This will allow timely recognition of resistant strains of *Klebsiella* spp and prescribe adequate etiological therapy, which will significantly improve the quality of treatment of patients and will prevent the spread of resistant strains of bacteria in the medical establishments.

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PATHOGENIC POTENTIAL OF COMMENSAL *ESCHERICHIA COLI* ISOLATED FROM ADULTS IN SAINT PETERSBURG

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Escherichia coli is one constitute a component of the natural microbiota of warm-blooded animals including humans. At the same time commensal *E.coli* is a dynamic population and in some cases is capable to cause ex-

traintestinal diseases, sometimes leading to morbidity and mortality. The aim of this work was to study of genetic diversity and assess of pathogenic potential of commensal *E. coli* isolated from healthy adults in Saint Petersburg. 300 *E. coli* strains were collected from fecal samples of 50 St. Petersburg's inhabitants. *E. coli* strains were isolated using Endo agar and identified by biochemical tests. Determination of four major phylogenetic groups and identification of virulence genes were performed by using real-time, multiplex and simplex PCR. Seven genes typical for ExPEC *(fimH, pap, sfa, aer, afa, cnf1, hlyA)* were identified among the analyzed strains. The B2 phylogroup (47.1%) was leading among other groups: A (20.5%), B1 (9.0%) and D (23.4%). Each strain had at least one virulence gene. No strain had all seven studied genes simultaneously. The maximum number of genes in one strain was five. The prevalence of virulence genes was as follows: *fimH* (98.0%), *pap* (25.0%), *sfa* (8.0%), *aer* (33.8%), *afa* (5.6%), *cnf1* (11.0%), *hlyA* (10.0%). The strains of groups B2 and D harbored the virulence determinants significantly more frequently then the strains of groups A and B1. Our results showed that *E. coli* isolated from adults differ in their phylogenetic structure and harbour a greater variety of virulence genes. Our study revealed that commensal *E. coli* isolated from healthy humans constitute a substantial reservoir of genes related to the extraintestinal pathotypes. All seven tested virulence genes typical for ExPEC were detected and it's important that the prevalence of these genes was significantly higher among the isolates from healthy adults. So, the extraintestinal virulence genes (encoding the adhesins, toxins, persistence) were found not only in pathogens, but also in commensal microflora of healthy people. Previous reports indicated that virulence genes associated with extraintestinal pathogenesis in fact help the *E. coli* strains to colonize the human gut; therefore, they may be considered as a fitness factor and the virulence is a coincidental side effect.

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EXPRESS METHOD OF GROWING BACTERIA ON THE MEMBRANE OF ANODIC ALUMINIUM OXIDE

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The classical method of studying bacteria is the cultivation of microorganisms and study of their biological properties. However, this method is very long (several days). So it may not be used in cases when you need to quickly get the result. It can be surgery, sepsis, severe infection, etc. In these cases, the doctor will need a few hours to make a decision on the appointment of causal treatment.

We have developed a method of growing the isolated clones of bacteria from any biological material for 3 hours. The rapid growth of microorganisms is ensured due to the new culture medium. Each microbial cell is grown in a separate cell on a porous membrane of anodic aluminum oxide. After 3 hours of incubation reads visual information using a specially developed image sensor zoom. The visual image of the individual microcolony identified to the species created by special computer programs. The probability of coincidence of the results is 90%. With the help of a special counter counts the number of bacteria of each species in the studied sample. This is especially important in the study of biological material containing several types of microorganisms.

Thus, 3 hours after inoculation of biological material, we get the result about of species and quantitative composition of bacteria. A living culture of microorganisms can work with it further to explore other biological properties, including rapid determination of sensitivity to antibiotics.

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THE DRUG RESISTANCE MUTATIONS OF THE HEPATITIS B VIRUS AMONG HIV-INFECTED INDIVIDUALS

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Hepatitis B virus (HBV) is one of the most common hepatotropic viruses that can cause both acute and chronic course of the disease. One form of chronic viral hepatitis B is occult hepatitis B, characterized by the presence of HBV DNA in the liver and undetectable levels of HBsAg and HBV DNA in the peripheral blood. The co-infection of HBV with the human immunodeficiency virus (HIV) is facilitated by the common mechanisms and pathways of infection. Although the effect of HBV on the progression of HIV infection appears to be minimal, HIV affects the progression of liver fibrosis, increasing the risk of developing hepatocellular carcinoma and cirrhosis. The need for timely identification HBV variants carrying drug resistance mutations among HBV/HIV-coinfected patients.

The aim of our study was to evaluate the prevalence of HBV with drug resistance mutations among HBV/HIV-coinfected patients.

The material was blood plasma of 264 HIV-infected (HBsAg-) patients with virologic ineffectiveness of ARVT. A method for detecting HBV DNA with a low viral load based on a two-step PCR, followed by sequencing was used.

HBV DNA was detected in 89 (33.7%) patients. Based on the phylogenetic analysis it was shown that in this group the HBV subgenotypes are represented in the following ratios: D1 — 39.3%, D2 — 29.2%, D3 — 30.4%, C1 — 1.1%, respectively. In the analysis of nucleotide sequences in the viral polymerase reverse transcriptase domain significant amino acid substitutions (mutations described in the literature as determining the development of drug resistance to nucleotide/nucleoside analogues therapy) were found in 12.35% of patients. Including 9 patient was found to have significant amino-acid replacement in HBV polymerase gene (L180M, M204V) associated with the development of resistance to lamivudine, entecavir, telbivudine and tenofovir. Also in 5.6% of patients were found potentially significant (substitutions in the same significant positions of the polymerase gene, but not described in the literature) — for example L80F.

The obtained data on the prevalence of HBV drug resistance indicate the need for screening of patients with HBV/HIV-coinfection before starting the antiviral therapy.

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INCREASE OF SELECTIVE AND GROWTH PROPERTIES OF A NUTRIENT MEDIUM FOR IDENTIFICATION AND ACCUMULATION *TRICHOMONAS VAGINALIS*

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Currently, the detection of patients with trichomoniasis is decreasing. This effect can be caused by asymptomatic infection, imperfect methods of protozoa identification, low availability and quality of nutrient media. Therefore,

the improvement of selective diagnostic nutrient media for the detection of *T. vaginalis* is so important.

The purpose of the study was to increase the selectivity and growth properties of the nutrient medium for the *T. vaginalis* detection. It was necessary to select a concentration of amphotericin B in media with different content of horse serum and peptone enzymatic, which inhibits the growth of *Candida* spp., but doesn't affect the growth of *T. vaginalis*.

For the research, an experimental mediums (based on the SVT medium (RU FSS No. 2009/05982), produced by the Pasteur Institute) were prepared with different content of horse serum (10%, 20%, 40%), half of which were with enzyme peptone (12.8 g/l) and all of them without antimycotics. Two dilutions of amphotericin B in the range of concentrations of 0.5–50 μg/ml, as well as fluconazole at a concentration of 264 mg/ml (as in the SVT) were introduced into all experimental media. The strains of *T. vaginalis* (T1, T5, T7, T11) from the Pasteur Institute collection in concentration 0.5×10^6 cells/ml and the standard strain *Candida albicans* ATCC 24433 in concentration 10^7 cells/ml were sown in all medias in three recurrence. The incubation temperature was 35±1°C. Counting the number of cells was carried out using the Goryaev chamber every day for a week. After 24 hours, *C. albicans* were sowed onto the Müller–Hinton agar for testing the suppression of their viability with subsequent microscopy.

It was revealed, that optimal accumulation of *T. vaginalis* (2.5×10^6 cells/ml) and inhibition of *C. albicans* occurs at concentration of amphotericin B (2–0.5) μg/ml. The activity of fluconazole to *C. albicans* in these media was low. It should be noted, that in medias with a high content of horse serum, the accumulation of *T. vaginalis* increased sharply on the second day of the study, and their resistance to high concentrations of antimycotic (up to 20 μg/ml) was also observed. However, the viability of the cells was reduced in contrast to media with 10% horse serum and low concentrations of amphotericin B. The addition of enzymatic peptone to experimental media did not reveal a significant difference in the growth properties.

According to the results, an experimental medium containing 10% horse serum with amphotericin B in concentration 2 μg/ml was chosen for detection and accumulate *T. vaginalis*.

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ANALYSIS OF THE PHAGE SENSITIVITY OF MICROORGANISMS OF A MICROBIOTA OF A VAGINA

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Recently in connection with growth of detection of antibiotic resistant cultures, for treatment of infectious diseases even more often recommend to use bacteriophages. Bacteriophages don't give side effects in comparison with antibiotics and work is specific on microorganisms and exist in different pharmaceutical forms: liquid, gel and tableted. The solution of a question of application of a phage has to be based on results of testing of activity of medicine.

The aim of the study was comparative analysis of two options of phagus medicines for definition of a phagus sensitive of microorganisms of a microbiota of a vagina.

50 women who have addressed to laboratory on an outpatient basis for the purpose of receiving a bacteriological research of a vaginal microbiota have been examined. Bacteriological researches were conducted according to the standard recommendations. For identification of species of bacteria by MALDI-TOF MS method used a desktop mass spectrometer of Microflex with the MALDI Biotyper library (Bruker Daltonics Germany). As the tested medicines applied polyvalent liquid and gel forms of bacteriophages. The modified technique where the bacteriophage was applied with a print on culture a bacteriological loop (cm d = 0.5) bent at an angle of 90° lehas been developed for a gel form of a bacteriophage. Assessment of lytic activity of a phage was carried out on a five-point scale (by quantity of "crosses").

The sensitivity to bacteriophages has been defined at 45 women with violation of a microbiota of V. At the same time the high sensitivity to a liquid form of a bacteriophage has been found in 6 patients (13%). To a gel form the high sensitivity has been defined at 39 patients (87%), coincidence cases on sensitivity at both bacteriophages weren't observed.

The parallel research of sensitivity of microflora to liquid and gel forms at a bacterial vaginosis has shown that in 87% microorganisms were sensitive to a gel form while the sensitivity to liquid bacteriophages has been found in 13% of the bacteria inhabiting the offered modification of a research of activity of bacteriophages on a gel basis allows to dose bacteriophages in this pharmaceutical form and to receive comparable results with a classical technique.

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S. AUREUS/C. ALBICANS MONO- AND DUAL-SPECIES BIOFILMS

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Several studies have reported the co-isolation of *S. aureus* and *C. albicans* from numerous biofilm-associated diseases. These data indicate that these organisms have the capacity to interact with one another at the molecular level. The possibility of the development of polymicrobial biofilms, consisting of both fungi and bacteria, should be considered in pathogenesis of various infections.

The aim of our work was study of mono-and dual-species *S. aureus/C. albicans* biofilms, evaluation of distinctions between clinical and standard streins biofilms of both in static assays.

In the work used standard strains of *S. aureus* 25923 ATCC and *C. albicans* CCM 885, as well as clinical strains of them, isolated from patients with acute otitis media (*S. aureus* U14) and also from healthy carrier (*S. aureus* 609, *C. albicans* 609).

Overnight cultures was diluted 1:100 into fresh medium for biofilm assays. Static biofilm assays of O`Toole G. and Kolter R. (1998) in a 96 well dish was used. Biofilms were formed by adding both organisms 1:1 to either microtiter plates. The plate was then grown statically at 37°C overnight. The cultures removed with a multichannel pipette plate, the plate was rinsed 3–4 times with water, a 0.1% solution of crystal violet in water was added of to each well, incubated for 15 minutes at RT. Then 200 μL of 95% ethanol was added to each well and the plate was left to stand on the bench for 30 minutes. Finally the plate was read with a microplate reader Multiskan at 620 nm.

Biofilms density of *Staphylococcus* cultures was the highest in the clinical isolate *S. aureus* U14 (24% more

than standard strains *S. aureus* 25923 ATCC). Density of *C. albicans* 609 biofilm exceed the density of the standard strain *C. albicans* CCM 885 biofilm at 14.5%. However, dual bacteria-fungal biofilm *S. aureus* U14 + *C. albicans* 609 exceed density of biofilm *S. aureus* 25923 ATCC + *C. albicans* CCM 885 more than twice (208%). Dual-species biofilm isolated from throat healthy media, *S. aureus* 609 + *C. albicans* 609, did not differ in density from dual-species biofilm of reference cultures.

Thus, when coupled with cultivation yeast *C. albicans in vitro* aggressiveness of *S. aureus* clinical strain was increased. Carrier state yeast fungi, therefore, could serve as a risk factor for developing a more dense staphylococcal biofilm on the mucous membranes and worsen the course of the disease.

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MICROBIOLOGICAL MONITORING OF THE RESISTANCE OF HOSPITAL BACTERIAL FLORA WITHIN THE SYSTEM OF PREVENTION OF HEALTHCARE-ASSOCIATED INFECTIONS

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Effective implementation of disinfection activities in healthcare organizations (HCO) plays a great and, sometimes, even a critical role in the prevention of healthcare-associated infections (HAI). Formation and spread of microorganisms which are resistant to used chemical disinfectants in HCO substantially reduce the effect of disinfection measures. This, in turn, is the common reason of the high level of HAI incidence. The problem is aggravated by the development of antibiotic resistance among the strains which are resistant to DA (cross-resistance).

According to the requirements of Sanitary Regulations and Norms 2.1.3.2630-10, it is necessary to conduct monitoring of hospital bacterial flora sensitivity to disinfection agents (DA).

The assessment of sensitivity of microorganisms isolated from the objects of intrahospital environment of intensive care, intensive therapy and surgical units — 20 strains (29%), and from the pathological loci of in-patients — 50 strains (71%) resistant to various groups of antibiotics *(K. pneumonia, A. baumannii, P. aeruginosa, P. mirabilis, S. maltophilia)* — was carried out.

Testing was conducted according to the method described in Methodical Guidelines 3.5.1.3438-17 "Assessment of sensitivity to disinfection agents demonstrated by microorganisms circulating in healthcare organizations". To compare the resistance of hospital microorganisms with that of the microorganisms from the collection which demonstrate standard resistance to DA a collection strain *P. aeruginosa ATCC 27853* was used.

It was determined that 11 strains were resistant to cationic surface-active agents, 10 strains — to active oxygen, 6 strains — to the combination of quaternary ammonium compounds and active oxygen. 27 (38.7%) out of 70 strains of microorganisms isolated from external environment objects and patients were resistant to used DA.

The identity of resistant strains isolated from external environment and patients serves as evidence of cross-contamination and leads to the spread of resistant strains among patients which, in turn, determines the need to improve the organization of preventive activities.

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GENETIC VARIANTS OF RESISTANCE DETERMINANT TO SILVER IN EPIDEMIC STRAINS OF *ACINETOBACTER BAUMANNII*

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Silver-containing dressings are widely used in burns and leg ulcers for prevention and treatment infection. Heavy metal pump CzcA of RND transporters family predicted roles in the efflux both toxic cations including silver and some antibiotics from bacterial cells. Molecular epidemiology and surveillance of outbreaks for last decades indicate that the most resistant *Acinetobacter baumannii* belong to two globally disseminated clonal lineages, GC1 and GC2. Strains of GC2 are epidemic as for Far East and Indochina. Our study was designed in order to clarify an evolution of silver resistance determinant in clinical *A. baumannii*.

Multiple-resistant strains from different geographic locations were selected. List of strains included *A. baumannii* AYE (GC1, epidemic in France for past years) and strains of GC2: ACICU isolated in an outbreak (Italy), sturdy-biofilm forming 1656-2 (South Korea), LY9 and BJ5 recovered in Southern and Northern China hospitals, consequently and strain of sequence-type ST2 (Institut Pasteur typing scheme) endemic in Perm in 2010–2011. Sequences of *czcA* were retrieved from GenBank database for *in silico* comparative analysis using BLAST.

Surprisingly, *czcA* gene on chromosomes of *A. baumannii* GC1 and GC2 spread over the world is presented as two prevailing alleles only (allele 1 in AYE, ACICU, 1656-2 and allele 2 in LY9, BJ5 and Perm). Even di- and multinucleotide variants on positions 213–215 ACC or TTT, 897–898 TG or AC, 945–948 CCGT or TAAA and 951–952 AG or TA have been related to distinct allele (nucleotide numbers from start codon ATG).

The important mechanism of *A. baumannii* survival under silver presence is expressed in an extreme decrease in the genetic heterogeneity of encoding sequence.

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COMMENSAL STRAINS OF *ESCHERICHIA COLI* AND BETA-LACTAM RESISTANCE

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According to the World Health Organization, the antimicrobial resistance (AMR) remains a huge worldwide problem of our time which we yet have to overcome. One of the ways to contain the antibiotic resistance is to monitor a circulation of resistant strains of microorganisms, as well as genes that determine the AMR. Studies in recent years have shown a high level of resistance in *Klebsiella pneumoniae* and *Escherichia coli*, the causative agents of nosocomial and community-acquired infections. However, resistant strains also may be a part of gastrointestinal microbiota in healthy individuals.

Antimicrobial susceptibility of 511 commensal *E. coli* strains isolated from faeces of children in age groups from 1 month to 17 years old living in St. Petersburg to 9 groups of antibiotics have been studied by disco-diffusion method. The resistance mechanisms among strains insusceptible

to beta-lactams have been studied by PCR with electrophoretic detection with specific primers to beta-lactamases encoding genes.

39.3% of isolated strains were resistant to 1 and more classes of antimicrobials, while 16.6% of isolates were characterized by multiple resistance (resistant to 3 or more classes of antibiotics). Resistant and multiresistant strains were isolated equally often from children of all age groups. 29.5% of the strains were not susceptible to ampicillin, while 11.2% were insusceptible to cephalosporins. It was established that the resistance mechanism to ampicillin is associated with the production of beta-lactamase molecular classes of TEM, SHV and OXA; to cephalosporins — CTX-M, TEM, SHV and AmpC. The most common genes are beta-lactamases of molecular class TEM (22.7%) and CTX-M (9.6%). Simultaneous production of several beta-lactamases was found in 8.4% of strains. *E. coli* strains producing beta-lactamases, unlike strains that do not produce them, are statistically significantly more often resistant to other groups of antibiotics (quinolones, aminoglycosides, chloramphenicol, tetracycline).

The results indicate that colonization of an intestine by resistant strains of *Enterobacteriacae* starts in early childhood. In the context of the widespread use of antibiotics in medicine, veterinary, agriculture and food industry, such strains persist for a long time in the microbiota of children and adults, making them potential sources of resistance determinants for enteropathogens causing the acute intestinal and septic infections.

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VIRULENCE GENES AND PHYLOGENETIC GROUPS OF COMMENSAL STRAINS OF *ESCHERICHIA COLI*

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The species *Escherichia coli (E. coli)* is globally distributed. Representatives of the species live in the distal intestine of almost every person on the planet, as well as in the intestines of mammals, amphibians and birds, participating in the biotransformation of nutrients and the synthesis of biologically active molecules. Among the non-pathogenic members of the species various pathotypes are found, which cause diseases of intestinal tract (enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAggEC)) and extraintestinal infections (uropathogenic *E. coli* (UPEC), meningitis-associated *E. coli* (MNEC)). They are characterized by a variety of virulence factors. Studies of the evolution of intraspecific diversity revealed seven phylogenetic groups, four of which are the main (A, B1, B2, D).

The aim of the study was to determine the phylogenetic profile of the population of *E. coli* commensal strains, to identify genetic determinants of known virulence factors and to compare their prevalence in the genomes of *Escherichia* in different phylogenetic groups.

511 strains of *Escherichia coli* isolated in 2012–2014 were studied. Strains were isolated from children faeces in age groups from 1 month to 17 years old living in St. Petersburg, without diarrhea and urinary tract infections, and studied with the PCR using electrophoretic detection with specific primers to genes encoding virulence factors and markers of phylogenetic groups.

The studied population of *E. coli* was represented by strains of phylogroup A — 33%; B1 — 7%; B2 — 34%; D — 26%. The genome of commensal strains contains virulence genes of EPEC (2.5%), EAggEC (4.5%). Strains with EPEC virulence genes are more common in phylogenetic group B1(18.9%), whilst strains with EAggEC virulence genes are more common in phylogroup D (12.4%). Virulence genes of EHEC, ETEC, EIEC were not identified.The genome of commensal strains contains some genes of UPEC virulence (*hlyB* — 20.9%; *cnf* — 17.4%; *pap* — 29.5%; *sfa* — 19.8%; *aer* — 20.0%). Genes of toxins *(hlyB, cnf)* and adhesins *(pap, sfa)* are encountered more frequently, with statistical significance, in strains of phylogenetic group B2.

The study showed that genes encoding virulence factors for some pathotypes of *E. coli* are also found in the genomes of the commensal *E. coli* strains and the probability of their detection among representatives of different phylogroups is inconsistent.

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THE SEROPREVALENCE *H. PYLORI* INFECTION IN DIFFERENT GEOGRAPHICAL REGIONS

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H. pylori colonizes more than 50% of humans worldwide. It causes unnoticed chronic gastritis in all carriers and represents a major risk factor for peptic ulcer disease and gastric cancer. It is known that a higher prevalence of *H. pylori* infection will lead to a higher overall prevalence of upper gastrointestinal disease. However the form of such disease may be dictated by socioeconomic and environmental factors as well as the habits and traditions of the people living in different geographical regions.

The aim of our work was to study the seroprevalence infection caused by toxigenic *H. pylori* strains among residents of different geographical regions.

We examined residents of the North-West region of the Russian Federation, Central Asia, Guinea and North Vietnam. Age of the examined was from 20 to 50 years. IgG screening for *H. pylori* and *Cag A H. pylori* antibodies was performed using ELISA method with test-system produced DRG (Germany), Biohit (Finland).

In the inhabitants of the North-West region of the Russian Federation, the seroprevalence of infection caused by toxigenic *H. pylori* strains was 55.59±1.2%. The lowest was the infection of *H. pylori* in North Vietnam — 43.75±8.7%. The highest percentage of *H. pylori* infection was found in Guinea and Central Asia — 85.11±5.2% and 84.00±3.7%, respectively.

An uneven prevalence of infection caused by toxigenic *H. pylori* strains was found among residents of different geographical regions. An increasing process of migration of the population can lead to the spread of infection and the exchange of *H. pylori* strains, specific for the particular regions. This suggests the necessity for further epidemiological studies of *H. pylori* infection in different geographical regions.

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INFLUENCE OF CHOLESTEROL ON THE GROWTH OF *STAPHYLOCOCCUS* spp.

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The aim of research was investigation of the effect of cholesterol (C) on the growth kinetics of *Staphylococcus aureus* and *S. epidermidis*.

We used *S. aureus* and *S. epidermidis* from ATCC collection, which were cultured in meat-peptone broth with

the addition of C at concentrations of 3, 5, 7 and 9 mmol/l. After 24 hours every hour an optical density of the broth at 580 nm was measured. The concentration of C was determined in samples before and after cultivation. The concentration of C was determined in 37 patients, which were included in 3 groups: 1 — "classical" staphylococcal infection (abscess, phlegmon, carbuncle, mastitis, hydradenitis); 2 — secondary infection of wounds with staphylococci; 3 — "not staphylococcal" infections. To determine the level of C in the culture medium or serum, an enzymatic method was used. Statistical processing of data was carried out using the paired version of Student's t-test.

It was found that C in all concentrations does not have a bactericidal effect on *Staphylococcus* spp. Before cultivation of *S. aureus* the level of C was 3.16±0.06 and after — 2.69±0.04 mmol/l ($p < 0.05$). Such decrease may be due to the fact that *S. aureus* includes in its metabolism the disrepared diphosphate necessary for the synthesis of the cell wall. Under cultivation of *S. aureus* in the presence of C the accumulation of biomass was more intense than in a medium without C. A direct relationship between the accumulation of the biomass of the microorganism and the level of C was shown. In assessing the kinetics of growth of *S. epidermidis*, a similar picture was established. A feature of *S. epidermidis* was an increase in the biomass of cells in a stationary growth phase in the presence of 7 mmol/l of C.

In patients of the 1st group the level of C was 4.6±0.3; 2nd — 3.28±0.26; 3rd — 4.10±0.37 mmol/l. In general, the level of C in patients of the compared groups corresponds to the age norm. However, in patients of the 2nd group concentration of C significantly differs from the values of the 1st group. We assume that in a secondarily infected wound the processes metabolism of microorganisms proceed more intensively, as a result of which C can be utilized more by staphylococci, which leads to decrease in its concentration.

Thus, staphylococci are able to include in their metabolism human C, which may be necessary for them for plastic purposes.

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SENSITIVITY OF BIOFILM CULTURES *KLEBSIELLA* spp. TO CIPROFLOXACIN

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In the study researched the effect of 10-, 100-, 1000-fold values of the minimum inhibitory concentration (MIC_{90} = 2 μg/ml, literature data), of the antimicrobial preparation ciprofloxacin on *Klebsiella* spp. autostrains isolated from coprological probes of kids under 5 years old. The experiment included 47 biofilm-forming *Klebsiella* spp. cultures (28 strains of *K. pneumoniae* and 19 isolates of *K. oxytoca*). A study of the ability of clinical strains to form a biofilm, as well as the influence of a number of concentrations of antibiotic on mature (48-hour) biofilm was carried out in sterile polystyrene plates in a microvolume. Mature biofilm cultures were incubated with ciprofloxacin during the 12 hours under standard conditions with a preliminary purification from plankton cells. The results were considered by optical density of the dye-1% crystal-violet bound to the film on a spectrophotometer at a wavelength of 492 nm. The biofilm formation coefficient was calculated as the ratio of the average value of the optical density of the sample to the average value of the optical density of the negative control. The value of the coefficient ≥ 2.1 was taken as positive.

Biofilms formed by *K. oxytoca* autostems when exposed to ciproflocacin at concentrations exceeding 100- and 1000-fold the MIC_{90} were completely destroyed. When exposed of 10-fold the MIC_{90}, the cells adhering to the surface of the wells formed biofilms that were preserved in 30% of *K. oxytoca* isolates. Among biofilms formed by strains of *K. pneumoniae* 48.3% were insensitive to a 10-fold concentration of ciproflocacin. 35.7% out of this insensitive isolates were insensitive to a 100-fold concentration of the antibacterial drug. In addition, a strain of *K. pneumoniae* was detected, which biofilm was not destroyed by a 1000-fold concentration (2000 μg/ml) of ciprofloxacin. The zone of inhibition of growth of this strain to ciprofloxacin, which investigated by the disco-diffusion method was absent; the strain was characterized as resistant.

Mature biofilms of strains of *K. pneumoniae* were significantly less damaged by exposure to selected concentrations of the antimicrobial drug, ciprofloxacin, compared to *K. oxytoca* isolates.

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THE CORRELATION BETWEEN BIOFILM-FORMATION ABILITY OF *KLEBSIELLA* spp. AUTOSTRAINS AND ANTIBIOTIC SENSITIVITY OF PLANCTONIC CELLS

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The former study is related to planktonic cells *Klebsiella* spp.(n = 117) isolated from coprological probes of kids with disbiotic disorder. These cells were isolated using disc-diffusion method to examine the correlation between their sensivity to 11 antibiotics and ability to form firm biofilms in the wells of polystirol microplate.

The study revealed that isolates of *K. pneumoniae* had more autostrains able to form biofilms that *K. oxytoca* (n = 84, 72.6% and n = 33, 60.6% respectively). More frequently strains were resistent to amoxicillin (*K. oxytoca* — 9%, *K. pneumoniae* — 26%). The insufficient share of biofilm structures can be explained by vast spread of antimicrobial medication.

All studied autostrains of *K. oxytoca* did not reveal resistence to the majority of antimicrobial medication like imipenem, ertapenem, meropenem, cefepimum, ciprofloxacin, levofloxacin. Strains of *K. oxytoca* which do not form biofilms were completely sensitive to tetracycline, chloramphenicolum, moxifloxacin, doxycycline. In this, intestinal isolates of *K. oxytoca* which form biofilms lowered their sensivity up to 5% (tetracycline, chloramphenicolum, moxifloxacin) and 10% (doxycycline).

K. pneumoniae strains did not reveal resistance to imipenem, ertapenem. Isolates of *K. pneumonia* which do not form biofilms were completely sensitive to moxifloxacin, chloramphenicolum and meropenem. Biofilm-forming strains had lesser sensitivity up to 8.2; 3.6; 3.3; 1.6% respectively. Sensitivity of *K. pneumoniae* was 95.7% to levofloxacin. Sensitivity of *K. pneumoniae* autostrains was 95.7% and for biofilm-forming strains was lowered up to 10.4%. Sensitivity of non-biofilm isolates of *K. pneumoniae* to doxycycline and ciprofloxacin was 91.3%, and for non-biofilm — 84.4; 73.8% respectively.

The study revealed that planktone cells *Klebsiella* spp. are able to form biofilm what makes them resistent to most common antibiotics.

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FERTILIZATION FAILURE IN HEIFERS INFECTED BY UREAPLASMA DIVERSUM

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Ureaplasma diversum is an opportunistic pathogen in cattle, but colonization of the respiratory tract by this ureaplasma, and its carriage in the reproductive tract may lead to the serious diseases. It also may be the cause of abortion and stillbirth in cattle.

The aim of this study was to estimate the fertilization effectiveness of in heifers into relation with *U. diversum* carriage in the vulval vestibule. All 20 heifers in the study group were from the same dairy farm from Leningradskaya oblast. The samples were collected from the vulval vestibule by cotton swab. At the sample collection vulvar mucous appearance was estimated. The *U. diversum* carriage was detected by real-time PCR assay with diagnostic system "Ureaplasma diversum Amp" (St. Petersburg Institut Pasteur, Russia).

In the group of 20 heifers, 13 had symptoms or granular vulvovaginitis, including yellowish-gray pustules on the mucous. No other reproductive disease symptoms were detected in any heifer. The carriage of *U. diversum* was detected in 15 animals. The granular vulvovaginitis is commonly associated with *U. diversum* carriage in heifers and cows, but the symptoms of this disease are nonspecific and frequently may be associated with other diseases, for example with bovine rhinotracheitis, that is very widespread in cattle. No association was detected between granular vulvovaginitis symptoms and *U. diversum* carriage in study population.

The effectiveness of fertilization was estimated in all heifers. The average number of inseminations leads to fertilization in heifers without carriage was 1.2, but in infected heifers it was 1.9, and the difference between two groups was statistically significant ($t = 0.36$; $p < 0.002$). The fertilization failure was more frequent in heifers with *U. diversum* carriage. Twelve heifers from this group were fertilised at first insemination, two heifers in the same group were fertilized at second insemination and one heifer was inseminated six times before fertilization. Into the group without *U. diversum* carriage all but one of heifers were fertilized in the first insemination an one heifer in second insemination.

The loss in fertilization effectiveness leads to the economic burden in dairy farms due to costs of repeated inseminations and animal management. The appropriate diagnosis of *U. diversum* carriage in heifest may improve dairy farm productivity.

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ANTIBIOTIC-RESISTANT *KLEBSIELLA PNEUMONIAE* IN THE GUT MICROBIOTA OF HEALTHY INDIVIDUALS

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Klebsiella pneumoniae causes a wide range of infectious diseases including pneumonia, urinary tract infections, bacteremia and liver abscesses. Previously, it was believed that *K. pneumoniae* can cause serious infections primarily in people with decreased immunity, but the recent emergence and spread of hypervirulent strains has increased the number of people susceptible to these infections, including healthy ones. In addition, strains of *K. pneumoniae* are becoming more resistant to antibiotics, which creates special difficulties in treatment. Strains of the genus *Klebsiella* quite often colonize the mucous membrane of the distal gastrointestinal tract of children and adults, being part of the gut microbiota.

The aim of this study was to reveal frequency of occurrence of *K. pneumoniae* in intestinal microbiota of clinically healthy adults and children and to define antimicrobial susceptibility of isolated strains.

The microbiota content of 180 people aged from 1 month to 65 years was studied by quantative bacteriological method according to OST 91500.11.0004-2003 "Protocol of management of patients. Intestinal dysbacteriosis." *K. pneumoniae* strains were isolated in 25.0% (95% CI:19.2–31.8) of samples in quantaties exceeding 10^5 CFU/g. The susceptibility of these strains to 7 groups of antibiotics (penicillins combined with inhibitors, cephalosporins, quinolones, aminoglycosides, tetracycline, chloramphenicol, nitrofurans) was studied by disc diffusion method. 51.1% (95% CI:37.0–65.0) of the isolates were resistant to one or more antimicrobials. Multiple resistance (resistance to 3 or more classes) was found in 11.0% (95% CI:4.8–23.5) isolates. The highest resistance was observed to amoxicilline/clavulanic acid (31.1% of strains), the lowest — to amikacin (4.4% of strains). No strains resistant to carbapenems were found. Resistance to other antimicrobials ranged from 8.9% (chloramphenicol) to 22.2% (gentamicin).

The study showed that the intestinal microbiota of every fourth clinically healthy person contains strains of *K. pneumoniae*, half of which are resistant to one or more antimicrobials. At the same time, more than 10.0% of *K. pneumoniae* strains isolated from healthy people have multiple antibiotic resistance. Such strains can serve as a reservoir of determinants of resistance to other enterobacteria, including pathogens of acute intestinal infections.

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ANTIMICROBIAL RESISTANCE MECHANISMS IN BACTERIA STRAINS ISOLATED FROM FARM ANIMALS

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The aim of the work was to study the quinolone and β-lactame resistance mechanisms in *Salmonella* and opportunistic bacteria strains isolated from farm animals.

We determined the quinolones and β-lactams susceptibility and resistance mechanisms in 482 *Salmonella* and 144 strains of opportunistic bacteria (*E. coli, Klebsiella* spp.).

For 6 *Salmonella* strains (3 *S.* Enteritidis and 3 *S.* Infantis), resistant to fluoroquinolones the mutations in the QRDR region of *gyrA* gene were detected by amplification and sequencing of this DNA region (Kosyreva et al., 2012). The extended-spectrum β-lactamases (molecular classes A and C) were determined by PCR with specific primers (Dallenne et al., 2010). 132 strains of *Salmonella* were resistant to quinolones (27.4%), 41 of them (8.5%) had high level resistance to ciprofloxacin (MIC 6–32.0 mg/l). Sequencing of the *gyr*A of some resistant *Salmonella* isolates have been identified three types of single point mutations. In two *S.* Enteritidis the mutation was noted in 83 position (Serine replacement by Phenylalanine), in one strain — in 87 position (Asparagine replacement by Glycine). Three *S.* Infantis strains had the replacement of Asparagine by Tyrosine in 87 position.

9 strains of *Salmonella* (1.9%) were resistant to extended-spectrum cephalosporins. According the beta-lactamase inhibitor susceptibility tests, five of them were classified as ESBL-producers, 4 strains — as AmpC-producers. ESBL CTX-M was detected in strains of *S.* Haifa isolated from chicken samples and *S.* Derby isolated from the imported pork heart. AmpC cephalosporinase CMY was produced by two strains of *S.* Kentucky, isolated in 2006 and 2009 from imported poultry products, as well as two *S.* Dublin isolated in 2005 from the internal organs of a fallen calf and cow.

In opportunistic bacteria (*E. coli* and *Klebsiella* spp.) 22 strains were resistant to extended spectrum cephalosporins, 15 of them produced ESBL according the beta-lactamase inhibitor susceptibility tests. The class of detected β-lactamases was established in 11 strains. In *K. pneumoniae* and *K. ozenae* isolated from the milk of cows sick with mastitis ESBL CTX-M1 were detected. In *E. coli* isolated from calves suffering from diarrhea, was detected ESBL CTX-M1 and CTX-M9. So, our study has confirmed the circulation of *Salmonella* and other *Enterobacteriaceae* strains resistant to clinical significant antibiotics (fluoroquinolones and cefalosporines) in animal farms.

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DATA ANALYSIS OF MASS-SPECTRAL *KLEBSIELLA PNEUMONIAE* PROFILES TO PREDICT OF CARBAPENEM-RESISTANT STRAINS

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The increase of the number of enterobacteria with resistance to carbapenems has become a global problem. A rapid method for predicting resistance to carbapenems is needed, the results of which will be obtained even before the sensitivity to antibiotics is determined. In recent years, has been developing the trend, related to the increase in the MALDI-TOF mass spectrometry potential by bioinformatic approach to typing bacteria at the level of strains.

The study's aim was the data analysis of the *K. pneumoniae* mass spectra for protein biomarker discovery that make it possible to predict the detection of strains with OXA-48 and NDM-1 carbapenemase activity.

We used archived spectra obtained for the routine identification of isolates from hospital patients of St. Petersburg in 2015–2017. Digital data of 67 raw spectra, selected by identification results at the *K. pneumoniae* species level, were exported to the "BioNumerics" software. The created classifier was used to identify of new seven OXA-48 and eight NDM-1 strains pre-characterized by PCR, and 16 sensitive to meropenem *K. pneumoniae* strains. The biomarker peaks were designated by comparing their molecular weights with the data of plasmid proteins *K. pneumoniae* in the NCBI and UniProtKB bases with using the ExPASy portal.

The cluster analysis results of 67 spectra were used to create a model, that consist of six classes. The aggregate efficiency of the classifier was 89.6%. The spectra of group #4 had a marker peak m/z = 5996 Da, which was comparable in molecular weight to the protein pKF140-142 of plasmid pKF3-140. The marker peak m/z = 6096 of group #2 was designated as a plasmid protein according it coincidence on molecular weight with the protein UUU_02980 of plasmid pKPt2. Sixteen sensitive strains were mainly classified in group #2, but their spectra lacked a plasmid peak m/z = 6096. Eight NDM-1 strains were assigned to different groups, however their spectra showed either the peak of the plasmid protein m/z = 6096 or the peak m/z = 5936 which was also identified as a plasmid protein according to the protein of the outer membrane receptor protein of the plasmid pF77. All OXA-48 strains were assigned to group #4 and their spectra contained the peak of the plasmid protein m/z = 5996.

It has been suggested that the mass spectra of carbapenem-resistant *K. pneumoniae* strains may contain peaks attributed to the plasmid-encoded proteins. Such small plasmid proteins, which molecular weight don't correspond to the carbapenemases, even so, can appear as predict biomarkers of carbapenemase activity of strains.

10. NEW CHEMOTHERAPY FOR THE TREATMENT OF INFECTIOUS DISEASES

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STUDY OF CYTOTOXIC AND ANTIPROLIFERATIVE ACTIVITY OF FUNGICIDAL SAPONIN TAUROSID Sx1 ON TRANSFORMED MAMMALIAN CELLS

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Saponins taurosids from the Crimean ivy are capable of enhancing the immune response in mice to HIV surface glycoproteins and influenza virus. It was shown that saponin taurosid Sx1 has a fungicidal activity against *Candida* spp. The aim of our work was to determine cytotoxic properties of saponin taurosid Sx1 on mammalian transformed cells such as MT-4 lymphoblastoid cell line and Vero fibroblast-like cell line.

The triterpene saponin taurosid Sx1 with the structure 3-O-α-Lrhamnopyranosyl(1→2)-α-L-arabinopyranoside hederagenin isolated from the Crimean ivy *Hedera taurica* Carr. *(Araliaceae)*, lymphoblastoid tumor cells of MT-4 line and fibroblast-like cells of the Vero line were used in the study. The saponin toxicity was determined with a methyltetrazolium test (MTT).

The effects of taurosid Sx1 taken in 0.019–50.0 μg/ml concentrations on MT-4 cells were assessed. The saponin concentration of 3.13 μg/ml was shown not toxic — the number of surviving cells was 81.56%. The marked toxic effects were observed with saponin concentrations 25 and 50 μg/ml — the number of surviving cells were 68.10% and 30.43%, correspondently. For Vero cells the non-toxic saponin concentration was 0.78 μg/ml (the number of surviving cells was 84.14%). Regarding Vero cells, taurosid Sx1 exhibited cytotoxic properties at lower concentrations — at 6.25 μg/ml. The number of surviving cells was 44.15%.

Cytotoxic concentrations of taurosid Sx1 from *Hedera taurica* Carr. *(Araliaceae)* are similar to the cytotoxic concentrations of triterpene saponins from plants such as *Albizia procera* and *Lysimachia thyrsiflora* L. Saponins from these plants exhibited cytotoxic and anti-proliferative properties for transformed and normal mammal cells at concentrations close to the cytotoxic concentrations of taurosid Sx1. This result allows us to consider taurosid Sx1 as a potent anti-fungal, anti-viral and immunomodulating agent, but also as a anti-proliferative substance possessing potential antitumor effects.

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ANTIMICROBIAL AND IMMUNOMODULATING ACTIVITY OF A TOPICAL GEL CONTAINING ACTIVE PEPTIDE COMPONENTS ON THE MODEL OF EXPERIMENTAL BACTERIAL VAGINITIS

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Common methods of therapy of bacterial vaginitis are not effective due to the spread of antibiotic resistance, side effects of antibiotics and insufficient immune response. One of modern approaches to the treatment of vaginitis are based on the use synthetic analogues of natural peptides. The objective of the study was to analyze the antibacterial and immunomodulatory effects of gel preparations based on chemically synthesized peptides on the model of experimental vaginitis.

White outbred female mice were infected *per vaginum* by pathogens: *Streptococcus agalactiae* and *Staphylococcus aureus* for 5 days. Then gels containing antimicrobial peptide pentadefenin (P), immunostimulating peptide alloferon (A) and both compounds were administered to the animals (groups P, A and PA, respectively) for 5 days. The control group (C) of the infected mice did not receive therapy. During course of therapy, the composition of the vaginal microbiocenosis was assessed using a bacteriological method and quantitative PCR. The concentration of IgA in vaginal lavages and IgM in serum were determined by ELISA.

Experimental vaginitis was accompanied by a change in the vaginal biocenosis: the number of lactobacilli decreased and the content of *Gardnerella* sp., *Prevotella* sp., and *Porphyromonas* sp. increased. Because of the therapy, a gradual decrease in the vaginal contamination of pathogenic bacteria occurred. Infection with *S. agalactiae* and *S. aureus* was observed in-group C throughout the observation period. The laboratory signs of bacterial vaginosis in the C group did not disappear, unlike other groups.

The drug P showed maximum antistreptococcal and antistaphylococcal effect only in the course of treatment. However, it acted only bacteriostatically and after its cancellation (day 9), the number of pathogenic bacteria became greater than in the group C. In groups P and PA, pathogenic bacteria practically disappeared, but this occurred only on the 9–14 days of the experiment. The antimicrobial effect of a correlated with an increase in the concentration of bacteriospecific IgA in the vaginal lavages and IgM in serum. Elimination of pathogenic bacteria occurred without the development of bacterial vaginosis, complications after antibiotic therapy or infection. Peptide P, as a bacteriostatic, should be used for a long time. The effect of peptide A is manifested only after the formation of a specific immune response. Thus, the maximum therapeutic effect should be expected in case of A and P mixture application.

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ANTIMICROBIAL ACTIVITY OF SYNTHETIC ANALOGOUS OF CAPRINE PEPTIDES BACTENECINS TOWARDS DRUG-RESISTANT BACTERIA

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Antimicrobial peptides (AMPs) of the innate immune system are unique molecules, providing human and animals host defense, and prototypes of novel drugs to fight

bacteria, resistant to conventional antibiotics. However, some cytotoxicity of the peptides towards host cells limits their use in medicine and points to the necessity of creation of AMPs analogs with optimized features. Our work is aimed to the analysis of the antimicrobial activity of structural analogs of proline-rich AMPs of the domestic goat Capra hircus leukocytes — bactenecins ChBac3.4, ChBac5 and ChBac7.5 against drug-resistant clinical isolates of gram-negative bacteria (*Pseudomonas aeruginosa* MDR 522/17, *E. coli* ESBL 531/17, *Acinetobacter baumannii* 7226/16, *Klebsiella pneumonia* 344/17) and examination of their hemolytic properties towards human erythrocytes. The broth microdilution assay was used to evaluate the minimal inhibitory concentrations (MIC) of chemically synthesized peptides, and it was shown that truncated variants of ChBac5 (1–23 — sequence from the 1st to 23rd amino acid residues) and ChBac3.4 (1–14) exerted a low activity in comparison with that of the full length peptides, while the peptide ChBac3.4 (1–19) had a significantly higher efficacy against all tested bacteria. We found that adding a fragment Arg-Phe-Arg to the peptides N-termini increased the antibacterial properties of the full length ChBac3.4, and to a much lesser extent of the truncated bactenecins. A significance of the His-including region (14–18) of ChBac3.4 has been explored: the peptide with modification in this region and a lack of His residue possessed a potent antimicrobial activity. The highest antibacterial effect was observed in the case of a chimeric peptide including N-terminal fragment of ChBac7.5 and a cystein-containing fragment of protegrin 1 (MICs of 0.5–4 microM). Analysis of the hemolytic activity of the studied AMPs revealed that all the peptides do not cause lysis of human erythrocytes in a range of concentrations from 1 to 100 microM, except of the chimeric peptide that induced a significant lysis of red blood cells. The structural-activity analysis of caprine bactenecins revealed most promising AMPs with potent antibacterial activity and a lack of the cytotoxic effects for human cells (in particular, analogs of ChBac3.4 with modification in 14-18 amino acids region) that point to the prospect of the further investigation of caprine batenecins aimed to the creation the novel pharmaceuticals to combat antibiotic-resistant bacteria.

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ANTI-INFLAMMATORY EFFECT OF ITRACONAZOLE IN PATIENTS WITH ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS

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The aim was to study the dynamics of immunological parameters in patients with ABPA on the background of the antifungal therapy.

The study included 11 patients with ABPA at the age from 29 to 78 years (median — 36 years). Allergological examination included skin tests with an allergens *A. fumigatus* ("Allergopharma", Germany). The levels of total IgE ("Polignost", Russia) and specific IgE (sIgE) to fungal allergens ("Alcor Bio", Russia) in serum were determined by enzyme immunoassay. Spontaneous production of interferon-γ (IFNγ) was determined in the culture supernatant of cells without the addition of inducers. To assess the mitogen-induced production of IFNγ, blood cells were incubated for 24 hours with PHA at a concentration of 50 mg/ml ("Sigma" USA). The production of IFNγ, activated by the allergen *A. fumigatus* ("Alcor Bio", Russia) at a concentration of 10 μg/ml, was determined on day 6. The resulting supernatants were used to determine spontaneous and induced IFNγ production by enzyme immunoassay using commercial test systems ("Vector-Best", Russia).

The prick test with *A. fumigatus* was positive, levels of sIgE to *A. fumigatus* (Me 1.56 (0.36÷10.56) IU/ml) and total IgE (Me 986 (873÷1695) IU/ml) were elevated in all ABPA patients. In the analyzed cases, according to the chest CT scans, focal and segmental lung infiltrations were detected in 6 (55%) patients, bronchiectasis — in 5 (45%). During the study, patients with ABPA were treated with itraconazole at a dose of 400 mg per day. In all patients after of therapy significant clinical effect was noted: decrease in dyspnea and cough, improvement in the lung function, and positive dynamics in chest CT scans. At a re-examination at 12 weeks, all patients had a statistically significant decrease in the level of sIgE to *A. fumigatus* (Me 0.66 (0.01÷5.24) IU/ml, $p = 0.003$) and total IgE (Me 540 (73÷613) IU/ml, $p = 0.003$). Was identified increased ability of blood cells to produce IFNγ in response to PHA stimulation of the blood cells (1914 (1294÷2232) vs 910 (852÷1648) pg/ml, $p = 0.004$) and to induction by the *A. fumigatus* allergen (48.0 (24.0÷61.0) vs 19.0 (2.0÷34.0) pg/ml, $p = 0.001$). The absolute number of eosinophils decreased ($p = 0.05$).

The tendency towards normalization of the immunological profile of patients in association with clinical signs improvement indicates the successful use of antifungal therapy in patients with ABPA.

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SAPONIN TAUROSID Sx1 ADMINISTRATION ENHANCES ANTIBODY PRODUCTION IN MICE, CHALLENGED WITH INFLUENZA VIRUS OR IMMUNIZED WITH INFLUENZA GRIPPOL® VACCINE

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Several saponins demonstrate antiviral and immune potentiating properties. In this work the influence of saponin Taurosid Sx1 on the anti-hemagglutinin (H) antibodies production has been studied in influenza virus (IV) challenged or GRIPPOL® vaccinated mice.

BALB/c mice were challenged intranasally with 50 μl (10 LD_{50}) of the A/WSN/1/33(H1N1) virulent strain or immunized with polymer-subunit GRIPPOL® vaccine season 2005/2006. A standard vaccine dose contained per 5 μg of H1 and H3, 11 μg of H from the IV type B. Taurosid Sx1 saponin was derived from *Hedera taurica* Carr. (Araliaceae). The blood serum levels of anti-H antibodies had been determined by Hemagglutination Inhibition (HI) test with the virulent A/WSN/1/33(H1N1) strain or standard kits of IV diagnostic strains (DS). Mice were vaccinated intramuscularly (i.m.) with 0.1 ml 10-times diluted vaccine. Control group was given isotonic sodium chloride saline solution (ISS). Within 3 days after vaccination or the virulent IV challenge animals were given 200 μg/mouse/day of saponin orally. Control mice were given ISS. Statistical analyses was based on a middle means of the reverse titers of anti-H antibodies calculations (M±m), and an unpaired two sample Student-t test. P values of $P \leq 0.05$ were considered as significant (*).

A significant increase of anti-H antibody titers in mice challenged with the virulent A/WSN/1/33(H1N1) strain and treated with Taurosid Sx1 was seen on the day 4, compare with the control group (128.0±19.6* and 80.0±15.1, correspondently). By the day 14 the difference was not significant. When vaccinated mice were given Taurosid Sx1, the rising of anti-H1 antibody titers was not significant on the day 4, but increased significantly 10 times to compare with the control group antibody titers by the day 14 (1280.0±286.2* and 120.0±25.3, correspondently). Oral administration of Taurosid Sx1 stimulated also production of antibody against IV type B hemagglutinin of DS on the day 4 (100.0±20.0* versus 40.0±0.0 in control). Use of the saponin did not influence significantly on the anti-H3 antibody production.

This study has shown that oral administration of 200 μg/mouse/day Taurosid Sx1 within 3 day after virulent IV challenge or GRIPPOL® vaccination selectively stimulates development of anibodies specific to IV type B hemagglutinin and anti-H1 anibodies generated by both virus infection and vaccination.

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ANTIBACTERIAL ACTIVITY OF SYNTHESIZED COMPOUNDS FROM DIFFERENT CLASSES OF CHEMICALS

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The main problem in bacteriology is a constant increase a number of antibiotic-resistant strains of bacteria. Therefore, the purpose of our work is the study of new developed chemicals.

We investigated the antibacterial effect of three groups of compounds derived from synthetic and natural substances: 2 groups are based on fluoroquinolonic acid (30 substances) and derivatives of 1.2,4-triazoles (25 substances). The third compound (CH-II) is synthesized from natural substances (there is a patent). It contains organic components: pyrogallol, glycerol, succinic acid, tannins, gallic acid, mannose, fructose, myo-inositol, glucose, ribitol and microelements and minerals includes about 60 names. As a model, we used reference strains of microorganisms and isolated in hospitals (with multiple resistance): *Esherichia coli*, *Klebsiella pneumonia, Pseudomonas aeruginosae, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Moraxella catarrhalis, Corynebacterium diphtheriae*. The sensitivity to preparations was studied by the method of the minimum inhibitory concentration (MIC) in accordance with the recommendations of EUCAST 8.0.

Several substances from the first group of compounds showed very good results with reference strains: MIC for all representatives of *Enterobacteriales* was less than 0.1 mg/l, for *Staphylococcus* spp. less than 0.5 mg/l, *E. faecalis* 2 mg/l, *M. catarrhalis* 0.1 mg/l, *C. diphtheriae* 1 mg/l. All reference and hospital strains were sensitive to this group.

Several substances from the second group of compounds also showed good results: MIC for different representatives of *Enterobacteriales* was different, but did not exceed 0.3 mg/l, for *Staphylococcus* spp. less than 0.5 mg/l, *E. faecalis* 4 mg/l, *M. catarrhalis* 0.2 mg/l, *C. diphtheriae* 2 mg/l. For all reference strains, the results were in the sensitivity zone. The best results among hospital strains were obtained on Grampositive bacteria: they were all sensitive.

The compound CH-II has good bactericidal properties. However, its MIC for all bacteria is slightly higher and is within the limits of intermediate values. This does not preclude the recognition of CH-II as an effective compound, since it is theoretically and practically (in toxicological researches) shown to be harmless when using the concentrations obtained.

Thus, the new groups of compounds obtained have a good antibacterial activity at MIC and can in the future take a worthy place among drugs with antibacterial action.

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DEVELOPMENT OF MAGNETICALLY CONTROLLED ANTIBACTERIAL COMPLEX EFFECTIVE AGAINST BIOFILMS

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The problem of effective therapeutic treatment of chronic and acute inflammation caused by the microbial biofilms development and vital activity is still actual today due to the resistance of bacteria to the most frequently antibiotic substances used in the medical practice. For recent years there are the most promising to use nanocomposite materials that can penetrate into the biofilms internal environment in a lightweight way. In particular magnetic particles in the composition of antibacterial agents can increase its effectiveness. Thus, the purpose of this work was to develop a new composite material with magnetically controlled properties and high antimicrobial activity.

As the main frame material was chosen amorphous calcium carbonate which entrapped ciprofloxacin. Composite matrix also consists of magnetite nanoparticles giving it magnetic properties. The active substance targeted release from nanocomposite occurs through calcium carbonate magnetoinductive recrystallization under the high frequency magnetic field action.

Experimental studies conducted on biofilms of two types of bacteria (gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli*) also proved a well-manifested composite based on calcium carbonate and magnetite antibacterial effect. The antibiotic in its initial form showed less effect on bacteria compared to the drug with immobilized antibiotic: the difference was 24–38% against *E. coli* and 50–76% against *S. aureus*. The antibacterial effect was also exerted by unentrapped nanocomposite particles without antibiotic which makes it possible to judge the possibility of providing a synergistic effect.

Thus, increasing the effectiveness of antibiotic substances on bacterial biofilms can be achieved (1) due to the nanocomposite magnetic targeting into which it is entrapped and its maximum localization in the inflammation focus, (2) due to the composite magnetic attraction into the biofilm and easier antibiotic penetration into biofilm because of its partial disintegration and (3) due to the synergistic effect in the form of biofilm local alkalization.

Further research is aimed at optimizing the method of obtaining an effective magnetically controlled drug and a comprehensive study of its impact on bacteria species biofilms the most important in medical practice as well as to assess its biocompatibility.

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A STUDY OF THE POTENTIAL OF SPIDER SILK USE FOR THE DEVELOPMENT OF ANTIBACTERIAL DRUGS

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Spidroin is the natural material possessed unique properties such as biocompatibility, noninflammatory property, controllable biodegradation, hydrophobicity. It can be used as functional biomaterial after processing and post-processing. In recent years the functional hybrid materials based on spidroin have been developed for the various biomedical and nanotechnological applications including drug delivery, tissue engineering, nanostructured optics, nanoelectronics, sensors, filtration, surface modification. In this study a hybrid consisting of spider silk and metal oxides that can generate active molecular forms as a result of interaction with peptides was investigated. Their biological properties were studied.

Hybrid materials were obtained by precipitation of nanoparticles of tungsten and molybdenum oxides from hydrosol. The natural silk was obtained in insectarium where the spiders *Linothele fallax* are grown. A preliminary study was conducted on the activation of the web hybrids in a contact with two types of the test-microorganisms: gram-positive *Staphylococcus aureus* 209 R and gram-negative *Escherichia coli* XL-1. The study of antibacterial properties of hybrid silk-based material was carried out by agar diffusion method.

The spider silk and their composites had a greater impact on the gram-positive type of bacteria itself. The results obtained for the hybrid material are comparable to the concentration dependence of the effect of nanoparticle solutions on bacterial cells. Moreover, a tendency for the synergistic effect of spider silk with deposited composite metal oxides on it was observed. In addition, there was a color change zone the dense medium. The colored area is considered as the result of interaction of a silk-based composite with the peptides of the medium and the exogenous proteins secreted by bacteria.

The results show a positive trend that requires further study to verify the possibility of creating a new biomaterial as effective antibacterial complex.

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CAPSULE SPECIFIC POLYSACCHARIDE DEPOLYMERASES OF *KLEBSIELLA PNEUMONIAE* BACTERIOPHAGES: IMPLICATION IN TYPING AND TREATMENT

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To overcome the carbohydrate barrier of bacteria, many bacteriophages use specific enzymes, polysaccharide-depolymerases (PS-dep), which destroy bacterial polysaccharide capsules, thereby ensuring the adsorption of the phage at the outer membrane receptors, the penetration of phage DNA, and the lysis of the bacterial cell. Phage depolymerases are an attractive and promising means for controlling pathogenic bacteria, such as *K. pneumoniae*, whose main virulence factor is a pronounced polysaccharide capsule.

The aim of the work is to characterize the specificity and anti-bacterial (anti-virulence) potential of polysaccharide depolymerases encoded by capsule specific *K. pneumoniae* bacteriophages.

We cloned and expressed genes PS-dep of the phages KpV71, KpV74 and KpV79, lytic for *K. pneumoniae* of capsule types K1, K2 and K57, respectively, into the *E. coli* cells. The recombinant proteins Dep_kpv71, Dep_kpv74, and Dep_kpv79 were isolated and purified and the PS-degrading activity of the recombinant proteins was demonstrated. The spectrum of activity of PS depolymerases against to *K. pneumoniae* strains of different phenotypes and genotypes was determined. It was shown that recombinant proteins are more specific to polysaccharides of the corresponding types than "parent" phages.

It was found that the depolymerases Dep_kpv74 and Dep_kpv79 are specific glycosidases that cleave the *K. pneumoniae* polysaccharides of capsular types K2 and K57 by β-glucoside and β-galactoside bonds, respectively, to form monomers and dimers of the tetrasaccharide repeating unit of the polysaccharide. Protein Dep_kpv74 is a bifunctional protein and, in addition to β-glucosidase activity, determines, as assumed, the phage binding with the primary bacterial receptors, the capsular polysaccharides.

In vitro and *in vivo* experiments showed that treatment of virulent hypermucoid strains of K2- or K57-type *K. pneumoniae* with Dep_kpv74 or Dep_kpv79, respectively, leads to a significant decrease in *K. pneumoniae* strain virulence in mice and ensures the survival of animals in the development of *K. pneumoniae*-sepsis.

In conclusion, the obtained data testify to the perspectives of using of phage PS depolymerases for *K. pneumoniae* capsular typing, as well as for treatment of *K. pneumoniae*-infections.

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DEVELOPMENT OF A NEW ANTI-INFLUENZA COMPOUND BASED ON CAMPHOR SCAFFOLD

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Despite success in chemotherapy and vaccine development, influenza remains a hard-to-control infection due to high genetic variability and long-term complications after the acute stage leading to a "hidden" or secondary mortality caused not by the influenza virus itself but by virus-induced secondary processes. The use of antiviral compounds leads to the rapid emergence of resistant strains. Therefore, the development of new anti-influenza drugs with new targets and other mechanisms of action is an important task of medical science and practical public health worldwide.

We identified a group of derivatives of natural terpenoids that exhibit a high level and a wide spectrum of activity against influenza viruses. Among them, camphecene (1.7,7-trimethylbicyclo [2.2.1] heptane-2-ylidene-aminoethanol) is one of the most active, possessing virus-inhibiting properties against influenza viruses A and B, both *in vitro* and in experiments on laboratory animals. The selectivity index (chemotherapeutic index) for influenza virus was 74–661, depending on the type and subtype of the virus, the protection index in animal experiments was 67% for influenza A and 89% for influenza B. The mechanism of camphecene activity was the suppression of fusogenic activity of the viral hemagglutinin,

which prevents the process of fusion of the viral and cell membranes. Using sequential passages, a laboratory selection of camphecene-resistant strains was carried out and the resistance-conferring mutation V458L located in the HA2 subunit was localized. When comparing the properties of the control and camphecene-resistant strains, it was shown that the pathogenicity of the latter for animals was at least 50 times lower than for a strain passaged without camphecene. No mortality was observed in group of animals inoculated with the resistant virus regardless of the infectious dose of the virus.

Thus, camfecine is a new promising anti-viral compound with a different mechanism of action and a different target as compared to those already used in the clinic.

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COMBINED ANTIBACTERIAL ACTIVITY OF ANTIMICROBIAL PEPTIDES AND ANTISEPTIC AGENTS

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Antimicrobial peptides (AMPs) are potent bactericidal molecules of innate immune system effective against antibiotic-resistant bacteria.

In this study we investigated the action of highly membranolytic AMP protegrin-1 (PG1) and of a goat bactenecin 3.4 kDa (ChBac3.4) from bactenecins' family, which members possess also intracellular targets, in combinations with a number of antiseptic agents against antibiotic-resistant clinically isolated bacteria *Escherichia coli* ESBL 521/17 (resistant to ampicillin, amoxicillin/clavulonic acid, cefotaxime, ceftazidime, cefixime, aztreonam, netilmicin, ciprofloxacin, trimethoprim/sulfamethoxazole), *Pseudomonas aeruginosa* MDR 522/17 (meropenem, ceftazidime, cefixime, amikacin, gentamycin, netilmicin, ciprofloxacin, colistin), *Klebsiella pneumoniae* ESBL 344/17 (ampicillin) and *Acinetobacter baumannii* 7226/16 (imipenem, gentamicin, tobramycin, ciprofloxacin, trimethoprim/sulfamethoxazole), *Staphylococcus aureus* 1399/17 (ampicillin, oxacillin, gentamicin, amikacin, ofloxacin), *Staphylococcus epidermidis* isolates 9/17, 10/17, 24/17, 33/17 (various fluoroquinolones), and against laboratory ampicillin-resistant strain *Escherichia coli* ML-35p.

Based on fractional inhibitory concentration indices (FICI) assessed by checkerboard titration (FICI ≤ 0.5 synergy; 0.5 < FICI ≤ 1 additivity; 1 < FICI ≤ 2 independent action; FICI > 2 antagonism), AMPs and sodium hypochlorite were found to be antagonistic. Most numerous and prominent cases of synergy were revealed in combinations of AMPs with poviargolum (a colloidal silver preparation), that corresponds with previous studies on AMPs and silver nanoparticles interaction. In combination with dioxidin PG1 showed synergy against gram-positive bacteria and ChBac3.4 against *A. baumannii* 7226/16 and *K. pneumoniae* ESBL 344/17. Prontosan was synergistic with AMPs against gram-positive bacteria and with PG1 also against *E. coli* ML-35p. Etidronic acid, that was shown to inhibit β-lactamases, acted synergistically with AMPs against *E. coli* strains and *S. aureus* 1399/17 and in case of ChBac3.4 also against *A. baumannii* 7226/16 and *S. epidermidis* 33/17. Besides sodium hypochlorite other cases was mostly additive. Using resazurin metabolic marker we found that dioxidin and prontosan significantly hasten the effect of PG1, and poviargolum of both AMPs, on the metabolic activity of bacteria. Thus, combined use of AMPs with antiseptics has perspectives.

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EVALUATION OF THE EFFECTIVENESS OF PNEUMOCOCCAL VACCINES FOR PREVENTION OF COMMUNITY-ACQUIRED PNEUMONIA IN SERVICEMEN

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In 2011 13-valent pneumococcal conjugate vaccine "Prevenar 13" (PCV13) was licensed in our country. It is more immunogenic, induces stronger immunity than non-conjugate polysaccharide vaccines Pneumo 23 and Pneumovax 23 (PPV23). In this regard, PCV13 appears to be preferable for the prevention of pneumonia in military personnel than PPV23 which is used now.

The aim of the study was to conduct testing of PCV13 for the prevention of pneumonia in military teams, to compare the epidemiological effectiveness of conjugate and non-conjugate polysaccharide pneumococcal vaccines.

The study was conducted in 3 groups of conscripts. In the first group of soldiers of 571 people, 407 (71.3%) were vaccinated with PCV13. In the second group of 663 people, 472 (71.2%) were vaccinated with pneumococcal polysaccharide vaccines (PPV23). The third group, which included 493 unvaccinated soldiers, formed a comparison group. All military personnel were recruits of the same age (18–22 years) and were in comparable conditions of service and life.

During 5 months of follow-up, the incidence of community-acquired pneumonia among vaccinated by PCV13 was 4.5 times less than in the comparison group, and among vaccinated by PPV23 — 2.8 times less ($p < 0.001$). The index of effectiveness of PCV13 (4.5) was 1.6 times higher than that of PPV23 (2.8). The indicator of the effectiveness of PCV13 made up of 77.7%, PPV23 — 64.3%. Thus, the epidemiological efficiency of PCV13 was 1.2 times higher than that of PPV23.

In the first and second groups of servicemen, the incidence of pneumonia was lower than in the comparison group, not only among vaccinated, but also among unvaccinated. Moreover, the incidence of pneumonia among those not covered by vaccination in the group where PCV13 was used was 1.6 times lower than in the group where PPV23 was used ($p < 0.001$), which is explained by the formation of a stronger collective immunity preventing the circulation of pneumococci during vaccination with conjugated vaccine.

On the basis of the data obtained, it is preferable to vaccinate recruits a month before the call and conscripts, not vaccinated before the call to the armed forces of the RF, by pneumococcal conjugate vaccine.

ПРАВИЛА ДЛЯ АВТОРОВ

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Статья должна описывать результаты законченного исследования. Допускается объем статьи до 20 машинописных страниц, включая рисунки, таблицы. Статья должна содержать: 1) введение; 2) материалы и методы; 3) результаты исследований; 4) обсуждение результатов; 5) благодарности.

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Описание статьи из журнала:

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Salina T.Yu., Morozova T.I. Immunological methods in differential diagnostics. Tuberculosis and Lung Diseases, 2011, vol. 88, no. 11, pp. 50–53.

Описание статьи из книги (монографии):

Шурыгина И.А., Чеснокова М.В., Климов В.Т. Псевдотуберкулез. Новосибирск: Наука, 2003. 320 с.

Shurygina I.A., Chesnokova M.V., Klimov V.T. Pseudotuberculosis. Novosibirsk: Nauka, 2003. 320 p.

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Turenne C.Y., Wallace R., Behr M.A. Mycobacterium avium in the postgenomic era. Clin. Microb. Rev., 2007, vol. 20, no. 2, pp. 205–229.

Goodman J.W., Parslow T.G. Immunoglobulin proteins. Basic and Clinical Immunology. Ed. Stites D.P., Terr A.I., Parslow T.G. Appletion & Lange, 1994, pp. 66–79.

Ссылки на литературные источники в тексте статьи, в рисунках и таблицах обозначаются арабскими цифрами в квадратных скобках [1, 2, 3,...]. Не допускаются ссылки на диссертации, авторефераты диссертаций, публикации в сборниках, методические документы местного уровня. Количество источников не ограничено. В каждой ссылке приводятся все авторы работы. Неопубликованные статьи в список не включаются.

Обозначения, сокращения и единицы измерения

Для сложных терминов или названий, наиболее часто используемых в тексте статьи, можно ввести (в круглых скобках после первого упоминания полного названия термина) не более 3–5 нетрадиционных сокращений. Узаконенные международными номенклатурами сокращения используются в соответствующей транскрипции. Например, для термина «интерлейкин» используется сокращение «IL», а не русскоязычный вариант «ИЛ»; аналогично этому используются сокращения: «TNF», а не «ТНФ» или «ФНО»; «CD», а не «СД». Названия микроорганизмов приводятся в оригинальной транскрипции с использованием курсива (*E. coli, Streptococcus pyogenes*). Единицы измерения приводятся без точки после их сокращенного обозначения, регламентированного международными правилами (с, ч, см, мл, мг, kDa и т.д.).

Оформление иллюстративного материала

Иллюстративный материал должен быть оригинальным, т.е. ранее нигде не опубликованным. Общее количество иллюстраций (таблиц и рисунков) не должно превышать восьми. При большем количестве иллюстраций их публикация оплачивается автором. Публикация цветных иллюстраций (независимо от их количества) также оплачивается автором.

Размеры иллюстраций:

- максимальная высота — 210 мм
- максимальная ширина для 1 столбца — 82 мм, для 2 столбцов — 170 мм

Таблицы. Каждая таблица предоставляется отдельным файлом. Таблицы нумеруются арабскими цифрами отдельно от нумерации рисунков (графиков и фотографий). Название печатается над таблицей. Весь текст на русском языке, содержащийся в таблице, включая единицы измерения, должен быть переведен на английский язык; при этом перевод следует помещать в ячейку с соответствующим русским текстом отдельной строкой. Название таблицы и текст примечания к ней также должны быть переведены на английский язык и приведены под русским текстом с новой строки. Для пометок в таблицах следует использовать одну или несколько (*). Пояснения печатаются после соответствующего количества (*) под таблицей. Единицы измерения, при необходимости, включаются в заголовки строк или столбцов.

Рисунки (графики и фотографии). В тексте статьи названия рисунков (графиков, фотографий) и таблиц размещаются сразу после абзаца, где на них дается первая ссылка. Все рисунки нумеруются последовательно арабскими цифрами по мере их включения в текст статьи. Названия рисунков и подписи к ним выносятся в виде списка в отдельный файл. В списке указываются: номер рисунка, название (с большой буквы), текст примечаний (для микрофотографий должно быть указано увеличение). Подписи к рисункам даются краткие, но достаточно информативные. Названия рисунков и примечаний к ним, нарисуночные подписи, текст легенды должны быть переведены на английский язык и размещены под соответствующим текстом с новой строки. Рисунки могут быть представлены в графических форматах с расширением .tif (разрешение не менее 300 dpi при 100% масштабе), .eps или .ai. Изображения, встроенные в документы Word, не принимаются. Графики и диаграммы предоставляются вместе с таблицами, на основе которых они были созданы, или с численными обозначениями показателей, отображаемых соответствующими графическими элементами (столбиками, секторами и т.п.) в виде файлов с расширениями .doc или, предпочтительнее, .xls.

Плата за публикацию статей

При соблюдении правил публикация статей в журнале «Инфекция и иммунитет» является бесплатной для авторов и учреждений, в которых они работают. Редакция может потребовать оплату в следующих случаях: 1) за публикацию цветных иллюстраций; 2) при большом количестве иллюстративного материала (свыше 8 иллюстраций).

Подготовка статей

При предоставлении статьи авторы должны руководствоваться требованиями, приведенными в нижеследующих пунктах. Статья может быть отклонена, если она им не соответствует.

1. Направляя статью в журнал, авторы гарантируют, что поданные материалы не были ранее опубликованы полностью или по частям, в любой форме, в любом месте или на любом языке. Так же авторы гарантируют, что статья не представлена для рассмотрения и публикации в другом журнале. С момента принятия статьи к печати в журнале «Инфекция и иммунитет» приведенный в ней материал не может быть опубликован авторами полностью или по частям в любой форме, в любом месте и на любом языке без согласования с руководством журнала. Исключением может являться: 1) предварительная или последующая публикация материалов статьи в виде тезисов или короткого резюме; 2) использование материалов статьи как части лекции или обзора; 3) использование автором представленных в журнал материалов при написании диссертации или книги. Воспроизведение всего издания или части любым способом запрещается без письменного разрешения издателей. Нарушение закона будет преследоваться в судебном порядке. Охраняется Законом РФ № 5351-1 «Об авторском праве и смежных правах» от 09.07.93 г.
2. Файл отправляемой статьи представлен в формате .doc, .docx, .rtf.
3. Помимо файла со статьей, предоставлены следующие файлы:
   1) Файл с метаданными (при загрузке в систему ему присваивается имя «Метаданные»):
      - фамилия, имя, отчество, ученая степень, ученое звание, должность автора, ответственного за дальнейшую переписку с редакцией (на русском и английском языках);
      - название учреждения, где работает ответственный автор (в русском и официально принятом английском вариантах);
      - почтовый адрес для переписки с указанием почтового индекса (на русском и английском языках);
      - телефон, факс (с указанием кода страны и города), e-mail;
      - фамилия и инициалы остальных соавторов, их ученые степени, ученые звания, должности;
      - полное название статьи, направляемой в редакцию;
      - количество страниц текста, количество рисунков, количество таблиц;
      - раздела журнала, для которого предназначена данная работа: «Лекции», «Обзоры», «Оригинальные статьи», «Краткие сообщения», «В помощь практическому врачу»;
      - дата отправления работы.
   2) Отсканированная копия файла с метаданными подписанная всеми авторами (при загрузке в систему ему присваивается имя «Подписи авторов»).

3) Титульный лист (при загрузке в систему ему присваивается имя «Титульный лист»), по форме:
   - название статьи (без использования каких-либо сокращений, на русском и английском языках);
   - фамилия, имя, отчество, ученая степень, ученое звание, должность каждого из соавторов статьи (полностью, на русском и английском языках);
   - подразделение и учреждение, в котором выполнялась работа; в случае, если авторами статьи являются сотрудники разных учреждений, то последние нумеруются по порядку, начиная с единицы, и соответствующая цифра размещается после фамилии автора, представляющего данное учреждение; для маркировки авторов в англоязычной части статьи вместо цифр используются латинские буквы (a, b, c, d и т.д.);
   - сокращенное название статьи для верхнего колонтитула (не более 35 символов, включая пробелы и знаки препинания, на русском и английском языках);
   - не менее 6 ключевых слов на русском и английском языках;
   - адрес для переписки с указанием номера телефона, факса и адреса e-mail.
4) Резюме (при загрузке в систему ему присваивается имя «Резюме»). Предоставляется в виде одного абзаца без ссылок и специфических сокращений. Объем — не менее 300 слов. Резюме в полном объеме представляется также в переводе на английский язык. В отдельных случаях, по решению редакционной коллегии, может быть затребован развернутый вариант резюме на английском языке.
5) Рисунки, если они есть — каждый отдельным файлом (при загрузке в систему каждому рисунку присваивается имя «Рисунок_Порядковый номер рисунка. Название рисунка»).
6) Файл в формате .doc, .docx, .rtf со списком, в котором указываются: номер рисунка, название (с большой буквы), текст примечаний (для микрофотографий должно быть указано увеличение). Подписи к рисункам даются краткие, но достаточно информативные.
7) Таблицы, если они есть — каждая отдельным файлом (название каждой таблицы должно быть приведены заголовком в файле с самой таблицей).
8) Файл с цитируемой литературой (при загрузке в систему ему присваивается имя «Литература») в виде таблицы из четырех столбцов (альбомная ориентация), где:

Порядковый номер ссылки	**Авторы, название публикации и источника, где она опубликована, выходные данные**	**Ф.И.О., название публикации и источника на английском языке**	**Полный интернет-адрес (URL) цитируемой статьи и/или ее DOI**
Размещаются в таблице в алфавитном порядке, вначале русскоязычные, затем на языках с латинской графикой	Указывать по библиографическому стандарту, представленному выше	Официальное англоязычное название публикации и источника, где она опубликована — для русскоязычных статей. В редких случаях, когда не существует официальных англоязычных названий, редакция просит предоставлять их перевод, обозначая его красным цветом шрифта. **Для англоязычных публикаций и источников в этом столбце ставится прочерк**	В том случае, если информация о статье не размещена на официальном сайте издания, допустимо использовать URL статьи со сторонних сайтов, в т.ч. системы **www.e-library.ru**. DOI статьи приводится в квадратных скобках после URL-адреса

4. Текст должен быть набран с одинарным межстрочным интервалом; используется кегль шрифта в 14 пунктов; для выделения используется курсив, а не подчеркивание; все ссылки на иллюстрации, графики и таблицы расположены в соответствующих местах в тексте, а не в конце документа.
5. Текст соответствует стилистическим и библиографическим требованиям.
6. Если вы отправляете статью в рецензируемый раздел журнала, то вы согласны с требованиями слепого рецензирования, подробнее о котором можно узнать на сайте журнала **(http://iimmun.ru)** в рубрике **«Рецензирование»** раздела **«О журнале»**.

АВТОРСКИЙ УКАЗАТЕЛЬ

ПРЕДМЕТНЫЙ УКАЗАТЕЛЬ

Иллюстрации к статье «Продукция биофильмов клиническим штаммами возбудителя туберкулеза» (авторы: О.Б. Огарков, А.Е. Суздальницкий, П.А. Хромова, Т.А. Цыренова, Н.А. Сокольникова, С.Н. Жданова, М.Е. Кощеев) (с. 435–440)

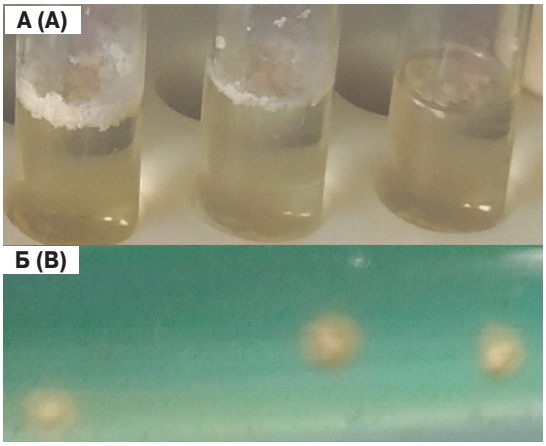

Рисунок. А. Градации в интенсивности продукции БФ (слева на право: ++++, +++, ++). Б. Морфология колоний штаммов МБТ, способных к продукции БФ (колонии имеют R-форму с выпуклым центром окруженным диском вторичного роста с фестончатым краем)

Figure. A. BF production intensity scale (left to right: ++++, +++, ++). B. Morphology of MBT strain colonies capable of BF production. Colonies adopted an R-shape with a convex center surrounded with a disc of secondary growth bearing a scalloped edge

Иллюстрация к статье «Результаты молекулярной детекции и характеристика вирусов гриппа и других возбудителей респираторных инфекций в России, сезон 2017–2018 гг.» (авторы: А.А. Соминина, Д.М. Даниленко, А.Б. Комиссаров, А.В. Фадеев, М.М. Писарева, М.Ю. Еропкин, Н.И. Коновалова, П.А. Петрова, А.А. Штро, К.А. Столяров, Л.С. Карпова, Е.И. Бурцева, А.В. Васин) (с. 473–488)

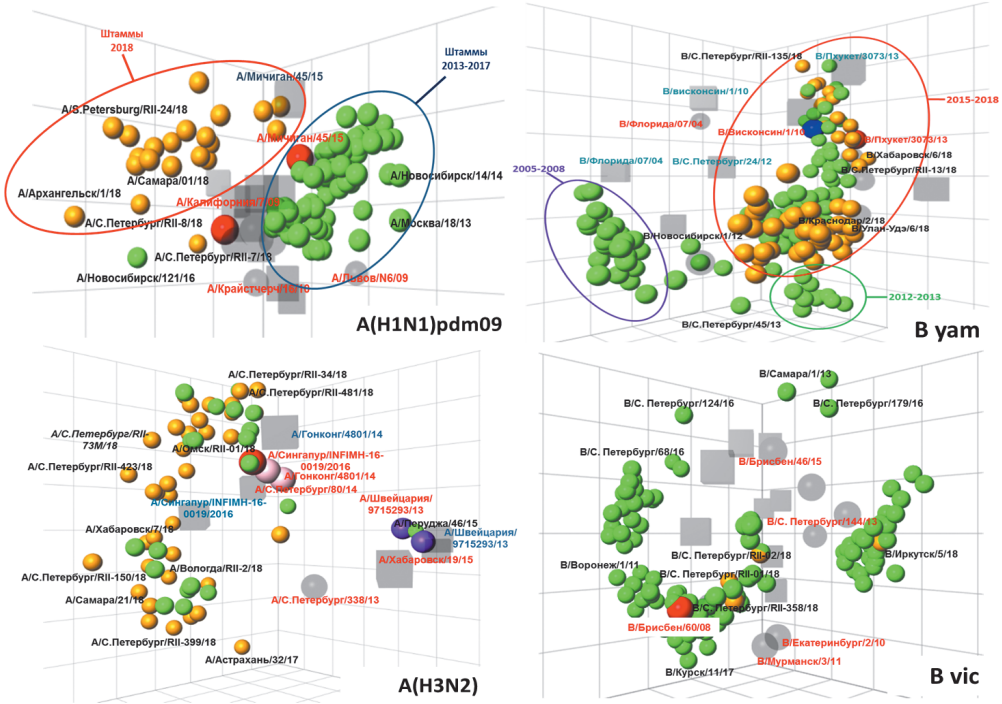

Рисунок 7. Антигенное картирование вирусов гриппа A(H1N1)pdm09, A(H3N2) и вирусов гриппа В Викторианской и Ямагатской разновидностей, циркулировавших в сезоне 2017–2018 гг. в России

Figure 7. Antigenic mapping of A(H1N1)pdm09, A(H3N2) as well as Victoria and Yamagata B influenza vuruses circulating during 2017–2018 season in Russia

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