St. Petersburg Pasteur Institute, Russia
Laboratory of Molecular Epidemiology and Evolutionary Genetics

Activity report, 2019-2020: TUBERCULOSIS AND OTHER MYCOBACTERIA

Researchers of laboratory involved in these activities

Head of Laboratory: Igor Mokrousov

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PROJECTS AND COLLABORATIONS

Funded Projects

– Russian Foundation for Basic Research project №17-54-30020 «A personalized approach to fight the HIV and drug resistant TB epidemic in Irkutsk, Siberia» (project PI – O. Ogarkov, Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk, Russia), 2017-2019.

- Russian Foundation for Basic Research project 18-04-01035 "Investigation of the role of the repeat element IS6110 in the micro- and macroevolution of Mycobacterium tuberculosis phylogenetic lineage 2" (PI - E. Shitikov, Center of Physico-Chemical Medicine, Moscow), 2018-2019

- Russian Foundation for Basic Research, Project #19-04-00263 "Pathogenomic features and epidemic potential of highly resistant strains of ancient sublineage of Mycobacterium tuberculosis Beijing genotype" (PI – I. Mokrousov), 2019-2020

- Joint project with National Institute for Public Health and Environment (RIVM, Bilthoven, Netherlands) on drug resistant tuberculosis, 2018-2020 (co-PI: I. Mokrousov and R. Anthony). 2018-2021

– Russian Science Foundation, Project 19-14-00013 ("Uneven evolutionary and epidemic trajectory of the paradoxical ancient subtype of the East Asian lineage of Mycobacterium tuberculosis: stochastic fluctuations or causative correlations?" PI – Igor Mokrousov), 2019-2021.

– Russian Science Foundation, Project 19-15-00028 ("Development of new efficient compounds against drug resistant Mycobacterium tuberculosis taking into account the population structure of the pathogen" PI – Anna Vyazovaya), 2019-2021.

- Project supported by PTR program of Institut Pasteur Paris "Transcriptional Response for AntimiCrobial Resistance detection in TB" (Coordinator - An van den Bossche, Belgium; Russian PI- Igor Mokrousov). 2019-2021.

- Russian Foundation for Basic Research, project 20-04-00686 "Deep machine learning methods in Mycobacterium tuberculosis genomics for the building of an open platform for the analysis of the pathogen's evolutionary signatures" (PI - E. Shitikov, Center of Physico-Chemical Medicine, Moscow), 2020-2022

- Russian Foundation for Basic Research, project 19-515-55009 (joint project co-funded by National Natural Science Foundation China) "Integral insight into development of drug resistant tuberculosis in adults versus children: impact of bacterial strain and surrounding

microbiome" (PI - Dr Zhdanova, Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk, Russia), 2020-2022

International collaborations

National Institute for Public Health and the Environment, RIVM (2018-2021), Beijing Children's Hospital, China (2017-2021), North Estonian Medical Centre (Tallinn Estonia), Biomedical Research and Study Centre, University of Riga (Latvia), Stephan Angeloff Institute of Microbiology and Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences (Sofia, Bulgaria), National TB Reference Laboratory, University Hospital Shefqet Ndroqi (Tirana, Albania), Department of Applied Microbiology, Institute of Microbiology, Faculty of Biology, University of Warsaw (Poland), Instituto de Investigação do Medicamento, Faculdade de Farmácia, Universidade de Lisboa (Lisboa, Portugal), Instituto de Investigación Sanitaria Gregorio Marañón (Madrid, Spain).

National collaborations

Central Research Institute for Epidemiology (Moscow), Omsk State Medical University, Scientific Center of Family Health and Reproductive Problems (Irkutsk), Ural Research Institute of Phthisiopulmonology (Ekaterinburg), Anti-tuberculosis dispensaries in Kaliningrad, Petrozavodsk (Karelia), Syktyvkar (Komi), Murmansk, Pskov.

MAJOR RESEARCH RESULTS

Genomic analysis of clinical *M. bovis* BCG isolates

Background: The only licensed live Bacille Calmette-Guérin (BCG) vaccine used to prevent severe childhood tuberculosis comprises genetically divergent strains with variable protective efficacy and rates of BCG-induced adverse events. The whole-genome sequencing (WGS) allowed evaluating the genome stability of BCG strains and the impact of spontaneous heterogeneity in seed and commercial lots on the efficacy of BCG-vaccines in different countries. Our study aimed to assess sequence variations and their putative effects on genes and protein functions in the BCG-1 (Russia) seed lots compared to their progeny isolates available from immunocompetent children with BCG-induced disease (mainly, osteitis).

Results: Based on the WGS data, we analyzed the links between seed lots 361, 367, and 368 used for vaccine manufacture in Russia in different periods, and their nine progeny isolates recovered from immunocompetent children with BCG-induced disease. (Fig. 1) The complete catalog of variants in genes relative to the reference-genome (GenBank: CP013741) included 4 synonymous and 8 nonsynonymous single nucleotide polymorphisms, and 3 frameshift deletions. Seed lot 361 shared variants with 2 of 6 descendant isolates that had higher proportions of such polymorphisms in several genes, including *ppsC*, *eccD5,* and *eccA5* involved in metabolism and cell wall processes and reportedly associated with virulence in mycobacteria. One isolate preserved variants of its parent seed lot 361 without gain of further changes in the sequence profile within 14 years.

Conclusions: The background genomic information allowed us for the first time to follow the BCG diversity starting from the freeze-dried seed lots to descendant clinical isolates. Sequence variations in several genes of seed lot 361 did not alter the genomic stability and viability of the vaccine and appeared accumulated in isolates during the survival in the human organism. The impact of the observed variations in the context of association with the

development of BCG-induced disease should be evaluated in parallel with the immune status and host genetics. Comparative genomic studies of BCG seed lots and their descendant clinical isolates represent a beneficial approach to better understand the molecular bases of efficacy and adverse events during the long-term survival of BCG in the host organism.

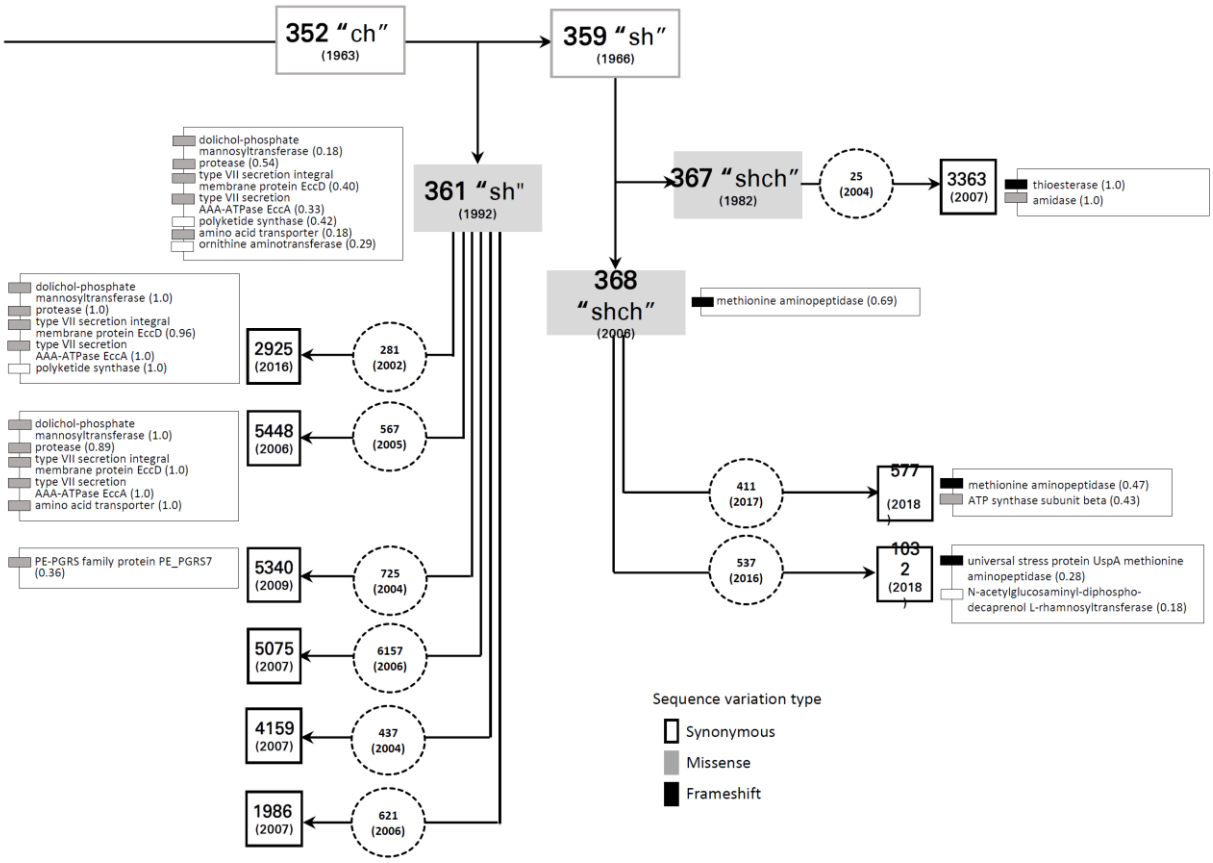


Figure 1. Schematic view of relationships between vaccine strain BCG-1 (Russia) parent seed lots, commercial vaccine lots, and their progeny clinical strains.

Rectangle boxes correspond to the original seed lots with dates of lyophilization shown in parentheses. Rectangle shadowed boxes depict the sequenced seed lots (dates of lyophilization shown in parentheses). Dashed circles show commercial vaccine lots (not available for sequencing) used for immunization with dates of inoculation in parentheses. Square boxes mark sequenced BCG clinical isolates (dates of BCG culture isolation shown in parentheses). Gray-scale small boxes depict sequence variants in genes related to particular proteins (proportions shown in parentheses) in seed lots 361, 367, 368, and their progeny clinical isolates.

Molecular insight into *Mycobacterium tuberculosis* bedaquiline resistance

Emergence and spread of multi- and extensively drug resistant (MDR/XDR) *Mycobacterium tuberculosis* strains is a global concern and novel drugs are required. Here, we analyzed genetic variation underlying development of *M. tuberculosis* resistance to bedaquiline in the Russian province of Kaliningrad with a high rate of primary MDR-TB (30.5%). We hypothesized that whole-genome sequencing analysis of consecutive isolates from the same patient spanning long time period would permit to gain comprehensive and quantitative view on the real-time mycobacterial adaptation to the human host.

Bedaquiline susceptibility testing is only in the process of implementation in Russia and the phenotypic susceptibility data were not available for the studied isolates neither in the regional laboratory (Kaliningrad) nor in the supervising reference center (St. Petersburg). However, mutations in *Rv0678c*, *atpE*, *pepQ* genes were robustly correlated with bedaquiline resistance in the previous studies (Somoskovi et al., 2015; Veziris et al., 2017; Zimenkov et al., 2017; de Vos et al., 2019) and we considered them as sufficiently reliable genotypic proxy of the bedaquiline resistance.

In total, 43 *M. tuberculosis* isolates were recovered from 11 patients infected with mainly XDR strains. All isolates were assigned to the Beijing genotype. Nine and two patients were infected with the Russian successful clone B0/W148 and Central Asia Outbreak strain, respectively. We identified ten unique mutations in the bedaquiline resistance genes in 6 patients. In particular, five mutations were frameshift and three mutations had no or little effect on the protein structure. Mutations in the efflux involved gene *Rv0678* were found in isolates from 5 patients, and a mutation in *atpE* coding for the bedaquiline target ATP synthase, was detected in one case. Both heteroresistance and fluctuating prevalence of mutations observed in both genes including emergence of mutations several months after stop of treatment (Fig. 2).

Effects of the other mutations in *Rv0678* and *atpE* on protein structures were assessed using *in silico* structural analysis of electrostatic potential around mutated amino acids and accessible surface area (Fig. 3).

In terms of molecular epidemiology, the studied collection was totally dominated by the Beijing genotype, furthermore its Russian and Central Asian epidemic clones B0/W148 and CAO that both demonstrated a capacity to rapidly acquire bedaquiline resistance mutations. This can have serious negative impact on the MDR/XDR-TB control in this region as well as Russia and former Soviet Union countries, on the whole, where these MDR/XDR-associated strains are endemically dominant.

Importantly, the bedaquiline treatment was efficient only in the patients whose *M. tuberculosis* isolates did not harbor any bedaquiline resistance mutations. In contrast, treatment outcome was negative in all patients with *Rv0678* or *atpE* mutant isolates, regardless of were these mutations pre-existing or emerged, dominant or minor, with or without significant effect on the protein structure.

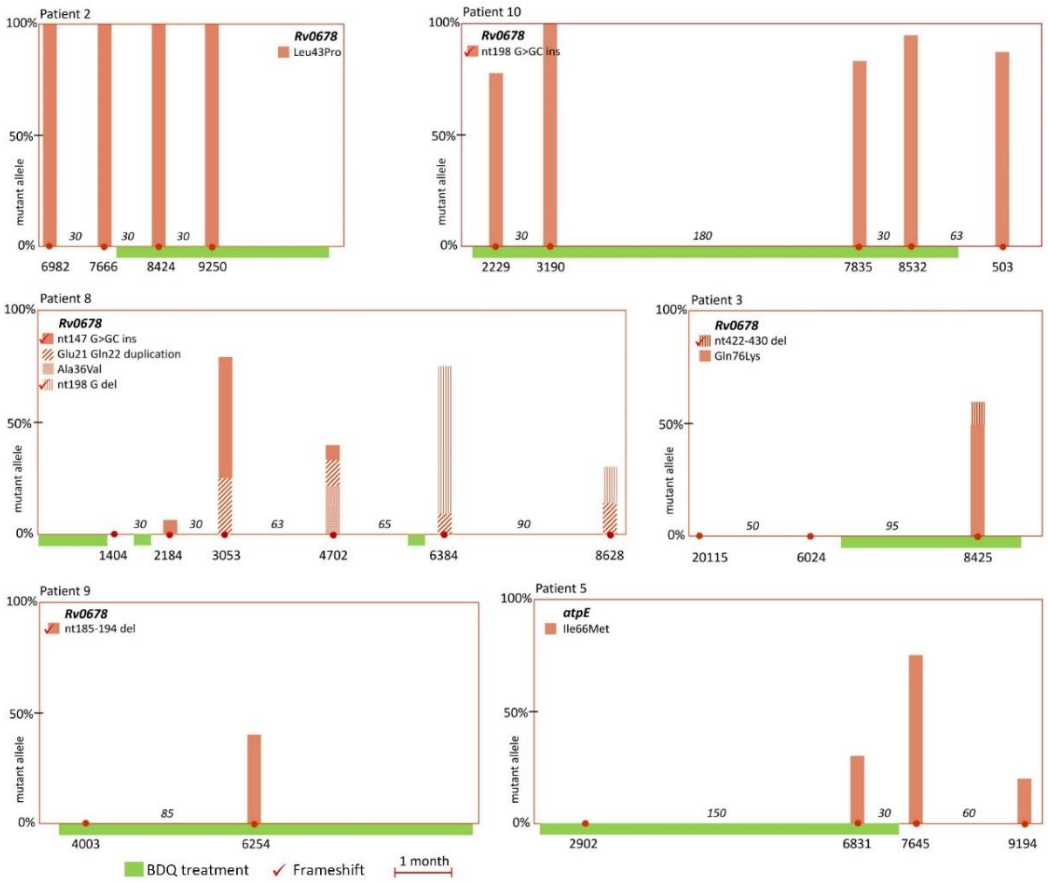


Figure 2. Timelines of treatment of the XDR TB patients with negative treatment outcome whose consecutive *M. tuberculosis* isolates harbored *Rv0678* or *atpE*.

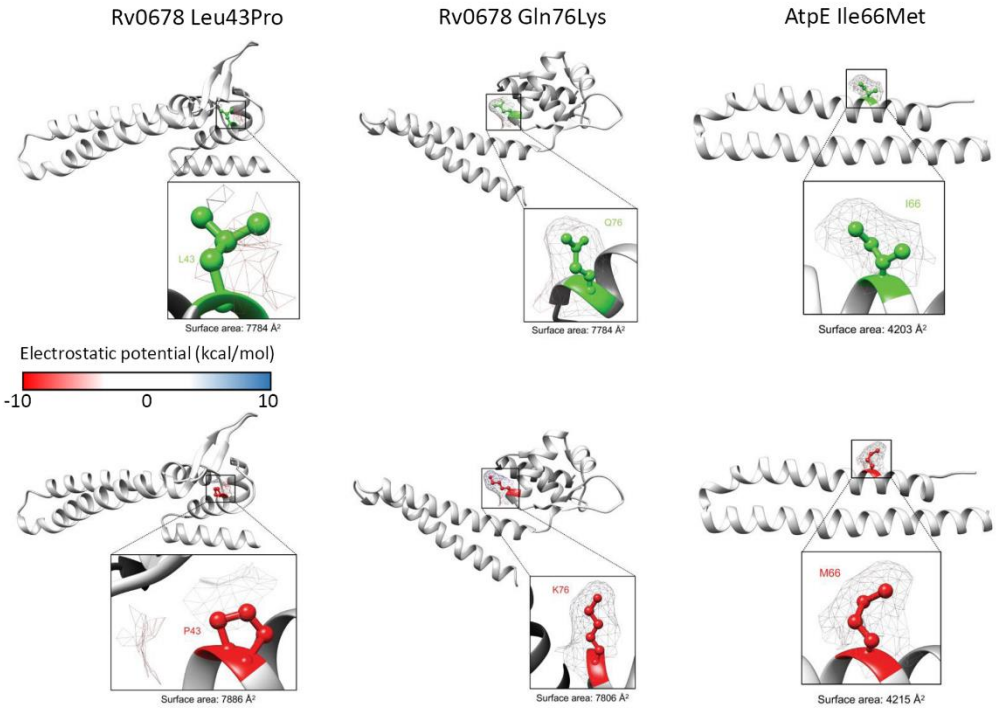


Figure 3. Structures of the MmpR5 (Rv0678) and AtpE (Rv1305) proteins in wild type and mutant forms for some of the identified substitutions in *M. tuberculosis* isolates. **A**. Rv0678 Leu43Pro; **B**. Rv0678 Glu76Lys; **C**. Rv1305 Ile66Met.

Molecular insight into *Mycobacterium tuberculosis* perchlozone resistance

Perchlozone® (4-thioureido-iminomethylpyridinium perchlorate [PCZ]) is a new thiosemicarbazone approved in Russia, along with bedaquiline and delamanid, for treatment of MDR/XDR tuberculosis (TB). The drug was also approved for use or is in the process of registration in some other countries. PCZ is similar to thiacetazone (TAC) and differs from it by the side chain attached to the thiosemicarbazone moiety. Thiacetazone (TAC) was formerly used in combination with isoniazid to treat patients infected with MDR *M. tuberculosis* strains but was removed from the antitubercular chemotherapy due to its secondary toxic effects. TAC is a prodrug that is activated by the flavin-containing monooxygenase EthA to exert its antimycobacterial activity, and mutations in *ethA* are associated with TAC resistance in *M. tuberculosis*. Upon activation, TAC binds to the HadA component of the HadABC dehydratase complex, leading to inhibition of mycolic acid biosynthesis. PCZ is a prodrug that is activated by EthA and inhibits the HadABC complex. EthA is known to activate second-line drugs ethionamide (ETH) and prothionamide (PTH) whereas *ethA* or *ethR* mutations were described as one of the ETH/PTH resistance mechanisms.

In this study, we aimed to gain insight into the molecular basis of PCZ resistance including dynamic changes in *M. tuberculosis* genome during long-term treatment. To this end, we applied next-generation, whole-genome sequencing to the isolates consecutively recovered from patients who received PCZ as part of their chemotherapy regimen.

This prospective study included patients admitted in 2018-2019 to the regional tuberculosis dispensary, Kaliningrad, Russia, whose treatment regimen included PCZ. Multiple *M. tuberculosis* isolates were recovered during PCZ treatment, and the bacterial DNA was subjected to WGS followed by bioinformatics analysis.

In total, 35 isolates were recovered from 9 patients who received PCZ (2-6 isolates from each, median 4 isolates per patient). First available isolates were resistant to 6 to 11 drugs, including ETH or PTH. One patient was MDR, one was pre-XDR and 7 were XDR.

We identified mutations in the genes putatively associated with PCZ resistance, *ethA* and *hadA.* The most frequent one was a frameshift *ethA* 106GA>G (7 of 9 patients) and most of the other mutations were also likely present before PCZ treatment. In one patient, a frameshift mutation *ethA* 702CT>C emerged after 6 months of PCZ treatment (Fig. 4).

In conclusion, the frequent presence of cross-resistance mutations to both PCZ and ETH/PTH presents an especially worrisome finding of this study. This situation raises a major concern with regard to the non-efficiency of PCZ in the treatment of a significant number of MDR-TB cases whose isolates may be additionally resistant to ETH/PTH. In view of the high and increasing burden of MDR-TB in Russia, ETH and PTH are frequently used to treat such patients. The ETH/PTH regimen can take at least 18-24 months as recommended by national and international guidelines and ETH/PTH resistance can emerge quite frequently in clinical isolates, especially in MDR isolates.

Treatment regimens of the XDR-TB patients include several drugs and the presence of PCZ resistance mutations is not necessarily associated with treatment failure. However, the inclusion of additional and non-effective drug in the treatment regimen is impractical and may be adverse for patient's health and well-being. To adequately assess the association of the identified mutations with PCZ resistance, an implementation of the phenotypic PCZ susceptibility testing is urgently needed. A large prospective study of the diverse *M. tuberculosis* collection is warranted to formulate the recommendations for optimal use of PCZ taking into consideration possible ETH/PTH resistance of the isolates.

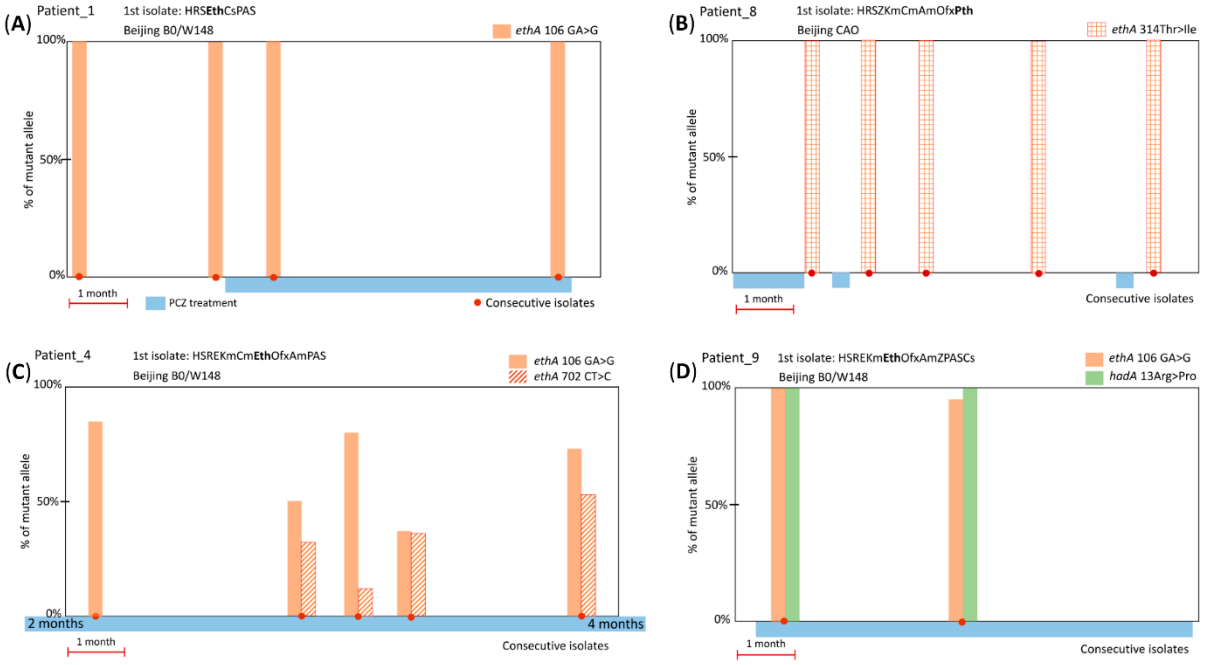


Figure 4. Representative examples of timelines of PCZ treatment, and *ethA* or *hadA* mutations in *M. tuberculosis* isolates. (a) Frameshift *ethA* 106 GA>G mutation present in PCZ pre-treatment isolates (5 patients); (b) Substitution *ethA* 314ACC>ATC/Thr>Ile (2 patients); (c) Frameshift *ethA* 702 CT>C emerged during long-term PCZ treatment, in addition to the likely pre-existing *ethA* 106 GA>G (1 patient); (d) Substitution *hadA* 13CGG>CCC/Arg>Pro and frameshift *ethA* 106 GA>G (1 patient).

Central Asia Outbreak Clade (project in collaboration with Federal Research and Clinical Centre of Physical-Chemical Medicine, Moscow)

Central Asia Outbreak (CAO) clade is a branch of the *Mycobacterium tuberculosis* Beijing genotype that is associated with multidrug-resistance, increased transmissibility and epidemic spread in parts of the former Soviet Union. Furthermore, migration flows bring these strains far beyond their areas of origin. We aimed to find a specific molecular marker of the Beijing CAO clade and develop a simple and affordable method of its detection. Based on the bioinformatics analysis of the large *M. tuberculosis* whole-genome sequencing (WGS) dataset (n=1398), we identified IS*6110* insertion in the *Rv1359-Rv1360* intergenic region as a specific molecular marker of the CAO clade. We further designed and optimized a multiplex PCR method to detect this insertion. The method was validated in silico with recently published WGS dataset from Central Asia (n=277) and experimentally, with *M. tuberculosis* isolates from European and Asian parts of Russia, former Soviet Union and East Asia (n=319). The developed molecular assay may be recommended for rapid screening of retrospective collections and for prospective surveillance, when comprehensive but expensive WGS is not available or practical. The assay may be especially useful in the high MDR-TB burden countries of the former Soviet Union and in the countries with respective immigrant communities.

Highly resistant M. tuberculosis strains of the early ancient sublineage of the Beijing genotype in Russia

The *Mycobacterium tuberculosis* Beijing genotype is a clinically and epidemiologically important lineage further subdivided into ancient/ancestral and modern strains. In our

previous study in western Siberia, we identified VNTR-based clusters within the early ancient sublineage of the Beijing genotype characterized by an unexpectedly high rate of extensive drug resistance (XDR). Here, we analyzed next generation sequencing data in order to gain insight into genomic signatures underlying drug resistance of these strains. In total, 184 genomes of the Beijing early ancient sublineage from Russia, China, Japan, Korea, Vietnam, Thailand were used for phylogenetic analysis. The drug-resistant profile was deduced genotypically. The Russian isolates were distributed in two clusters and were all drug resistant, mainly pre-XDR and XDR. The largest of these clusters included only Russian isolates from remote locations in both Asian and European parts of the country (Fig. 5). All isolates had a quadruple drug resistance (to isoniazid, rifampin, ethambutol and streptomycin) due to the 6-mutation signature (KatG Ser315Thr, KatG Ile335Val, RpoB Ser450Leu, RpoC Asp485Asn, EmbB Gln497Arg, RpsL Lys43Arg). In most samples, it was complemented with additional and different *pncA*, *gyrA*, or *rrs* mutations leading to the pre-XDR/XDR genotype. Phylogenomic analysis suggests a distant origin of this Russian resistant cluster in the early 1970s but location and circumstances are yet to be clarified.

Circulation of these resistant isolates with identical mutational signature in very distant locations of Russia underlines an importance of their close monitoring in view of the potentially wider dissemination.

In *M. tuberculosis* research, an SNP barcode system for lineages, genotypes and subtypes is being developed but it is not clear how new codes can be proposed and agreed upon by the scientific community. Undoubtedly, it would be desirable to assign specific SNP-barcodes to the newly identified and epidemiologically significant clusters. However, the existing barcode system is of low-resolution for the Beijing genotype. Analysis of more strains from more locations will hopefully pave the way to the more comprehensive and more discriminatory classification.

In order to study the evolution and the hypothetical origin of these Russian resistant clusters on a larger time-scale, a further study is warranted with geographically more diverse and more exhaustive collections including susceptible and pre-MDR isolates.

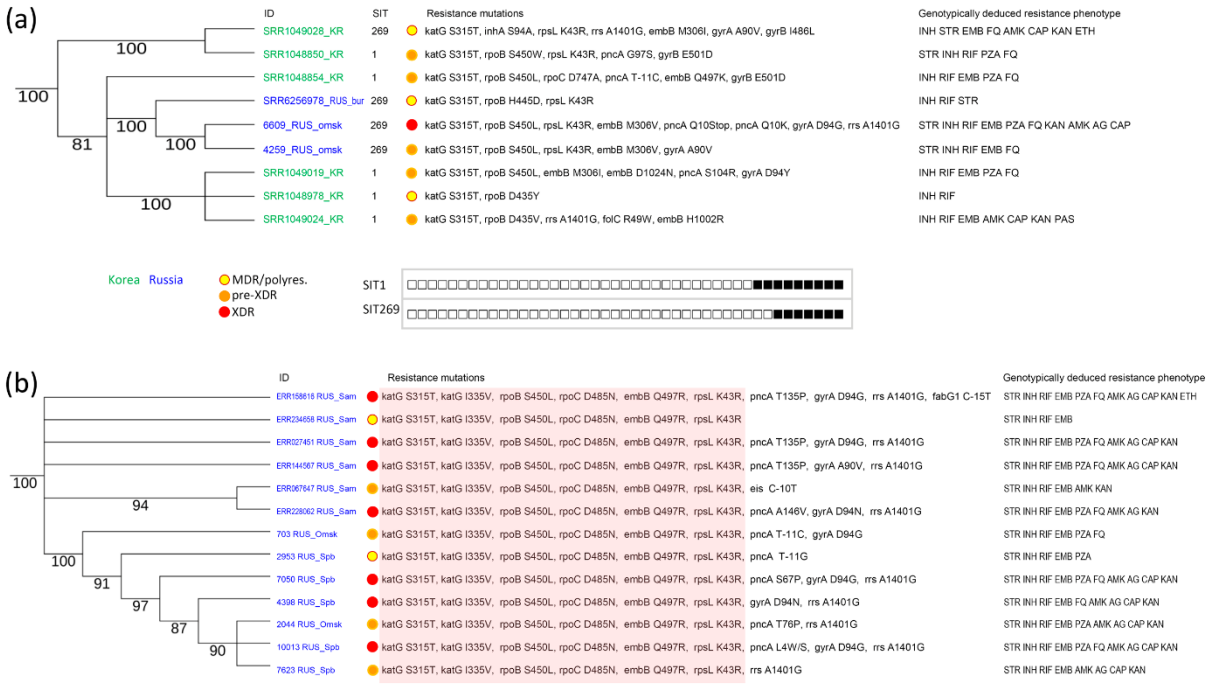


Figure 5. Enlarged view and additional information on clusters with Russian isolates.

Molecular epidemiology of tuberculosis in the Komi Republic, northwestern Russia

The local situation with tuberculosis (TB) is shaped by the complex interplay of multiple factors related to both human host and *Mycobacterium tuberculosis*. We hypothesized that TB epidemiology in the rural regions in the Soviet Union was impacted by construction of the Gulag camps and significant incoming migration.

The Komi Republic is located in the northernmost part of the European Russia. The population is ~850,000, with a population density of 2.1 people per sq.km. The urban population is 78%, and the main ethnic groups are Russians (60%) and Komi (25%). The industrial development of Komi in the 20th century started with construction of the forced-labor concentration camps in 1929 which impacted the demography of the region in three ways. First, the population increased 4-fold during the last 100 years from 207,000 in 1926 to 830,000 in 2019. The so-called "camp cities" of Ukhta, Vorkuta, Pechora, Inta, and Sosnogorsk emerged from the Gulag camps in the 1930s-1956. Their population initially consisted of the released prisoners and workers employed in the forced-labor camps and grew due to incoming migration from other Russian regions. Second, ethnic population structure dramatically changed as the percentage of the Finno-Ugric Komi people decreased from 92% in 1926 to 24% in 2010, as a result of influx from other Russian regions, urbanization, and Gulag-driven industrial development. Third, the particular feature of the Soviet (and Russian) penitentiary system is that the prisoners are sent to prisons outside their regions of residence. In Komi, the former prisoners mostly stayed in the Komi region after their release and thus make a visible proportion of the current population of the region.

In this study, In this study, more than half (56.2%) of *M. tuberculosis* isolates were assigned to the Beijing genotype, which is a characteristic feature of other regions of northwestern Russia and Russia as a whole where the Beijing genotype was identified in 40-70% of the local populations in most regions (Fig. 6). MDR was detected in 30.8% isolates; eight were extensively drug resistant. The main Beijing subtypes B0/W148 and 94-32 differed in the MDR rate, 83.3% and 27.2%, respectively. The non-Beijing isolates represented five genotypes (LAM, Ural, Haarlem, X, T). The proportion of Beijing B0/W148 in the "camp" cities was twice as large as in other districts of the Komi Republic. The *M. tuberculosis* population in the Komi Republic in northern Russia is relatively heterogeneous and is represented by the global genetic families Beijing, T, LAM, Ural, and Haarlem. The Beijing genotype is predominant (56.2%), and its prevalence rate increased by 14.3% over the last decade. The Beijing strains mainly belong to the major Russian clusters of its modern sublineage - B0/W148 (32.9%) and 94-32 (61.1%). Circulation of the MDR isolates of the Beijing genotype and especially its B0/W148 cluster critically and adversely impacts the current situation with the MDR-TB in the Komi Republic. The increased prevalence of the MDR-associated Beijing B0/W148 in the urban setting on the whole, and in the "camp cities" (transformed from the Gulag camps), in particular, highlights an increased transmission capacity of this successful Russian variant of *M. tuberculosis*. A long-term continuous molecular monitoring of the *M. tuberculosis* population is required to confirm the found differences and to predict the epidemic trends of tuberculosis in this region in the northern Russia.

Figure 6. Percent of the Beijing genotype in *M. tuberculosis* local populations across Federal Districts (https://en.wikipedia.org/wiki/Federal_districts_of_Russia) and regions of the Russian Federation

***M. tuberculosis* population structure in Albania** (in collaboration with Dr. Silva Tafaj, National Reference Laboratory in Mycobacteria, Tirana, Albania)

Albania is a Balkan country with moderate to low incidence of tuberculosis (TB) and very low prevalence of drug resistant TB. Here, we analyzed a country-wide multi-year *Mycobacterium tuberculosis* collection in order to detect possible dynamic trends of TB in Albania, with a focus on drug resistance and endemic/epidemic clones.

In total, 743 isolates collected in 2007 to 2011 were divided into 107 spoligotypes and 351 MIRU-types. Based on the MIRU-VNTR phylogenetic analysis, the isolates were assigned to the following lineages/families: animal ecotypes (5 *M. bovis* and 2 *M. caprae* isolates), Lineage 2 (5 Beijing isolates), Lineage 3 (1 CAS-Delhi isolate) and, mostly and overwhelmingly, Lineage 4 (Cameroon, Uganda, Ghana and related; NEW-1-related; Ural, Haarlem, LAM, S, TUR; and unclassified isolates). Most of the isolates (452/743) were intermediately located on the global VNTR tree and did not cluster with any reference profile; they were distantly related to different families within Lineage 4 and we designated them as "unclassified L4" isolates. The significantly higher proportion of drug resistance was observed in (i) Beijing genotype compared to all other isolates (60%, P=0.008), (ii) "unclassified L4" compared to all other isolates (13.9%, P=0.04) and (iii) SIT2936 compared to other "unclassified L4" (34.3%, P=0.0006). Analysis of the yearly collections revealed (i) some decrease of the large heterogeneous "unclassified L4" from 65% to 57%; (ii) steadily increasing gradient of LAM from 3.4 to 13.3%; (iii) stable prevalence of Haarlem (15-20%); and (iv) decrease of TUR with only 1.1% in 2011. Most of the LAM (33/49) and Beijing (3/5) isolates belonged to the VNTR types specific for Russia and former Soviet Union countries.

Our results highlight a peculiar nature of *M. tuberculosis* population in Albania that is dominated by local and unclassified genotypes within Lineage 4, and also features European genotypes and epidemically relevant clones originating from the former Soviet Union

countries. At the same time, these imported clones remain drug susceptible and prevalence of drug resistance on a whole is low.

Species diversity of non-tuberculous mycobacteria in patients with mycobacteriosis in the North-Western Federal District of Russia

Aim of the study: to analyze the structure and trends in non-tuberculous mycobacteria (NTBM) causing diseases in the North-Western Federal District of the Russian Federation.

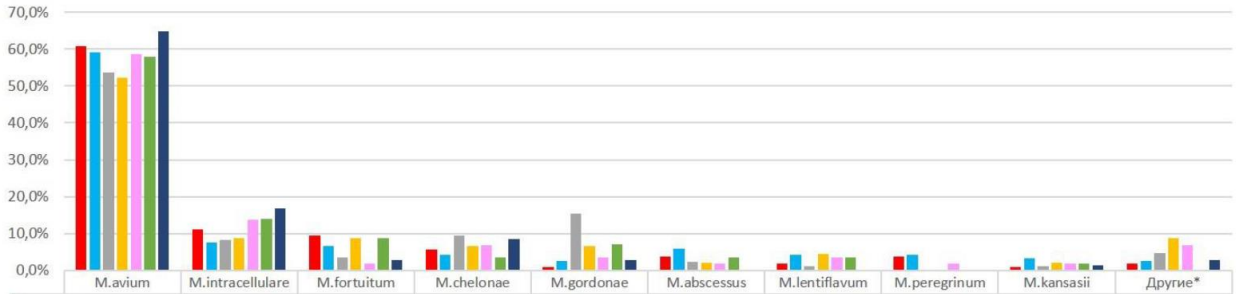
In total, 745 clinical NTM strains were identified. All clinical strains were isolated from patients with mycobacteriosis in 2012–2018. Analysis of the structure of the NTM population showed the predominance of the *M. avium* species (56.4%) (Fig. 7).

During the treatment of patients from St. Petersburg and the Leningrad region, 585 strains of NTM were isolated, and 340 (58.1%) belonged to the *M. avium*. Less frequently, other types of slow- and fast-growing NTM were detected: *M. intracellulare* (11.3%), *M. fortuitum* (6.7%), *M. chelonae* (5.8%) and *M. gordonae* (5.0%) and others (fig. 1). In the Kaliningrad region, over the same period, among 61 NTM strains 61% *M. avium*, 11% *M. fortuitum*, 10% *M. gordonae*, were found. In the Republic of Karelia, as well as in the Vologda, Pskov, and Novgorod regions, a smaller proportion of *M. avium* was isolated. In the Republic of Karelia *M. avium* (39%), *M. gordonae* (26%) were found. In the Pskov region 48% *M. avium*, 16% *M. fortuitum*, 12% *M. peregrinum* were isolated; in the Vologda Oblast - 40% *M. avium*, *M. fortuitum* and *M. abscessus* 20% each, *M. intracellulare* and *M. peregrinum* 10% each; in the Novgorod region - 50% *M. avium*, 25% each *M. kansasii* and *M. intracellulare*. In contrast to these regions, where *M. avium* is the dominant species, other types of NTM have predominated in the Komi Republic and the Arkhangelsk region: *M. lentiflavum* (44%) and *M. gordonae* (34%) dominated in the Komi Republic and Arkhangelsk respectively.

Over the past 12 years, there has been an increase in the detection of NTM in immunocompetent and HIV-infected individuals in St. Petersburg. For the period 2006-2011 only 22 *M. avium* strains and 20 strains of other NTM species (*M. intracellulare*, *M. fortuitum*, *M. kansasii*, *M. abscessus*, and *M. peregrinum*) were isolated, while for the period 2012-2018 were identified already 306 clinical isolates of *M. avium* and 224 isolates of the large group of NTM (*M. intracellulare*, *M. fortuitum*, *M. chelonae*, *M. gordonae*, *M. abscessus*, *M. lentiflavum*, *M. peregrinum*, *M. kansasii*, *M malmoense*, *M. xenopi*, *M. celatum*, *M. smegmatis*, *M. marinum*, *M. scrofulaceum*, *M. szulgai*) (Fig.1). This can be explained by the fact that doctors began to pay more attention to the etiological diagnosis of mycobacterial infections, especially with the increase of the prevalence of immunosuppressive conditions (drug therapy, pulmonary diseases, HIV infection). Secondly, the introduction of molecular methods for the mycobacteria identification into clinical laboratory practice played an important role, which greatly simplified the NTM identification scheme. The steady increase in the detection of NTM demands the formation of national clinical guidelines for the diagnosis of mycobacteriosis of various localization and registration of each case of NTM identification.

Unlike other regions of Russia, in the North-Western region, there was a consistently low level of detection of *M. kansasii* and *M. xenopi*. For the last 12 years in St. Petersburg and Leningrad region, the variety NTM in patients with different immune status has been growing but the portion of *M. avium* remains to be stably high exceeding 50%.

Thus, our data indicate a change in the distribution of NTM species throughout the entire period of the study, possibly as a reflection of migration processes or a different level of examination of risk groups.



	M.avium	M.intracellulare	M.fortuitum	M.chelonae	M.gordonae	M.abscessus	M.lentiflavum	M.peregrinum	M.kansasii	Другие*
2012	60,7%	11,2%	9,3%	5,6%	0,9%	3,7%	1,9%	3,7%	0,9%	1,9%
2013	59,2%	7,5%	6,7%	4,2%	2,5%	5,8%	4,2%	4,2%	3,3%	2,5%
2014	53,6%	8,3%	3,6%	9,5%	15,5%	2,4%	1,2%		1,2%	4,8%
2015	52,2%	8,7%	8,7%	6,5%	6,5%	2,2%	4,3%		2,2%	8,7%
2016	58,6%	13,8%	1,7%	6,9%	3,4%	1,7%	3,4%	1,7%	1,7%	6,9%
2017	57,9%	14,0%	8,8%	3,5%	7,0%	3,5%	3,5%		1,8%	
2018	64,8%	16,9%	2,8%	8,5%	2,8%				1,4%	2,8%

Figure 7. Frequency of the occurrence of NTM species in patients with mycobacteriosis in St. Petersburg and the Leningrad region (2012-2018). Note: * Others: slow-growing mycobacteria - *M. malmoense*, *M. xenopi*, *M. celatum*, *M. marinum*, *M. scrofulaceum*, *M. szulgai*; fast growing mycobacteria - *M. smegmatis.*

PUBLICATIONS IN PEER-REVIEWED JOURNALS (Pubmed, Web of Science):

ARTICLES IN ENGLISH: 24 (13 - in Q1)

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