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Research paper

Current topics of molecular mycobacteriology☆

Igor Mokrousov*

Laboratory of Molecular Epidemiology and Evolutionary Genetics, St. Petersburg Pasteur Institute, St. Petersburg 197101, Russia

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ABSTRACT

The 2nd St. Petersburg Symposium on Tuberculosis and Mycobacteria: Molecular Approach, was held in St. Petersburg, Russia on 5–6 December 2018. A special issue of *Infection, Genetics and Evolution* will publish articles based on the selected presentations. In this paper, I will discuss some of the hot topics of molecular mycobacteriology highlighted at this meeting that I had the pleasure to organize and honor to chair. The symposium addressed interrelated fundamental and applied issues of modern mycobacteriology such as molecular evolution and phylogenomics, host-microbe interactions and pathogenesis, coevolution of *M. tuberculosis* with humans, new genomic and postgenomic technologies. Molecular methods for TB diagnostics and drug resistance detection are supported by WHO and whole genome/next generation sequencing presents a comprehensive approach. At the same time, cost and implementation of new methods for direct analysis of clinical samples and/or in low-resource settings remain a great challenge. A due attention was also given to the medically important non-tuberculous mycobacteria. Assessment of spectrum of the circulating mycobacterial species in the Russian Federation and the countries of the European Union was presented and the underlying reasons of the observed diversity were discussed. To conclude, the symposium became a multidisciplinary event that was useful to promote networking and exchange of knowledge and experience. The next (third) symposium was planned to be organized in 2021.

1. Introduction

Tuberculosis (TB) is ancient disease whose traces can be found in paleopathological samples and classical books. For example, it was described as phthisis by Hippocrates, 400 BCE, and as *xulao bing* (weak consumptive disease) by the Emperor Shennong of China, 2700 BCE. As recent as 120 years ago TB mortality was 500/100,000 in Russia and this level was recorded in Europe in 17th–18th centuries. Tuberculosis is a re-emerging disease with high or very high incidence in many parts of the world, as well as aggravating HIV-TB coinfection rates (World Health Organisation, 2018a, 2018b). Tuberculosis epidemics is a global concern for public health and world economy as was highlighted at the Global Ministerial Conference on Ending TB in the Sustainable Development Era on 16–17 November 2017 in Moscow, Russia and at the United Nations General Assembly high-level meeting "United to end tuberculosis: an urgent global response to a global epidemic" on September 26, 2018. *Mycobacterium tuberculosis* is not only medically important pathogen that accompanied humans since our early evolution but an interesting biological species. An impressive advance in molecular studies of *M. tuberculosis* and other mycobacterial species was achieved thanks to implementation of next generation and omics approaches.

Back in the history, in September 2014, within the St. Petersburg Ecological forum, a Symposium on Tuberculosis and Mycobacteria took place. It was a one-day event organized by St. Petersburg Pasteur Institute and St. Petersburg Institute of Phthisiopumonology. Along with Russian participants, it was attended by six experts from Germany, UK, France, Sweden, and Japan. It was the first event on the molecular tuberculosis research with a relatively visible international component ever held in Russia. Four years later, 2nd St. Petersburg Symposium on Tuberculosis and Mycobacteria: Molecular Approach was organized on 5–6 December 2018, within the frames of the quinquennial anniversary conference of St. Petersburg Pasteur Institute. International conference "Molecular bases of epidemiology, diagnosis, prevention and treatment of infectious diseases" was dedicated to the 110 years since establishment of the institute and 95 years since its naming after Louis Pasteur. On a side note, there is a tacit disagreement about the year of establishment of the institute while an earlier date of 1908 was recently officially accepted instead of the traditional 1923 (Alekseeva and Totolian, 2018). In my personal opinion, this claim is supported by the newly found historical documents and thus justified.

The 2nd St. Petersburg Symposium on Tuberculosis and

☆ Special Issue *St. Petersburg Symposium on TB & Mycobacteria*

* Corresponding author at: St. Petersburg Pasteur Institute, 14 Mira str., St. Petersburg 197101, Russia.
E-mail address: imokrousov@mail.ru.

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Mycobacteria was devoted to the advanced molecular studies in the area of tuberculosis and diseases caused by nontuberculous mycobacteria. The official language of the Symposium was only English which was quite exceptional for such events organized in Russia (indeed a simultaneous Russian translation was provided). The symposium consisted of the oral sections: Evolution and phylogenomics, Whole genome sequencing and personalized medicine; Molecular diagnosis and molecular epidemiology; Nontuberculous mycobacteria; Virulence and resistance (http://pasteur110.ru/wp-content/Program-of-Symposium-eng.html). In total, 35 oral talks were delivered by 26 foreign and 9 Russian speakers representing academic institutes and universities, federal research institutions, national reference laboratories, and WHO. Participants came from 24 countries, in particular, Russia, USA, Japan, Thailand, South Africa, and 14 EU countries.

What I present below is by no means a comprehensive description of all meeting but a personal account of some of the hot topics and thought-provoking presentations.

2. Evolution and co-evolution

Population structure of *M. tuberculosis* is influenced by multiple factors, including those related to the pathogen itself, host genetics and environmental factors in a wide sense. Their relative weight is yet to be determined. The availability of thousands of *M. tuberculosis* genomes allows not only to infer emergence and spread of the pathogen but also to identify genome loci associated with major evolutionary changes as was demonstrated by Iñaki Comas (Spain). Genetic drift can contribute to the genomic diversity of the pathogen mirrored at the transcription and methylation levels. Overall, this analysis revealed new bacterial factors associated with virulence and drug resistance. Somewhat paradoxically, it was suggested that manifestation of the advantageous genetic variants leading to high strain fitness, nonetheless depends on local TB control programs (Comas et al., 2018). Indeed the structure of local *M. tuberculosis* populations is shaped by multiple factors, related to the host, pathogen itself, and the environment. In its turn, this latter may be determined by nature (climate) and humans (social and economic conditions) (Fig. 1). Deteriorated public health system presents a perfect condition for selection and subsequent spread of resistant epidemic strains (Sinkov et al., 2018). On the other hand, a complex human genetic background is perhaps a key to understanding why strains epidemic in one country do not so easily spread in the other human population that had had a long history of coadaptation with its endemic strains (Pérez-Lago et al., 2016). There is a sharp difference between two kinds of receiving human populations in their response to an imported strain: (a) a naïve population without previous TB history (a rare situation nowadays); (b) an "experienced" population that had had a long coadaptation with local endemic strains. Introduction of *M. tuberculosis* strains to the TB-naïve population may have decimating consequences as we know from historical examples of indigenous populations in North America (Bellamy, 1998) and Siberia (Dabernat et al., 2014). An important line of studies presents those that focus on both genotypes of infecting *M. tuberculosis* strains and genotypes of the same patients. Subsequently, human allelic data are stratified according to the *M. tuberculosis* genotypes or lineages and potential associations can be looked for. An apparent challenge is a recruitment of a sufficiently large number of patients. On the other hand, development of TB infection is under multigenic control in humans and focus only on few genes or SNPs presents an outdated approach.

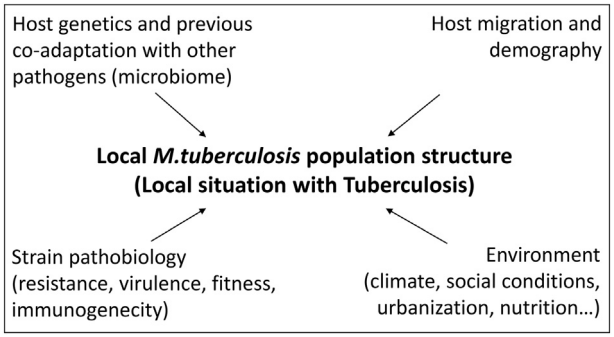

Fig. 1. Non-exhaustive representation of multiple and interacting factors that influence *M. tuberculosis* local population structure.

In this view, remarkable results of the genomic study in a cohort of TB patients in Chiangrai, northern Thailand were presented by Prasit Palittapongarnpim. The genomes of *M. tuberculosis* isolates from 1170 patients were sequenced. In parallel, genomes of the same patients were subjected to the high density SNP arrays. Most of *M. tuberculosis* strains were assigned to two lineages: Indo Oceanic (lineage 1) and East Asian (lineage 2). Few novel sublineages within lineage 1 were identified, especially in remote populations. The patients mostly belonged to three genetic groups, identified by principal component analysis, and three ethnic groups. The subgroup of patients infected by lineage 2 was especially heterogeneous; this may be interpreted as historically recent introduction of the Beijing genotype in Thailand and not yet sufficient time for co-adaptation with certain human groups. In contrast, lineage 1 is endemic in Thailand which explains the above findings. A strong correlation was found between the *M. tuberculosis* genotypes and human ethnicity and GWAS identified genes associated with particular *M. tuberculosis* genotypes (Palittapongarnpim et al., 2018). Important issues to be considered under such studies: (i) heterogeneous population of *M. tuberculosis* even within the lineage, and thus importance to look more closely at particular clusters; but (ii) when the data are stratified into subgroups, the sample size is reduced and statistical significance is hard to achieve. A study of the *INFG* alleles in Russian population had unexpected results when healthy controls were compared to the Beijing B0 infected patients and other Beijing infected patients. Comparison of allelic and genotype frequencies revealed a trend in difference for INFG +874 alleles between these different groups. Beijing B0/W148 subgroup had rate of "TB-protective" TT genotype even higher than in healthy controls. On the other hand, other Beijing and non-Beijing genotypes "correctly" had lower rate of the TT genotype than controls. However, that difference was non-significant and replication of that study on a large cohort is warranted (Mokrousov et al., unpublished data).

Spoligotyping is quite old (not to say outdated) method of *M. tuberculosis* genotyping. A low discriminatory capacity is one of its apparent limitations. In contrast, MIRU-VNTR typing (especially, 24 loci format) presents a much more powerful approach. Both methods and their respective online tools (first of all, SITVIT_WEB and MIRU-VNTRplus) present already classical references in modern molecular mycobacteriology. The uncritical use of these well known online tools and resources is a serious problem when their indications are taken dogmatically, as final and absolute truth (Mokrousov, 2018). Serious limitations of spoligotyping make the spoligotype-based decision rules and clade/subclade assignment inadequate in the non-negligible number of cases (Fig. 2).

Further, use and abuse of minimal array of classical references that conveniently support long-lasting clichés present another serious problem. For example, it is a common saying that Beijing genotype is genetically homogeneous. However, there are important and distinct clusters within it, in particular: (i) New York City epidemic strain W in the 1990s, (ii) Gran Canaria strain with ongoing and increasing circulation, (iii) successful Russian strain Beijing B0/W148. Further, in many articles, Beijing genotype is termed as "escape" variant, hypervirulent, multidrug-resistant, highly transmissible/clustered. However, these features are variable among epidemic, endemic and sporadic strains (Lasunskaia et al., 2010; Yang et al., 2012; Mokrousov et al., 2018). The classification matters because different *M. tuberculosis* families differ in the pathogenic potential and evolutionary pathways (e.g. see Reiling et al., 2013). A confusing terminology, misclassification and false

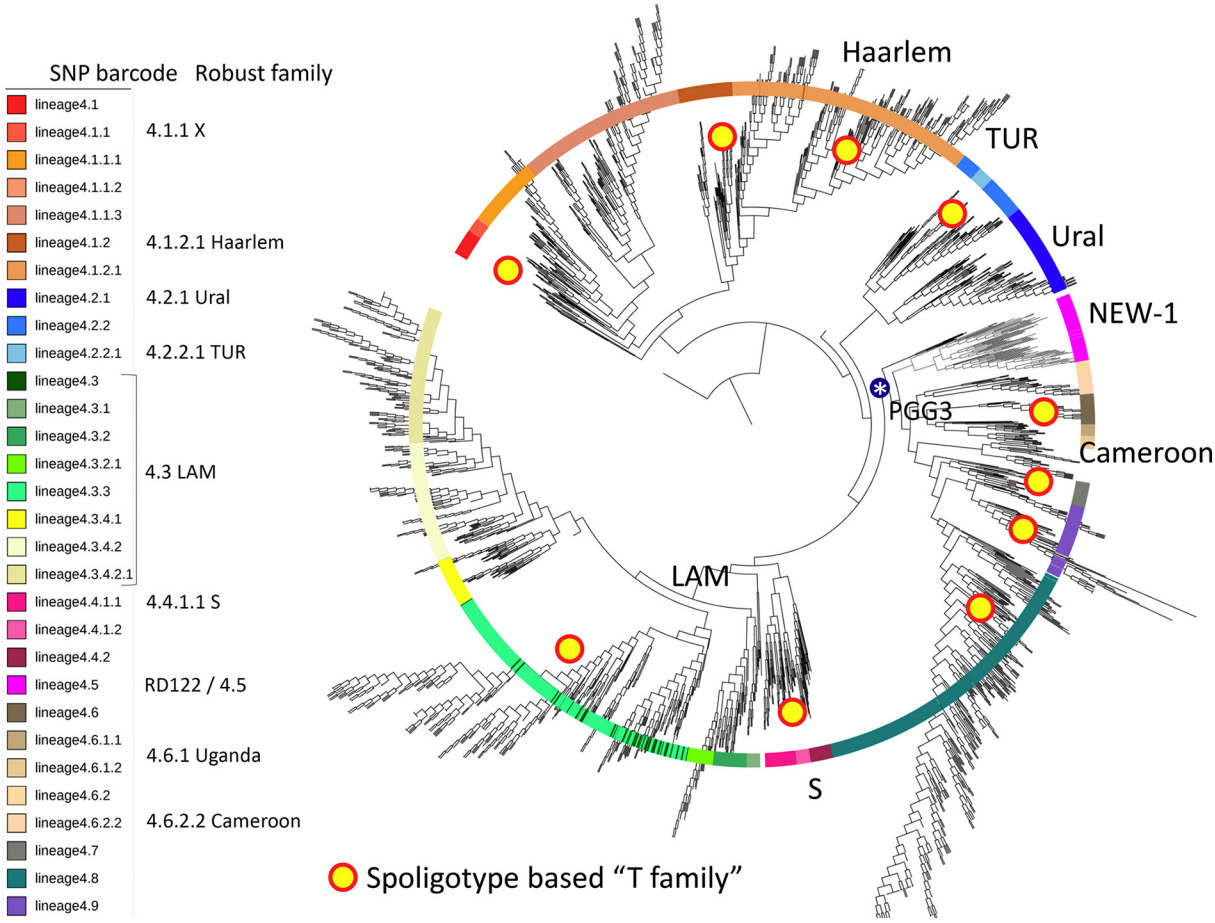

Fig. 2. WGS/NGS based tree of *M. tuberculosis* lineage 4 (Euro-American lineage) shows lack of phylogenetic sense of the "T family".
SNP barcode designation (Coll et al., 2014) is complemented with family names when available. Spoligotype-defined “T family” isolates are shown by red/yellow dots. The original dendrogram was taken from Mokrousov et al. (2017). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

clustering make a scientific discussion meaningless, and some courses for improvement of the situation may be considered: (i) a cautious definition of the clade status as ‘unknown’ to be applied for more SIT, (ii) renaming some clades based on the already established correlation with robust markers (already done for some cases in new version SITVIT2 [Couvin et al., 2018]); (iii) removing an option to build phylogenies from spoligotyping data or more explicit warning that such trees are scientifically unsound (Mokrousov, 2018).

3. Genomic and molecular epidemiology

Nowadays NGS/WGS appears to be the key to the doors of the top/elite journals with IF > 10/20, although, in my opinion, the added value to the previous knowledge is not always apparent. NGS/WGS was the theme of the special section focused on its practical implementation. Indeed, this approach starts to be used in national reference laboratories in some of the EU countries for monitoring of circulating strains and rapid and comprehensive detection of drug resistance. Unfortunately, the high burden countries, such as Russia, are very far from even partial implementation of these methods in the routine work of national reference centers not to say regional laboratories.

Stellenbosch University in Cape Town is one of the leading research centers in Africa and globally in the field of molecular epidemiology of tuberculosis. An impressive database of both strains and their molecular profiles (in particular, IS*6110*-RFLP) has been collected here since mid-1990s. A recent reanalysis by means of NGS was definitely instrumental to gain unprecedented insight into long-term evolution of the local endemic/epidemic clusters. Anzaan Dippenaar (South Africa) presented an in-depth study of the long-term 23 years evolution of one important *M. tuberculosis* IS*6110* RFLP cluster to understand transmission and strain diversity in the Cape Town area. WGS analysis subdivided this cluster into several subclusters that acquired drug resistance mutations (beyond MDR) on multiple instances. It was concluded that IS*6110* RFLP typing underestimated the complexity of this 23-year outbreak and there was a continuous circulation and reintroduction of this *M. tuberculosis* cluster in the community setting (Dippenaar et al., 2018). In spite of powerful NGS approach it was also recognized that even WGS is not always sufficient to confirm epidemiological links or assess outbreak directionality in high TB burden settings. It may be noted that this situation resembles Russian epidemic clone Beijing B0/W148 that is also characterized by a distinctive and conserved IS*6110*-RFLP profile, widespread in Russia, but can be subtyped by certain SNPs and hypervariable VNTR loci. Another interesting example of the NGS based reanalysis of longitudinal data is a study of the Scandinavian Cluster 2/1112–15: of 234 non-synonymous (NS) monomorphic SNPs found, 23 were in virulence related genes and mutation was in a gene associated with hypervirulence (Folkvardsen et al., 2018).

Molecular epidemiology, and more recently, genomic epidemiology improved our understanding of the transmission dynamics of *M. tuberculosis* in a population. However, in many countries, including many with a high burden of TB, systematic genomic epidemiology cannot be implemented in near future. In contrast to the above total WGS analysis of all isolates, a simplified approach to optimize tracing of the trans-border spread of *M. tuberculosis* is promoted by Dario Garcia de Viedma

(Spain). This alternative approach combines the initial use of WGS (anyway) in order to identify strain/clone-specific SNPs followed by development of allele-specific PCR assays to detect those SNPs (García De Viedma and Pérez-Lago, 2018; Pérez-Lago et al., 2016). An established decentralized multinational network of surveillance points permits to 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. This allows to discriminate transmissions after arrival of migrants to the host countries from independent importations from their countries of origin. However, in any case, an initial step of this scheme still requires WGS analysis and a comprehensive in silico validation of the detected SNPs to confirm their specificity. Also, it may be noted that the major line of development of molecular assays is a closed-tube, real-time format rather than classical PCR followed by gel-electrophoresis. However, utility of this latter is understandable in view of limited resources in quite many countries.

Availability of the powerful bioinformatics resources along with sufficient motivation (global importance of the disease) in recent years have led to development of online resources and databases, aimed to comprehensively deliver information on both patients and infecting strains. Andrei Gabrielian and Alex Rosenthal (USA) presented TB Portals program as an outcome of the data driven multinational consortium against drug resistant tuberculosis (Rosenthal et al., 2017). The TB Portals has an ambitious objective to collect, analyze, standardize, and present anonymized clinical, laboratory, and socioeconomic data, bacterial genomes, and radiological data (CXR and CT). The TBPP database currently has > 1300 published (22,250 total) cases, 75% of which are MDR or XDRTB, as well as 730 published (1300 total) sequenced and annotated *M. tuberculosis* genomes with the patient record. The ongoing TBPP projects focus on study of clinical significance of *M. tuberculosis* population structure: (i) genomic signatures for TB relapse and reinfection, and (ii) comparative analysis of *M. tuberculosis* strains isolated from sputum vs. lung surgery material. Thanks to the advanced bioinformatics, such projects are being developed in different parts of the world, and their critical and unbiased survey is needed such as one published 3 years ago (Faksri et al., 2016).

In Russia, total WGS remains a future and more classical typing schemes are being used, with focus on special settings. One such setting is represented by the so called closed towns that were quite widespread in the Soviet Union, and frequently constructed around strategic military oriented productions. As a consequence, they were characterized by restricted conditions for residency and outgoing/incoming migration (https://en.wikipedia.org/wiki/Closed_city). In return, their inhabitants, at least those belonging to the technical or scientific elite, could enjoy conditions of the 'golden cage' even though in the totalitarian environment (Riehl and Seitz, 1988). After the collapse of the Soviet Union, their situation has changed in many aspects including worsening of the social and economic conditions. Regarding epidemiological situation, given the limited conditions of the population migration, such cities, to some extent, can serve as a model for the formation and propagation of new forms of causative agents of infectious diseases including tuberculosis. 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. Epidemiological situation with TB is characterized by high level of TB/HIV co-infection. Analysis of molecular features of *M. tuberculosis* isolates from patients in Novouralsk was presented by Tatiana Umpeleva (Ekaterinburg, Russia). This study revealed a preferential circulation of the most transmissible variants, in particular, MDR Beijing B0/W148 cluster, thus leading to high levels of primary MDR among newly diagnosed patients. At the same time, new clonal subtypes are emerging in this area and characterized by particular molecular signatures (Umpeleva et al., 2019).

4. Molecular diagnostics and drug resistance

Molecular methods for diagnosis of TB and detection of drug resistant TB are being widely introduced worldwide and it is important that such methods be formally endorsed by the national and international authorities. WHO policies on molecular methods for diagnosis of tuberculosis were presented by Alexey Korobitsyn (WHO, Switzerland). According to WHO, molecular methods play critical role in global fight against TB (Gilpin et al., 2018). 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 (developed since mid-1990s) are recommended as rapid diagnostic tests for detection of resistance to isoniazid, rifampin, fluoroquinolones and amikacin. DNA sequencing is becoming increasingly important as a reference method for detecting mutations associated with resistance to the first- and second-line anti-TB drugs (Korobitsyn, 2018). The Guidelines for use of NGS technologies for detection of drug resistant TB were recently jointly prepared by WHO and FIND. These Guidelines contain information on all available on the market NGS platforms along with data analysis algorithms, both open source and commercial tools. Specificity and sensitivity of using particular mutations as markers of resistance is presented in detail for main first- and second-line drugs (but not for streptomycin, ethambutol and new anti-TB drugs).

A nice example of the multicenter collaborative effort is exemplified by the ReSeqTB, a data sharing platform aggregating genotypic, phenotypic and associated meta-data for MTBC in order to adequately understand the value and weight of the particular molecular mechanisms of resistance (Starks et al., 2015). Even for the long used drugs some novel insights continue to be reported, e.g. about complex nature of the isoniazid resistance (Ghodousi et al., 2019). However to be just, although these global and in-depth efforts to detect the minor variants are definitely remarkable, only limited number of resistance codons are prevalent in the high burden areas and cover most of the resistant strains. This was true 20 years ago (Van Rie et al., 2001) and this remains true today, at least, for the first-line drugs (Dymova et al., 2014; Ergeshov et al., 2017).

Continued and increasing circulation of the drug resistant *M. tuberculosis* strains is not only a public health concern but a motivation for the development of new approaches to early detection of such strains. An Van den Bossche (Belgium) presented a novel RNA based drug susceptibility testing assay of *M. tuberculosis*. The test is defined as a next generation diagnostic test based on quantification of drug specific RNA biomarkers. The underlying principle is that a brief exposure to anti-TB drug triggers specific transcriptional responses in susceptible, but not in resistant, strains. The pilot study analysed global transcriptional response of two *M. tuberculosis* strains to 10 anti-TB drugs which was determined using RNAtaqSeq. Subsequently, a set of highly responsive genes was selected for each drug and RNA targeting probes were designed. Next, the RNA based DST method was developed in 96 well format and the assay was proven to be efficient for isoniazid. Theoretically, the drug resistance profile of up to 14 drugs can be determined (Van den Bossche et al., 2018). The assay is postulated to have the advantage over long culture dependent steps, while the resistance phenotype is detected independent of the specific genetic mechanism of resistance. However, one should note that in any case the grown culture is needed for analysis. Also, the cost of such assay, especially, keeping in mind high transport and taxes cost for consumables and very high cost for equipment will be a challenge in a bureaucracy/corruption afflicted countries (some of which correspond to those with high burden of MDR-TB). Further technological steps should focus on validation with clinical isolates of different genetic background and from high burden areas, as well as on direct assessment with clinical samples.

Emergence of MDR and XDR is also a major motivation of development of new anti-TB compounds, even if such new drugs are very expensive and are not devoid of side effects. Bedaquiline (BDQ) is an effective drug for the treatment of MDR/XDR TB allowing up to 85%

cure rate in complex therapy. As with other drugs, BDQ resistance can be developed via acquisition of mutations in *mmpR* and *atpE* genes. However, the clinical significance of mutations is still unclear due to an insufficient number of clinical isolates, characterized by phenotypic and molecular methods. Phenotypic and genotypic study of resistance to this drug during treatment with BDQ presented by Danila Zimenkov (Moscow, Russia) revealed that intermediate resistance emerged by selection of *mmpR* mutations, while a high level resistance was caused by substitutions in AtpE. These results also raise the question of reliability of the currently used BDQ critical concentrations for 7H11 agar (0.25 mkg/ml) and Bactec MGIT 960 (1 mkg/ml) tests (Peretokina et al., 2018). In addition to the fundamental issue of clinical significance of particular mutations, there is a practical issue of (non) emergence of such resistant isolates in different parts of the same country, e.g., their rarity in St. Petersburg (V. Zhuravlev, personal communication) compared to the increased prevalence in Moscow (Zimenkov et al., 2017) although this difference may be explained by the fact that only few reference laboratories perform DST to these drugs in Russia.

The non-common features of the development of bedaquiline resistance during the treatment of MDR-TB patient were analysed in the study of Margaretha de Vos in high-burden setting in Khayelitsha, South Africa. The systematic gain and loss of *Rv0678* variants in post-BDQ-treatment isolates was identified. This highlights the complex pattern of *M. tuberculosis* evolution as the concentration of BDQ decreases in the patient (long half-life). Alternatively, this may be due the emergence of existing BDQ-resistant *M. tuberculosis* from lesions which rupture after continuation of treatment without BDQ (de Vos et al., 2018). This shows once again that a real *M. tuberculosis* population in a TB patient is far more complex, heterogeneous and compartmentalized than one observed in vitro.

The above said highlights an importance of the systemic omics approach to study infecting agents in their real environment, i.e. human body. The vast majority of microbes exist as part of complex polymicrobial biofilm communities attached to host and environmental surfaces. A number of studies have shown that in the experimental in vivo model, *M. tuberculosis* can form biofilm-like structures in lungs. The aim of the study presented by Oleg Ogarkov (Irkutsk, Russia) was to demonstrate the role of tuberculomas satellite microbiota as an example of polymicrobial biofilm in lungs of TB patients, as a possible cause of unexpected defaulted treatment. The study of clinical *M. tuberculosis* strains demonstrated that < 5% of them were able to produce mature biofilms (pellicle) on a liquid medium. It was shown on in vitro model that about of 50% clinical *M. tuberculosis* strains can coexist together with *Bacillus licheniformis*, also isolated from sputum of TB patient. Furthermore, the bacilli had a high tolerance to streptomycin, ethionamide, isoniazid and ethambutol. The 16S rRNA metagenome study of several tuberculomas has shown that quantity of *M. tuberculosis* genomes were < 3% in all cases whereas majority were represented by Gram-positive *Firmicutes* such as, *Staphylococcaceae* and a small proportion of Gram-negative bacteria. Anti-TB therapy may be confronted with not only *M. tuberculosis*, but with polymicrobial biofilm communities whose drug resistance may differ from the resistance of standalone *M. tuberculosis* strains (Ogarkov et al., 2018).

5. Nontuberculous mycobacteria

Although tuberculosis was the main theme of many reports (and the Symposium on a whole), a due attention was also given to the nontuberculous mycobacteria (NTM) that cause mycobacterial diseases in humans. Although geographical distribution of the NTM species differs globally, NTM pulmonary disease is not notifiable in most of the world (Zweijpfenning et al., 2018; Prevots et al., 2017). Therefore noncommon approaches are needed to adequately describe the disease burden, temporal and geographic distribution and associated mortality.

M. avium subsp. *hominissuis* (MAH) is a human pathogen that causes *M. avium* complex (MAC) lung disease. Circulation of the MAH strains between the human body and the environment was suggested as a life style of this mycobacterial species. Despite its clinical significance, the genetic mechanisms underlying local adaptation of this pathogen are unknown due to a lack of population-wide genomic data. Tomotada Iwamoto (Japan) presented results of analysis of the large WGS dataset of *M. avium* strains from different countries. In particular, it was found that the origin of trehalose biosynthesis genes differed between Asian and non-Asian MAH populations. Furthermore, a transmission of alleles encoding *mce* ("mammalian cell entry") proteins was observed between populations within East Asia (Yano et al., 2017). As a new concept, a model for the life cycle of *M. avium* was presented 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 (Iwamoto, 2018).

The immune responses against *M. avium* and *M. tuberculosis* look similar, but the host genetic control of susceptibility to and severity of the diseases caused by these pathogens is different. This control, in both mice and humans, involves numerous interacting genes, which makes the whole picture complicated (reviewed in Ignatov et al., 2012). Two presentations from the laboratory of Alexander Apt (Moscow, Russia) communicated results of the immunogenetics studies of *M. avium* infection in mice models. Mice of the I/St strain are extremely susceptible to *M. 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, it was demonstrated that genetic susceptibility of the host determines the pattern of lung pathology (Linge et al., 2019). In particular, allelic differences in the H2-A molecule were shown to be involved, albeit moderately, in control to *M. avium* infection.

M. 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. 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. Tomasz Jagielski (Poland) presented results of the multicenter project on *M. kansasii* genotyping. The highest recovery of *M. kansasii* from respiratory samples was in Poland (36%) and Slovakia (35%). The subtype I represented the vast majority of *M. kansasii* clinical isolates worldwide. Furthermore, the genetic diversity of the *M. kansasii* population showed regional variations. Since all detected *M. kansasii* subtypes (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 (Bakuła et al., 2018). In fact, this raises a question about clinical significance of this genotyping scheme and highlights an importance of moving to the WGS based approach for genotyping that could hopefully reveal a functionally meaningful genetic diversity.

Two complementing presentations focused on the assessment of spectrum of the circulating mycobacterial species in the Russian Federation (Ustinova et al., 2018) and countries of the European Union (Nikolayevskyy et al., 2018). The reasons underlying an observed diversity at within country level and/or between countries may be related, in particular, to environmental protection strategies that may differ in different countries. A study design focused on active disease cases may be another reason. Overall, there is a known trend of the increasing NTM prevalence in case of decreasing TB burden, compare, for example, some EU countries or North America with increasing prevalence of NTM (Menzies and Nahid, 2013; Martínez González et al., 2017) against Siberia where NTM clinical cases are extremely rare

(Dymova and Alkhovik, 2015; O. Ogarkov, personal communication).

6. Miscellanea and conclusions

The Poster session included 26 posters (by 15 foreign and 11 Russian presenting authors) on the various topics of diagnosis of tuberculosis and determinants of drug resistance including new anti-TB drugs, assessment of genetic diversity of local populations of *M. tuberculosis*, results of long-term epidemiological studies, implementation of state-of-the-art methods of NGS/WGS in the routine practice of reference centers, analysis of genotypes of strains from patients with TB-HIV coinfection, assessing the functional role of certain mutations in the transmission capacity of strains. The evaluation committee formed of the 4 leading experts in the fields of mycobacterial genomics, molecular epidemiology, drug resistance and immunology decided to award two Best Poster Awards to Margaretha de Vos (Stellenbosch University, Cape Town, South Africa) and Irina Linge (Central Institute for Tuberculosis, Moscow, Russia). The subjects of their posters reflect key directions for the development of research in modern mycobacteriology, namely (1) use of whole genome sequencing for understanding the development of resistance to new anti-TB drugs and (2) interaction of mycobacteria with a host organism.

One of the sessions was devoted to the satellite event of the symposium, a 2nd meeting of the FATE (Fight Against Tuberculosis in Central and Eastern Europe; https://fate-consortium.org/ [Jagielski, 2017]) consortium chaired by Tomasz Jagielski and attended by participants from Albania, Bulgaria, Estonia, Latvia, Poland, Russia, Slovenia, Spain and UK. The ongoing initiatives (e.g. *M. kansasii* global genotyping project; bilateral Polish-Lithuanian *M. tuberculosis* project) and possibilities for new multicenter and bilateral projects were presented and discussed.

More information on the symposium can be found in the Supplementary file and online at http://www.pasteurorg.ru/article/369/2861/International-Symposium-on-Tuberculosis-and-Mycobacteria-took-place-in-St-Petersburg and http://pasteur110.ru/wp-content/Program-of-Symposium-eng.html.

To conclude, the 2nd St. Petersburg Symposium on Tuberculosis and Mycobacteria addressed the interrelated fundamental and applied issues of the modern mycobacteriology such as, molecular evolution and phylogenomics, pathogenesis, host-microbe interactions, coevolution of *M. tuberculosis* with humans, new genomic and postogenomic technologies and their implementation into routine practice. The symposium became a multidisciplinary event useful to promote networking and exchange of knowledge and experience. The next (third) symposium was planned to be organized in St. Petersburg in 2021.

Acknowledgments

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Appendix A. Supplementary material

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