

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 coinfecting 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%).

*Funding: this study was supported by Russian Foundation for Basic Research (grant 17-04-00367).*

### 6.39

doi: 10.15789/2220-7619-2018-4-6.39

#### LOOKING INSIDE THE FOREST: FROM CLASSICAL GENOTYPING OF MYCOBACTERIUM TUBERCULOSIS TO WHOLE GENOME SEQUENCING IN HIGH MULTIDRUG RESISTANCE SETTINGS

J. Perdigão

*Research Institute for Medicines (iMed.U LISBOA), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal*

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) super-clusters 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.

### 6.40

doi: 10.15789/2220-7619-2018-4-6.40

#### ADVANCES IN THE STUDY OF MOLECULAR BASIS OF RESISTANCE TO NEW ANTI-TB DRUGS

I.V. Peretokina<sup>1</sup>, L.Yu. Krylova<sup>1</sup>, O.V. Antonova<sup>2</sup>, M.S. Kholina<sup>2</sup>, D.A. Gryadunov<sup>2</sup>, E.Yu. Nosova<sup>1</sup>, D.V. Zimenkov<sup>2</sup>

<sup>1</sup>*Moscow Research and Clinical Center for Tuberculosis Control, Moscow, Russia;* <sup>2</sup>*Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia*

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 mg/kg/ml with the mode at 0.12 mg/kg/ml. Isolates with mutated *mmpR* gene possessed MIC range of 0.12–4.00 mg/kg/ml with mode at 0.25 mg/kg/ml. Heteroresistant isolates had an intermediate MICs from 0.12 to 2.00 mg/kg/ml. Four isolates with *AtpE* substitutions (D28N, A63P, A63V) had bedaquiline MICs of 4.00 and 8.00 mg/kg/ml. The MICs distributions of wild-type and mutated isolates on 7H11 media had the distinct border between 0.06 mg/kg/ml and 0.12 mg/kg/ml: most of the strains with a MIC of  $\geq 0.12$  mg/kg/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 mg/kg/ml) and Bactec MGIT 960 (1 mg/kg/ml) tests.

*This work was financially supported by the Russian Science Foundation (grant no. 14-50-00060).*

### 6.41

doi: 10.15789/2220-7619-2018-4-6.41

#### THE IMPLEMENTATION OF NEXT-GENERATION SEQUENCING FOR EPIDEMIOLOGICAL STUDIES AND DRUG RESISTANCE INVESTIGATIONS IN MICRO-EPIDEMICS INVOLVING PEDIATRIC TUBERCULOSIS PATIENTS

I. Pole<sup>1,2</sup>, I. Ozere<sup>2,3</sup>, I. Norvaisa<sup>2</sup>, R. Ranka<sup>1,3</sup>

<sup>1</sup>*Latvian Biomedical Research and Study Centre, Riga, Latvia;* <sup>2</sup>*Riga East University Hospital, Centre of Tuberculosis and Lung Diseases, Riga, Latvia;* <sup>3</sup>*Riga Stradiņš University, Riga, Latvia*

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 3<sup>rd</sup> 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 IS6110 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.

*This study was supported by Latvian National Research program VPP "BIOMEDICINE".*

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doi: 10.15789/2220-7619-2018-4-6.42

## MOLECULAR EPIDEMIOLOGY OF TUBERCULOSIS IN LATVIA

R. Ranka<sup>1,2</sup>, I. Pole<sup>1,3</sup>, S. Markovska<sup>1</sup>, I. Ozere<sup>2,3</sup>, V. Riekstina<sup>3</sup>, I. Norvaisa<sup>3</sup>

<sup>1</sup>Latvian Biomedical Research and Study Centre, Riga, Latvia;

<sup>2</sup>Riga Stradiņš University, Riga, Latvia; <sup>3</sup>Riga East University Hospital, Centre of Tuberculosis and Lung Diseases, Riga, Latvia

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 char-

acterize 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 IS6110 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.

*This study was supported by the Latvian National Research program VPP "BIOMEDICINE".*

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doi: 10.15789/2220-7619-2018-4-6.43

## FUNCTIONAL RELEVANCE OF MYCOBACTERIUM TUBERCULOSIS DIVERSITY: FROM GENOTYPES TO IMMUNE RESPONSES AND DISEASE SEVERITY

M. Saraiva

*i3S — Instituto de Investigação e Inovação em Saúde, Porto, Portugal; IBMC — Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal*

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-