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-