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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-