INTRODUCTION

Tuberculosis remains one of the major problems in public health (1). During the last 10 years the tuberculosis incidence and mortality rate in Kazakhstan decreased by 2.4 times and 6 times, respectively. Despite the decreasing rate of incidences in Kazakhstan last years, the rate of multidrug-resistant (MDR) forms is increasing.

According to WHO European Region report, Kazakhstan is 1 of 18 countries with a high rate of MDR tuberculosis (2). Some previous studies have been performed in Kazakhstan using genotyping and sequencing methods (3-5).

It is extremely important to examine sensitive and resistant strains with different mutations in genes encoding drug metabolism among M. tuberculosis strains from the different geographic regions.

MATERIALS AND METHODS

• Prepared genomic libraries of 7 MDR strains were sequenced on Roche 454 GS FLX Titanium NGS platform at the Centre for Life Sciences, NLA-NU.

• FastQC was applied to analyze reads quality and adapters were trimmed using Trimmomatic v.0.38 to truncate low quality reads.

• De novo assembly has been performed by Velvet v.1.2.10.

• Gene prediction and annotation were carried out with Genome Annotation Service PATRIC, using RAST toolkit.

• High quality reads were then mapped to the M. tuberculosis H37Rv genome [GenBank AL234356.3] using GS Reference Mapper v.2.8. The alignment files were subjected to local realignment and de-duplication using GS Reference Mapper v.2.8 and MUmmmer v.3.23. Identified genomic variants were annotated using in-house prepared Python script.

• Comparative phylogenetic analysis has been performed by Maximum-Likelihood method based on Tamura-Nei model in MEGA X.

CONCLUSION

We performed comprehensive bioinformatics analysis of whole-genome data from 7 MDR isolates. We identified genomic variants (SNPs and InDels) in de novo assembled and annotated whole-genomes and specific/novel variants in drug-resistant genes of MDR strains circulated in Kazakhstan.

These findings may provide additional source for justification of drug sensitivity before clinical treatment as well as the basis for expansion of the current reference MTB database with genetic variability among different drug sensitivity isolates. Moreover, these results may provide supplementary information in further fundamental investigation of virulence and transmissibility patterns of MDR strains.

The whole-genome sequencing for 7 MDR isolates produced 667,052,381 paired reads with average read length 520 bp. The mean coverage ranged between 21-51X, and the mapping quality 94.43-97.1%. From 4385 to 4473 coding sequences CDSs and 44 tRNAs, 4 rRNA have been identified. Number of SNPs and InDels ranging from 1581 to 1952, and from 213 to 297, respectively (Table 1). Spoligotyping analysis based on NGS data revealed all MDR strains as Beijing genotype. Phylogenetic analysis (based on rpoB gene) of the five MDR strains in comparison with seven susceptible/drug-resistant strains (H37Ra, H37Rv, KZN 2407, CDC1551 and other) has been showed clustering on two main clusters (Figure 2). Among all isolates we detected several new genetic variants in drug-resistance genes which are not described/unknown in antibiotic resistance databases (ResFinder v.3.0; CARD; CASTB). Circular maps for each MDR isolate have been created (Figure 3 – representation of circular map for isolate MDR-1280).

REFERENCES


Table 3. Annotated of known and unknown variants in antimicrobial resistance genes by ResFinder.

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