GENOTYPE AND PHENOTYPE CHARACTERISTICS OF RESISTANCE TO ANTIMYCOBACTERIAL DRUGS OF 8 M. TUBERCULOSIS STRAINS, ISOLATED FROM PATIENTS WITH NEWLY DIAGNOSED PULMONARY TUBERCULOSIS

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Abstract

The article presents the experience of whole-genome sequencing of M. tuberculosis (MBT) strains.

Materials and methods. 8 MBT isolates were tested. The strains were obtained from patients with newly detected pulmonary tuberculosis (NDPTB). Critical concentrations of anti-mycobacterial drugs (AMBD): H; R; E; Z; Lfx; Mfx; Am; cm; Lzd; Bdq; Dlm and Cfz were determined using the BACTEC MGIT system. Sequencing of MBT strains was performed on Illumina MiSeq equipment at the laboratory of microbiology and biochemistry of the National Institute of phthisiology and pulmonology (NIPP). The obtained whole-genome sequences of MBT strains were deposited in fastq files and analyzed using the PhyResSE program (https://bioinf.fz-borstel.de/mchips/phyresse/) with the bioanalytical solution package. The panel of this package includes gene targets involved in the development of MBT to AMBP. Phylogenetic information and a certain spoligotype make it possible to obtain neutral single nucleotide polymorphisms. The genetic profile of the drug was performed according to the WHO mutation catalog (https://www.who.int/publications/i/item/9789240028173).

The results. The dominant in Ukraine Beijing genotype was represented by 7 strains. The non-beijing group included 1 strain: Ural. 7 MBT strains belonged to the Beijing family, Lineage 2 phylogenetic line with the same spoligotype indices. The analysis of mutations according to the "Catalogue of mutations of the M. tuberculosis complex and their association with drug resistance", WHO, 2021, allowed all detected mutations to be divided into categories - associated with resistance, associated with resistance - intermediate, not associated with resistance, with uncertain significance. Comparison of the resistance profile with the corresponding genotype showed that isolates, belonging to the Beijing genotype, usually carried MDR/pre-XDR mutations.

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All MBT strains with resistance to R had mutations found in the rpoB gene. The canonical amino acid substitution of rpoB S450L, associated with a high level of resistance to R with a non-significant decrease in the viability of the strains, was identified in 6 isolates. Another clinically significant mutation in rpoB (S435Y) was found in 2 cases and only in representatives of the Beijing family. Mutations of the categories: not associated with resistance (rpoC (E1092D)) and of uncertain significance: rpoC (I491V), rpoB (H674N), rpoB (V496A), were detected only in combination with "strictly associated" positions in phenotypic-resistant isolates of the genotype Beijing. The canonical single amino acid substitution katG S315T was detected in all isolates selected for sequencing. All 8 strains resistant to H showed mutations in genes associated with resistance to the drug. Mutations in the key Bdq resistance genes - atpE and mmpR - were not detected in any genome. In 1 strain of MBT, a C154R mutation associated with Lzd resistance was detected in the rplC gene. Similarly, in 1 strain of MBT, a mutation in ddn (D30S0) was detected with an as yet undetermined significance of association with resistance to Dlm.

Conclusions. The study provided information on the genetic diversity of modern MBT isolates in TB patients. The predominance of the Beijing genotype testified to the stable population structure of the TB pathogen and the preservation of trends of high levels of the spread of TB forms of infection on the territory of the country.

The combination of phenotype and molecular genetic data made it possible to give a more complete assessment of DR isolates. A large share of MDR was caused by mutations that did not have a negative effect on the viability and transmissibility of the pathogen, indicating a large epidemic reservoir of MBT strains with MDR/pre-XDR.

The highly transmissible strains of Beijing carried mostly single uncombined R and H mutations, which were associated with a high level of resistance.

Key words: genetic diagnostics, sequencing, pulmonary tuberculosis.

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