AN EXPERIENCE IN INTRODUCTION OF M. TUBERCULOSIS WHOLE GENOME SEQUENCING ON ILLUMINA MISEQ PLATFORM IN THE LABORATORY OF MICROBIOLOGY AND BIOCHEMISTRY AT NSC PPA NAMS OF UKRAINE

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Abstract

The aim: to obtain reliable and representative information on the genetic profile of drug resistance (DR) in clinical isolates of MTB MDR, PRE-WDR and WDR in Ukraine isolated from patients with new cases and relapses using the modern new generation sequencing method.

Materials and methods. Samples with an advanced spectrum of phTMS resistance were selected for the study. The selection of strains took place in the regions gradually and consistently. A total of 75 strains were selected, among which 31 were resistant to at least one of the new drugs. Whole sequencing was performed for 3 IR, 18 MDR, 38 pre-WDR and 16 WDR of MTB strains. All strains have high reliability mutations in accordance with the M. tuberculosis Complex (WHO, Catalogue of Mutations in Mycobacterium tuberculosis Complex and their association with drug resistance, 2023). The genome analysis was performed using MTBseq.

Results and discussions. The phenotypic examination data showed a high level of resistance MTB to the ATD of the I-st and II-nd line. For all MTB isolates, mutations were related to resistance and phTMS results. The results of whole genome sequencing of 75 MTB isolates showed the largest number of identical mutations that determine the resistance to H in the *kat*G - p.Ser315Thr gene (73/75; 93.0 %). The second mutation, which was most commonly found in the MTB studied isolates, was determined by the mutation associated with R – resistant p.Ser450leu (60/75; 80.0 %) in the *rpo*B gene. 44 MTB isolates (58.6 %) contained a mutation p.Lys43Arg in *rps*L gene. There were also often options in *emb*A, *gyr*A gyrB та *rrs*1 genes. Of the mutations associated with MTB DR, the most commonly found missens variants that lead to amino acid replacement in coded protein.

When comparing the resistance profiles obtained in phenotypic and genetic testing (whole sequencing method), a number of differences were found in models of insulation stability. For the drugs of the 1-line, the consistency between the two methods was within 3 percent for R and E, 1.0 % for Z and completely coincided for H. High level of concordance was also noted for Q, Am and Cm-no more than 5, 0 %. The opposite situation has been for Km and new and re-profiled drugs (Bdq, Lzd, Dlm and Cfz) with a high level of difference in test results in two methods. Among 75 TB patients we found that 69 (92.0 %) were infected with MTB strains Beijing. 7 isolates were classified as belonging to the Central Asian outbreak (CAO), 33 isolates were classified as belonging to the Central Asia Subline (Central Asia), and 28 as those belonging to Europe/Russian W148 Outbreak. One isolate - with an indefinite subline. The remaining 6 (8.0 %) patients were infected with other MTB sublines belonging to the L4 Euro-Emerican genotype: Latin American-Mediterranean (LAM)-2 isolates, two isolates-Haarlem and one belonging to the Ural subline.

Conclusions. The study provides information about the genotypic diversity of modern clinical isolates of M. tuberculosis in patients with TB. The prevalence of the Beijing genotype testifies to the stable population structure of the TB pathogen and preserving the trends of high levels of spread of DR forms of infection in Ukraine.

Key words: M. tuberculosis, gene-phenotypic diagnostic methods, new generation sequencing.

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