THE METHOD OF TARGETED NEXT GENERATION SEQUENCE USING THE DEEPLEX MYC-TB REAGENT SET — A PROSPECTIVE APPROACH IN DIAGNOSING *M. TUBERCULOSIS* AND DETERMINING ITS DRUG RESISTANCE

O. A. Zhurylo, A. I. Barbova, N. I. Barbova, L. M. Sladkova

Abstract

The aim of the study is to use tNGS to identify MBT in clinical isolates and sputum samples. This method will be compared with the effectiveness of the culture method and fTMS, whole-genome sequencing (WGS) and GeneXpert MTB/Rif Ultra. To evaluate the effectiveness of tNGS in the diagnosis of TB and detection of drug-resistant gene mutations.

Materials and methods. 75 MBT isolates were selected from strains stored in the laboratory.

In addition, 70 sputum samples taken for the study were collected from adult patients with suspected tuberculosis. BACTEC and GeneXpert systems were used for cultivation. All bacterial isolates were confirmed as MBT using MBP 64 antigen detection kits.

The phenotypic method and molecular genetic testing using the GeneXpert system and Xpert MTB/Rif Ultra cartridges with simultaneous detection of MBT-complex DNA and determination of resistance to R were carried out in accordance with the order of the Ministry of Health of Ukraine No. 1462 dated 06.27.2019.

The tNGS study was performed using Deeplex Myc-TB kits to detect MBT DNA and identify mutations associated with resistance to H, R, Eto, Z, E, S, Km, Am, Cap, Lfx, Mfx, Lzd, Cfz and Bdq. tNGS was performed according to SOPs. Analysis of sequencing data files in FASTQ format was performed using the Deeplex Web Application.

Results. fTMS showed that the resistance rate of 9 drugs ranged from 2.7% in Am to 46.7% in H. According to the results of tNGS-based TMS, the resistance level ranged from 5.3% in Am to 46.7% in R and H, and the difference between the resistance rate of tNGS and fTMS in each drug did not exceed 6.0%. For 75 MBT isolates, the average concordance between tNGS and fTMS was 92.3% for all nine drugs, ranging from 66.7% in Am to 100% in H, Mfx, and Lfx.

The average agreement between tNGS and WGS in the detection of drugs of nine drugs was 97.02%. The sensitivity and specificity of tNGS in predicting DR MBT were 100% and 91.7% for R, 100% and 100% for H, 93.8% and 100% for E, 96.2% and 100% for S, 100% and 100% for Mfx, 100% and 100% for Lfx, 100% and 100% for Lfx, respectively. 94.7% for Am, 100% and 100% for Km, and 39.1% and 100% for Eto. The prognostic values of LS, with the exception of Am, were 100%. Predictive values of drug sensitivity were greater than 95.0%, except for E and Eto. The majority of MBT isolates resistant by WGS but susceptible by tNGS were due to DR mutations detected by WGS that were not captured by tNGS.

Using all research methods, MBT was detected in 27 samples out of 70 sputum samples, which accounted for only 38.5% of cases. Further analysis of the data showed that with negative results of culture using MG studies in Xpert MTB/Rif Ultra cartridges and the tNGS method, an additional 15 samples containing MBT were simultaneously detected, i.e. the number of positive results increased by 21.4% compared to similar results by the culture method. Only the tNGS method additionally made it possible to detect the TB pathogen in another 14 (20.0%) sputum samples that were negative according to the results of routine tests used in practical laboratories of the country for the diagnosis of TB. That is, with the help of the methods of testing sputum samples for TB, the presence of MBT was detected in 80.0% of cases. Compared to other applied methods, tNGS was 1.3 times more effective than the MG method in Xpert MTB/Rif Ultra cartridges in the Gene Xpert system and 1.3 times higher than the level of positive results obtained by the seeding method.

The effectiveness of both MG-methods in detecting MBT with resistance to R in sputum samples was identical and amounted to 15.5%. 1.8 times more samples containing R-susceptible MBT were detected by the MG

method using GeneXpert MTB/Rif Ultra cartridges than by the tNGS method, 41.4% and 22.9%, respectively. This difference in indicators is statistically significant. The main reason drug resistance is not detected by tNGS is that some rare mutations are not included in the detection targets of tNGS. These include rpoB Leu545Met, katG Gly593Asp, ahpC-48G > A, embA -12 C > T, embB Asp354Ala, etc. Among them, rpoB Leu545Met, embA -12 C > T and embB Asp354Ala were observed by us in several isolates of MBT. These mutational variants may be prospectively considered for inclusion in future optimizations of the tNGS method.

Conclusions. The potential of tNGS to transform routine practice in the field of TB diagnosis and treatment is very significant. The high level of concordance between tNGS and WGS/fTMS, combined with its cost-effectiveness, suggests that tNGS may be a competitive alternative and may lead to a change in the main directions of TB diagnosis in the future: instead of relying on sequential MBT drug testing, a one-step comprehensive assessment may simplify the diagnostic process.

One-step comprehensive assessment has several clinical advantages: it allows early detection of TB and its resistance profiles, and provides broad coverage of resistance targets for commonly used clinical drugs.

tNGS demonstrates reliable and rapid performance in the detection of TB and associated MBT and can be directly applied to clinical samples, positioning it as a promising approach for laboratory TB testing.

Key words: targeted sequencing of the next generation, *M. tuberculosis*, implementation of the method.

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Oleksandr A. Zhurilo
SO "National scientific center phthisiology, pulmonology and allergology named after F.G. Yanovskyi National academy of medical sciences of Ukraine"
Head of the Laboratory of Microbiology and Biohemistry
Doctor of medicine, professor
10, M. Amosova str., Kyiv, 03038, Ukraine
Тел/факс: 38044 275-54-30, microbio@ifp.kiev.ua