COMPARISON OF GENEXPERT MTB/RIF AND GENOTYPE SYSTEMS WITH MTBDRPLUS STRIPS FOR DETECTION OF MUTATIONS, ASSOCIATED WITH *M. TUBERCULOSIS* RESISTANCE TO RIFAMPICIN IN TUBERCULOSIS

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Summary

The aim was to analyze the level of compliance of two molecular genetic methods GeneXpert MTB/RIF and GenoTypeDR*plus* in determining the drug resistance of *M. tuberculosis* to rifampicin when detecting mutations in the RRDR region associated with drug resistance.

Object and methods. We studied strains of *M. tuberculosis* with resistance to rifampicin, which was detected by any of the studied molecular genetic methods. 96 sputum samples were taken. Sputum smears were examined for the presence of acid-fast bacteria by microscopy after staining with the Ziehl-Neelsen method. The material was inoculated into Middlebrook 7H9 broth and on Lowenstein-Jensen medium. The liquid medium was incubated in the BACTEC MGIT system. An immunochromatographic test was used to identify the strains. The drug susceptibility test of *M. tuberculosis* to rifampicin was performed using the BACTEC MGIT system. The GeneXpert MTB/RIF test was performed according to the manufacturer's instructions. The GenoTypeDR*plus* assay was performed on decontaminated and concentrated sputum samples. The process was carried out in three stages: DNA extraction from the processed sputum sample; amplification of the RRDR region by PCR; hybridization of the PCR product to specific oligonucleotide probes immobilized on the test strip. For sequencing, *M. tuberculosis* DNA isolation was performed using the QIAamp® DNA Mini Kit. The DNA concentration was measured on a Denovix Quantus spectrophotometer. Targeted panel amplification was performed using the Deeplex Myc-TB kit. Amplicon purification was performed using Agencourt AMPure XP magnetic beads. Quantitative analysis of the purified amplification products was performed using a Qubit fluorometer. *The M. tuberculosis* DNA library was prepared for sample sequencing using the Nextera XT DNA library preparation kit. The library used 5.0 µl of input DNA at a concentration of 0.2 ng/µl. Sequencing was performed on MiSeq equipment with the library normalization and denaturation protocol.

Results and discussion. The GeneXpert MTB/RIF and MTBDR*plus* systems target the same 81 bp rifampicin resistance domain. (RRDR) subunits of bacterial RNA polymerase (*rpo*B) for mutation detection using DNA probes, i.e. there is a correspondence of probes to each other and an expected similarity of probe binding. We analyzed all sputum samples using GeneXpert MTB/RIF and GenoType MTBDR*plus* and phenotypic BACCTEC MGIT methods. The level of agreement between two molecular genetic methods for the detection of rifampicin-associated mutations in the RRDR region has been established. The RRDR 81bp region of the *rpo*B gene of mismatched cases was studied by sequencing.

GeneXpert MTB/RIF and GenoType DR*plus* matched the phenotypic method in 92.7% and 89.6% of cases of *M. tuberculosis* resistance, respectively. Complete agreement between the results of GeneXpert MTB/RIF and GenoTypeMTBDR*plus* was observed in 92.7% of cases. GeneXpert MTB/RIF and GenoType DR*plus* showed a similar pattern of binding failure of wild type probes (WT-probes) when scanning the 81 bp region (RRDR-domain), which leads to stability diagnostics through probe failure software. Sequencing of the RRDR region of "mismatched" strains showed that

GeneXpert probes detected seven "mismatched" cases correctly, and GenoTypeDRplus was erroneous in all cases. GeneXpert has demonstrated greater accuracy in R-resistance detection for mismatched isolates compared to GenoTypeDR*plus*. GeneXpert MTB/RIF has a number of other benefits over GenoTypeDR*plus*. GeneXpert MTB/RIF is relatively easier to implement, biosafety requirements are minimal, study times are shorter, and the study process is more automated, resulting in less human error and more reproducible results. Given these facts and the results of GeneXpert MTB/RIF found in the study, it is recommended that GeneXpert MTB/RIF be used to detect MDR-TB.

Conclusions. Sequencing of the 81bp RRDR region of mismatched *M. tuberculosis* strains showed that GeneXpert MTB/RIF performed more accurately than GenoTypeDR*plus* in detecting mutations associated with rifampicin resistance.

GeneXpert MTB/RIF is relatively easier to perform, biosafety requirements are minimal, time to study is shorter, and the study process is more automated, resulting in less human error and greater reproducibility of results, so it is reasonable to use it for the detection of multidrug-resistant tuberculosis.

The Deeplex Myc-TB analytical solution delivers a wealth of insightful information from antimycobacterial drug resistance markers and speeds up data analysis with easy-to-use software.

Key words: tuberculosis, Mycobacterium tuberculosis, drug resistance, GeneXpert MTB/RIF and GenoTypeDR*plus* molecular genetic systems, BACTEC MGIT phenotypic system, sequencing.

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