Detection of the multidrug-resistant phenotype in *Mycobacterium tuberculosis* clinical isolates, by using Genotype MTBDR*plus* and Whole Genome Sequencing

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The study was financed by the National Science Center «DAINA» Programme (2017/27/L/NZ6/03279) and by Research Council of Lithuania «DAINA» Programme (S-LL-18-4)

Background: With an approximately 10 million new cases and 1.4 million deaths every year, tuberculosis (TB) remains a major global health challenge. Worldwide in 2019, *ca.* 400,000 people had multidrug-resistant (MDR) TB.
The aim of the study was to compare the capacity of two molecular approaches, i.e. multiplex PCR combined with hybridization assay and whole genome sequencing (WGS), both commercially available, for the detection of resistance of *Mycobacterium tuberculosis* to isoniazid (INH) and rifampicin (RIF), defining the MDR phenotype in TB.

Methods: The study included 80 *M. tuberculosis* (54 MDR and 26 drug-susceptible; DS) isolates, recovered from as many Polish (*n*=52) and Lithuanian (*n*=28) patients, over a 2-year period (2018-2019). Conventional drug susceptibility testing (DST) was performed using the currently WHO-approved BACTEC MGIT 960 system (BD, USA) and/or 1% proportion method on Löwenstein-Jensen medium. Genomic DNA was extracted using PureLink Genomic DNA Mini Kit (ThremoFisher Scientific, USA) or using a modified cetyltrimethylammonium bromide method. For molecular determination of drug resistance, mutation profiling was performed using either GenoType MTBDR*plus* assay (Hain Lifescience, Germany) or Whole Genome Sequencing analysis. For the latter, raw reads obtained from Illumina NovaSeq 6000 sequencer were quality checked and assembled into contigs. Mutation profiles were established based on multiple sequence alignments constructed using Clustal Omega, on homologs of *katG* and *rpoB* genes, and *inhA* promotor region from the *M. tuberculosis* H37Rv reference strain. An isolate was considered as drug-resistant, if a relevant mutation was detected, according to the surveyed literature.

Results: The sensitivities of the Genotype MTBDR*plus* and WGS-based approach for the detection of INH-resistance were congruent and reached 97.5% (78/80).

Two isolates designated as INH-resistant upon routine DST did not contain any non-synonymous mutation in the *katG* gene or *inhA* promotor region, as shown with the GenoType MTBDR*plus* and WGS analysis.
For RIF detection, the sensitivity of Genotype MTBDR*plus* and WGS was 100% (80/80) and 98.8% (79/80), respectively. Only one phenotypically RIF-resistant isolate harbored S531L mutation in the RpoB protein, detected only with the Genotype MTBDR*plus* kit.

Conclusions: Overall, high sensitivities of Genotype MTBDR*plus* assay and sequence analysis for the detection of INH and RIF resistance in TB support their use for large-scale screening of MDR phenotype in TB.