31st ECCMID Online 9 – 12 July 2021

ESCMID EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

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

Zofia Bakuła[1], Mikołaj Dziurzyński[2], Przemysław Decewicz[2], Edita Vasiliauskienė[3,4,5], Laima Vasiliauskaitė[3,4,5], Katarzyna Roeske[1], Petras Stakėnas[3], Uršulė Miknevičiūtė[5], and Tomasz Jagielski[1]

[1]Department of Medical Microbiology, Institute of Microbiology, Faculty of Biology, University of Warsaw, Poland;
[2]Department of Environmental Microbiology and Biotechnology, Institute of Microbiology, Faculty of Biology, University of Warsaw, Poland;
[3]Department of Immunology and Cell Biology, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania;
[4]Department of Physiology, Biochemistry, Microbiology and Laboratory Medicine, Institute of Biomedical Sciences, Vilnius University, Lithuania
[5]Centre of Laboratory Medicine, Tuberculosis Laboratory, Vilnius University Hospital Santaros klinikos, Vilnius, Lithuania

OBJECTIVES

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.

MATERIALS & 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 ClustalW, on homologs of *katG*, *rpoB, inhA* genes, and *inhA* promotor region from the reference strain. An isolate was considered as drug-resistant, if a relevant mutation was detected, according to the surveyed literature.

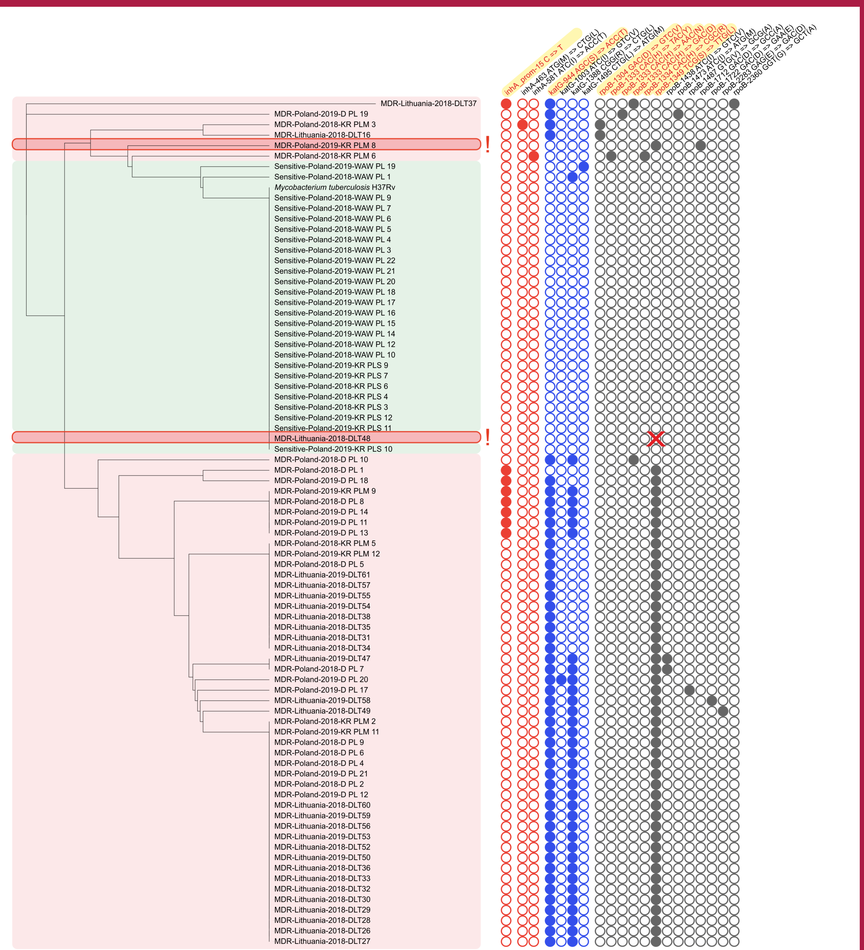

Figure 1. UPGMA cladogram based on the concatenated alignments of the differences in *inhA* promotor sequence and InhA, KatG, and RpoB protein sequences. Pink and green shadowed area of the cladogram refer to MDR and sensitive strains, respectively. Mutations covered with GenoType MTBDR*plus* are written in red on yellow background. Filled/empty circles inform about the presence/absence of mutations detected upon WGS. A circle marked with „X" represents mutation detected with GenoType MTBDR*plus* assay and missed with WGS. Isolates which gave inconsistent results between analysed methods are marked with „!".

RESULTS

The sensitivities and specificities of the Genotype MTBDR*plus* and WGS-based approach for the detection of INH- and RIF-resistance are shown in **Table 1**.

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 (**Figure 1**).

Only one phenotypically RIF-resistant isolate harbored S531L amicoacid change in the RpoB protein, detected only with the Genotype MTBDR*plus* assay (**Figure 1**).

Drug	Assay	Sensitivity [%]	Specificity [%]
INH	GenoType MTBDR*plus*	96.4%	100%
	WGS	96.4%	100%
RIF	GenoType MTBDR*plus*	100%	100%
	WGS	98.2%	100%

Table 1. Performance of the Genotype MTBDR*plus* and WGS-based approach for the detection of INH- and RIF-resistance.

CONCLUSION

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.

ACKNOWLEDGMENTS

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).

CONTACT INFORMATION

Tomasz Jagielski, PhD, DSc, Department of Medical Microbiology, Institute of Microbiology, Faculty of Biology, University of Warsaw,
I. Miecznikowa 1, 02-096 Warsaw, Poland.
E-mail: t.jagielski@biol.uw.edu.pl