Evaluation of conventional methods versus whole-genome sequencing and MYCOTBI for the detection of resistance to rifampicin and isoniazid in *Mycobacterium tuberculosis* complex strains from Lithuania and Poland

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OBJECTIVES

Rapid and accurate rifampicin (RIF) and isoniazid (INH) resistance detection is essential for treatment of multi-drug-resistant tuberculosis (MDR-TB). The objective of this study was to evaluate the performance of phenotype- and genotype-based drug susceptibility testing (pDST/gDST) methods for detection of RIF/INH resistance in relation to whole genome sequencing (WGS).

MATERIALS & METHODS

The study included 185 *Mycobacterium tuberculosis* isolates (124 MDR-TB; 61 drug-susceptible), recovered from patients in Lithuania (n=122) and Poland (n=63) (2018–2021). DST was performed using BACTEC MGIT-960 System and Löwenstein-Jensen (LJ) medium and MICs were determined using MYCOTBI plates. Line probe assay (LPA) was used for rapid resistance prediction. WGS was done with Illumina NovaSeq 6000 sequencer, and data were analyzed using either (i) bioinformatic pipeline or (ii) freely available software platforms (Mykrobe, TB Profiler).

RESULTS

The congruency of BACTEC/LJ with LPA was higher than with pipeline-based WGS results (98.9% [RIF]; 97.8% [INH] vs. 97.3% [RIF]; 97.3% [INH]). MIC ranges for genotypically susceptible isolates were ≤0.12–0.5 µg/mL and ≤0.03–0.06 µg/mL for RIF and INH, respectively (Figure 1 & Figure 2). The most prevalent *rpoB* mutation was S450L (79.8%), and majority of mutants demonstrated MICs of >16 µg/mL. *katG* S315T (85.3%) mutants and those with an extra inhA C-15T (12.1%) or *inhA* T-8A (0.8%) mutation had MICs within the range of 0.12–>4.0 µg/mL. Single *inhA* C-15T mutant had MIC of 1 µg/mL. There were four genotypically RIF-susceptible isolates with resistant phenotypes in Bactec/LJ of which three (KR-PLM-15; DLT-80; DLT76) resulted in low MICs of 0.5, ≤0.12, 0.25 µg/ml, respectively, and one isolate (D-PL-22) had MIC of 16 µg/ml. One *katG* S315T mutant (DLT49) elevated MIC only to 0.06 µg/ml, which could be attributed to technical errors. Three isolates without detected resistance conferring mutations resulted in high MICs to INH (DLT48 – 1 µg/ml, KR-PLM-8, and D-PL-22 – >4 µg/ml) which were congruent with Bactec/LJ results, and two isolates (DLT80; KR-PLM-15) with resistant phenotypes were within the MIC range of ≤0.03-0.06 µg/ml.

CONCLUSIONS

The overall discordance rate between pDST and gDST results was 3.8%. Five isolates were found that displayed unexplained discordant results. This may relate to heteroresistance, mixed infection or methodological flaws.

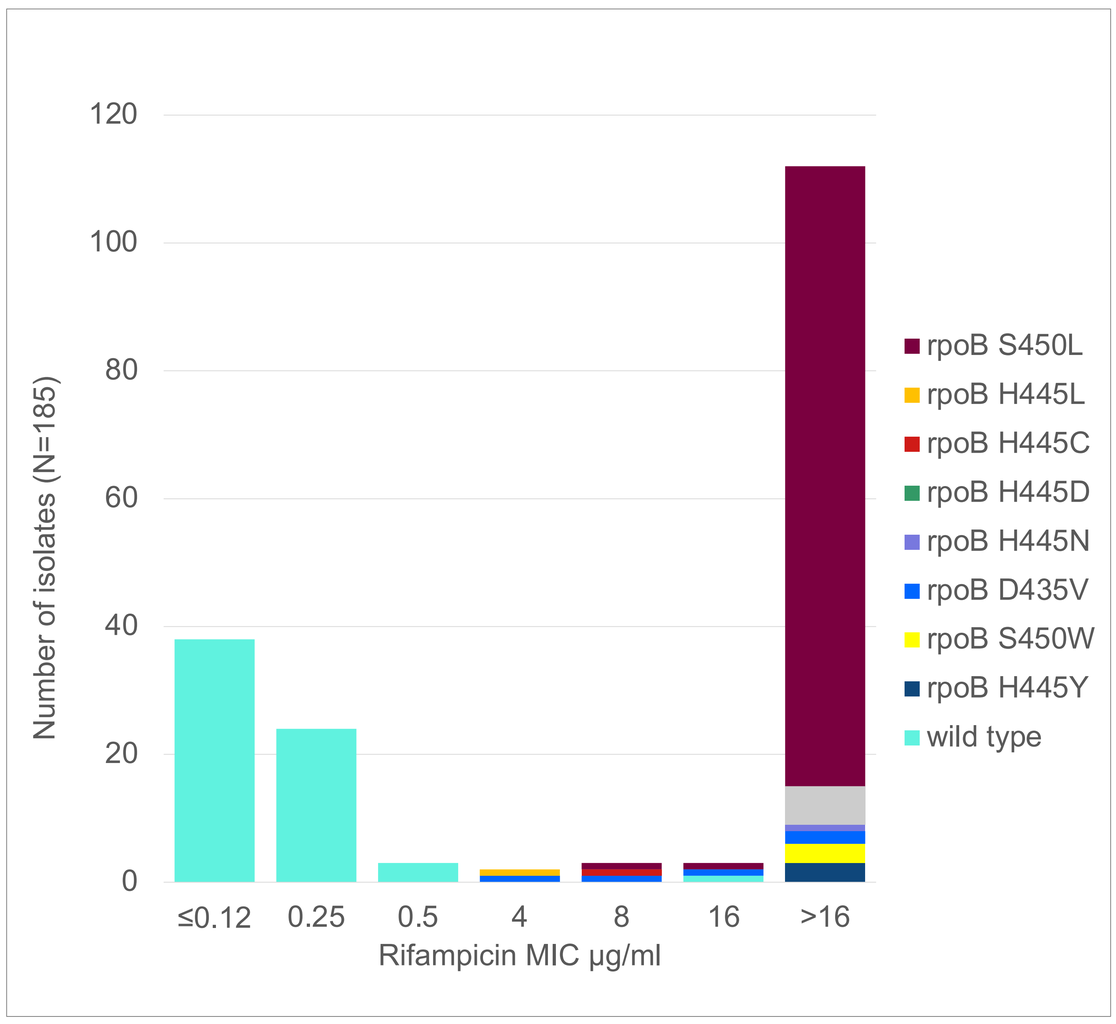

Figure 1. Rifampicin MIC distribution determined using 96-well plates (MYCOTBI, Thermo Scientific) and stratified by *rpoB* mutations.

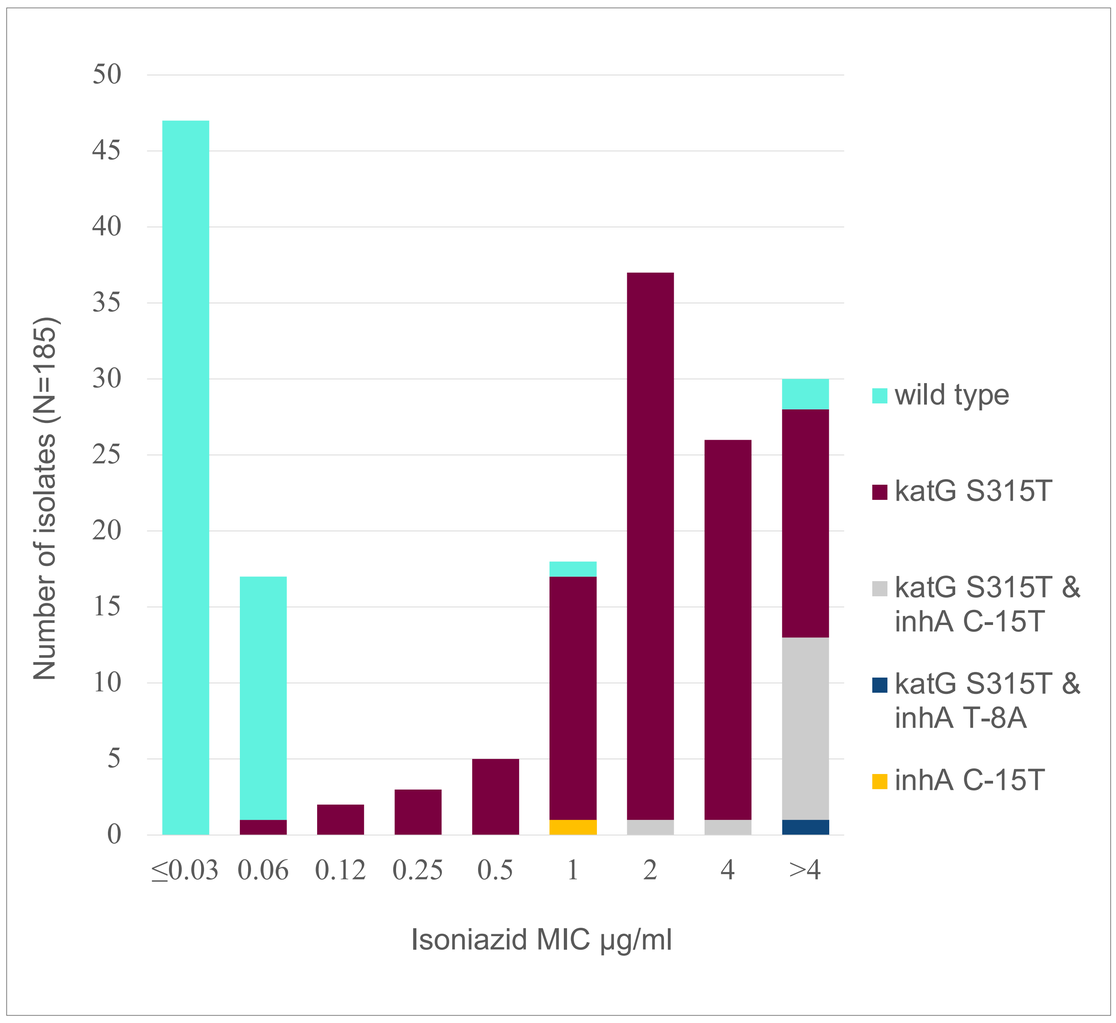

Figure 2. Isoniazid MIC distribution determined using 96-well plates (MYCOTBI, Thermo Scientific) and stratified by *katG* and *inhA* mutations.

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