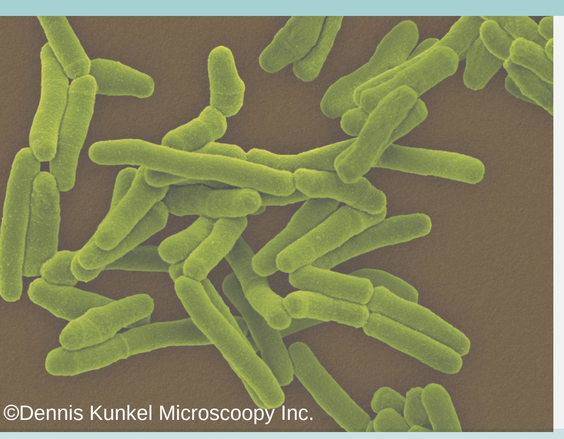
Detection of resistance to second line drugs in *Mycobacterium tuberculosis* using *in silico* approaches



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INTRODUCTION

In 2021, *ca.* half million people developed tuberculosis (TB) eligible for treatment with second-line drugs (SLDs). Yet relatively few studies evaluated the use of whole-genome sequencing (WGS) in prediction of TB susceptibility to SLDs.
The aim of the study was to compare the capacity of two *in silico* WGS-based approaches for the detection of resistance to SLDs in *Mycobacterium tuberculosis.*

MATERIALS AND METHODS

The study included 118 multidrug-resistant (MDR) and 60 drug-susceptible (DS) isolates, recovered from as many (178) Polish and Lithuanian patients between 2018 and 2021. Conventional drug susceptibility testing was performed using BACTEC MGIT 960. WGS was done with Illumina NovaSeq 6000 sequencer. Molecular determination of resistance to amikacin (AMK), capreomycin (CAP), kanamycin (KAN), moxifloxacin (MOX), and ofloxacin (OFX) was done with Mykrobe and TBProfiler. The latter application was also used to assess resistance to bedaquiline (BDQ), delamanid (DLM), ethionamide (ETH), and linezolid (LZD).

RESULTS

Both tools produced congruent results for all tested drugs, except for OFX and MOX. For those two fluoroquinolones the concordance of the results between phenotypic and genotypic assays was higher for Mykrobe (93.3% for OFX and 94.9% for MOX) than for TBProfiler (77.5% for OFX and 89.3% for MOX) (**Figure 1**).
Overall, the sensitivities of the *in silico* approaches varied across drugs and was the highest (100%) for MOX (assessed with Mykrobe), and BDQ, and the lowest (50%) for OFX (with TBProfiler), MOX (with TBProfiler), LZD, and DLM) (**Table 1**).

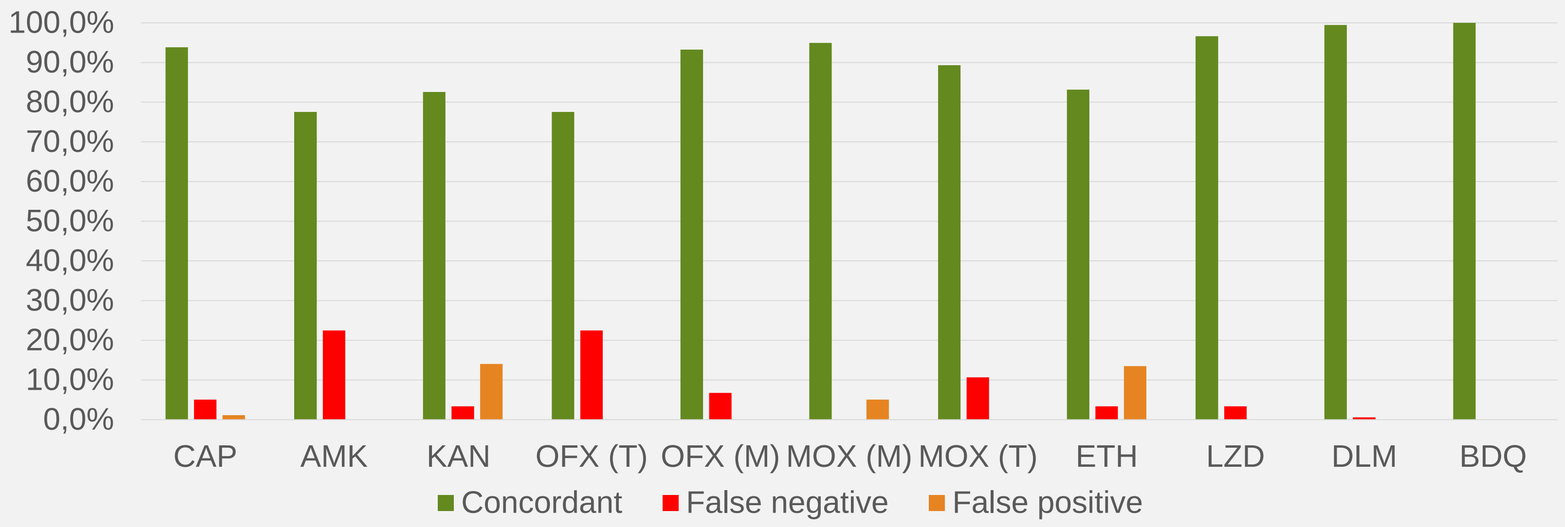
Table 1. Accuracy of *in silico* approaches in drug resistance prediction. If the results differed between Mykrobe (M) or TBProfiler (T), the name of the test is given in brackets. Red, orange and green shading indicate high (>95%), moderate (95-70%) and low (>70%) accuracy, respectively.

Characteristics	Tested drug (no. of isolates resistant to a tested drug)								
	CAP (22)	AMK (52)	KAN (40)	OFX (40)	MOX (19)	ETH (14)	LZD (6)	DLM (1)	BDQ (0)
Sensitivity (%)	71	56.5	87.0	50 (T); 76.9 (M)	100 (M); 50 (T)	70	50	50	100
Specificity (%)	98.7	100	84.7	100	94.6 (M);100 (T)	87.2	100	100	100
Positive predictive value (%)	91.7	100	61.5	100	67.9 (M); 100 (T)	36.8	100	100	100
Negative predictive value (%)	94.5	75.9	95.8	77.5 (T); 92% (M)	100(M); 89.3 (T)	96.5	96.6	99.4	100

CONCLUSIONS

Given relatively low sensitivities of Mykrobe and TBProfiler for the detection of resistance to SLDs in *M. tuberculosis*, performance of standard phenotypic tests is advised.

Figure 1. Percentage of concordant, false negative and false positive results, obtained with *in silico* approaches. If the results differed between Mykrobe (M) or TBProfiler (T), the name of the test is given in brackets.



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