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MECHANISMS OF RESISTANCE

Systematic Review of Whole-Genome Sequencing Data To Predict Phenotypic Drug Resistance and Susceptibility in Swedish *Mycobacterium tuberculosis* Isolates, 2016 to 2018

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ABSTRACT In this retrospective study, whole-genome sequencing (WGS) data generated on an Ion Torrent platform was used to predict phenotypic drug resistance profiles for first- and second-line drugs among Swedish clinical *Mycobacterium tuberculosis* isolates from 2016 to 2018. The accuracy was ~99% for all first-line drugs and 100% for four second-line drugs. Our analysis supports the introduction of WGS into routine diagnostics, which might, at least in Sweden, replace phenotypic drug susceptibility testing in the future.

KEYWORDS drug resistance prediction, high-throughput nucleotide sequencing, microbial drug resistance, microbial sensitivity tests, tuberculosis, whole-genome sequencing

Globally, *Mycobacterium tuberculosis*, the bacterium that causes tuberculosis (TB), remains a major public health issue, and the emergence of multidrug- and extensively drug-resistant TB (MDR-/XDR-TB) calls for improved disease control and diagnostic assays (1). In Sweden, however, the incidence has rapidly declined since the 1940s, and TB is now considered a rare disease, with 4.9 cases per 100 000 inhabitants in 2018 and an MDR rate of 3.1% among culture-confirmed cases (https://www.folkhalsomyndigheten.se/folkhalsorapportering-statistik/statistik-a-o/sjukdomsstatistik/tuberkulos/). In Sweden, the primary diagnostics of TB and the initial screening for drug resistance are performed at five regional TB laboratories. Culture-positive samples are subsequently sent to the National Reference Laboratory (NRL) for TB at the Public Health Agency of Sweden for further characterization, i.e., whole-genome sequencing (WGS) for epidemiological typing and detection of resistance mutations and extended phenotypic drug susceptibility testing (DST) of MDR-TB cases.

Based on previous studies, we decided to evaluate whether a basic catalogue of resistance mutations could be used to reliably predict phenotypic drug resistance and susceptibility (2–5). Since 2016, *M. tuberculosis* isolates sent to the NRL have routinely been subjected to WGS on an Ion Torrent platform (Thermo Fisher Scientific, Inc., Waltham, MA). In this study, all isolates sent to the NRL between 2016 and 2018 ($n = 1{,}313$; 65 samples were, however, excluded due to contamination [$n = 8$], replicates/retrospective isolates [$n = 56$], or no available phenotypic DST [$n = 1$]) were classified as genotypically resistant or susceptible based on the current version of the in-house mutation catalogue used for genotypic DST (see Data Set S1 in the supplemental material). Briefly, sequencing reads were mapped against a set of resistance genes (*katG*; *fabG1*; *inhA*, including the promoter region; *rpoB*; *embB*; *pncA*; *gyrA*; *gyrB*; *rrs*; *eis*, including the promoter region; and *tlyA*) derived from the reference genome H37Rv (GenBank accession no. NC_000962.3) (CLC Assembly Cell version 4.4.2; Qiagen,

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TABLE 1 Performance of whole-genome sequencing for prediction of resistance to first- and second-line *Mycobacterium tuberculosis* drugs

Drug concn (mg/liter)	No. of isolates testing as[a]: pR gR	pR gS	pS gR	pS gS	Sensitivity (% [95% CI])[j]	Specificity (% [95% CI])	Predictive value (% [95 % CI]) Positive	Negative	Accuracy
Isoniazid 0.1 (n = 1,233)	145	10[b]	0	1,078	93.5 (88.5–96.9)	100 (99.7–100)	100 (97.5–100)	99.1 (98.3–99.6)	99.2 (98.5–99.6)
Rifampin 1 (n = 1,235)	51	0	6[c]	1,178	100 (93.0–100)	99.5 (98.9–99.8)	89.5 (78.5–96.0)	100 (99.7–100)	99.5 (99.0–99.8)
Ethambutol 5 (n = 1,230)	18	2[d]	15[e]	1,195	90.0 (68.3–98.8)	98.8 (98.0–99.3)	54.5 (36.4–71.9)	99.8 (99.4–100)	98.6 (97.8–99.2)
Pyrazinamide 100 (n = 1,230)	47	4[f]	0	1,179	92.2 (81.1–97.8)	100 (99.7–100)	100 (92.5–100)	99.7 (99.1–99.9)	99.7 (99.2–99.9)
Ofloxacin 1 (n = 342)	10	0	0	332	100 (69.2–100)	100 (98.9–100)	100 (69.2–100)	100 (98.9–100)	100 (98.9–100)
Moxifloxacin 0.5 (n = 57)	4	0	4[g]	49	100 (39.8–100)	92.5 (81.8–97.9)	50.0 (15.7–84.3)	100 (92.7–100)	93.0 (83.0–98.1)
Kanamycin 2.5 (n = 55)	7	0	0	48	100 (59.0–100)	100 (92.6–100)	100 (59.0–100)	100 (92.6–100)	100 (93.5–100)
Amikacin 1 (n = 446)	1	0	0	445	100 (2.5–100)	100 (99.2–100)	100 (2.5–100)	100 (99.2–100)	100 (99.2–100)
Capreomycin 2.5 (n = 55)	6	0	0	49	100 (54.1–100)	100 (92.7–100)	100 (54.1–100)	100 (97.7–100)	100 (93.5–100)
Ethionamide 5 (n = 55)	4	3[h]	5[i]	43	57.1 (18.4–90.1)	89.6 (77.3–96.5)	44.4 (13.7–78.8)	93.5 (82.1–98.6)	85.5 (73.3–93.5)

[a]pR, phenotype resistant; gR, genotype resistant; gS, genotype susceptible; pS, phenotype susceptible.
[b]*katG*: Arg119Pro + Arg463Leu (1), Arg463Leu (3), Val1Gly + Glu3Val (1), wild type (WT) (5).
[c]*rpoB* mutations related to rifampin susceptibility in MGIT and resistance in LJ (Löwenstein-Jensen medium)-DST.
[d]*embB*: Leu499Pro + (C1602T) (1), WT (1).
[e]Nine of 12 strains resistant to 2.5 mg/liter ethambutol.
[f]*pncA*: Ala171Val (1), Asp8His (2); heteroresistance, *pncA*: Ala143Val (1).
[g]Three strains resistant to 0.25 mg/liter moxifloxacin.
[h]*inhA*: WT (3).
[i]*inhA*: C-15T (4), C-15T + Ile194Thr (1).
[j]CI, confidence interval.

Hilden, Germany). The extracted variants (CLC Assembly Cell version 4.4.2) were filtered (minimum frequency of reads calling single nucleotide polymorphisms, 25%; minimum frequency of reads calling indels, 80%), and the remaining variants were matched against the in-house database of resistance mutations. Phenotypic DST results from Bactec MGIT 960 (Becton, Dickinson Biosciences, Sparks, MD) were obtained from regional TB laboratories or the NRL. Statistical analysis was performed in STATA version 15.0 (STATA Corp., College Station, TX).

Overall, the sensitivity ranged between 90.0% (ethambutol) and 100% (rifampin) for the first-line drugs and was 100% for the second-line drugs (ofloxacin, moxifloxacin, amikacin, kanamycin, and capreomycin) (Table 1). Similarly, the specificity ranged between 98.8% (ethambutol) and 100% (isoniazid and pyrazinamide) for the first-line drugs and between 92.5% (moxifloxacin) and 100% (ofloxacin, kanamycin, amikacin, and capreomycin) for the second-line drugs. Ethionamide was included in the analysis but was less accurate than the other drugs.

For isoniazid, all isolates lacking a resistance mutation (gSusceptible) were classified as susceptible in the phenotypic DST (pSusceptible). Conversely, 10 isolates classified as gSusceptible were reported as phenotypically resistant (pResistant) (detailed data on all phenotypes and genotypes are reported in Data Set S2 in the supplemental material).

For rifampin, the discrepancies were limited to 6 gResistant isolates classified as pSusceptible. All of these isolates harbored a so-called disputed *rpoB* mutation, which typically tests as resistant in Löwenstein-Jensen medium but susceptible in the MGIT system (6, 7).

For ethambutol, 2 gSusceptible isolates were reported as pResistant, whereas 15 isolates that carried a mutation in *embB* codon 306 or 406 were classified as pSusceptible. We had MGIT results for ethambutol (2.5 mg/liter) for 12 of the latter isolates. Nine (75%) of 12 were resistant at 2.5 mg/liter, whereas the remaining 3 did grow in the MGIT tube but below the level (growth units $<$100) to be classified as resistant (see Data Set S2). This illustrates a stronger correlation between the *embB* mutations and resistance to the lower test concentration.

For pyrazinamide, 4 isolates reported as gSusceptible were classified as pResistant, and all of these harbored a nonsynonymous *pncA* mutation absent in our catalogue, which was discovered in a resistant subpopulation (heteroresistance) in 1 isolate.

Among the second-line drugs, no discrepancies were reported for ofloxacin, amikacin, kanamycin, or capreomycin. For moxifloxacin, 4 isolates carrying a mutation present in our catalogue were tested as susceptible at 0.5 mg/liter, but 3 of these were resistant at 0.25 mg/liter.

Ethionamide was distinguished by both false positives and false negatives, and more knowledge about the correlation between phenotype and genotype is needed for this drug.

The main limitations in this study are the incomplete mutation catalogue and the inability of the genotypic assay to detect subpopulations at a frequency <10%. This would typically result in isolates being classified as false susceptibles in the genotypic test; and for isoniazid and pyrazinamide, the discrepant isolates were exclusively on the false-negative side (pResistant but gSusceptible). This may be explained by limitations in the mutation catalogue but may also be due to resistant subpopulations not being detected by the genotypic test, as illustrated in one isolate tested for pyrazinamide resistance. Rifampin, ethambutol, and moxifloxacin were, on the other hand, characterized by a number of false positives (pSusceptible but gResistant). These discrepancies may, in turn, be attributed to the unclear role of "disputed" *rpoB* mutations (rifampin) and less optimized test concentrations for ethambutol (5 mg/liter) and moxifloxacin (0.5 mg/liter, but changed to 0.25 mg/liter in the latest WHO recommendation) (6, 8). Further studies are needed to clarify the relationship between genotype and phenotype, including the corresponding MICs. However, based on these results, we can conclude that it is possible to produce reliable predictions for first- and second-line drug resistance with an Ion Torrent platform using an in-house catalogue of resistance mutations. The small number of strains with second-line drug resistance is another study limitation. However, the specific aim was to determine the reliability of WGS to predict phenotypic resistance in the Swedish context, a country with low incidence where >95% of the clinical *M. tuberculosis* isolates are non-MDR. Based on our findings, it is arguable whether routine phenotypic DST of first-line drugs is necessary for gSusceptible isolates. In the near future, with further improvements in DNA isolation from liquid cultures and/or clinical specimens, this will have great implications for the algorithms used for both phenotypic and genotypic DST.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, XLSX file, 0.2 MB.

SUPPLEMENTAL FILE 2, XLSX file, 0.2 MB.

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