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Comparisons of whole-genome sequencing and phenotypic drug susceptibility testing for *Mycobacterium tuberculosis* causing MDR-TB and XDR-TB in Thailand

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ABSTRACT

Drug-resistant tuberculosis (TB) is a major public health problem. There is little information regarding the genotypic–phenotypic association of anti-TB drugs, especially for second-line drugs. This study compared phenotypic drug susceptibility testing (DST) with predictions based on whole-genome sequencing (WGS) data for 266 *Mycobacterium tuberculosis* isolates. Phenotypic DST used the standard proportional method. Clinical isolates of *M. tuberculosis* collected in Thailand between 1998 and 2013 comprised 51 drug-sensitive strains, six mono-resistant strains, two multiple-resistant strains, 88 multi-drug-resistant strains, 95 pre-extensively drug-resistant strains and 24 extensively drug-resistant strains. WGS analysis was performed using the computer programs PhyResSE and TB-Profiler. TB-Profiler had higher average concordance with phenotypic DST than PhyResSE for both first-line (91.96% vs. 91.4%) and second-line (79.67% vs. 78.20%) anti-TB drugs. The average sensitivity for all anti-TB drugs was also higher (83.13% vs. 72.08%) with slightly lower specificity (83.50% vs. 86.68%). Regardless of the program used, isoniazid, rifampicin and amikacin had the highest concordance with phenotypic DST (96.2%, 93.5% and 95.6%, respectively). Ethambutol, ethionamide and fluoroquinolones had the lowest concordance (87.34%, 81.44% and 73.85%, respectively). Concordance rates of ofloxacin (a second-generation fluoroquinolone), levofloxacin, moxifloxacin and gatifloxacin (third- and fourth-generation fluoroquinolones) were 91.79%, 76.62%, 72.64% and 57.35%, respectively. Discordance between phenotypic and WGS-based DSTs may be due, in part, to the choice of critical concentration and variable reproducibility of the phenotypic tests. It may also be due to limitations of the mutation databases (especially for the second-line drugs) and the analysis program used. Mutations related to fluoroquinolone resistance, especially the later generations, need to be identified.

1. Introduction

Tuberculosis (TB), a major infectious disease caused by *Mycobacterium tuberculosis*, accounts for 1.8 million deaths and 10.4 million new cases annually [1]. Resistance to anti-TB drugs is an increasing global health threat. Multi-drug-resistant (MDR) and extensively drug-resistant (XDR) TB strains are emerging. In 2016,

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the World Health Organization (WHO) estimated that there were 490 000 MDR-TB cases and 8000 XDR-TB cases worldwide [1].

Drug susceptibility testing (DST) is essential for detection and management of drug-resistant TB. Although phenotypic DST using the proportional method is regarded as the gold standard [2], this approach has a long turnaround time and there are some problems with interpretation of breakpoint values. Rapid identification of mutations conferring drug resistance is likely to be a superior approach. Commercial molecular assays such as GeneXpert and Line Probe Assays, which are able to identify members of the *M. tuberculosis* complex and simultaneously detect drug-resistance-associated mutations [3], are still limited in the number of tested genes and antibiotic. Advanced molecular techniques such as whole-genome sequencing (WGS) analysis are used increasingly in health care, and WGS is currently one of the most promising approaches for TB diagnosis, determining the complete DNA sequence. This provides definitive diagnosis, allows researchers to study TB transmission within a community, and provides sequences of genes that might be associated with drug resistance. However, WGS-based DST is currently of limited value due to the lack of databases of mutations associated with drug resistance. This is particularly true for the second-line anti-TB drugs.

WGS-based DST is likely to become the main test for TB diagnosis. A comparison of WGS-based DST and phenotypic DST is required to assess the clinical applicability of WGS. However, previous comparisons have largely focused on *M. tuberculosis* strains that are resistant to the first-line anti-TB drugs [4–6], and have investigated few *M. tuberculosis* strains resistant to the second-line anti-TB drugs [7,8]. Additional large collections of *M. tuberculosis* strains resistant to second-line anti-TB drugs, especially MDR-TB and XDR-TB strains, are needed to provide insight into the applicability of WGS in routine DST for TB.

Among the software tools available for WGS-based DST, TB-Profiler [9] and PhyResSE [10] stand out due to their relatively comprehensive databases covering both mutations and types of anti-TB drugs. Evaluation of these programs has rarely been undertaken with strains resistant to second-line drugs (i.e. MDR-TB and XDR-TB), or has used only a low number of such strains [11,12].

This study reports the use of drug-resistant *M. tuberculosis* strains in Thailand to compare the performance of WGS-based DST (using the software mentioned above) and the phenotypic proportional method.

2. Methods

2.1. M. tuberculosis isolates and setting

In total, 266 *M. tuberculosis* isolates were retrieved from stock cultures of clinical isolates deposited at the Drug-Resistant Tuberculosis Research Fund, Siriraj Foundation, under the patronage of HRH Princess Galyanivadhana, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. Strains included were 212 *M. tuberculosis* isolates from sputum of pulmonary TB patients and eight extrapulmonary samples other than cerebrospinal fluid (CSF) collected from 2003 to 2013. Also included were 46 *M. tuberculosis* isolates from CSF of tuberculous meningitis patients collected from 1998 to 2006. These comprised 51 drug-sensitive strains, six mono-resistant strains, two multiple-resistant strains, 88 MDR strains, 95 pre-extensively drug-resistant (pre-XDR) strains and 24 XDR strains. This study used leftover specimens with permission from the Director of Siriraj Hospital, Mahidol University. The study protocol was approved by the Ethical and Scientific Committees of the Faculty of Medicine Siriraj Hospital, Mahidol University (EC No. Si 029/2557).

2.2. Phenotypic DST

Phenotypic DST for anti-TB drugs was performed using standard proportional methods [13] on Middlebrook 7H10 agar plates. Drug concentrations used were 0.2 mg/L for isoniazid; 1.0 mg/L for rifampicin; 5.0 mg/L for ethambutol and ethionamide; 6.0 mg/L for amikacin and kanamycin; 2.0 mg/L for streptomycin, *p*-aminosalicylic acid, ofloxacin, levofloxacin, moxifloxacin and gatifloxacin; 3.0 mg/L for azithromycin and clarithromycin; and 1.0 mg/L for linezolid. *M. tuberculosis* H37Rv was used as the susceptible reference strain.

2.3. Culture of M. tuberculosis and extraction of genomic DNA

All *M. tuberculosis* isolates from stock culture were re-subcultured on to Löwenstein–Jensen media and incubated at 37°C for 4 weeks. Genomic DNA was extracted from multiple loopfuls of *M. tuberculosis* colonies using the cetyl-trimethyl-ammonium bromide-sodium chloride method [14].

2.4. Whole-genome sequencing

Sequencing of the *M. tuberculosis* isolates was performed at the Genome Institute of Singapore, Singapore. Genomic libraries were prepared according to the recommendations of the TrueSeq DNA sample preparation kit (Illumina, San Diego, CA, USA) for the MiSeq platform (Illumina), generating 250-bp read lengths, or NEBnext Ultra kit (Illumina, San Diego, CA) for Hiseq (Illumina) platform, generating 150-bp read lengths. The sequence data have been deposited in the Sequence Read Archive containing 293 Biosample Accession Nos. SAMN07236248–540 under Bioproject Accession No. PRJNA390471.

2.5. Bioinformatics and data analysis

The overall quality of sequence reads was checked using FastQC Version 0.11.3 [15]. All sequences with an average quality score above 36 were retained. Reads shorter than 36 bp and possibly contaminating adaptor sequences were excluded using Trimmomatic Version 0.33 [16]. Paired-end raw reads of each isolate were mapped to the *M. tuberculosis* H37Rv reference genome (GenBank Accession number: NC_000962.3) using BWA MEM Version 0.7.12 [17]. Samtools Version 0.1.19 [18] was used for SAM-BAM format conversion and sorting of mapped sequences. Local realignment of the mapped reads was performed using GATK Version 3.4.0 [19]. The stat reports were generated using GATK and Samtools, indicating that the average depth coverage of the mapped sequences was 118.88 ± 69.62 and the average mapping rate of the sequences was 97.79% ± 1.65%. This level of coverage and quality provides sufficient input data for WGS-based DST analysis software.

WGS-based DST analysis was performed using PhyResSE [10] and TB-Profiler [9] with default parameters and databases. The data were screened for mutations associated with resistance to 10 anti-TB drugs, including the first-line drugs isoniazid, rifampicin, ethambutol, pyrazinamide and streptomycin, and the second-line drugs ethionamide, fluoroquinolone, amikacin, capreomycin and kanamycin.

Descriptive statistics were used to describe the characteristics of the *M. tuberculosis* isolates. Sensitivity was calculated based on the ratio between resistance results detected by the software and the resistance results from the phenotypic DST. Specificity was calculated based on the ratio between susceptible results obtained from the software and the susceptible results from the phenotypic DST.

Table 1
Frequency of resistance against anti-tuberculosis (TB) drugs according to phenotypic drug susceptibility testing (DST) and whole-genome-sequence-based DST.

Anti-TB drugs	Phenotypic DST			PhyResSE		TB-Profiler	
	No. of strains tested (*n*=266)	No. of strains with resistant phenotype	Phenotypic resistance rate (%)	Detected resistant strains	Resistant rate (%) (*n*=266)	Detected resistant strains	Resistant rate (%) (*n*=266)
Isoniazid	261	204	78.16	198	74.44	210	78.95
Rifampicin	262	202	77.10	187	70.30	189	71.05
Ethambutol	229	121	52.84	152	57.14	161	60.53
Streptomycin	262	130	49.62	126	47.37	138	51.88
Pyrazinamide	ND	ND	ND	61	22.93	79	29.70
Amikacin	204	29	14.22	20	7.52	25	9.40
Kanamycin	204	34	16.67	7	2.63	29	10.9
Ethionamide	204	49	24.02	1	0.38	29	10.90
Fluoroquinolones				105	39.47	108	40.6
Ofloxacin	207	111	53.62				
Levofloxacin	201	66	32.84				
Gatifloxacin	204	20	9.80				
Moxifloxacin	201	60	29.85				
Azithromycin	13	13	100.00	NA	NA	NA	NA
Clarithromycin	108	70	64.81	NA	NA	NA	NA
p-amino salicylic	203	63	31.03	NA	NA	NA	NA
Linezolid	201	1	0.50	NA	NA	NA	NA
Capreomycin	ND	ND	ND	0	0.00	20	7.52

ND, not done; NA, not available.

3. Results

3.1. Characteristics of *M. tuberculosis* strains and phenotypic DST

Phenotypic DST results based on the standard proportion method (agar-based test) of 266 clinical *M. tuberculosis* isolates with a range of drug sensitivity/resistance phenotypes are given in Table 1. Isoniazid and rifampicin were the drugs against which the most resistance was seen, as most strains were MDR-TB. The least resistant drug was linezolid (one isolate, 0.5%) (Table 1).

3.2. WGS-based DST results using TB-Profiler and PhyResSE

Frequencies of resistance against each anti-TB drug, as inferred by TB-Profiler and PhyResSE, are listed in Table 1. TB-Profiler detected a higher number of resistance-conferring mutations than PhyResSE for all anti-TB drugs (Table 1). Distribution and frequencies of drug-resistance genes for the first- and second-line anti-TB drugs, and the most common mutations associated with each drug, are presented in Figs. 1 and 2. Some mutations were detected only by TB-Profiler, such as *kasA* (Gly312Ser). PhyResSE detected a higher number of resistant strains caused by the same mutation than TB-Profiler, such as *katG* (Ser315Thr) (173 vs. 162 strains) (Figs. 1 and 2, and Table 2). The discordance in detection of drug-resistant strains based on particular variants derived from different mutation sets and analysis pipelines is shown in Supplementary Table 1 (see online supplementary material).

3.3. Concordance rate, discordance rate, sensitivity and specificity of WGS-based DST compared with phenotypic DST

The concordance rates, discordance rates, sensitivity and specificity of WGS-based DST compared with phenotypic DST are shown in Fig. 3, Table 2 and Supplementary Table 2 (see online supplementary material). TB-Profiler had higher average concordance with phenotypic DST than PhyResSE for both first- (91.96% vs. 91.40%) and second-line (79.67% vs. 78.20%) drugs. The average sensitivity for all anti-TB drugs was also higher (83.13% vs. 72.08%) with slightly lower specificity (83.50% vs. 86.68%) (Table 2 and Supplementary Table 2, see online supplementary material). Compared with PhyResSE, TB-Profiler had higher concordance with phenotypic DST for six anti-TB drugs (rifampicin, streptomycin, ethambutol, kanamycin, ethionamide and moxifloxacin), but concordance was lower for a further five drugs (isoniazid, amikacin, ofloxacin, levofloxacin and gatifloxacin) (Table 2). The two programs detected slightly different numbers of mutations, as shown by the 88–98% concordance of WGS-based DST results between PhyResSE and TB-Profiler (Table 2 and Supplementary Table 1, see online supplementary material). Among the fluoroquinolones, resistance conferred by mutations in *gyrA* and/or *gyrB* was found in 91.79%, 76.62%, 72.64% and 57.35% of isolates for ofloxacin, levofloxacin, moxifloxacin and gatifloxacin, respectively (Fig. 2 and Supplementary Table 1, see online supplementary material).

Approximately 30% of the strains yielding discordant results for each drug were selected at random from stock cultures, and phenotypic DST was repeated. Ethionamide had the highest reproducibility rate (78.57%) between the first and the second (repeated) DST result, followed by ofloxacin (71.43%), moxifloxacin (60%) and ethambutol (30%) (Supplementary Table 3, see online supplementary material).

4. Discussion

Several WGS analysis tools are available for predicting drug-resistant TB [20]. PhyResSE and TB-Profiler are the two most promising among these [12,21]. This study evaluated WGS-based DST using these tools, compared with the phenotypic proportional method using MDR/XDR-TB strains in Thailand. TB-Profiler was previously found to have 100% concordance with phenotypic DST results for first-line drugs except pyrazinamide (95.3% concordance) [21]. Schleusener *et al.* [12] reported slightly lower concordance with phenotypic DST of TB-Profiler (94%) compared with PhyResSE (96%). However, these studies used a low number of drug-resistant strains, especially MDR and XDR-TB strains, in their evaluation, casting some doubt on the value of the tools for identifying resistant strains. This study also compared the performance of the two tools for detecting drug-resistant mutations in M/XDR strains. Both were found to have high but varied performance for detection of drug-resistant strains. TB-Profiler had better average concordance with phenotypic DST than PhyResSE. However, PhyResSE had higher concordance for five anti-TB drugs than TB-Profiler. Such differences derived in part from the differences between the programs in balance between sensitivity (detection of true drug-resistant strains) and specificity (avoiding

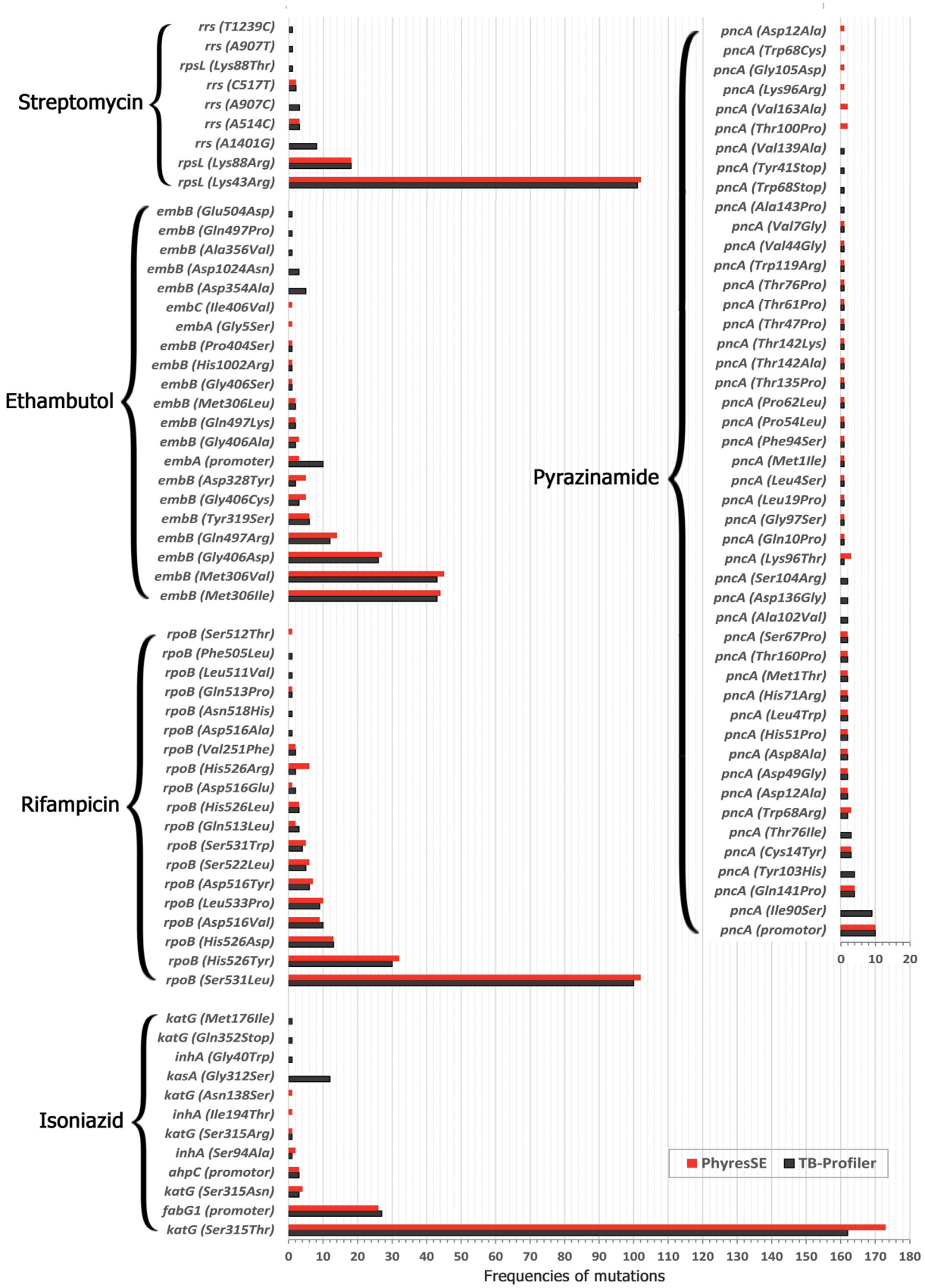

Fig. 1. Frequencies of mutations associated with resistance against first-line anti-tuberculosis drugs, as identified by TB-Profiler and PhyResSE.

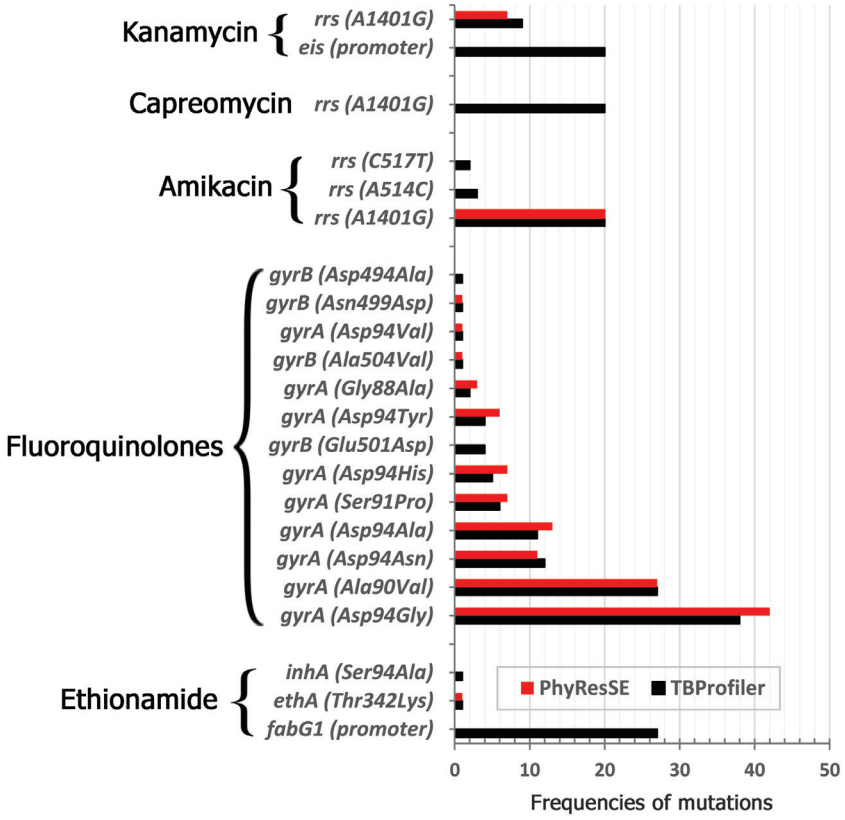

Fig. 2. Frequencies of mutations associated with resistance against second-line anti-tuberculosis drugs, as identified by TB-Profiler and PhyResSE.

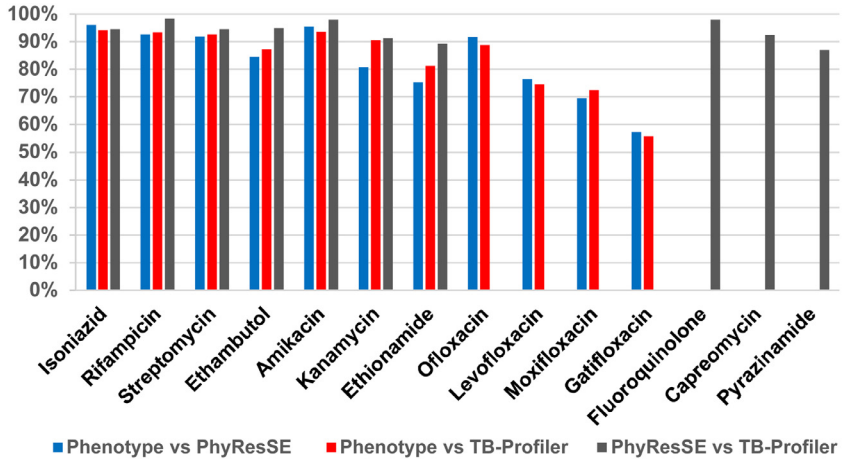

Fig. 3. Phenotypic–genotypic concordance rate of anti-tuberculous drug resistance.

false-positives). Differences in performance between the two tools also derived from the different mutation catalogues used. For example, *kasA* (Gly312Ser), which was found in the variant set of TB-Profiler, resulted in the detection of 12 *kasA*-mutated strains but none of these strains were detected by PhyResSE. Furthermore, the analysis pipeline of these programs also affected their sensitivity. For example, the same variant position, *katG* (Ser315Thr), was detected in 173 strains by PhyResSE but in only 162 strains by TB-Profiler. TB-Profiler used a larger variant set (>1294 variants) but employed high-stringency parameters in the pipeline, leading to identification of fewer resistant strains. PhyResSE included a smaller set with high-confidence variants (>374 variants), but used less stringent pipeline parameters, including those allowing detection of heterozygous resistant variants associated with the resistant subpopulation in the sample. Hence, both the genetic database used and the analysis pipeline affected the concordance rate in the two programs, leading to differences.

The coverage of WGS-based DST compared with phenotypic DST for the first-line anti-TB drugs was high but also varied: isoniazid (66.7% to 100%), rifampicin (83.3% to 100%), ethambutol (15.4% to 95.8%) and pyrazinamide (66.7% to 100%) [22]. In this study, the concordance rates between the WGS-based DST and phenotypic DST for the first-line anti-TB drugs were high, especially for isoniazid and rifampicin. For first-line drugs, concordance ranged from 87.3% to 97.2%, and for the second-line drugs, the range was from 57.4% to 95.6%. The discrepancies between WGS-based DST and phenotypic DST might be due to deficiencies in the genetic mutation databases used for interpretation [23], errors in phenotypic DST, especially the uncertain breakpoint values [12], inadequate sequence coverage in the WGS pipeline [12,21], and the fact that

Table 2
Performance comparisons between phenotypic drug susceptibility testing (DST) and whole-genome-sequence (WGS)-based DST.

Anti-TB drugs	Phenotype vs. PhyResSE					Phenotype vs. TB-Profiler					PhyResSE vs. TB-Profiler			
	Concordance rate (%)	Discordance rate (%)		Sens (%)	Spec (%)	Concordance rate (%)	Discordance rate (%)		Sens (%)	Spec (%)	Concordance rate (%)	Discordance rate (%)		
		S vs. R	R vs. S				S vs. R	R vs. S				S vs. R	R vs. S	R vs. R
Isoniazid	**96.17**	-	3.83	95.10	100.00	94.25	3.07	2.68	96.57	85.96	94.74	4.14	-	1.13[a]
Rifampicin	92.75	-	7.25	90.59	100.00	**93.51**	0	6.49	91.58	100.00	98.50	1.13	0.38	-
Ethambutol	84.72	8.73	6.55	87.60	81.48	**87.34**	8.73	3.93	92.56	81.48	95.11	4.14	0.75	-
Streptomycin	91.98	2.67	5.34	89.23	94.70	**92.75**	4.96	2.29	95.38	90.15	94.74	4.89	0.38	-
Pyrazinamide	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	87.22	9.77	3.01	-
Amikacin	**95.59**	-	4.41	68.97	100.00	93.63	1.96	4.41	68.97	97.71	98.12	1.88	-	-
Kanamycin	80.88	2.94	16.18	2.94	96.47	**90.69**	3.43	5.88	64.71	95.88	91.35	8.65	-	-
Ethionamide	75.49	0.49	24.02	0.00	99.35	**81.37**	3.92	14.71	38.78	94.84	89.47	10.53	-	-
Ofloxacin	**91.79**	2.42	5.80	89.19	94.79	88.89	4.83	6.28	88.29	89.58	NA	NA	NA	NA
Levofloxacin	**76.62**	20.40	2.99	90.91	69.63	74.63	22.39	2.99	90.91	66.67	NA	NA	NA	NA
Gatifloxacin	**57.35**	42.16	0.49	95.00	53.26	55.88	43.63	0.49	95.00	51.63	NA	NA	NA	NA
Moxifloxacin	69.65	25.37	4.98	83.33	63.83	**72.64**	24.88	2.49	91.67	64.54	NA	NA	NA	NA
Fluoroquinolone gr.	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	98.12	1.50	0.38	-
Capreomycin	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	92.48	7.52	-	-
Average (first-line)	91.40			90.63	94.04	91.96			94.02	89.40	95.77			
Average (second-line)	78.20			61.48	82.48	79.67			76.90	80.12	92.79			
Average (all drugs)	83.00			72.08	86.68	84.18			83.13	83.50	93.99			

TB, tuberculosis; Sens, sensitivity; spec, specificity; NA, not available; S, susceptible; R, resistant.
Concordance and discordance rates were calculated based on the resistance and susceptibility results from 266 *Mycobacterium tuberculosis* strains.
Bold numbers refer to the highest concordance rate between the phenotypic DST and the WGS-based DST, regardless of analysis program.
[a] Three isolates showed the same resistance result but due to different mutations.

some mutations confer only low-level drug resistance [22]. Concordance rates were highest (close to 100%) between phenotypic DST and WGS for the first-line drugs rifampicin and isoniazid, as also noted by a previous systematic review [22]. This suggests that WGS is a useful alternative or replacement for existing phenotypic and molecular DST methods for these two drugs.

Ethambutol had the lowest WGS-phenotype concordance (87.3%) among the first-line drugs. DST for ethambutol is difficult for several reasons, such as the narrow range of values between resistant and susceptible strains, and the possible presence of microcolonies that are difficult to detect visually [24]. Recently, WHO recommended that the critical concentration for ethambutol should be lowered [25]. This study used 5 mg/L as the recommended critical concentration for ethambutol. A previous study has reported only moderate sensitivity of *embB* gene sequencing to detect strains that are phenotypically ethambutol resistant [26]. Such resistant strains with mutation of *embB* codon 306 had minimum inhibitory concentration (MIC) values at or just above the critical concentration, reflecting borderline resistance, and MICs overlapping the critical concentration associated with phenotypic–genotypic discordance [27]. Based on the ethambutol phenotypic-genotypic discordant strains (29 strains), 20 strains were the phenotypic susceptible but had genotypic (based on WGS analysis) resistant result. Among these 20 strains, 11 were considered to be resistant based on *embB* Gly406Asp and six strains based on *embB* *Met*306Ile/Val. These mutations might be associated with a low level of resistance resulting in the discordance seen, a possibility supported by a recent study [28]. The phenotypic ethambutol DST was repeated in 10/20 discordant strains, and 70% were found to be concordant with the WGS results. The reproducibility of DST with ethambutol was lowest compared with other first-line drugs such as isoniazid and rifampicin [29]. Eighty percent intralaboratory reproducibility of ethambutol DST has been reported [30]. The lower reproducibility rate found in this study might be due to the proportion of borderline-resistant subpopulations and/or selective pressure due to the storage of stock samples in the freezer for 10 years. Besides the uncertain breakpoint of ethambutol and the low-level phenotypic resistance, low phenotype–WGS concordance rates for ethambutol were also associated with the low reproducibility of phenotypic DST, and these factors might be correlated.

A previous study has reported the applicability of WGS for rapid determination of drug resistance in MDR and XDR-TB patients [31]. Another study has found high concordance rates in phenotypic–genotypic comparisons for streptomycin, ofloxacin and amikacin [8]. Neither study used a high number of MDR/XDR-TB strains for evaluation and included only a few second-line drugs. This study found that the phenotypic-WGS-based DST concordance rates for each of eight second-line drugs varied. Concordance was highest for amikacin, ranging from 93.6% to 96%, and 80% of the amikacin-resistant strains possessed the mutation *rrs* A1401G, similar to previous studies [32]. This study also found that *rrs* A1401G caused cross-resistance to other second-line injectable drugs, including kanamycin and capreomycin, as reported in previous studies [33]. Although within the same antibiotic class (aminoglycosides), amikacin and kanamycin differ in structure, and different specific variants associated with resistance have been reported [34]. In the present study, TB-Profiler yielded higher concordance rates for amikacin than PhyResSE, and the sensitivity for detection of amikacin resistance by WGS-based DST was higher than for kanamycin resistance. Such differences, both between software and between drugs, were mainly due to the use of different mutation catalogues.

Ethionamide showed moderate concordance rates (81.37%), mainly due to isolates that were phenotypically resistant but WGS-based susceptible (78.95%, 30/38 discordant strains). This study used 5 mg/L as the critical concentration for ethionamide, and

the repeated phenotypic DST showed nearly 80% reproducibility. TB-Profiler detected only 38.8% of the strains that were phenotypically resistant to ethionamide, perhaps due to inadequacy of the mutation database. In ethionamide-resistant strains, mutations were most frequently found in the *fabG1* promotor, but these explained only 24.5% (12/49 strains) of ethionamide-resistant strains in this study. *EthA* is the major gene associated with ethionamide resistance [20]. However, only one ethionamide-resistant strain in this study had the *ethA* mutation. Therefore, ethionamide is one of the second-line drugs for which the mutation database requires improvement. *FabG1* encodes mycolic acid biosynthetic enzymes, and mutation of this gene confers cross-resistance to isoniazid, which is a structural analogue of ethionamide [35]. All phenotypic-WGS concordant ethionamide-resistant strains were cross-resistant to isoniazid.

There were many commonly used anti-TB drugs in the fluoroquinolone group. These include second-generation drugs (ofloxacin) and later-generation drugs (levofloxacin, gatifloxacin and moxifloxacin). Previous evaluations of phenotypic and genotypic DST of fluoroquinolone have been undertaken [8,36]. However, no study has evaluated specific drugs within the fluoroquinolone group, or compared the early (first and second) generations and later (third and fourth) generations of fluoroquinolone using WGS-based DST. This study found varying concordance rates between WGS-based DST and phenotypic DST for each fluoroquinolone. Concordance was higher (91.8%) for the early generations of fluoroquinolone (ofloxacin), based on mutations at *gyrA* and *gyrB* genes, than for the later generations (levofloxacin, gatifloxacin and moxifloxacin) (76.6%, 57.4 and 72.64%, respectively). Two points can be made here. First, the critical concentration is crucial for interpretation of DST for anti-TB drugs, including fluoroquinolone. WHO has suggested that a critical concentration of 2 mg/L used to test susceptibility to the newest quinolones is likely to be too high, and fails to capture strains with lower levels of resistance that might be clinically relevant, as they would be classified as susceptible [37]. Instead, 0.5 mg/L, based on M7H10, and 0.25 mg/L, based on MGIT, were suggested as critical concentrations for gatifloxacin and moxifloxacin, respectively, whereas 1 mg/L, based on M7H10, was suggested for levofloxacin [37]. These differing views could be relevant for this study: DSTs were performed for the second-line drugs before these recommendations were made in 2018, and used a critical concentration of 2 mg/L based on M7H10 for all fluoroquinolones. The lower WGS-phenotypic DST concordance rates found in the present study for the later generations of fluoroquinolones might be due to this. Determination of MIC values might be informative concerning these discrepant strains. Also, the reproducibility of phenotypic DSTs is partly affected by the susceptibility patterns: the repeated results showed approximately 60–70% reproducibility for ofloxacin and moxifloxacin. Second, lower concordance rates with phenotypic DST for the later generations of fluoroquinolones might be due to the use of older mutation catalogues derived from investigations on the early generations of fluoroquinolones. A previous study revealed that some strains containing *gyrA* or *gyrB* mutations resisted second-generation fluoroquinolones but were susceptible to the third and fourth generations [38]. Possibly, genetic variants conferring resistance might differ for different generations or types of fluoroquinolone. Knowledge of such variants could lead to personalized treatment of patients. Caution is required in assuming that a mutation conferring resistance to one member of a group of drugs will confer resistance to other members. This issue is especially important when optimizing the regimen for treatment of XDR-TB.

Approximately 70% of the isolates used in this study were M/XDR-TB strains. Such strains exhibited low resistance rates against some particular drugs, especially linezolid. Linezolid belongs to the oxazolidinones, a new class of antibiotics that bind to the 50S ribosomal subunit to interfere with protein synthesis [39]. Linezolid is therefore a promising choice for treatment of M/XDR-TB in Thailand.

In conclusion, concordance rates were higher between WGS-based DST and phenotypic DST for first-line anti-TB drugs, especially isoniazid and rifampicin, than for second-line drugs. WGS-based DST analysis programs differ in their mutation catalogues and analysis pipelines, thus affecting their performance in the detection of resistance. The current mutation catalogue is inadequate for inferring resistance against some generations of fluoroquinolones, especially the third and fourth generations. Certain second-generation fluoroquinolones do not exhibit strong cross-resistance compared with the third and fourth generations.

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Competing interests

None declared.

Ethical approval

The study protocol was approved by the Ethical and Scientific Committees of the Faculty of Medicine Siriraj Hospital, Mahidol University (EC No. Si 029/2557).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2019.04.004.

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