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Use of whole-genome sequencing to predict *Mycobacterium tuberculosis* drug resistance in Indonesia

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ABSTRACT

Objectives: Whole-genome sequencing (WGS) is rarely used for drug resistance testing of *Mycobacterium tuberculosis* in high-endemic settings. Here we present the first study from Indonesia, which has the third highest tuberculosis (TB) burden worldwide, with <50% of drug-resistant cases currently detected.
Methods: WGS was applied for strains from 322 human immunodeficiency virus (HIV)-negative adult TB patients. Phenotypic drug susceptibility testing (DST) was performed for a proportion of the patients.
Results: Using WGS, mutations associated with drug resistance to any TB drug were identified in 51 (15.8%) of the 322 patients, including 42 patients (13.0%) with no prior TB treatment (primary resistance). Eight isolates (2.5%) were multidrug-resistant (MDR) and one was extensively drug-resistant (XDR). Most mutations were found in *katG* ($n = 18$), *pncA* ($n = 18$), *rpoB* ($n = 10$), *fabG1* ($n = 9$) and *embB* ($n = 9$). Agreement of WGS-based resistance and phenotypic DST to first-line drugs was high for isoniazid and rifampicin but was lower for ethambutol and streptomycin. Drug resistance was more common in Indo-Oceanic lineage strains (37.5%) compared with Euro-American (18.2%) and East-Asian lineage strains (10.3%) ($P = 0.044$), but combinations of multiple mutations were most common among East-Asian lineage strains ($P = 0.054$).
Conclusions: These data support the potential use of WGS for more rapid and comprehensive prediction of drug-resistant TB in Indonesia. Future studies should address potential barriers to implementing WGS, the distribution of specific resistance mutations, and the association of particular mutations with endemic *M. tuberculosis* lineages in Indonesia.

1. Introduction

Drug-resistant tuberculosis (TB) threatens the global control of TB in many parts of the world. There were an estimated 580 000 cases of multidrug-resistant TB (MDR-TB) in 2015, with <50% being detected and even fewer receiving appropriate treatment [1,2]. Culture-based drug susceptibility testing (DST), the gold standard to diagnose drug-resistant TB, is technically difficult and takes about 4-6 weeks after the isolation of *Mycobacterium tuberculosis*. Furthermore, inconsistencies in DST results are widely reported, especially for ethambutol (EMB) and second-line drugs [3,4], whilst phenotypic DST for pyrazinamide (PZA) requires different protocols [5]. Molecular-based DST testing using the Xpert MTB/RIF assay can rapidly detect most rifampicin (RIF) resistance [6], but this assay has incomplete sensitivity for RIF, does not examine resistance for other TB drugs and may fail to detect heteroresistance [7,8].

Whole-genome sequencing (WGS) has been shown to be a potential tool for reliable prediction of the drug susceptibility phenotype of *M. tuberculosis* isolates within a clinically relevant timeframe [9]. However, so far WGS is mostly applied in well-resourced, low TB burden settings. Because of rapid advances in

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WGS technology and its decreasing cost and turnaround time, WGS is now becoming accessible in limited-resourced, high TB burden countries [10]. In the current study, WGS was applied to predict drug resistance in a selection of *M. tuberculosis* isolates from Indonesia, which has the third-highest TB burden in the world. In Indonesia, diagnosis of drug-resistant TB is challenging as *M. tuberculosis* culture is not routinely performed and DST is only available in certain reference laboratories. To date, the contribution and concordance of phenotypic and genotypic DST in Indonesia is unknown. This study aimed to describe resistance-conferring mutations to first- and second-line TB drugs in Indonesia using WGS and to examine their concordance with phenotypic DST and their distribution among different *M. tuberculosis* lineages.

2. Materials and methods

2.1. Selection of M. tuberculosis isolates and drug susceptibility testing

WGS was performed on a random sample of archived *M. tuberculosis* isolates from 322 human immunodeficiency virus (HIV)-negative adult patients (216 with pulmonary TB and 106 with meningeal TB) with complete medical information. Pulmonary TB patients had been diagnosed in Hasan Sadikin Hospital (Bandung, Indonesia) between 2012–2013 and in the TB-HIV Research Centre of Universitas Padjadjaran (Bandung, Indonesia) between 2013–2015. TB meningitis patients had been diagnosed at Hasan Sadikin Hospital between 2006–2013 [11]. Specimens from each patient were processed accordingly and were inoculated on solid Ogawa medium or in MODS (Microscopic Observation of Drug Susceptibility) liquid medium [12]. Positive cultures from each method were subcultured and aliquots were archived at −80 °C prior to DNA extraction.

Xpert MTB/RIF was available in the study setting only after 2012 and was accessible only for patients with suspected MDR-TB according to Indonesian national guidelines. Phenotypic DST was not performed routinely for all patients but only when requested by treating physicians. DST was performed in the provincial referral laboratory using the proportion method on Löwenstein–Jensen (LJ) medium at concentrations of 40.0 μg/mL for RIF, 0.2 μg/mL for isoniazid (INH), 2.0 μg/mL for EMB and 4.0 μg/mL for streptomycin (STR). Briefly, a 1.0 McFarland standard isolate suspension was serially diluted 10-fold (from 10^{-1} to 10^{-5}) in sterile distilled water. Dilutions 10^{-3} and 10^{-5} were inoculated, respectively, onto LJ slants with and without drugs, and were incubated at 37 °C. The results were read at 28 days and up to 42 days, depending on the control growth. An isolate was considered resistant to a given drug when growth of ≥1% above the control was observed in drug-containing medium. DST for second-line drugs was not available during patient inclusion for this study. Phenotypic DST was repeated for several isolates that had tested INH-susceptible but that harboured mutations conferring resistance to INH in the *katG*315 gene. In these cases, *M. tuberculosis* was subcultured from frozen isolates onto Ogawa slopes prior to DST. The study protocols for the inclusion of patients and for bioanalysis were approved by the Ethical Committee of the Faculty of Medicine of Universitas Padjadjaran.

2.2. Whole-genome sequencing and analysis

Frozen isolates were subcultured on Ogawa slopes and mycobacterial DNA was extracted for sequencing using UltraClean® Microbial DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA) following the manufacturer's protocol, or using the cetyltrimethylammonium bromide (CTAB) method of DNA purification. The concentration and purity of extracted DNA were measured using a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA) and the intactness of DNA was checked by agarose gel electrophoresis.

M. tuberculosis DNA was sequenced on an Illumina HiSeq 2000 instrument (Illumina Inc., San Diego, CA) using 2 × 100-bp paired-end reads. Sequencing was performed at BGI (Hong Kong). After sequencing, the raw FASTQ sequence reads were filtered, including removal of adapter sequences, contamination, and low-quality reads that had more than 10% N base calls or had a quality score ≤4 in more than 40% of the bases. Five TB meningitis strains and four pulmonary TB strains were contaminated, based on a low GC content and were excluded from further analyses. Sequencing coverage was determined using the FastQC v.0.10.1 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) quality control tool. The proportion of bases sequenced with a sequencing error rate of ≤1% per base ranged from 93% to 97% per genome. The average depth of coverage for the remaining 322 sequenced strains was 121.1, and the average percentage of bases covered by at least one read was 98.9%. The sequence reads were aligned to reference strain *M. tuberculosis* H37Rv (GenBank accession no. **NC_000962.3**) and variants were called using Breseq software v.0.27.1 [13]. Mutations with low-quality evidence (i.e. possible mixed read alignment) were not included.

2.3. In silico determination of drug resistance

Raw FASTQ sequencing files were uploaded to TB Profiler, an online tool to determine drug resistance in silico [10]. It uses raw sequence data as input and compares identified single nucleotide polymorphisms (SNPs) and indels to a curated list of 1325 drug resistance mutations and displays related output. The precision of TB Profiler's curated mutation catalogue for predicting resistance had been assessed using six geographically distinct data sets from China, Pakistan, Malawi, Portugal, Russia and Canada [10], and its accuracy compared with other in silico drug resistance prediction tools has been proven recently [14].

2.4. Phylogeny construction

A phylogenetic tree was constructed to determine the evolutionary relationship of the isolates. All 29 199 variable positions identified by breseq across the 322 *M. tuberculosis* sequences were extracted and concatenated into a single alignment. Solely for the purpose of creating the phylogenetic tree, SNPs occurring in PE/PPE genes and genes related to mobile elements (listed in Supplementary Table S1) were excluded to avoid any concern about inaccuracies in the read alignment in these parts of the genome. In addition, SNPs in an additional 40 genes previously associated with drug resistance [10] were also removed to exclude the possibility that homoplasy of drug resistance mutations would significantly affect the phylogeny [15]. After applying these filters to the initial set of 29 199 SNPs, the 28 544 remaining SNPs were used to construct the phylogenetic tree using PhyML v.3.0 [16] using the HKY85 model with four categories for the gamma model of rate distributions and 100 bootstraps.

To examine possible associations between *M. tuberculosis* lineage and drug susceptibility, the lineage was determined for each of the 322 strains using a 62-SNP barcode [17]. The resulting classification in the main *M. tuberculosis* lineages also served as a quality check for the generated maximum-likelihood phylogenetic tree, as it enabled us to validate that isolates belonging to the same lineage clustered together in the tree.

2.5. Statistical analysis

The χ^2 test was used to statistically test the association between *M. tuberculosis* lineage and drug susceptibility. Cohen's κ was used

to determine the level of agreement between WGS and phenotypic DST for first-line drugs. In addition, enrichment values were calculated for drug resistance per lineage based on the ratio of lineage-specific observed and expected occurrence of drug resistance. The ratios were visualised in a heat map as a measure of association between *M. tuberculosis* lineage and drug susceptibility.

3. Results

3.1. Patient characteristics

Patients were mostly young (median age 33 years, interquartile range 23.5–45.0 years), 51.9% were male and 16.1% reported a history of TB treatment. Using WGS, mutations associated with drug resistance to any TB drug were identified in 51 (15.8%) of the 322 patients; 42 (13.0%) were identified to have primary resistance, MDR-TB was present in 8 patients (2.5%), including primary MDR-TB in 5 patients (1.6%) and primary extensively drug-resistant TB (XDR-TB) in 1 patient (0.3%) (Table 1; Supplementary Table S2).

3.2. Mutations associated with drug resistance

Genetic variants in multiple genes associated with drug resistance in *M. tuberculosis* were identified by WGS (Table 2). A total of 29 isolates (9.0%) had mutations in genes associated with resistance to INH, including *katG* ($n = 18$), *kasA* ($n = 3$) and the promoter region of *fabG1* C-15T ($n = 9$). Nine isolates with mutations at the *fabG1* promoter site were predicted to have co-resistance to INH and ethionamide. The most common mutation in *katG* was Ser315Thr ($n = 14$), but the uncommon mutation *katG* Ser315Met was also found in two isolates, and *katG* Trp191Arg and *katG* Ala106Val were found in one isolate each. The *katG* Ser315Thr mutation was found in seven of eight MDR-TB isolates (Table 2).

RIF resistance-conferring mutations were identified in ten isolates (3.1%), all in the *rpoB* gene. Six common mutations distributed over codons 435, 445 and 450 were found, mostly as single mutations at one of these positions. Rare mutations at codon 432 and 441 were found together in one isolate. One MDR-TB isolate with *rpoB* Ser450Leu also harboured the well-known compensatory mutation at *rpoC* Leu527Val.

For EMB, nine isolates (2.8%) showed mutations in the *embB* gene, with the mutation Met 306Val being most common ($n = 4$), and rare mutations at codon 406 and codon 497 in three MDR isolates. PZA resistance-conferring mutations were identified in 18 isolates (5.6%). All mutations occurred as a single mutation in five codons in the *pncA* gene. For fluoroquinolones (FQs), mutations in *gyrA* codon 90 and 94 were identified in 4 isolates (1.2%), three with FQ monoresistance and one XDR-TB isolate. With regard to resistance to STR and injectable agents, mutations were found in the *rpsL* and *rrs* loci in nine isolates (2.8%). Mutations at *rrs* codon 492 were only found in STR-monoresistant strains. The XDR-TB isolate harboured a mutation at the *eis* promoter linked to kanamycin resistance.

Table 1
Presence of drug resistance mutations in 322 clinical *Mycobacterium tuberculosis* isolates detected by whole-genome sequencing.

Resistance pattern	No. (%) of strains
Susceptible to all drugs	271 (84.2)
Resistant to any drug	51 (15.8)
Resistant to first-line drugs	
Any first-line drug	48 (14.9)
Isoniazid	29 (9.0)
Rifampicin	10 (3.1)
Ethambutol	8 (2.5)
Pyrazinamide	18 (5.6)
Streptomycin	9 (2.8)
Resistant to second-line drugs	
Ethionamide	9 (2.8)
Fluoroquinolones	4 (1.2)
Amikacin	1 (0.3)
Kanamycin	1 (0.3)
Monoresistance	
Isoniazid	10 (3.1)
Rifampicin	1 (0.3)
Pyrazinamide	15 (4.7)
Streptomycin	3 (0.9)
Fluoroquinolones	3 (0.9)
Resistance to multiple drugs	
Multidrug-resistant (MDR)	8 (2.5)
Extensively drug-resistant (XDR)	1 (0.3)
Polyresistant[a]	10 (3.1)

[a] Defined as resistance to multiple drugs but not MDR/XDR.

3.3. Agreement of phenotypic and genotypic drug susceptibility testing to first-line drugs

Overall, there was considerable agreement between genotypic and phenotypic DST. Phenotypic DST for the first-line drugs RIF, EMB and STR was available for 103 isolates with WGS data and for INH it was available for 102 isolates, with resistance found to INH ($n = 17$), RIF ($n = 7$), EMB ($n = 6$) and STR ($n = 7$). Concordance between WGS and phenotypic DST was high for RIF ($\kappa = 0.865$; $P < 0.001$) and INH ($\kappa = 0.814$; $P < 0.001$) but was low for EMB ($\kappa = 0.712$; $P < 0.001$) and STR ($\kappa = 0.136$; $P = 0.148$). Agreement of genotypic and phenotypic DST for each drug is shown in Fig. 1.

The common mutations *katG* Ser315Thr and *fabG1* C-15T were highly predictive of phenotypic INH resistance in this setting (89.5% of the isolates with either one of these mutations were phenotypically resistant). Similarly, mutations in the RIF resistance-determining region (RRDR) of *rpoB* were highly predictive of resistance to RIF (100% of the isolates with a mutation were phenotypically resistant). Nine isolates showed drug resistance-associated mutations but were susceptible by phenotypic DST. In those isolates, mutations were found for STR at *rrs* C492T ($n = 3$) and A514C ($n = 1$) and *rpsL* Lys43Arg ($n = 1$); for INH at *katG* Ser315Thr ($n = 1$), *katG* Trp191Arg ($n = 1$), *fabG1* C-15T ($n = 1$) and *kasA* Gly312Ser ($n = 3$); and for EMB at *embB* Met306Val ($n = 1$) and Met306Ile ($n = 1$). Conversely, nine other isolates that were drug-resistant according to phenotypic DST ($n = 7$ for STR, $n = 2$ for RIF and $n = 1$ for EMB) showed no known drug resistance mutations using WGS. However, one isolate with phenotypic RIF resistance but without well-known drug resistance mutations harboured other mutations in *rpoB* (*rpoB* Cys681Gly and *rpoB* Pro1014Ser).

3.4. Phylogenetic distribution of drug resistance

Drug resistance rates and patterns differed significantly between different *M. tuberculosis* lineages (Fig. 2; Supplementary Table S3). Among 116 isolates belonging to the East-Asian lineage, 12 (10.3%) were genotypically drug-resistant compared with 36 (18.2%) of 198 Euro-American strains and 3 (37.5%) of 8 Indo-Oceanic strains ($\chi^2 = 6.258$; $P = 0.044$). Although fewer strains belonging to the East-Asian lineage had drug resistance mutations, they more often had multiple drug resistance mutations ($\chi^2 = 5.844$; $P = 0.054$). From the phylogeny, it was observed that most of the *pncA* mutations in isolates harbouring PZA resistance clustered together. The nine isolates with a *pncA* Thr87Met mutation were adjacent in the tree, and the same goes for the six isolates carrying the *pncA* His82Arg mutation, suggesting transmitted resistance. However, the isolates differed by more than 12 SNPs, the commonly used threshold for (recent) transmission [18]. The other three mutations occurred only once and in genetically distant strains.

Table 2
Distribution of drug resistance-associated mutations in 51 *Mycobacterium tuberculosis* isolates with any drug resistance identified by whole-genome sequencing.

Drug	Gene	Amino acid change	No. of isolates	No. of MDR-TB isolates
Isoniazid	*katG*	Ser315Thr	14	7[a]
		Ser315Met	2	0
		Trp191Arg	1	0
	fabG1	C-15T promoter	8	1
	kasA	Gly312Ser	3	0
	Double loci *fabG1* + *katG*	*C-15T* promoter + Ala106Val	1	1
Rifampicin	*rpoB*	His445Tyr	1	1
		His445Asp	1	1
		Asp435Val	1	1
		Asp435Tyr	2	2
		Ser450Leu	2	2[a]
		His445Cys	1	1
		Gln432Lys + Ser441Leu	1	0
	Double loci *rpoB* + *rpoC*	Ser450Leu + Leu527Val	1	1
Ethambutol	*embB*	Met306Ile	2	1[a]
		Met306Val	4	1
		Gly406Asp	1	1
		Gln497Lys	1	1
		Met306Val + Gly406Asp	1	1
Streptomycin	*rrs*	C492T	3	0
		A514C	1	1
	rpsL	Lys43Arg	5	3[a]
Pyrazinamide	*pncA*	His82Arg	6	0
		Thr87Met	9	1
		Ser66Pro	1	1
		Pro62Leu	1	0
		Ala171Val	1	1[a]
Ethionamide	*fabG1*	C-15T promoter	9	2
Fluoroquinolones	*gyrA*	Asp94Gly	1	0
		Ala90Val	1	0
		Asp94Asn	1	0
		Asp94Ala	1	1[a]
Amikacin	*rrs*	A514C	1	1
Kanamycin	*eis*	G-14A promoter	1	1[a]

MDR-TB, multidrug-resistant.
[a] Mutation occurred in the extensively drug-resistant (XDR) isolate.

4. Discussion

This is the first study to report the use of WGS on clinical *M. tuberculosis* isolates from Indonesia, showing its potential for clinical management and TB control in Indonesia. Indonesia has a huge gap between MDR-TB incidence and MDR-TB treatment. Together with China, India, Nigeria and the Russian Federation, it accounted for >60% of this gap on a global scale in 2015 [2]. Of the 10 000 estimated MDR- or RIF-resistant notified pulmonary TB cases in Indonesia in 2015, only an estimated 15% were started on treatment [2]. A large part of this gap can be explained by poor detection of drug resistance. Phenotypic DST is poorly accessible in remote parts of the country. WGS could offer a rapid and comprehensive diagnostic solution, especially with the introduction of portable platforms and the decreasing price and turnaround time of WGS [18], leading to quicker and more appropriate treatment.

In a random collection of patient isolates, it was found that 15.8% carried mutations associated with drug resistance to any TB drug, often in patients without a history of TB treatment. This is much lower than reported in an earlier study in Indonesia where resistance to first-line drugs was reported in 38.2% of 262 culture-positive samples, although these samples were collected over the whole country and resistance was determined by phenotypic DST [19]. In the current study, resistance mutations to all first-line drugs, FQs and second-line injectable drugs were found. MDR-TB was present in eight isolates (2.5%), which is lower than expected based on recent survey data [2], and XDR-TB was present in one isolate (0.3%).

In contrast to the findings from previous studies [19–21], in the current study drug resistance was observed to occur more often in strains belonging to the Indo-Oceanic lineage compared with the Euro-American and East-Asian lineages. On the other hand, strains belonging to the East-Asian lineage more often harboured multiple mutations. One possible explanation is that the East-Asian lineage, which includes Beijing strains, appears to have a higher mutation rate that could lead to accelerated acquisition of drug resistance mutations. It may be one of the reasons why this lineage has been repeatedly associated with drug resistance [22,23]. However, this would also lead to more resistance against a single drug.

Published studies from Indonesia have clearly shown disparities in the relative distribution of *M. tuberculosis* genotypes across the archipelago. In this article, we reported resistance mutations in a urban setting in Java where modern lineage 2 (East-Asian) and lineage 4 (Euro-American) are highly prevalent. These findings should be confirmed in other regions, especially eastern Indonesia, where lineage 1 (Indo-Oceanic) occurs at a substantially higher frequency [24–26]. Current catalogues of drug resistance mutations rely predominantly on data from modern lineages 2 and 4 [10,27]. A recent study from India, where lineage 1 and 3 predominate, reported putative novel resistance-conferring mutations in lineage 1 that had not previously been implicated in resistance [27], suggesting that the mutations underlying genotypic drug resistance differ by lineage.

WGS offers the possibility to learn more about the influence of the genetic background of strains on all aspects of drug resistance evolution in *M. tuberculosis*. WGS could provide insight into secondary compensatory mutations, not conferring resistance but reducing the fitness cost of the resistance mutation by interacting epistatically with it. The rate of acquiring new drug resistance mutations and the fitness costs of these mutations may vary as a function of the strain genetic background [27,28].

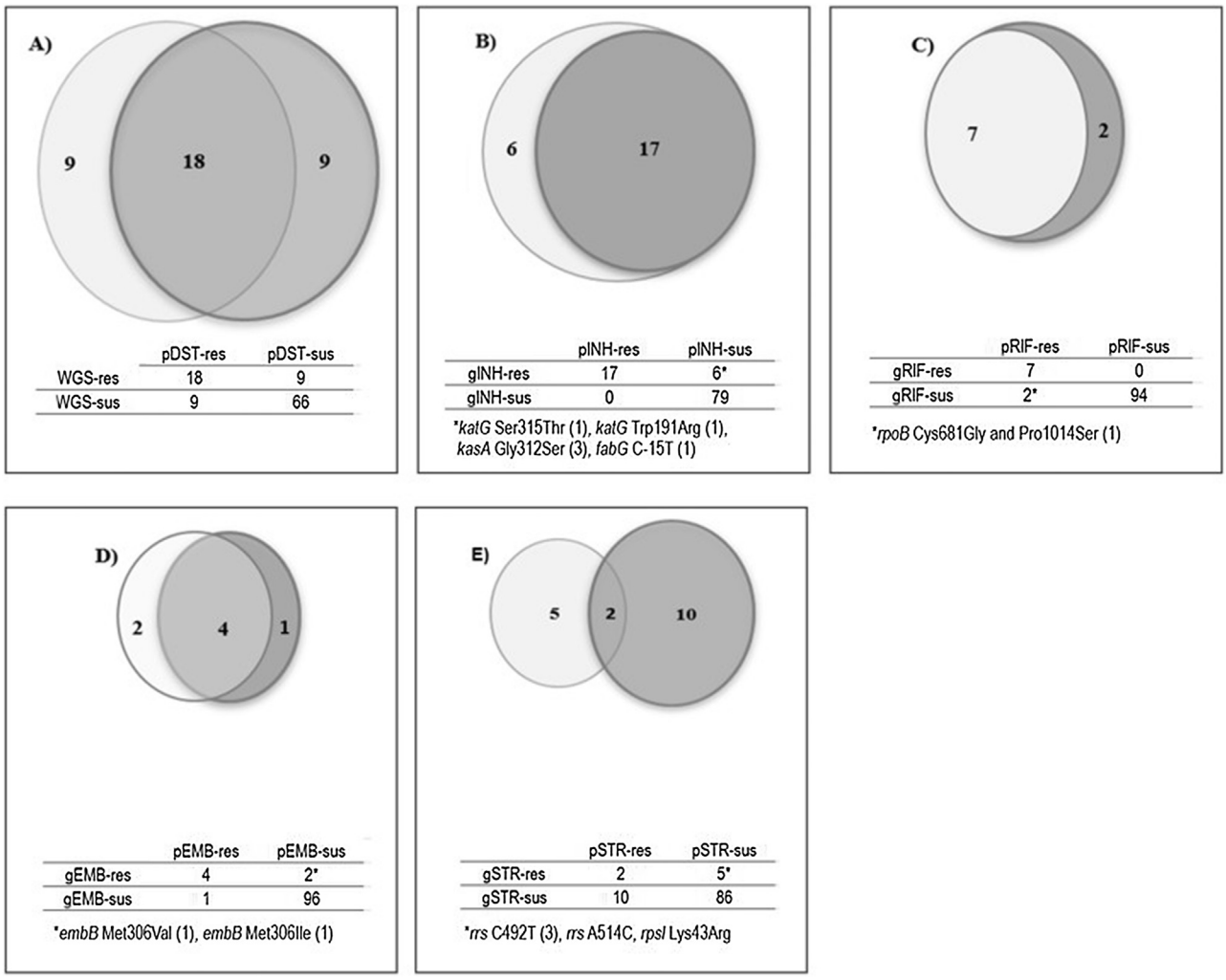

Fig. 1. Comparison of phenotypic drug susceptibility testing (DST) and drug resistance testing by whole-genome sequencing (WGS) for *Mycobacterium tuberculosis* isolates to (A) any first-line drug, (B) isoniazid (INH), (C) rifampicin (RIF), (D) ethambutol (EMB) and (E) streptomycin (STR). Light grey, drug resistance determined by WGS; dark grey, drug resistance determined by phenotypic DST. Data are presented as number of strains. n = 103 for RIF, EMB and STR and n = 102 for INH.

Most mutations associated with INH resistance were found in *katG* (62.1%) and the promoter region of *fabG1* (31.0%). Testing only for *katG* and *fabG1* promoter mutations would detect INH resistance in 89.6% of the INH-resistant isolates. All mutations associated with RIF resistance were found only in the RRDR of *rpoB*. This therefore confirms the usefulness of these three specific regions for prediction of INH and RIF resistance [29]. Conversely, *kasA* Gly312Ser and *katG* Trp191Arg mutations were found in phenotypically INH-susceptible isolates. KasA (β-ketoacyl ACP synthase), coded by the *kasA* gene, is an enzyme involved in mycolic acid synthesis. Mutations in the *kasA* gene have been associated with low-level INH resistance [30,31] but the role of these mutations in INH resistance remains unclear. Therefore, the significance of this gene in conferring drug resistance in Indonesia should be carefully assessed. Two isolates were phenotypically RIF-resistant but harboured mutations in *rpoB* that have not been associated with drug resistance before (Cys681Gly and Pro1014Ser), therefore their possible role in RIF resistance should be confirmed in other studies.

Regarding EMB, three loci of the *embB* gene (loci 306, 406 and 497) were identified, with mutations at locus 306 being the most frequent. Mutations in *embB* were present only in combination with other drug resistance mutations, in line with several studies that have demonstrated a strong association between *embB*306 mutations and INH-, RIF- or multidrug-resistant TB [32]. This finding suggests that *embB*306 mutations may have a selective advantage upon treatment with multiple drugs [33]. Regarding STR, isolates carried mutations in *rpsL* ($n = 5$) and *rrs* ($n = 4$). A common variant in *rpsL* (Lys43Arg) has been associated with high-level STR resistance [32] and this variant was indeed the most common in this study. Phenotypic STR resistance was only confirmed in two of seven genotypically STR-resistant isolates with DST results available. Three isolates had a single mutation in *rrs* codon 492; their relevance should be carefully interpreted since this mutation has been reported as a marker for the LAM3 genetic lineage of *M. tuberculosis* rather than for STR resistance [34].

In line with previous studies, concordance between WGS and phenotypic DST was good for INH and RIF [29] but had low agreement for EMB and STR [35,36]. There are several possible explanations for this finding. First, discordance was mainly found with uncommon genotypic mutations, which may be associated with low-level resistance that can be missed by conventional phenotypic DST. Second, the difference between the epidemiological breakpoint and the minimum inhibitory concentration (MIC) for EMB and STR is relatively small [35,36], which complicates phenotypic DST. Third, detection of phenotypic STR resistance in the absence of known mutations for STR resistance suggests the existence of other resistance mechanisms such as efflux pumps

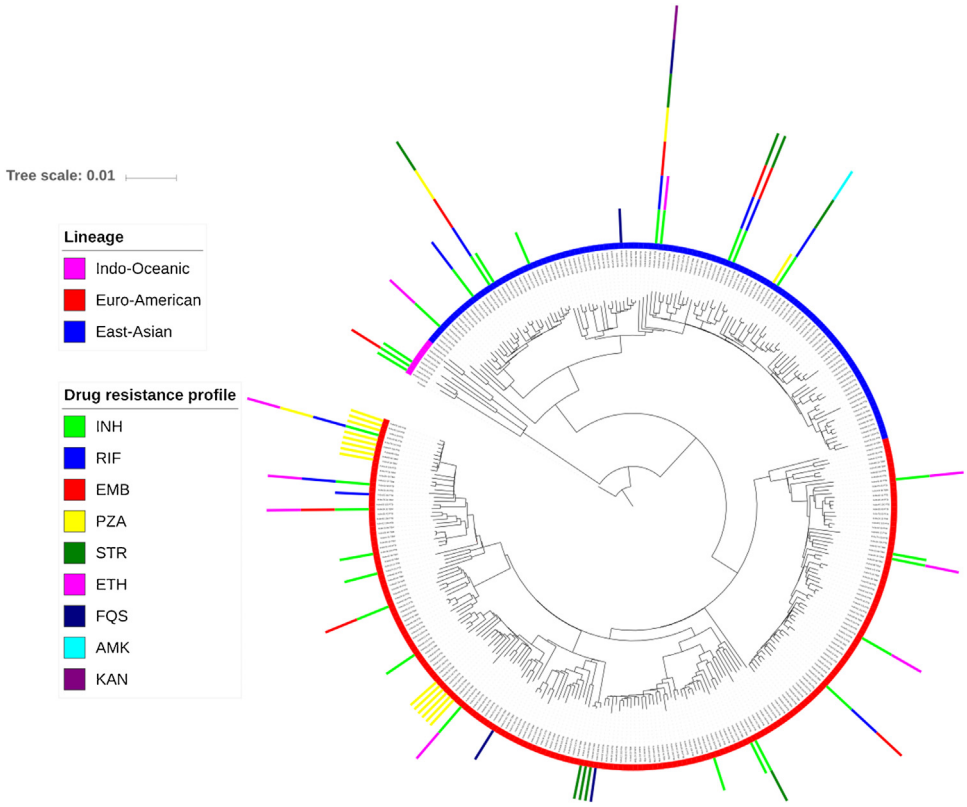

Fig. 2. Phylogenetic tree of 322 *Mycobacterium tuberculosis* isolates indicating drug resistance profiles and main lineages based on single nucleotide polymorphism (SNP) barcoding. INH, isoniazid; RIF rifampicin; EMB, ethambutol; PZA, pyrazinamide; STR, streptomycin; ETH, ethionamide; FQS, fluoroquinolones; AMK, amikacin; KAN, kanamycin.

[36]. Finally, we cannot exclude errors in phenotypic DST despite good quality control in the laboratory. In this regard, the fact that one isolate was phenotypically drug-susceptible with a high confidence *katG* Ser315Thr mutation for INH in WGS is a matter of concern since this mutation is associated with high-level resistance [29,37]. Given this high specificity, mutations at *katG*315 might even be used to assess laboratory quality for DST.

Second-line drug resistance was observed in a number of isolates. At present, there is no standard phenotypic DST for PZA and the second-line drugs in Indonesia, and evidence is limited on the performance of DST for these drugs [38], indicating once more the potential added value of WGS in this setting. Also, reported prior treatment for TB poorly corresponded with genotypic drug resistance, and primary drug resistance was common in this study population of Indonesian patients. Of concern, the only XDR-TB strain was found in a patient who had reported no prior TB treatment. This suggests that selective use of genotypic or phenotypic DST, targeting only those with previous treatment or other factors presumed to be associated with drug resistance, will result in many undetected resistant cases and ongoing transmission of drug-resistant strains [39]. This is further supported by the observation that 10 of 51 isolates with mutations associated with resistance to any drug were not phenotypically tested for drug susceptibility because they did not have these risk factors. WGS could help in early identification of resistance in these patients, limiting the spread of drug-resistant TB.

Molecular drug resistance for PZA mostly involves mutations in the *pncA* gene. There is no predominant drug resistance mutation in *pncA* but a range of diverse mutations across the gene, each associated with a different MIC [5]. Five different mutations in *pncA* were found in the isolates in this study, almost all in PZA-monoresistant strains. Most previous studies reported that PZA resistance is common in MDR-TB strains [40] although significant rates of PZA monoresistance have been reported in some settings [40–42]. A study in China showed that PZA monoresistance contributes to delay in the resolution of lung cavitation without affecting the sputum conversion and lesion elimination rates [41]. The non-essential nature of the *pncA* gene, such that it can accumulate various mutations without affecting the viability of the organism, might explain the spread of PZA-monoresistant strains. Two *pncA* mutations exclusively occurred in strains adjacent in the phylogenetic tree, suggesting possible transmission. However, the genetic difference between these strains was too large to conclude that this was indeed the case.

This study has several limitations. First, WGS was performed on a random sample of archived *M. tuberculosis* isolates so we cannot conclude that the proportions of drug resistance shown in this study are representative. This was a convenience sample collected from patients with pulmonary and meningeal TB. We estimate that several thousands of patients are treated for TB each year in Bandung. However, culture is not routinely performed and isolates are not archived. Therefore, our sampling fraction is likely to be <10%. Second, phenotypic DST was available for most genotypically resistant strains but only for a fraction of isolates without resistance mutations. As a consequence, specificity estimation for genotypic resistance was not possible. Third, sequencing was performed retrospectively on archived isolates, therefore it was not possible to evaluate time to diagnosis of drug resistance using WGS. Nevertheless, we for the first time highlight the potential benefit of using WGS to generate an in silico drug susceptibility profile in Indonesia and show that mutations associated with drug resistance are highly predictive for phenotypic resistance to RIF and INH in the region. Larger studies are needed to confirm the clinical relevance of several uncommon mutations found in this

strain collection. Given the fact that phenotypic DST is complex and slow and is poorly accessible in large parts of Indonesia, WGS could offer a rapid and comprehensive diagnostic solution. This technology is now more accessible with the introduction of portable platforms and an automatic bioinformatic pipeline as well as the decreasing price and turnaround time of WGS [43]. It could lead to quicker and more appropriate treatment in low-income settings where many still rely on empirical treatment regimens. This pilot study offers a good starting point to further evaluate the impact of WGS in the diagnosis, treatment, surveillance and control of drug-resistant TB in Indonesia.

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Competing interests

None declared.

Ethical approval

Health Research Ethics Committee, Faculty of Medicine, Universitas Padjadjaran (Bandung, Indonesia).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jgar.2018.08.018.

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