Discordance between Genexpert, Line Probe Assay and Drug Susceptibility Test in Assessing Drug-Resistant Tuberculosis

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Abstract

Background: Rapid molecular diagnostics have potentially revolutionized early detection of drug-resistant tuberculosis (DR-TB) in Indonesia. However, there is discordance between conventional culture using drug susceptibility test (DST) and rapid diagnostic tools using GeneXpert and line probe assay (LPA). This discordance result can cause confusion to clinician in determining diagnosis of DR-TB. **Objective:** This study aimed to identify discordance between GeneXpert, LPA, and DST. **Methods:** A retrospective study was conducted at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. Data were collected based on medical record between third to fourth quarter of 2018. Rifampicin resistant-tuberculosis (RR-TB) proven by GeneXpert, were further analyzed with second-line LPA and DST. Discordance result between it was analyzed using McNemar. **Results:** Among 81 patients diagnosed with DR-TB, 59 patients RR-TB were eligible in this study. There were 13 (22.0%) DST rifampicin result showed sensitive but resistant to GeneXpert. Among 53 samples from LPA, there were 3 (5.7%) result showed LPA fluoroquinolone resistant but sensitive to DST ofloxacin; 10 (18.9%) result has LPA fluoroquinolone sensitive but resistant to DST ofloxacin; 2 (3.8%) has LPA second-line injectable drug (LPA SLID) resistant but sensitive to DST kanamycin; 5(9.4%) has LPA SLID sensitive but resistant to DST kanamycin. The McNemar analysis showed discordance between GeneXpert and DST rifampicin was 13 (22.0%; p=0.046); LPA fluoroquinolone and DST ofloxacin was 13 (24.6%; p=0.042); LPA SLID and DST kanamycin was 7 (13.2%; p=0.183). No variable that can be used to analyze discordance result between GeneXpert and LPA. **Conclusion:** There is significant discordance between GeneXpert and DST; LPA fluoroquinolone and DST ofloxacin, while neither LPA SLID nor DST kanamycin shows no significant discordance.

Keywords: *Rapid test, conventional culture, drug susceptibility test, line probe assay, drug-resistant tuberculosis*

Introduction

Worldwide, tuberculosis (TB) is one of the top 10 causes of death and the leading cause of curable single infectious agent[1]. It is an infectious disease caused by the bacillus Mycobacterium tuberculosis (MTB)[2]. Now we stand at Sustainable Development Goals (SDGs) for 2030[3]. TB is the only disease ever declared a global emergency by the World Health Organization (WHO)[2]. Integral to this transition, the world community is launching accelerated fight against TB[3].

Globally in 2017, around 10.0 million people (range 9.0 – 11.1 million) developed TB disease in 2017: 5.8 million men, 3.2 million women, and 1.0 million children. Drug resistant-tuberculosis (DR-TB) continues to be a public health crisis[1]. The emergence of DR-TB is further complicating the situation and is threatening

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to jeopardize all the prior gains by global TB control programs in recent years[4,5]. The best estimate is that 558,000 people (range 483,000 – 639,000) developed TB that was rifampicin resistant-tuberculosis (RR-TB), the most effective first line drug, and of these, 82% had multidrug resistant-tuberculosis (MDR-TB). Globally, 3.5% of new TB cases and 18% of previously treated cases had MDR/RR-TB. Among cases MDR-TB in 2017, 8.5% were reported to have extensively drug resistant-TB (XDR-TB)[1].

DR-TB surveillance data show that an estimated 160,684 cases of MDR/RR-TB were detected and notified. Of these, a total of 139,114 people (87%) were enrolled on treatment with a second line-regimen. In spite of increased testing from 129.689 cases in 2016, but still only 25% of the estimated 558,000 people who developed MDR/RR-TB[1].

TB is an age old disease, but even today the diagnosis of TB remains elusive[6]. Urgent action is required to improve the quality of diagnosis for people with DR-TB[7]. Indonesia accounted for countries for 11% from 80% of the 3.6 million global gaps in the detection and treatment of TB cases. In 2017, Indonesian national study found that although about 80% of new cases were detected, 41% of these cases were not reported. Gaps between the estimated number of new cases and the number actually reported due to underdiagnoses. Closing gaps in detection require much higher coverage of drug susceptibility testing among people diagnosed with TB, reducing underdiagnoses of TB[1].

Only one in every six estimated cases was being detected worldwide. Gap remains of the estimated MDR/RR-TB cases still undetected[4]. For the diagnosis of TB, a large number of tests are available, each one having its advantages and disadvantages[2].

Culture based-methods remain the ‘‘gold standard’’ for TB diagnosis in developing countries as these techniques have been greatly improved and routinely used over the past decade. However, the time for bacteriological culture-based diagnosis of TB may require several weeks to months[8]. To address such delay in TB diagnosis as well as to discretely upgrading the speed and quality of MTB diagnostic accuracy[2,8].

Worldwide emphasizing the need to be considered for the early detection of MTB which involves the detection of the mutation in specific genes imparting against resistance[4,8]. Molecular methods have led to the development of rapid and reliable diagnostic and drug susceptibility testing[2]. GeneXpert and Line Probe Assay (LPA) are two which approved standard molecular diagnostic methods that have been developed for the rapid detection of drug resistance by scanning the DNA for associated mutations[4]. GeneXpert is an urgent necessity for tests that can quickly diagnose TB[6]. Introduction of newer and rapid diagnostic tools have increased the detection of RR-TB cases[4]. RR-TB is crucial for proper control of TB disease[9]. In the present study comparative analysis of the conventional method and molecular method like LPA for diagnosis of MTB and detection of MDR TB is carried out[2].

However, there is a discordance between conventional culture with DST and rapid diagnostic tools with GeneXpert and LPA. This discordance result can cause confusion to the clinician in determining diagnosis DR-TB. Here, this study was observed the rapid molecular diagnostic validity of GeneXpert and LPA whether the results will or will not be in accordance with the results given by DST.

Methods

Study Design and Setting: A retrospective study conducted between third to fourth quarters of 2018 at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. The subjects were 81 patients diagnosed with DR-TB. Only 53 patients were eligible for this study (figure 1).

Figure 1. Study flow diagram

This study reviewed based on medical records of diagnostic tools in DR-TB. DR-TB definition adapted from WHO guidelines. This study obtained data on demographics, gender, age, comorbidities, acid fast bacilli (AFB), GeneXpert, second line LPA, and DST. The inclusion criteria were men and women aged between 20 and 65 years. The exclusion data were that patient sample with incomplete GeneXpert, LPA, and DST data.

Procedure: Each sample data was initially examined with smear AFB sputum specimens. AFB data

were conducted from microbiology sputum examination result in the medical record. Positive AFB stains were quantified into 4 groups as scanty (1–9 AFB/100 fields), 1+ (1–9 AFB/10 fields), 2+ (1–9 AFB/fields), and 3+ (>9 AFB/fields).

Then sputum specimens were screened for DR-TB. Diagnostic tests for DR-TB disease include rapid molecular tests and culture based methods. The rapid test for diagnosis of TB currently recommended by WHO is the GeneXpert and LPA.

GeneXpert data were conducted from medical records. GeneXpert can provide results within 3 days, and was for diagnosis of pulmonary TB in adults. RR-TB proved by GeneXpert. GeneXpert RR-TB result was quantified into 4 groups as very low, low, medium, and high.

The further analyzed with second-line LPA. Second-line LPA data were collected from TB03. Second-line LPA sample comes from Balai Besar Laboratorium Kesehatan (BBLK) Surabaya. Second-line LPA can provide results within 14 days. Second-line LPA result was targeting two drugs for its resistance, LPA fluoroquinolone (LPA FQ) and LPA second line injection drug (LPA SLID).

DST was performed on the culture-based methods to identify *Mycobacterium tuberculosis* complex (MTBC) strain. DST has carried to all the culture positive samples by Standard Proportion method. The drugs were used for DST, rifampicin, Isoniazid, ethambutol, streptomycin, kanamycin, amikacin and ofloxacin (Ofl), for DR-TB detection. DST form the current reference standard and can take up to 12 weeks to provide results[2].

We compared DST as the gold standard with GeneXpert. We wanted to see concordance or discordance for detection of rifampicin resistance. We compared DST with second-line LPA. We wanted to see concordance or discordance for detection of fluoroquinolone and second line injection drug resistance.

Statistical Analysis: All of the patient's data were collected on Microsoft Excel. Categorical variables were expressed as an absolute number. Statistical analysis was analyzed using IBM SPSS software 20.0 (IBM Corp., Armonk, NY, USA) for Windows. The cross-tabulation with McNemar formula was performed to analyze concordance or discordance between GeneXpert, second line LPA, and DST. The probability levels $p < 0.05$ were considered as statistically significant.

Results

The study has enrolled 81 patients diagnosed with DR-TB at study entry. Only 59 patients with RR-TB were eligible in this study.

Their demographic data are summarized in Table 1. In this research, baseline characteristic stratified by gender, approximately 69.5% of patient were men. The mean age of patients was 44.47 ± 11.27, range between the youngest ages was 22 years, while the oldest was 62 years. Around 72.9% of patients self-reported had common comorbidities. The most frequent patient comorbidities were smoking (54.2%) and diabetes mellitus (38.9%).

Table 1. Characteristic demographic

Variable	n (%)
Gender	
Men	41 (69.5)
Women	18 (30.5)
Age	
≤ 60 years old	56 (94.9)
> 60 years old	3 (5.1)
Comorbidities	43 (72.9)
Diabetes Mellitus	23 (38.9)
Hypertension	6 (10.2)
HIV/AIDS	1 (1.7)
Smoking	32 (54.2)
Alcoholic	5 (8.5)

Sputum microscopy AFB showed 74.5% has a positive result. GeneXpert was stratified according to four categories very low, low, medium, and high rifampicin resistance. Most of sample 42.4% has a medium result. Second line LPA and DST were stratified according to two categories resistant and sensitive. 53 samples were analyzed for DR -TB by second-line LPA by targeting two drugs for its resistance, LPA fluoroquinolone (LPA FQ) and LPA second line injection drug (LPA SLID). It was found that 84.7% of samples were found to be sensitive by second-line LPA. Also, only 5.1% of the

samples were found to be resistant by second-line LPA. DST component was isoniazid rifampicin, ethambutol, streptomycin, kanamycin, amoxicillin, and ofloxacin. Resistant DST rifampicin was 78.0%, resistant DST kanamycin was 8.5%, and resistant DST Ofl was 18.6%. The other laboratory result is summarized in Table 2.

Table 2. DR-TB based on laboratory modality (GeneXpert, LPA, and DST)

Classification	Frequency (%)	Resistant (%)	Sensitive (%)
Based on AFB			
Negative	10 (16.9)		
Scanty	5 (8.5)		
1+	13 (22.0)		
2+	18 (30.5)		
3+	13 (22.0)		
Based on GeneXpert			
Very low	6 (10.2)		
Low	15 (25.4)		
Medium	25 (42.4)		
High	13 (22.0)		
Based on LPA			
MTB positive	53 (89.8)		
LPA FQ		3 (5.1)	50 (84.7)
LPA SLID		3 (5.1)	50 (84.7)
Based on DST			
Isoniazid		38 (64.4)	15 (25.4)
Rifampicin		46 (78.0)	13 (22.0)
Ethambutol		14 (23.7)	39 (66.1)
Streptomycin		13 (22.0)	40 (78.0)
Kanamycin		5 (8.5)	54 (91.5)
Amoxicillin		6 (10.2)	53 (89.8)
Ofloxacin		11 (18.6)	48 (81.4)

There were 13 (22.0%) DST result showed sensitive rifampicin but resistant form GeneXpert. Among 53 samples from second line LPA, there were 2 (5.7%) result showed resistant to fluoroquinolone (FQ) but sensitive to ofloxacin from the DST; 10 (18,9%) result has FQ sensitive but resistant to ofloxacin from DST; 2 (3.8%) has second line injectable drug (SLID) resistant but sensitive to kanamycin from DST; 5 (9.4%) has sensitive SLID but showing kanamycin resistant from DST.

Table 3. Concordance and discordance between GeneXpert on DST and second-line LPA on DST

Diagnosis DR-TB	Resistant (%)	Sensitive (%)	Concordance (%)	Discordance (%)	p
GeneXpert RR	46 (78.0)	13 (22.0)	46 (78.0)	13 (22.0)	0.046
LPA FQ	0 (0.0)	3 (5.7)	40 (75.4)	13 (24.6)	0.042
	10 (18.9)	40 (75.4)			
LPA SLID	0 (0.0)	2 (3.8)	46 (86.8)	7 (13.2)	0.183
	5 (9.4)	46 (86.8)			

The analysis showed discordance between GeneXpert and DST rifampicin was 13 (13,2%) samples with (p=0.046). Discordance between LPA fluoroquinolone and DST ofloxacin was 13 (24.6%) samples with (p=0.042); LPA SLID and DST kanamycin was 7 (13.2%) samples with (p=0.183). There is no variable that can be used to analyze the discordance result between GeneXpert and second line LPA (table3).

Discussions

In the recent years, major importance has been given on rapid diagnosis and quick initiation of accurate treatment for DR-TB[8]. Resistance to anti-TB drugs can occur when these drugs are misused or mismanaged[10]. Late diagnosed DR-TB leading to diagnostic delay with associated exacerbation of transmission, amplification of resistance, and increased mortality[11]. Precise and early diagnosis of DR-TB is extremely beneficial as it interrupts further transmission of the disease and avoids addition of life-saving drugs and consequently increases of drug resistance. It also avoids unnecessary cost of administration and occurrence of serious side effects of second line anti-TB drugs in case one is dealing with drug sensitive MTB strains[2].

The best method of diagnosing an infectious disease is to demonstrate the causative organism in representative samples of tissue or fluid by either staining and or by culture. Sputum microscopy by the AFB staining is accepted worldwide as the first line test as it is simple, convenient, rapid, inexpensive and can be done in field condition. However, it is less sensitive as it requires bacterial load at least 10^4 bacilli per ml of sputum to be positive and it may be falsely positive in many conditions including environmental mycobacterial infection[6]. AFB cannot distinguish between dead and live bacteria and is unable to identify different species of MTB[2]. Culture is the reference golden standard for TB diagnosis but they are time consuming. Culture and drug-susceptibility testing (DST) using solid media can take up to 8–12 weeks for results and faster liquid-based culture techniques still take at least 4–6 weeks[5]. DST based on the estimation of growth or no growth of an MTB strain in the presence of a single critical concentration of one drug. The critical concentration of an anti-tuberculosis drug represents the lowest concentration of the drug in the medium that indicates clinically relevant resistance if growth is observed. Susceptible wild-type strains are inhibited by this concentration. Resistance is defined if over 1% of the bacterial population of a strain is able to grow[2].

The development of rapid molecular diagnostic tests for the identification of MTB and drug resistance has consequently become a research and implementation priority[5]. Rapid and accurate diagnosis of pulmonary TB remains a great challenge. There is an urgent necessity for tests that can quickly diagnose TB. Hence the most promising approach was to demonstrate remnants of the TB bacilli in representative samples. Detecting even small amounts of bacterial DNA was feasible due to the development of various molecular diagnostic tests for TB[6].

Molecular techniques have revolutionized the diagnosis of pulmonary tuberculosis (PTB), as well as DR-TB. Rifampicin resistance is considered as surrogate marker of DR-TB[6]. GeneXpert and LPA are recommended for diagnostic testing for the presence of MTB and detection of mutations associated with rifampicin resistance[12].

GeneXpert is a novel integrated diagnostic system for major change in the speed, simplicity and accuracy of not only diagnosis of TB but also drug resistance to rifampicin in TB, which is accepted as a surrogate for DR-TB. The rapidity and robustness of diagnosis in-turn breaks the chain of transmission in addition to early institution of treatment and improved chances for cure[13]. GeneXpert can simultaneously identify MTB and rifampicin resistance within two hours. The GeneXpert has been approved by the WHO in 2013. It adopted a GRADE system approach to arrive at recommendations on the diagnostic value of the assay in PTB patients on therapy for less than seven days. This test has the potential to dramatically reduce the time to diagnosis and the time to initiation of effective therapy[6,13].

GeneXpert advantage is simple and automated to perform with minimal training, is not prone to cross-contamination, and requires minimal biosafety facilities[4,6]. GeneXpert requires a reliable power supply and operating temperatures below 30°C. Sputum should be of good quality, and it should be concentrated by usual laboratory methods[6]. The specificity and sensitivity of GeneXpert for detection of rifampicin resistance are

more than 98% and 99%, respectively[4,14].

GeneXpert is designed to identify rifampicin resistance[6]. Rifampicin is a widely used first line anti TB drug that works by inhibiting the mycobacterial ribonucleic acid (RNA) synthesis[4]. The vast majority (around 95 to 98%) of rifampicin resistance associated mutations using DNA probes in an 81-bp region (codons 507 to 553) of the RNA polymerase β subunit (rpo-β) gene known as the rifampicin resistance determining region (RRDR). Rifampicin resistance detection in the GeneXpert is based on hybridization or the absence of five molecular beacon probes complementary to the wild type sequence of rpo-β gene[14]. Most of the RIF resistance mutations are of the first kind hence are easily detected[15].

In our study, there is discordance between GeneXpert and DST Rif, but the percentage is low. The accuracy for identification of rifampicin resistance was 98%. However, a study done in Swaziland demonstrated that the assay may not be able to detect wild type mutations for rifampicin resistance outside the rpo-β I491F domain[6,4]. GeneXpert detected four samples discordant with culture DST. On sequencing, two showed mutations [517,519 [dual mutation] and 533CCG], while two others had no mutation in the RRDR. The mutations at probe ends might be missed[13].

Another study revealed mixed MTB infections have been suggested to be responsible for false negative and positive results for rifampicin resistance. Several studies have reports of mixed infections, its interference with drug resistance detection. For the same reason, GeneXpert cannot be used for assessing the emergence of rifampicin resistance during treatment. Hetero-resistance MTB populations is often suggested to be responsible for discordant DST results[13].

LPA are rapid molecular diagnostics that can detect MTB and drug resistance. Although LPAs are more technically complex and take longer to perform than the GeneXpert. First line LPA detect drug resistance by identifying mutations in the rpo-β, katG, and inhA genes. By targeting mutations in the 81-base pair "core region" of the rpo-β gene, more than 95% of all Rifampicin resistant strains can be detected. Although mutations in katG and inhA account for approximately 80-90% of INH resistant strains[5].

LPA targeting resistance to second line anti TB drugs are under evaluation. Second line LPA detect fluoroquinolone (moxifloxacin) and SLID (amikacin, kanamycin and capreomycin) resistance by identifying mutations in gyrA, and rrs. Sequencing of the gyrA, and rrs genes was performed on a representative sample of isolates with discrepant second line LPA and DST results. These assays detect mutations in the gyrA gene as fluoroquinolone resistance and detected mutation in rrs gene as kanamycin, Amk, and Cm resistance[11].

In the present study we have performed a comparison of the conventional method of DST and a newer molecular method that is LPA for the detection of DR-TB from the sputum samples[2]. In Indonesia we have only check second-line LPA. The second-line LPA detects resistance to fluoroquinolones (LPA-FQ) and second-line injectable drugs (LPA-SLID), and it may be used as an initial test for second-line drug resistance[6]. The results of LPA were then compared to the DST, which is still a gold standard[2]. A positive result is reliable for detection of drug-resistant TB but a negative result may not always rule out the presence of drug-resistant TB, and that should be confirmed by conventional culture and drug sensitivity test (DST)[6].

LPA is capable of indicating hetero-resistance but the limit of detection of LPA is at least $5x10^3$ bacilli per ml of sample, hence the bacillary load would need to be high enough for detection using reverse hybridization by LPA. The LPA also may not detect all mutations at position 533; the probes are so designed that the mutation does not always affect the loss of binding of probes[13].

Another study discordance between LPA and DST may be due to DNA extraction protocol of LPA. In LPA, DNA extraction was done directly from the sputum samples; hence even the DNA of dead bacilli may have contributed. Higher percentage of TB positive in LPA may be attributed to the limitation of the technique that cannot differentiate between live and dead bacilli[2].

Depending on the specific region interrogated by SL-FL and SL-LPA, one or more follow-up diagnostic actions are either recommended or suggested as optional to better guide the choice of the treatment regimen. The decision to perform the optional follow-up diagnostic actions should be guided by considerations on the individual patient's risk for resistance and by the

prevalence of resistance in the specific geographical setting, as these factors affect the positive predictive value of the test[16]. Genotypic testing is much faster than phenotypic methods, as these are not growth based tests. Drug sensitivity test results by solid Lowenstein Jansen media has a turnaround time of up to 84 days, liquid culture up to 42 days, LPA up to 72 hours and GeneXpert by 2 hours[6].

However, it should be remembered that a positive result suggest but a negative result do not exclude TB as well as DR-TB. At present GeneXpert and LPA has not totally replaced the traditional smear and culture for TB[6]. Molecular tests are not recommended for treatment monitoring. DST may be used during treatment to assess for any acquisition of additional resistance or reinfection. Given that decisions on the treatment of patients depend to an important degree on the bacteriological findings[12].

Conclusions

Bacteriological examinations in patients with DR-TB include sputum smear microscopy, culture and DST as well as rapid test such as GeneXpert and LPA. Genotypic assays though offer rapidity and most often a good sensitivity when the probes designed can cover all possible mutations responsible for resistance but could give false positive results due to detection of mutations not responsible for resistance. There is significant discordance between GeneXpert and DST; LPA fluoroquinolone and DST ofloxacin, while neither LPA SLID nor DST kanamycin shows no significant discordance.

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Author's Contribution

All authors contributed toward data analysis, drafting and revising the paper, gave final approval of the version to be published and agree to be accountable for all aspects of the work.

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Data Availability: The dataset used and/or analyzed during the current study are available from corresponding author on reasonable request.

Ethics Statement

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia (*1491 / KEPK / IX / 2019*).

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