



# COMPARISON BETWEEN GENOTYPE MTBDRSL VER 2.0 ASSAY AND PHENOTYPIC METHOD ON RIFAMPICIN RESISTANT *MYCOBACTERIUM TUBERCULOSIS*

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## ABSTRACT

**Objective:** Multi-drug resistance tuberculosis remains a crisis and threats to world health. Line Probe Assay is a molecular techniques for rapid diagnosis of second line drugs MDR-TB. But there is less sufficient information on the clinical viability of this test and a lack of evidence in the diagnosis of MDR-TB use of this Line Probe Assay technique in Indonesia. The aim of this study is to determine performance of line probe assay GenoType MTBDRsl VER 2.0 assay on rifampicin-resistance *Mycobacterium tuberculosis* isolates compared with

phenotypic Lowenstein Jensen drug susceptibility test method (DST) as a gold standard. **Methods :** This research was used cross sectional study with analytic observational approach from January- June 2020. After being examine with Xpert MTB/RIF, a total of 26 rifampicin resistance *Mycobacterium tuberculosis* isolates were collected and tested with GenoType MTBDRsl VER 2.0 assay and phenotypic Drug Susceptibility Test (DST). Statistic analysis were performed to determine sensitivity, specificity, PPV, NPV and accuracy of second line tuberculosis drugs.

**Results:** Our study found three fluoroquinolone resistant *Mycobacterium tuberculosis* isolates (11.5%) and one amikacin resistant *Mycobacterium tuberculosis* isolate (3,8%). The performance of GenoType MTBDRsl VER 2.0 assay is shown 100 % (95% CI 84,6-100 % ) sensitivity, 100% (95% CI 39,8-100 %) specificity, 100% (95% CI %) PPV, 100% (95% CI %) NPV and 100% (95% CI 86,8-100 %) accuracy.

**Conclusion:** GenoType MTBDRsl VER 2.0 assay showed a high performance compared with phenotypic method to determine resistance of fluoroquinolone and Second Line Injectable Drugs.

**Keywords:** Line Probe Assay, GenoType MTBDRsl ver 2.0, Drug Susceptibility Test, MDR-TB, Second Line Injectable Drugs

## INTRODUCTION

One of infectious disease that still remain as global health problem is tuberculosis. *Mycobacterium tuberculosis* was the causative agent of tuberculosis and often infected lungs. Tuberculosis was placed as one of the top 10th disease in the world that lead to the death.<sup>1,2</sup> During the year, an active tuberculosis person will be infected 10 to 15 other people by closing contact. Tuberculosis can be cured and prevented through proper diagnosis and treatment.<sup>2</sup> In 2018, 10 million people was suffered with TB. Because of this disease 1.5 million people lost their life and children fell ill with tuberculosis around one million and died with TB estimated as 230,000 include co-infection HIV-TB.<sup>3</sup>

Multi-drug resistant tuberculosis remains a crisis and threats to world health. There were 558,000 new cases of rifampin and around 82% suffered with MDR-TB.<sup>2</sup> Indonesia was on 2nd order of high tuberculosis cases in the world, after India.<sup>2</sup> The new tuberculosis cases number in Indonesia was 420.994 cases.<sup>4</sup> In Palembang, South Sumatra, the MTB- DR's incidence rate is 1.4%.<sup>5</sup>

The second-line drug susceptibility test for MDR-TB can be completed by two methods, Lowenstein Jensen solid culture DST and MGIT liquid culture.<sup>6,7</sup> Lowenstein Jensen solid culture DST protocols taken 3-4 month for resistance result.<sup>7</sup> whereas MGIT liquid culture had 42 days.<sup>8</sup> Both cultures were much slower when compared to Line Probe Assay. WHO recommended MTBDRsl VER 2.0 assay as a rapid diagnostic for determining the susceptibility of *Mycobacterium tuberculosis* drugs in acid fast bacilli positive specimens or in isolates from acid fast bacilli negative specimens. Both cultures were much slower when compared with Line Probe Assay.<sup>9,10</sup> GenoType MTBDRsl VER 2.0 assay is a DNA-STRIP qualitative in vitro test to identify *Mycobacterium tuberculosis* complex and determined resistance to SLID which detected mutation in *rrs* gene

for amikacin resistance, *eis* gene for kanamycin resistance, also *gyrA* and *gyrB* gene for fluoroquinolone resistance. Diagnosis of second line MDR TB with GenoType MTBDRs/ VER assay 2.0 can be detected within 24-48 hours (1-2 days) much faster when compared with DST.<sup>10</sup>

However, in various countries, especially in Indonesia, there is less sufficient information on the clinical viability of this test, and there is a lack of evidence in the diagnosis of MDR-TB use of this Line Probe Assay technique.<sup>11,12</sup> It can minimize MDR-TB treatment time with correct diagnosis in a short time by using a 9-11 month Shorter Therapy Regimen (STR).<sup>11</sup> Therefore, further research is needed to compare the performance of Line Probe Assay with the gold standard Drug Susceptibility Test (DST) method especially in Indonesia, the second-tier tuberculosis world.

## Material and methods

This study used cross sectional design with observational approach.

### Specimens collection isolates

The research included 324 isolates of *Mycobacterium tuberculosis* strains were examined by Xpert MTB/RIF in Dr. Mohammad Hoesin Hospital, Palembang, South Sumatera, from January – June 2020 to finding for rifampicin resistant *Mycobacterium tuberculosis* strains. There were 26 isolates of rifampicin resistant *Mycobacterium tuberculosis* obtain and 298 isolates rifampicin sensitive *Mycobacterium tuberculosis* were excluded after Xpert MTB/RIF examination. Those 26 isolates were delivered to Palembang Health Center Laboratory as referral laboratory Indonesian Health Ministry of Sumatera region for testing MTBDRs/ Ver 2.0 assay. Microscopic of sputum smear showed that 26 isolates were all acid fast bacilli positive. Procedure of the N-acetyl-cysteine-NaOH was used to decontaminate the samples according to international guidelines.<sup>13</sup> The phenotypic method as the gold standard was used to compare the drug sensitivity and specificity of the corresponding strains with MTBDRs/ VER 2.0 assay. This research was approved by Committee of Ethical Medicine faculty of Universitas Airlangga with the number 82/EC/KEPK/FKUA/2020.

**Xpert MTB/RIF** Xpert MTB/RIF procedure was done to acid fast bacilli positive specimens in Mohammad Hoesin Hospital Palembang according to WHO guidelines.<sup>14</sup>

**DST method.** The critical concentrations for kanamycin, 30 µg/ml; for amikacin, 40 µg/ml; for ofloxacin, 4 µg/ml were used in DST culture Lowenstein Jensen.

**DNA Extraction** Thermal lysis was done to extract DNA genome from isolates and also for decontaminated specimens. DNA extraction was also used GenoLyse (Hain Lifescience) from clinical specimens as alternative.<sup>8,11</sup>

### GenoType MTBDRs/ VER 2.0 assay procedures

A 500 µl DNA genome was used to perform GenoType MTBDRs/ Ver 2.0 assay. Until receiving the results, a DNA portion was kept refrigerated (4°C). The pellet of saved DNA was centrifuged for 5 minutes at 13,000 G and 5 µl DNA was put in to 45 µl of amplification mix and got cycling 42 of PCR being amplified, after that hybridization and read strip test steps were followed. As quality assurance, H37Rv isolates was used in MTBDRs/

VER 2.0 assay for each running.<sup>8,11</sup> Amplification and hybridization of DNA extracted from isolates was performed according to the manufacturer's instruction

### Statistical analysis.

Statistical analysis were carried out to know the performance of MTBDRs/ VER 2.0 assay using Epi Info statistics to determine sensitivity, specificity, PPV, NPV and accuracy.

## RESULT

### Data of gender and age from isolates base on PMDT (Programmatic Management of Drug Resistant Tuberculosis)

Sputum samples containing rifampicin resistant *Mycobacterium tuberculosis* in this study were collected 26 isolates after Xpert MTB/RIF testing. There were four isolates resistance to second-line tuberculosis drug (15,3%). Three isolates were fluoroquinolone resistance and one isolate was amikacin resistance. Meanwhile, 22 isolates were susceptible to second line tuberculosis drug (84,7%). The composition isolates based on gender were 15 males (57,7%) and 11 (42,3%) females. The isolates frequency based on age includes 2 people in the age group of 20-29 years old (7,7%), 9 people of 30-39 years old (34,6%), 6 people of 40-49 years old (23,1%), 7 people of 50-59 years old (26,9%), and 2 people of 60-69 years (7,7%)

### Performance of GenoType MTBDRs/ VER 2.0 assay

The results of the susceptibility test for fluoroquinolone and amikacin (SLID) on rifampicin resistant *Mycobacterium tuberculosis* using the GenoType MTBDRs/ VER 2.0 assay showed four isolates (4/26) which were resistance of second line drug tuberculosis (both FQ and SLID). These result are shown in table 1. Whereas, in table 2 are shown that genotypic performance of MTBDRs/ Ver 2.0 assay against reference DST for both FQ and amikacin as second-line TB drugs, were 100% (84,6-100 %) sensitivity, 100% (39,7-100) specificity, 100% PPV, 100% NPV, and 100 % (86,8-100 %) accuracy with P.Value < 0,05. Our study found one isolate (1/26) had resistance to amikacin and three isolates (3/26) had resistance to fluoroquinolone. These result are shown in table 3. The performance of GenoType MTBDRs/ VER 2.0 assay for each FQ and amikacin resistance were showed in table 4. In our study, GenoType MTBDRs/ VER 2.0 assay for FQ performance reported 100% (29,2 - 100 %) sensitivity, 100 % (85,2-100 %) specificity, 100% PPV, 100% NPV, and 100% (86,8-100 %) accuracy with P.Value < 0,05. As for amikacin, GenoType MTBDRs/ VER 2.0 performance, assay showed 100% (2,5-100 %) sensitivity, 100%(86,8-100 %) specificity, 100% PPV, 100% NPV, and 100% (86,8-100 %) accuracy with P.Value < 0,05.

### Mutation associated with ofloxacin and amikacin resistance of TB drugs

The mutations frequency associated with ofloxacin and amikacin resistance of TB drugs among 26 result of rifampicin resistant MTB testing by GenoType MTBDRs/ VER 2.0 assay and concordant DST were being analysed. One isolates was missing of wild type 2 (25%) and associated with mutation A90V in *gyrA* gene. While two were missing of wild type 3 (75%) and associated with mutation D94G in *gyrA* gene. This mutation was more frequent in associated with FQ resistance. Mean while in our study, one mutation A1401G in *rrs* gene

was detected in amikacin resistance. The hybridization patterns on strip of GenoType MTBDRsl VER 2.0 Assay compared with the reference DST are shown in tabel 5.

## DISSCUSSION

The rifampicin resistance *Mycobacterium tuberculosis* DNA isolates used in this study had a composition based on gender, most of which were male around 57.7%. We were tracked the data of patients from PMDT (Programmatic Management of Drug Resistant Tuberculosis). This findings of gender group was similar with a study conducted in Taiwan, male were 74.4%.<sup>15</sup> This result was also accordance with WHO that men dominate TB infection cases in the world.<sup>16</sup> Previous research has shown that the social role and activity dynamics of men were higher than those of women. The risk factor for the transmission of *Mycobacterium tuberculosis* is higher in men.<sup>17</sup> The hormone estrogen in women is more able to activate the immune response against TB infection than the hormone testosterone in male.<sup>17-19</sup> Men also smoke a lot and consume alcohol. So that the possibility of TB is greater. Biological mechanisms allow smoking and alcohol to ruin system of body's immune and increased TB infection.<sup>20</sup> Cigarettes can result inhibitory effect on nitric oxide synthase which was irreversible. Alveolar macrophage needs those enzyme to inhibit the replication of *Mycobacterium tuberculosis*.<sup>20</sup> Iron can bind to nitric oxide syntase to produce radicals of toxic that disrupt alveolar macrophages which was increased by smoking. Cigarettes also might reduce the effectiveness lung immune alveolar macrophages ability by altering the pro-inflammatory cytokine cells expression'.<sup>20</sup>

The sample pattern of *Mycobacterium tuberculosis* rifampicin resistant isolates based on age 30-39 years was 34.6% and 50-59 years old was 26.9%. This findings was in accordance with WHO report data in 2014 with the dominance of productive age as TB patients.<sup>21</sup> This cause is associated with the transmission of TB which allows productive age with high activity and social roles, exposure to cigarette smoke and environmental pollution, making it easier for the to infect. Exposure to these bacteria in youth can cause reactivation at productive age. Comorbid factors and other immunocompromising conditions also support the cause of active bacteria, for example Diabetes Mellitus, heart disease and so on.<sup>22</sup> Examination of rifampicin resistance *Mycobacterium tuberculosis* isolates using MTBDRsl VER 2.0 assay was able to detect mutations of four genes, *gyrA* and *gyrB* (fluoroquinolone resistance), *eis* (kanamycin resistance) and *rrs* (amikacin resistance). The results of this study showed that isolates resistance to second line TB drug were four isolates (15.38%) with fluoroquinolone resistance were three isolates (11.5%) showed mutation in *gyrA* gene, while one isolate resistant to amikacin (3.8%) showed mutation in *rrs* gene.

According examination by MTBDRsl ver 2.0 in this study, there was found that one sample had a mutation of the *gyrA* MUT1 A90V gene. This mutation was also found in Gardee et al study in 2016 with 25 isolates and Gao et al research in 2018 with 14 isolates.<sup>8,9</sup> We were also found that two samples had mutations in the *gyrA* gene MUT3C D94G which shown fluoroquinolone resistance. This mutation was also reported in previous study Gardee et al on 21 isolates and Gao et al 18 isolates.<sup>8,9</sup> Futhermore, our study reported one sample had mutations in the *rrs* MUT1 A1401G gene. This result was also in accordance with the other study that found



*rrs* MUT1 (A1401G) from 37 isolates amikacin resistance was the main dominance of the *rrs* gene mutation.<sup>8</sup> Whereas mutation of *gyrB* gene and *eis* gene was not found in this research.

GenoType MTBDRsl VER 2.0 assay performance in this research showed 100% specificity, 100% sensitivity, 100% PPV, 100% NPV and 100% accuracy in 26 *Mycobacterium tuberculosis* rifampicin resistance isolates for ofloxacin and amikacin. This was almost the same with the previous study result which were 100% sensitivity, 98.9% specificity, 97.7% PPV, 100% NPV and 99.3% accuracy for fluoroquinolone and for amikacin; the sensitivity was 100%, specificity 98.5%, PPV 90.9%, NPV 99.0% and accuracy 97.8%.<sup>9</sup> The other study on 353 MDR-TB specimens, MTBDRsl VER 2.0 assay sensitivity was 80.5% (95% CI: 72.0-87.4%) for fluoroquinolone resistance and 80.7% (95% CI: 67, 7-89.5%) for SLID resistance (amikacin, kanamycin and capreomycin) while the specificity of the MTBDRsl VER 2.0 assay testing was 100% (95% CI: 98.5-100.0%) to detect fluoroquinolone resistance and 99.3% (95% CI: 97.3–99.9%) for SLID resistance. The PPV in Gao et al's study was 100% (95% CI: 95.0-100.0%) for FQ and 95.8% (95% CI: 84.6–99.3%) for second-line injection agents whereas the NPV was 91.6% (95% CI: 88.2-94.1%) for fluoroquinolone resistance and 96.4% (95% CI: 93.5-98.1%) for SLID resistance.<sup>8</sup>

This study result also was concordance with other previous studies evaluating MTBDRsl VER 2.0 assay in 228 rifampicin resistant *Mycobacterium tuberculosis* strains isolates which compared with phenotypic DST. The comparative result of Tagliani et al study as a previous study were shown performance sensitivity 83,6%, 100% specificity, 100 % PPV and 92,8% NPV for fluoroquinolone resistance and 86,4 % sensitivity, 90,1% specificity, 94,1% PPV and 78,5% NPV for amikacin (SLID) resistance.<sup>23</sup>

## Conclusion

GenoType MTBDRsl VER. 2.0 assay showed a high performance for specificity and sensitivity as DST to detect FQ and SLID resistance. It could be used as rapid diagnostic to identify resistance second line drugs in MDR-TB patients and helps the clinician as diagnostic tool to determine therapy of MDR-TB. The limitation of this study that there was small amount isolates from single center study. So it requires multicenter study in Indonesia to get a bigger amount isolates.

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## CONFLICT INTEREST

There was no conflict interest.

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**Table 1.** Detection of drug resistance for second-line tuberculosis drug (both FQ and SLID) between GenoType MTBDRsl VER 2.0 assay with DST in rifampicin resistant *Mycobacterium tuberculosis* isolates

GenoType MTBDRsl ver 2.0 assay	DST second line TB drugs (FQ and SLID)		
	S (isolates)	R (isolates)	Total (isolates)
Sensitive	22	0	22
Resistant	0	4	4
Total	22	4	26

DST: Drug Susceptibility Drug; FQ: Fluoroquinolone; SLID: Second line Injectable Drug ; R : Resistance; S: Sensitive

**Table 2.** GenoType MTBDRsl VER 2.0 assay performance against the reference DST for second-line tuberculosis drug (both of fluoroquinolone and Second line Injectable Drug).

Performance of MTBDRsl VER 2.0 assay	Sensitivity*	Specificity*	PPV*	NPV*	Accuracy*
Second line TB drug (both FQ and SLID)	100% (84,6- 100%)	100%(39,7- 100%)	100%	100%	100%( 86,8- 100%)

- Confidence Interval 95% with P.Value < 0,05; FQ: Fluoroquinolone; SLID: Second line Injectable Drug

**Table 3.** Detection of drug resistance for FQ and SLID between GenoType MTBDRsl VER 2.0 assay with DST in rifampicin resistant *Mycobacterium tuberculosis* isolates

Detection of Drug Resistance	DST S (isolates)	DST R (isolates)	MTBDRsl VER 2.0 assay S	MTBDRsl VER 2.0 assay R
FQ Ofloxacin	23	3	23	3
SLID Amikacin	25	1	25	1

DST: Drug Susceptibility Drug; FQ: Fluoroquinolone; SLID: Second line Injectable Drug ; R : Resistance; S: Sensitive

**Table 4.** GenoType MTBDRsl VER 2.0 assay performance against the reference DST for each fluoroquinolone and Second line Injectable Drug.

Performance of MTBDRsl VER 2.0 assay	Sensitivity*	Specificity*	PPV*	NPV*	Accuracy*
FQ Ofloxacin	100% (29,2-100)	100 % (85,2-100)	100%	100%	100% (86,8-100)
SLID Amikacin	100% (2,5-100)	100% (86,8-100)	100%	100%	100% (86,8-100)

- Confidence Interval 95% with P.Value < 0,05; FQ: Fluoroquinolone; SLID: Second line Injectable Drug

**Table 5.** Hybridization patterns on strip of GenoType MTBDRsl VER 2.0 assay compared with the reference DST.

AFB: Acid Fast Bacilli ; DST; Drug Susceptibility Test; FQ; Fluoroquinolone;

OFX: Ofloxacin

Samples ID	Phenotypic DST	AFB	GenoType MTBDRsl VER 2.0 assay				Frequency (n=isolates)
			Missing wild probe	Mutation probe	Mutant	Interpretation	
744SS	OFX Resistant	3+	<i>gyr A</i> WT2	<i>gyr A</i> MUT1	A90V	FQ Resistant	1
45 SS	OFX Resistant	2+	<i>gyr A</i> WT3	<i>gyr A</i> MUT3	D94G	FQ Resistant	1
841SS	OFX Resistant	1+	<i>gyr A</i> WT3	<i>gyr A</i> MUT3	D94G	FQ Resistant	1
2SS	amikacin Resistant	2+	-	<i>rrs</i> MUT1	A1401 G	Amikacin Resistant	1