ORIGINAL ARTICLE

Diagnostic Performance of Genotype MTBDR*sl* Assay (Version 2) for Detecting 2nd Line Drug Resistance TB Compared to Sensititre MycoTBI MIC in MDR-TB Patients

Piamlarp Sangsayunh, MD[1], Thanyanuch Sanchat, MD[1], Karntheera Sangkaew, BSc[2], Waraporn Thuansuwan, MSc[2]

[1] Pulmonary Department, Central Chest Institute of Thailand, Nonthaburi, Thailand; [2] Medical Laboratory Unit, Central Chest Institute of Thailand, Nonthaburi, Thailand

Objective: The diagnostic test aimed to compare the GenoType MTBDR*sl* assay (version 2) and minimum inhibitory concentration (MIC) using a Sensititre MycoTBI MIC plate to detect quinolone and aminoglycoside resistance.

Materials and Methods: One hundred thirty-three remaining samples from individuals diagnosed with multidrug-resistance tuberculosis (MDR-TB) and having tested positive for TB cultures between September 2016 and August 2021 were included in the present study.

Results: The sensitivity, specificity, PPV, and NPV using GenoType MTBDR*sl* version 2 compared to MIC of levofloxacin at greater than 0.5 µg/mL were 87.5% (95% CI of 67.6 to 97.3), 98.16% (93.5 to 99.8), 91.30% (72.0 to 98.9), and 97.2% (92.2 to 99.4). Similarly, for moxifloxacin at 2 mg/dL or greater for resistance, the values were 95.4% (95% CI of 77.2 to 99.9), 98.2% (93.6 to 99.8), 91.3% (72.0 to 98.9), and 99.0% (95.0 to 100). The sensitivity, specificity, and diagnostic performances of GenoType MTBDR*sl* version 2 compared to the MIC of amikacin of greater than 4 µg/mL and kanamycin of greater than 5 µg/mL were 100% (95% CI of 54.1 to 100), 99.2% (95.7 to 100), 99.2% (42.1 to 99.6), and 85.7% (97.1 to 100). Two location mutations at D94G and D94 N/Y were related to a high level of fluoroquinolone with median MIC values of 8 µg/mL of levofloxacin and greater than 4 of moxifloxacin. The mutation at C-14T did not cause resistance in phenotypic patterns.

Conclusion: GenoType MTBDR*sl* (version 2) and MIC determination using Sensititre MycoTBI MIC plate are consistent with each other.

Keywords: Sensititre MycoTBI MIC plate; MTBDR*sl* assay (version 2); Quinolone resistance; Aminoglycoside resistance

Received 4 August 2023 | Revised 22 December 2023 | Accepted 2 January 2024

J Med Assoc Thai 2024;107(1):21-6

Website: http://www.jmatonline.com

Drug-resistant tuberculosis, particularly pre-XDR-TB, poses a significant problem. Understanding drug susceptibility and selecting appropriate medication can reduce mortality rates and control further spread. A gold standard method for the diagnosis of drug resistance is the drug sensitivity test (DST). This method takes a considerable amount of time. As an alternative, molecular tests such as GenoType MTBDR*sl* have been introduced for diagnosing drug resistance to quinolone and aminoglycoside. They play a vital role in helping to separate a group of patients from multi drug-resistance tuberculosis (MDR-TB)[1].

Correspondence to:

Sangsayunh P.
Pulmonary Department, Central Chest Institute of Thailand,
74 Tiwanon Road, Nonthaburi 11000, Thailand.
Phone: +66-2-5470999
Email: Piamlarp@yahoo.com

How to cite this article:

Sangsayunh P, Sanchat T, Sangkaew K, Thuansuwan W. Diagnostic Performance of Genotype MTBDR*sl* Assay (Version 2) for Detecting 2nd Line Drug Resistance TB Compared to Sensititre MycoTBI MIC in MDR-TB Patients. J Med Assoc Thai 2024;107:21-6.
DOI: 10.35755/jmedassocthai.2024.1.13932

Finding quinolone resistance can be quickly used to adjust the current treatment. GenoType MTBDR*sl*, now widely used as version 2, is more specific and covers fluoroquinolone-resistant locations at gyrA and gyrB and aminoglycoside resistance positions at rs and eis[2]. The lowest concentration of drugs that can inhibit tuberculosis growth, or minimum inhibitory concentration (MIC), is one way to detect drug resistance. Sensititre MycoTBI MIC plate (ThemoFisher Scientific, Waltham, USA)[3] is a dry microdilution plate containing lyophilized antibiotics, with concentrations prepared and quality controlled by the manufacturer. At times, there was discordance between phenotypic and genotypic results. The objective of the present research was to establish a more robust correlation between GenoType MTBDR*sl* version 2 and Sensititre MycoTBI MIC plate, specifically aiming to determine resistance of levofloxacin, moxifloxacin, and the aminoglycoside group.

Materials and Methods

The diagnostic study was conducted between September 2016 and August 2021. Collected

Table 1. Correlation of the level of resistance by MycoTB MIC plate compared to standard local drug susceptibility test (DST) by absolute concentration method in quinolone resistance

Drug	APM critical concentration (µg/mL)	MycoTB MIC (level of resistance) (µg/mL)	MycoTB MIC plate result	No of isolate resistance on standard DST	No of isolate susceptible on standard DST	% Sensitivity (95% CI)	% Specificity (95% CI)	Spearman correlation method (r)
Moxifloxacin	0.5	>0.5	Resistance	22	3	91.6% (73.0 to 99.0)	96.9% (90.5 to 99.3)	0.87
		≤0.5	susceptible	2	95			
	2.0	≥2	Resistance	22	3	100% (84.6 to 100)	97.0% (91.0 to 99.3)	0.92
		<2	susceptible	0	97			
		>2	Resistance	11	14	100% (71.5 to 100)	85.0% (91.0 to 99.3)	0.62
		≤2	susceptible	0	97			
levofloxacin	1	>1	Resistance	22	3	95% (68.8 to 97.5)	96.9% (94.1 to 100)	0.89
		≤1	susceptible	1	96			

APM=agar proportion method; CI=confidence interval

sputum was done in confirmed MDR-TB patients referred for treatment of MDR-TB at the Central Chest Institute of Thailand. The Ethical Approval statement was approved by the Human Research Ethics Committee, Central chest institutes of Thailand between December 4, 2019 and December 3, 2020, and between January 27, 2021 and January 26, 2022. Two hundred fifteen samples were routinely cultured using Lowenstein-Jensen (LJ) media.

• The positive cultures were processed to detect drug sensitivity using two techniques. Firstly, the absolute concentration method was employed, which was a standard local technique to identify susceptibility. This method was performed locally by CCIT. Secondly, another technique called Sensititre MycoTBI MIC plate was used to determine the lowest concentration of drugs that can inhibit tuberculosis growth known as the MIC.

• Drug susceptibility to levofloxacin, amikacin, and kanamycin was defined as CLSI MIC break-points, of 1 or less, 4 or less, and 5 or less µg/mL, respectively. However, there were various definitions of moxifloxacin resistance. The present study chose cut-off MIC for moxifloxacin resistance under correlation between MIC and DST. A high level of quinolone resistance definition was defined as 4 µg/mL or more, correlating with Chien's study[5]. No proper CLSI MIC breakpoint was defined for capreomycin resistance.

• The positive cultured samples were used to identify mutant codons using by GenoType MTBDR*sl* version 2 (Hain Lifescience, Germany). GenoType MTBDR*sl* version 2 enabled to detect mutations of quinolone and aminoglycoside resistance by commercial NAT.

Statistical analysis

Descriptive analysis was used to analyze the number of quinolone resistance and aminoglycoside resistance in Sensititre MycoTB MIC and GenoType MTBDR*sl* (version 2). The correlation of moxifloxacin resistance between DST and Sensititre MycoTB MIC was analyzed by the Spearman correlation method. Sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) with 95% confidence interval (CI) were used to analyze the correlation of MycoTB MIC and GenoType MTBDR*sl* (version 2) by Stata Statistical Software, version 15.1 (StataCorp LLC, College Station, TX, USA).

Results

There were 215 cases of MDR-TB between September 2016 and August 2021, ranging in age from 21 to 89 years old, classified as 127 males and 88 females. Seventy-six samples were excluded because of baseline negative culture. Five patients were unable to perform a MIC test because there were not enough specimens to process. There was one case with an invalid result in GenoType MTBDR*sl* version 2 detection process. There were 133 remaining samples for study. There were higher specificity and correlation in moxifloxacin resistance between DST and cut-off MIC of 2 µg/mL or greater than with cut-off concentration greater than 2 µg/mL or greater than 0.5 µg/mL according to Table 1.

From the MIC procedure, 24 samples (18.0%), six samples (4.5%), and six samples (4.5%) showed resistance in levofloxacin, amikacin, and kanamycin, respectively. There were 22 resistance samples (16.5%) in the moxifloxacin drug under cut-off MIC of 2 µg/mL or greater. From GenoType MTBDR*sl* (version 2), the 23 samples (17.2%) and seven samples (5.2%) that showed resistance in quinolone and aminoglycoside, respectively. Two cases were detected with quinolone resistance at GenoType

Table 2. Abnormal band detection of quinolone resistance by GenoType MTBDR*sl* (version 2) in 23 samples*

Probe detection	Mutation position	Sample of probe detection; n (%)	MIC level (µg/mL)			
			Levofloxacin		Moxifloxacin	
			IQR (Q1, Q3)	Median	IQR (Q1, Q3)	Median
gyrA MUT1	A90V	6 (26.0)	2 (2, 4)	3	2 (2, 4)	2
gyrA MUT2	S91P	1 (4.4)	-	2	-	2
gyrA MUT3A	D94A	1 (4.4)	-	2	-	2
gyrA MUT3B	D94N/Y	2 (8.6)	4 (4, 8)	6	4 (4, 8)	6
gyrA MUT3C	D94G	7 (30.4)	6 (2, 8)	4	2 (2, 4)	4
gyrA MUT3D	D94H	1 (4.4)	-	4	-	2
gyrA MUT1+3B+3C	D94G+D94N/Y+A90V	1 (4.4)	-	2	-	<0.06
gyrA MUT3B+3C	D94G+ D94N/Y	2 (8.6)	>0 (8, >8)	8	>0 (4, >4)	4
gyrA MUT1+2	A90V+ E540V	1 (4.4)	-	4	-	4
Loss WT1	N/A	1 (4.4)	-	4	-	2
gyrB MUT1	N538D	0 (0.0)	-	-	-	-
gyrB MUT2	E540V	0 (0.0)	-	-	-	-

MIC=minimum inhibitory concentration; IQR=interquartile range; N/A=not applicable
* Resistance criteria: Moxifloxacin MIC levels ≥2 µg/mL, Levofloxacin MIC >1 µg/mL

MTBDR*sl* (version 2), but no moxifloxacin resistance at Sensititre MycoTB MIC at level MIC 0.06, 0.06 µg/mL, and one case at Sensititre MycoTB MIC at level MIC 4 µg/mL but not at GenoType MTBDR*sl* (version 2). The Sensitivity, specificity, PPV, and NPV of GenoType MTBDR*sl* version 2 compared to MIC of moxifloxacin at MIC levels of 2 µg/mL or greater were 95.4% (95% CI 77.2 to 99.9), 98.2% (95% CI 93.6 to 99.8), 91.3% (95% CI 72.0 to 98.9), and 99.0% (95% CI 95.0 to 100), respectively. The three levofloxacin resistance samples were detected at Sensititre MycoTB MIC at level MIC 2, 4, and 8 µg/mL but not at GenoType MTBDR*sl* (version 2), and two cases in GenoType MTBDR*sl* (version 2) but not Sensititre MycoTB MIC at MIC level 0.25, 0.5 µg/mL. Sensitivity, specificity, PPV, and NPV, using GenoType MTBDR*sl* (version 2) compared to the MIC of levofloxacin were values of 87.5% (95% CI 67.6 to 97.3), 98.16% (95% CI 93.5 to 99.8), 91.30% (95% CI 72.0 to 98.9), and 97.2% (95% CI 92.2 to 99.4), respectively. One sample detected amikacin and kanamycin resistance at GenoType MTBDR*sl* (version 2) but not at Sensititre MycoTB MIC at MIC level of amikacin 1 µg/mL and kanamycin 0.5 µg/mL. Sensitivity, specificity, and diagnostic performances of GenoType MTBDR*sl* (version 2) compared to the MIC of amikacin were 100% (95% CI 54.1 to 100), 99.2% (95% CI 95.7 to 100), 99.2% (95% CI 42.1 to 99.6), and 85.7% (95% CI 97.1 to 100), respectively. Sensitivity, specificity, PPV, and NPV of GenoType MTBDR*sl* (version 2) compared to MIC of kanamycin 100% (95% CI 54.1 to 100), 99.2% (95% CI 95.7 to 100), 99.2% (95% CI 42.1 to 99.6), and 85.7% (95% CI 97.1 to 100), respectively. From the GenoType MTBDR*sl* (version 2), quinolone resistance traits were detected with a single defined mutation band of gyrA MUT3C, followed by gyrA MUT1, and no resistance was observed at the gyrB band. There were four cases of multi-mutation bands such as one case with mutation band gyrA MUT1/gyrA MUT3B/gyrA MUT3C and two cases with gyrA MUT3B/gyrA MUT3C according to Table 2.

Based on aminoglycoside resistance data from the GenoType MTBDR*sl* (version 2), resistance characteristics were primarily associated with rrs MUT1, which showed a proportion of resistance according to Table 3.

The most common mutation position of the quinolone resistance was single mutation, which found in seven cases (30.4%) at codon D94G, corresponding to probe gyrA MUT3C, median MIC to moxifloxacin 4.0, interquartile range (IQR) min-max smaller than 0.06 to 4, and MIC to levofloxacin 4.0, IQR min-max smaller than 0.25 to 8. The second most common mutation position was at A90V (probe position gyrA MUT1), median MIC to moxifloxacin 2.0, IQR min-max of 2 to 4, and MIC to levofloxacin 3.0, IQR min-max 0.5 to 4 with six cases (26%).

Discussion

Diagnosis of MDR-TB in Thailand is performed by a molecular test that also gives out early detection of *Mycobacterium tuberculosis* disease and drug

Table 3. The proportion of aminoglycoside resistance with abnormal band detection by GenoType MTBDR*sl* (version 2) in 23 samples*

Probe detection	Mutation position	Sample of probe detection; n (%)	MIC level (µg/mL)					
			Capreomycin		Kanamycin		Amikacin	
			IQR (Q1, Q3)	Median	IQR (Q1, Q3)	Median	IQR (Q1, Q3)	Median
rrsMUT1	A1401G	6 (85.7)	5 (5, 10)	5	0 (>16, >16)	>16	0 (>16, >16)	>16
rrsMUT2	G1484T	0 (0.0)	-	-	-	-	-	-
eisMUT1	C-14T	1 (14.3)	-	1.2	-	1	-	0.5

MIC=minimum inhibitory concentration; IQR=interquartile range

* Resistance criteria: Amikacin MIC levels >4 µg/mL, Kanamycin MIC >5 µg/mL

resistance, so there is a high proportion of baseline negative culture, which is 35%. There were limitations in data collection due to COVID situation, leading to delays in research operations, and only 133 patients could be included in the present study. Because the Central Chest Institute of Thailand is a referral center for patients with MDR TB, there were high amount of quinolone resistance cases in the present study such as 17.1% of levofloxacin and 17.9% of moxifloxacin by way of MIC under Sensititre MycoTBI MIC plate. Therefore, statistic cannot reflect resistance population in Thailand.

The previous study, which compared phenotypic DST in 228 samples using a Sensititre MycoTBI MIC plate, showed that the agar proportion method (APM) critical concentration for moxifloxacin was 2.0 µg/mL[3]. For ofloxacin, kanamycin, and amikacin, there were 2.0 µg/mL, 5.0 µg/mL, and 5.0 µg/mL, respectively. The study had a definition of resistance that was more than APM critical concentration[3]. The sensitivity and specificity of moxifloxacin susceptibility/resistance correlation between Sensititre MycoTB plate compared to DST in the study were 98.4 and not applicable, respectively. Another study showed sensitivity and specificity of moxifloxacin correlation at APM MIC 2.0 µg/mL were 95.8% and 81.3% and at APM MIC 0.5 µg/mL was 100% and 79.5%[4]. However, the conditional agreement of the study defined susceptibility as lower than or equal to APM critical concentration plus 1 doubling dilution, or the critical concentration was considered as susceptibility[4]. Resistance was considered equivalent to or higher than APM critical concentration. This is different criteria from other studies. The other study of 111 samples divided the levels of moxifloxacin resistance in Sensititre microtiter plates as susceptible for MIC of 0.5 µg/mL or lower, low-level resistance for MIC of 1 to 2 µg/mL, and high level for MIC of 4 µg/mL or greater. There were various definitions of moxifloxacin resistance in different study[5]. The study compared GenoType MTBDR*sl* version 2 and phenotypic DST[3] and found sensitivity and specificity of quinolone at 93% (95% CI 83.3 to 97.2) and 98% (95% CI 95.1 to 99.4), and diagnostic accuracy at 97.0% (95% CI 93.9 to 98.5). Individual drug susceptibility, sensitivity and specificity were 100% (95% CI 78.5 to 100) and 90.9% (95% CI 62.3 to 98.4) in levofloxacin and 100% (95% CI 78.5 to 100) and 90.9% (95% CI 62.3 to 98.4) in moxifloxacin. The study showed sensitivity, specificity, and diagnostic accuracy of the aminoglycoside group using amikacin, kanamycin, and capreomycin were 88.9% (95% CI 78.8 to 94.5) and 91.7% (95% CI 86.5 to 95.0) and 90.9% (95% CI 86.5 to 94.0). Individual drug susceptibility, sensitivity and specificity were 96.0% (95% CI 86.5 to 98.9) and 92.2% (95% CI 87.1 to 95.4) in kanamycin.

The level of cut-off moxifloxacin resistance varied. The present study chose the definition of Lee et al.[4] study for cut-off MIC to detect moxifloxacin resistance because the authors found the highest correlation between moxifloxacin resistance at MIC of 2 µg/mL or greater by Sensititre MycoTBI MIC plate and standard local DST. There were high sensitivity and specificity of GenoType MTBDR*sl* (version 2) and Sensititre MycoTB in moxifloxacin at 95.4% and 98.2% in the present study. There were few studies about the MIC of levofloxacin. The present study showed high specificity of levofloxacin MIC at 98.16% compared to GenoType MTBDR*sl* version 2. It means that GenoType MTBDR*sl* (version 2) can suggest using levofloxacin treatment in the regimen when no mutation band of GenoType MTBDR*sl* was detected. Sensitivity and specificity comparisons between GenoType MTBDR*sl* (version 2) and MIC of aminoglycoside drugs in the present study were high, at 100% and 99.2% in both amikacin and kanamycin, making the method reliable.

The previous study in the United States found 13% of cases in the D94G position, followed by A90V at 10% of cases[6]. Another study found

common resistance mutation at position A90V at 27.2%, followed by mutation D94N in band gyrA mut3B, and D94A in band gyrA mut3A in 11%, 7.6%, respectively. Resistance with more than one position in the present study was observed at 17.6%, with the most common D94G/D94N/Y, but this was different from the previous study (A90V/D94N/94Y)[7]. The present study observed that codon 94 was related to a high level of fluoroquinolone resistance, which was the same as the previous study, especially in multi-location mutation at D94G and D94 N/Y with median MIC values of 8 µg/mL of levofloxacin and greater than 4 of moxifloxacin[8]. Another mutation point in the present study could not evaluate high-low level resistance because of data limitations. The previous study of 111 sputum found that mutations at the D94G and D94N positions of gyr A and G512R of gyr B were associated with high-level MFX resistance, while D94A mutation is associated with low-level MFX resistance[5]. However, cases in the present study were susceptible to moxifloxacin and levofloxacin but detected with a mutation position of D94G, which differed from the other study[5]. Aminoglycoside resistance at codon A1401G (probe rrs MUT1) caused high resistance to amikacin, and kanamycin and had a MIC value of more than 16 mg/dL, which is the same as the present study[9,10]. Mutation at C-14T (probe eisMUT1) did not cause resistance in phenotypic patterns in the present study but studies found that the mutation at eis showed resistance to kanamycin[9,10].

Conclusion

GenoType MTBDR*sl* version 2 can accurately indicate the resistance of quinolones for levofloxacin and moxifloxacin, when compared to MIC results obtained through the Sensititre MycoTBI MIC plate method.

What is already known on this topic?

The cutoff point of MIC under Lee's criteria, which is MIC of 2 µg/mL or greater, for detecting moxifloxacin resistance showed the highest correlation with standard local DST. The use of GenoType MTBDR*sl* version 2 was beneficial in detecting resistance to both moxifloxacin and levofloxacin.

What does this study add?

Few studies have focused on the correlation between GenoType MTBDR*sl* version 2 and levofloxacin, with most studies focusing on moxifloxacin. This study is particularly interesting as it examines the correlation with levofloxacin, which is commonly used in Thailand.

Funding disclosure

The present study was support by Medical Service of Ministry of Public Health of Thailand.

Conflicts of interest

The authors declare no conflict of interest.

Reference

1. World Health Organization. WHO consolidated guidelines on tuberculosis: Module 4: Treatment: Drug-resistant tuberculosis treatment [Internet]. 2022 [cited 2023 Oct 22]. Available from: https://www.who.int/publications/i/item/9789240007048.
2. HAIN LifeScience, GenoType MTBDRsl VER 2.0 instructions for use. Document IFU-317A-01 [Internet]. Nehren, Germany: HAIN LifeScience; 2015 [cited 2023 Oct 22]. Available from: http://www.hain-lifescience.de/en/instructions-for-use.html.
3. Hall L, Jude KP, Clark SL, Dionne K, Merson R, Boyer A, et al. Evaluation of the sensititre MycoTB plate for susceptibility testing of the Mycobacterium tuberculosis complex against first- and second-line agents. J Clin Microbiol 2012;50:3732-4.
4. Lee J, Armstrong DT, Ssengooba W, Park JA, Yu Y, Mumbowa F, et al. Sensititre MYCOTB MIC plate for testing Mycobacterium tuberculosis susceptibility to first- and second-line drugs. Antimicrob Agents Chemother 2014;58:11-8.
5. Chien JY, Chiu WY, Chien ST, Chiang CJ, Yu CJ, Hsueh PR. Mutations in gyrA and gyrB among Fluoroquinolone- and Multidrug-Resistant Mycobacterium Tuberculosis isolates. Antimicrob Agents Chemother 2016;60:2090-6.
6. Farhat MR, Jacobson KR, Franke MF, Kaur D, Sloutsky A, Mitnick CD, et al. Gyrase mutations are associated with variable levels of fluoroquinolone resistance in Mycobacterium tuberculosis. J Clin Microbiol 2016;54:727-33.
7. Tagliani E, Cabibbe AM, Miotto P, Borroni E, Toro JC, Mansjö M, et al. Diagnostic performance of the new version (v2.0) of genotype MTBDRsl assay for detection of resistance to fluoroquinolones and second-line injectable drugs: A multicenter study. J Clin Microbiol 2015;53:2961-9.
8. Sirgel FA, Warren RM, Streicher EM, Victor TC, van Helden PD, Böttger EC. gyrA mutations and phenotypic susceptibility levels to ofloxacin and moxifloxacin in clinical isolates of Mycobacterium tuberculosis. J Antimicrob Chemother 2012;67:1088-93.
9. Georghiou SB, Magana M, Garfein RS, Catanzaro DG, Catanzaro A, Rodwell TC. Evaluation of

genetic mutations associated with Mycobacterium tuberculosis resistance to amikacin, kanamycin and capreomycin: a systematic review. PLoS One 2012;7:e33275.
10. Campbell PJ, Morlock GP, Sikes RD, Dalton TL, Metchock B, Starks AM, et al. Molecular detection of mutations associated with first- and second-line drug resistance compared with conventional drug susceptibility testing of Mycobacterium tuberculosis. Antimicrob Agents Chemother 2011;55:2032-41.