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Performance of line probe assay and phenotypic drug susceptibility testing in detecting drug-resistant tuberculosis

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ABSTRACT

Background: Tuberculosis (TB) still threatens human beings when drug-resistant tuberculosis (DR-TB), such as rifampicin-resistant TB, isoniazid-resistant TB, multidrug-resistant TB (MDR-TB), pre-extensively drug-resistant TB, and extensively drug-resistant TB increases continuously. The drug susceptibility testing (DST) is important to detect DR-TB for TB treatment.

Objectives: The study aimed to assess first-line line probe assay (FL-LPA) performance of screening MDR-TB and detecting DR-TB on phenotypic drug susceptibility testing.

Materials and methods: A laboratory-based study was performed at Cho Ray Hospital from August 2023 to August 2024. The sputum samples of presumptive TB were inoculated in *Mycobacterium* growth indicator tube (MGIT). Positive inoculum was examined in acid-fast bacilli (AFB) by Ziehl-Neelsen microscope. Cord-forming AFB were yielded to FL-LPA to identify *Mycobacterium tuberculosis* complex (MTBC); detect rifampicin-resistant TB, isoniazid-resistant TB, and MDR-TB. The identified MTBC was subjected to FL phenotypic DST (performed by BACTEC MGIT 960) with SIRE kit, considering gold standard to assess FL-LPA performance. The detected multidrug and/or rifampicin-resistant TB (MDR/RR-TB) were subjected to the second-line MGIT DST including ethionamide, amikacin, levofloxacin, and linezolid to screen pre-extensively drug-resistant TB and extensively drug-resistant TB.

Results: Among 1853 samples inoculated, 621 positive MGIT tubes seen cord-forming AFB on Ziehl-Neelsen smear were performed to FL-LPA. Out of 621 LPA tests, 304 MTBC (61 isoniazid-resistant TB, 20 rifampicin-resistant TB, and 243 susceptible TB) were detected and compared to FL phenotypic DST. The excellent agreements between FL-LPA and FL phenotypic DST for detecting rifampicin-resistant TB, isoniazid-resistant TB, and MDR-TB were greater than 98%; kappa at 0.89 and above (p<0.001); with sensitivity values at 88.9% and above; specificity values at greater than 99%. For FL-MGIT DST, 101 (33.2%) were drug-resistant to at least one anti-TB agent, 81 (26.6%) to streptomycin, 60 (19.7%) to isoniazid, 20 (6.6%) to rifampicin. Among 20 MDR/RR-TB (2 rifampicin mono-resistant-TB and 18 MDR-TB) performed second line phenotypic DST, 25% resistance to ethionamide, and 100% susceptibility to amikacin, levofloxacin, and linezolid.

Conclusion: The performance of FL-LPA to detect rifampicin-resistant TB, isoniazid-resistant TB, and MDR-TB agreed perfectly with phenotypic DST. The reaffirmed critical concentration of isoniazid, rifampicin and levofloxacin would be used to screen DR-TB on population.

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Introduction

TB incidence increased to the highest cases of 7.5 million since 1995.[1] Furthermore, DR-TB such as rifampicin-resistant TB (RR-TB), isoniazid-resistant TB (HR-TB), MDR-TB (TB resistance to both RIF and INH), pre-extensively drug-resistant TB (MDR/RR-TB resistance to any fluoroquinolone),[2] and extensively drug-resistant TB (MDR/RR-TB resistance to any fluoroquinolone and at least one additional Group A drug such as bedaquiline or linezolid) still causes severe obstacles to TB treatment. MDR/RR-TB have reported 410,000 new cases and 160,000 deaths in 2022.[1] On the End TB strategy, presumptive TB were recommended to receive the rapid tests to detect TB and DR-TB from DST playing an important role for chemotherapy, treatment response and surveillance of emerge drug resistance.[3] FL-LPA is also recommended as an initial rapid test to detect resistance to RIF and INH within 48 hours.[4] LPA of Genoscholar NTM + MDRTB Detection Kit (NIPRO Corporation, Osaka, Japan) had sensitivity, specificity at 96.5, 97.5 for detecting RR-TB, and 94.9, 97.6 for HR-TB while Genotype MTBDR*plus* was reported the higher sensitivity, specificity at 98.2, 97.8 for detecting RR-TB and 95.4, 98.8 for HR-TB.[5] Genotype MTBDR*plus* identifies the most significant mutations of the *rpoB* gene (coding for β-sub-unit of the ribonucleic acid polymerase); the *katG* gene (coding for the catalase-peroxidase) and promoter region of the *inhA* gene (coding for nicotinamide adenine dinucleotide enoyl-acyl carrier protein reductase) to detect RR-TB, high and low level HR-TB, respectively.[6,7] The second-line (SL) LPA test has not been recommended for DST due to their detecting injectable anti-TB drugs which are not including the shorter oral regimen for treating MDR/RR-TB recently.[8,9] Whereas phenotypic culture-based DST with turnaround time about 2 weeks as the gold standard which is available for new and repurposed Group A drugs to treat MDR/RR-TB and detect pre-extensively drug-resistant TB (pre-XDR-TB), and 3.8% as extensively drug-resistant TB (XDR-TB).[10]

Vietnam was ranked in two of three WHO global lists of high-burden countries for TB and MDR/RR-TB with 9200 MDR/RR-TB incident cases in 2020.[1] Wrohan *et al*. reported 88% MDR-TB, 8.2% as pre-extensively drug-resistant TB, and 3.8% as XDR-TB on high-risk populations in Ha Noi and Thanh Hoa, Northern, Vietnam that was performed by Xpert MTB/RIF, FL-LPA, and DST in 2022.[11] So, an effort to improve testing and diagnosis of DR-TB is the prior challenge worldwide and in Vietnam particularly. We analyzed data between August 2023 and August 2024 for a retrospective laboratory-based study to detect drug-resistant TB at the Microbiology department in Cho Ray Hospital. The FL-LPA evaluated the detection performance of rifampicin, isoniazid, and multi-drug resistance of TB based on the gold standard of phenotypic conventional culture-based DST that screened drug resistance, multi-drug resistance, pre-extensive drug resistance, and extensive drug resistance of TB, as well. The data of our study reported the practice of screening DR-TB according to the updated definition of drug resistance.[12]

Materials and methods

A laboratory-based study was performed at Cho Ray Hospital, from August 2023 to August 2024. Positive MGIT cultures of sputum were performed by Ziehl-Neelsen staining. Smear of inoculum was covered with hot 0.3% Carbol Fuchsin-Phenol in 10 minutes, de-colored with 3% acid alcohol for 3 minutes, and counterstained with 0.3% Methylene Blue for 1 minute.[13] Rinsing slightly was performed after each steps before microscopic examination for cord formation of AFB (Figure 1).[14,15]

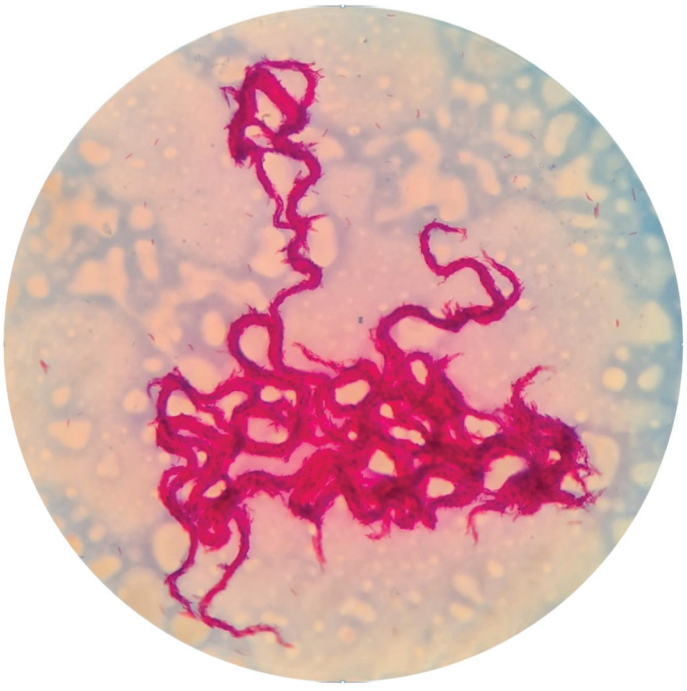

Figure 1. *Cord-forming acid-fast bacilli in liquid media on Ziehl-Neelsen microscopy.*

The inoculum with AFB cord formation was subjected to FL-LPA of GenoType MTBDR*plus* version 2.0 (Hain Life science, Nehren, Germany) to identify MTBC and detect RR-TB, HR-TB, and MDR-TB according to the procedure of manufacture.[6] Genolyse DNA extraction prepared with 1 mL of inoculum from positive MGIT was centrifuged for sediment and suspended with 100 µL of Lysis Buffer at 95 °C for 5 minutes, centrifuged at 13,000 rpm with 100 µL Neutralization Buffer for 100 µL of supernatant. A mixture including 10 µL of Amplification Mix A, 35 µL of Amplification Mix B and 5 µL of extracted DNA was amplified on CFX96 Real-Time system (Bio-rad, USA) in 15 min at 95 °C for denaturation; 10 cycles of 30 seconds at 95 °C and 2 minutes at 65 °C; 20 cycles including 30 seconds at 90 °C, 40 seconds at 50 °C, 40 seconds at 70 °C, and 8 minutes at 70 °C. Reversed hybridization was performed on membrane strip with 20 µL of PCR products with 20 µL of DEN solution, 1.0 mL of HYB solution, 1.0 mL of STR solution, 1.0 mL of RIN solution, 1.0 mL of conjugate and 1.0 mL of subtract solution for binding to probes targeting the most commonly occurring mutations (MUT), and wild-type (WT) probes.

Results were interpreted based on presence of bands for loci of *rpoB*, *katG* and *inhA* genes; presence of all WT probe bands for a sensitive classification of gene; absence of one or more WT bands indicating the strain has resistance to a specific drug and the absence of a WT band accompanying by the presence of a MUT probe band; presence of bands at both WT and MUT probe sites indicating either a heterogeneous test strain with partial resistance; or a mixed culture where at least one of the strains harbors a mutation. Deviation from the WT banding pattern for *rpoB, katG* and *inhA* probes indicated rifampicin resistance, high- and low-level Isoniazid resistance, respectively.

Detected MTBC by FL-LPA was yielded to DST on the MGIT BACTEC 960 against FL anti-TB agents of SIRE kit (Becton Dickinson, USA) considered as gold standard to evaluate FL-LPA performance. For preparing Mycobacterial suspension from MGIT positive, a tube observed positivity of day one to two was inverted and stood within ten minutes for sediment. For a positive observation of day three to five, tube was diluted five-fold.[16] Each MGIT tube for DST was added 0.8 mL of SIRE Supplement, 0.5 mL of inoculum suspension and 100 µL of reconstituted drug solution for the critical concentration of Streptomycin (STR) 1.0 µg/mL, INH 0.1 µg/mL, RIF 0.5 µg/mL, Ethambutol (EMB) 5 µg/mL.[3,17,2] The growth-control tube was added 800 µL of SIRE Supplement and 500 µL of inoculum suspension diluted 1:100 ratio (100 µL of inoculum : 9.9 mL BACTEC Diluting Fluid) without anti-TB drugs. After inoculation, the tube was incubated at 36±1°C in BACTEC MGIT 960 where fluorescence is detected automatically based on the growth of the bacteria in the presence of the drug about 4 to 13 days for both line DST.[16] The result was compared to a growth control (400 growth unit) when growth unit value in the drug-containing tube was less than 100 as susceptible (S) and greater than or equal 100 as resistant (R).

Detected MDR/RR-TB were subjected to the second line (SL) anti-TB agents (Sigma-Aldrich, Germany) including the critical concentration of amikacin (AMK) 1.0 µg/mL, ethionamide (EDT) 5.0 µg/mL, linezolid (LZD) 1.0 µg/mL, levofloxacin (LVX) 1.0 µg/mL (a later generation fluoroquinolone) and one GC tube.[2] The rates of drug resistance to anti-TB agents were evaluated and interpreted RR-TB, MDR-TB, pre-XDR-TB (MDR/RR-TB combined with LVX resistance), XDR-TB (MDR/RR-TB combined with both resistance to LVX and LZD).[2]

Quality assurance

The assays in this study including Genotype MTBDR*plus* version 2, first- and second-line MGIT DST testing were performed the internal control with negative control for each sample and positive control of MTB H37Ra for each batch;[18] accredited external quality assurance performance by Integrated Quality Laboratory Services, France; and certified method verification by the laboratory center of AIDS Clinical Trials Group and International Maternal Pediatric Adolescent AIDS Clinical Trials.

Statistical analysis

STATA 17.0 (StataCorp, College Station, Texas, USA) was used in this study. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of LPA were evaluated performance assay. The respective 95% confidence interval (CI) was computed using the Clopper–Pearson method. The agreement of resistant detection of two assays compared with kappa; p-value < 0.05 was considered statistically significant.

Results

Among 1853 tubes alarmed culture positives from MGIT 960 system, 621 were seen with cord formation on Ziehl-Neelsen AFB smears and performed to FL-LPA. Out of 621 LPA tests, 304 MTBC including 61 isoniazid-resistant-TB, 20 rifampicin-resistant TB, and 243 susceptible TB were detected and compared to FL-MGIT DST which was subjected to SIRE kit. Among 304 FL-MGIT DST, 203 were susceptible whereas 60, 20, and 18 were resistant to INH, RIF, and MDR-TB, respectively. A total of 18 MDR-TB and 2 rifampicin mono-resistant-TB were performed further in the SC-MGIT DST. There were only 5 ETD-resistant TB whereas AMK-resistant TB, LVX-resistant TB, LZD-resistant TB, pre-XDR-TB, and XDR-TB were not found (Figure 2).

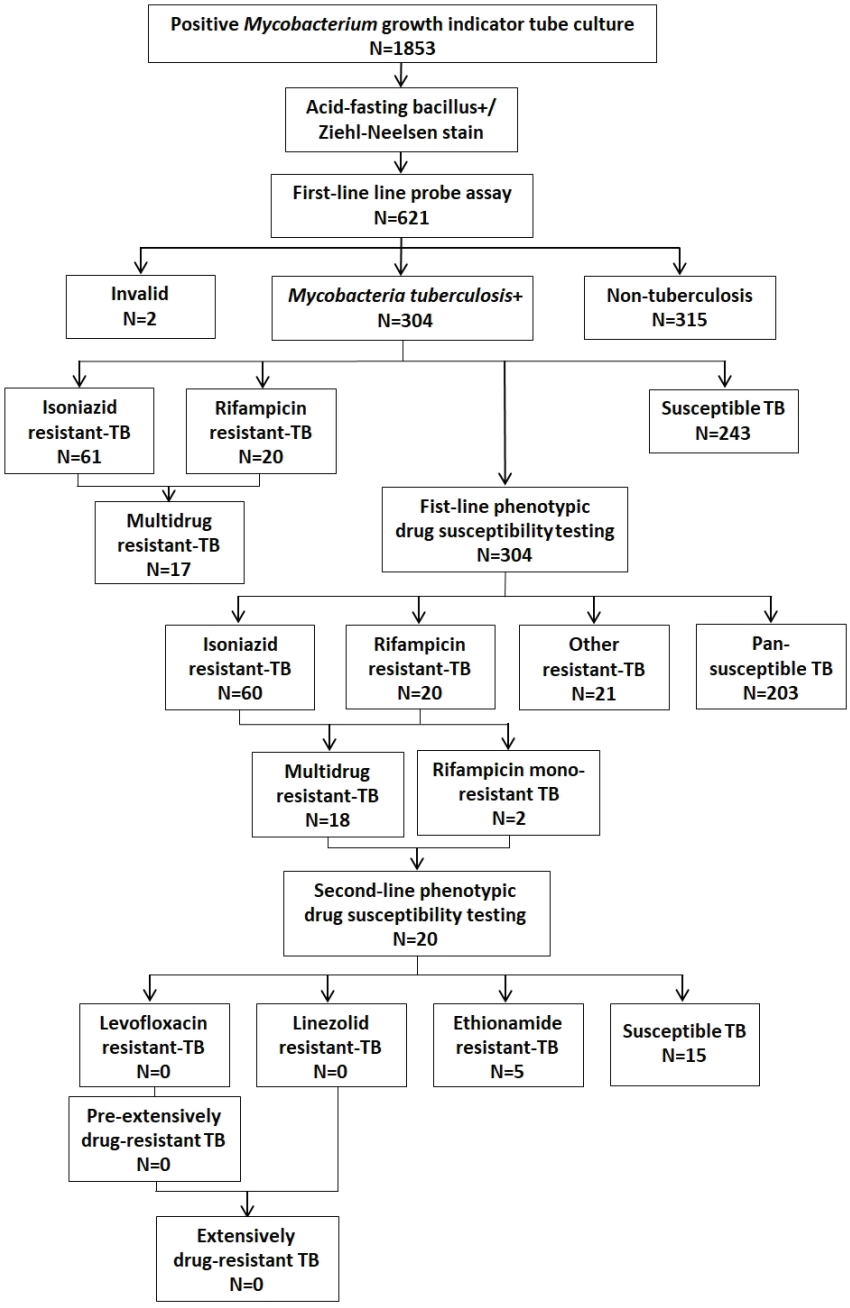

***Figure 2**. Flowchart of screening drug-resistant tuberculosis by line probe assay and phenotypic drug susceptibility testing.*

LPA was compared to the gold standard of FL-MGIT DST for the performance of detecting RR-TB, HR-TB, and MDR-TB. All high values of sensitivity at 88.9% and above; with specificity values being greater than 99%. HR-TB shows the highest values at 98.3% (95%CI, 91.1-99.9) for sensitivity, 99.2 (95%CI, 97.1-99.9) for specificity, 96.7% (95%CI, 88.7-99.6) for PPV, and 99.6 (95%CI, 99.7-99.9) for NPV. While RR-TB showed 90.0% (95%CI, 70.0-97.2) for both sensitivity and PPV; and 99.3 (95%CI, 97.5-99.8) for both specificity and NPV. MDR-TB was found at 88.9% (95%CI, 65.3-98.6), 99.6 (95%CI, 99.7-99.9), 94.1% (95%CI, 71.3-99.9, and 99.2 (95%CI, 97.1-99.9) for sensitivity, specificity, PPV, and NPV; respectively. There were excellent agreements at approximately 99% between LPA and MGIT DST with kappa values at 0.89, 0.97, and 0.91 ($p<0.001$) for RR-TB, HR-TB, and MDR-TB, respectively (Table 1).

Table 1. Performance of LPA compared with phenotypic DST for detecting TB resistance to Rifampicin, Isoniazid, and multidrug in TB.

	Anti-TB drugs	Susceptibility	First line MGIT DST		Sensitivity (95% CI)	Specificity (95% CI)	PPV% (95% CI)	NPV% (95% CI)	Agreement %	Kappa (*p* value)
			Resistant	Susceptible						
LPA	RIF	Resistant	18	2	90.0	99.3	90.0	99.3	98.7	0.89
		Susceptible	2	282	(70.0-97.2)	(97.5-99.8)	(70.0-97.2)	(97.5-99.8)		(<0.001)
	INH	Resistant	59	2	98.3	99.2	96.7	99.6	99.0	0.97
		Susceptible	1	242	(91.1-99.9)	(97.1-99.9)	(88.7-99.6)	(99.7-99.9)		(<0.001)
	RIF and INH (MDR-TB)	Resistant	16	1	88.9	99.6	94.1	99.2	98.8	0.91
		Susceptible	2	240	(65.3-98.6)	(97.7-99.9)	(71.3-99.9)	(97.1-99.9)		(<0.001)

***Note**: CI: confidence interval, DST: drug susceptibility testing, INH: isoniazid, LPA: line probe assay, MDR-TB: multidrug-resistant tuberculosis, MGIT: mycobacterium growth indicator tube, NPV: negative predictive value, PPV: positive predictive value, RIF: rifampicin.*

The mono-resistant and multidrug resistant strains were shown the banding pattern by LPA in Table 2. The frequency of inferred mutation was *rpoB* WT3 (2), *rpoB* WT4 (2), *rpoB* WT7 (4), *rpoB* WT8 (7) for RR-TB and *katG* WT (2), *inhA* WT1 (2) for HR-TB. For mono HR-TB, the mutation S315T1 associated with *katG* MUT1 of 39 for high level HR-TB was higher than mutation C15T associated with *inhA* MUT1 of 19 for low level HR-TB. While the frequency of *rpoB* mutation was D516V (5), H526Y (2), H526D (2), S531 (1) for RR-TB.

Table 2. Gene mutation pattern detected in drug resistant *Mycobacterium tuberculosis* strains by first-line line probe assay of Genotype MTBDR*plus* version 2.

Gene	Gene regions or associated mutations	Band MUT (mutation) WT (wild Type)	Rifampicin monoresistance	Isoniazid monoresistance	Multidrug resistance
rpoB					
	506-509	*rpoB* WT1			
	510-513	*rpoB* WT2			
	513-517	*rpoB* WT3			2
	516-519	*rpoB* WT4			2
	518-522	*rpoB* WT5			
	521-525	*rpoB* WT6			
	526-529	*rpoB* WT7			4
	530-533	*rpoB* WT8	2		5
	D516V	*rpoB* MUT1	1		4
	H526Y	*rpoB* MUT2A			2
	H526D	r*poB* MUT2B			2
	S531L	*rpoB* MUT3			1
katG					
	315	*katG* WT		2	
	S315T1	*katG* MUT1		28	11
	S315T2	*katG* MUT2		2	1
inhA					
	0.9375	*inhA* WT1		2	
	-8-	*inhA* WT2			
	C15T	*inhA* MUT1		13	6
	A16G	*inhA* MUT2			
	T8C	*inhA* MUT3A			
	T8A	*inhA* MUT3B			

Among 304 MTBC subjected to MGIT DST against the first-line drugs including STR, INH, RIF, and EMB; the overall drug resistance to at least one anti-TB agent was 101 (33.2%). The highest resistance proportion of STR mono-resistant-TB was 81 (26.6%) whereas 60 (19.7%) to INH, 20 (6.6%) to RIF and 1 (0.3%) to EMB. INH mono-resistant-TB was higher than RIF mono-resistant-TB at 16 (5.2%) and 2 (0.7%). There were 18 (5.9%) MDR-TB including 1 (0.3%) pan-resistance. Of 20 MDR/RR-TB yielded to SL-DST, 75% susceptible to panel of AMK, ETD, LVX and LZD were found. Only 25% ETD-resistant TB were detected from 5 MDR-TB with resistance to STR. MDR-TB was detected resistance to neither LVX nor LZD. Among 18 MDR-TB, there was neither pre-XDR TB nor XDR-TB found. (Table 3).

Table 3. Drug resistant tuberculosis detected by the first-and second-line phenotypic drug susceptibility testing.

Resistance classification	Number of detections N (%)	First-line MGIT DST (N=304)				Second-line MGIT DST (N=20)				Number of detections N (%)
		STR	INH	RIF	EMB	AMK	ETD	LVX	LZD	
Drug-resistance	101 (33.2)	81 (26.6)	60 (19.7)	20 (6.6)	1 (0.3)	0 (0)	5 (25)	0 (0)	0 (0)	
4 pan-susceptible	203 (66.8)	S	S	S	S					
Rifampicin mono-resistance	2 (0.7)	S	S	R	S	S	S	S	S	2 (10)
Isoniazid mono-resistance	16 (5.2)	S	R	S	S					
Mono-resistance, not INH/RIF	38 (12.5)	R	S	S	S					
Poly-resistance with INH resistance	27 (8.9)	R	R	S	S					
Multidrug resistance	15 (4.9)	R	R	R	S	S	S	S	S	10 (50)
		R	R	R	S	S	R	S	S	5 (25)
	2 (0.7)	S	R	R	S	S	S	S	S	2 (10)
	1 (0.3)	R	R	R	R	S	S	S	S	1 (5)
Pre-extensively resistance		S/R	R	R	S/R	S	S	R	S	0
Extensively resistance		S/R	R	R	S/R	S	S	R	R	0

***Note**: MGIT: mycobacterium growth indicator tube, DST: drug susceptibility testing, STR: streptomycin, INH: isoniazid, RIF: rifampicin, EMB: ethambutol, AMK: amikacin, ETD: ethionamide, LVX: levofloxacin, LZD: linezolid, R: resistant, S: susceptible.*

Discussion

The importance of DST for treatment was raised globally,[10] and reaffirmed the critical concentrations of isoniazid (INH) at 0.1 µg/mL, rifampicin (RIF) at 0.5 µg/mL and Levofloxacin (a fluoroquinolone) at 1.0 µg/mL for MGIT DST to reduce the rate of risk of being misclassified susceptibility of anti-TB agents .[3,16] Nevertheless, capacity of DST for MTB requires sophisticated laboratory infrastructure, proficient staff, good practice of quality assurance.

The study described the high LPA sensitivity of 90% and specificity of 99.3% for detecting RR-TB which are comparable to the previous report of Shah et al. in 2009 in Vietnam and a study in Uganda.[19] However, our findings were lower than sensitivity but higher than specificity in other studies on samples with smear positives in Uganda,[20] Peru,[21] India;[22] and from South Africa where LJ was used on the MDR-TB population;[23] Namibia [24] due to differences in their high-risk population or previously treated. This study showed higher sensitivity and specificity than studies reported by Mohamed et al. in 2020, [25] Hussain *et al*. in 2024,[26] in Ethiopia on popular including positive smear and the proportion LJ used as a gold standard.[22] Yadav et al. reported sensitivity and specificity were greater than 97% on smear-positive in 2013,[22] and compared with sequencing testing in 2021.[27] The current study described the LPA with very high sensitivity and specificity of detecting HR-TB of 98.3% and 99.2% which were higher than previous studies in South Africa,[25] India,[28] where LJ was considered the gold standard. The LPA sensitivity and specificity for MDR-TB of 88.9% and 94.1%, respectively seen similarly with studies in Peru.[21] However, Meaza et al. had a perfect sensitivity and specificity of 100% and some previous studies also showed higher values in Ethiopia,[22, 29] in India on presumptive MDR-TB size,[22] Uganda on smear positives.[20] MDR-TB (5.9%) detection was lower in Northwest Ethiopia.[29] Our study showed excellent agreements between FL-LPA and FL-MGIT DST at 98.7% (k=0.89), 99% (k=0.97), and 98.8% (k=0.91) for detecting RR-TB, HR-TB, and MDR-TB; respectively which were higher than a studies Wondale *et al*. for detecting RR-TB (k=0.49) and HR-TB (k=0.66), and lower for MDR-TB (k=1).[30]

The mono-resistant and multidrug resistant strains were shown the banding pattern by LPA. The Inferred mutation was 19 due to absence of wild type probes. The high mutations associated with *katG* MUT1 and *inhA* MUT1 were 39 and 19, respectively. For mono HR-TB, the mutation S315T1 associated with *katG* MUT1 was higher than mutation C15T associated with *inhA* MUT1. While the frequency of *inhA* mutation was D516V (5), H526Y (2), H526D (2), S531 (1). This also seen in the previous studies in high TB burden countries where DR strains transmitted continuously.[19,31]

Among 304 MTBC were subjected to MGIT DST against the first-line drugs including STR, INH, RIF, and EMB; The overall drug resistance to at least one anti-TB agent was 101 (33.2%), the highest value of resistance to

STR at 81 (26.6%) whereas 60 (19.7%) to INH, 20 (6.6%) to RIF, at least 1 (0.3%) to EMB and 5.9% for MDR-TB. INH mono-resistant-TB was higher than RIF mono-resistant-TB at 16 (5.2%) and 2 (0.7%). Our study can be comparable with previous studies in South Vietnam which found 26.3%, 19.4%, 16.6%, 2%, 1.1%, and 1.8% for resistance to at least one drug, STM, INH, RIF, EBM, and MDR in 2006;[32] and 19.8%, 3.4%, 2.5% and 3% for resistance to INH, RIF, EBM, and MDR in 2022 in Ca Mau province, Vietnam.[33] The slightly lower prevalence than their study may have come from 20 years ago when MDR-TB incident cases had not increased at the time of this study and Cho Ray located at a more crowded than Ca Mau may have detected a higher prevalence. In Ethiopia, RIF mono-resistant-TB, pan-susceptible was approximately 0.9% and 66.0% in Northwest Ethiopia, 2021.[29] Tutik *et al*. reported high proportions of DR-TB at 64.4%, 78%, and 14% for INH, RIF, and EMB while STR is the same at 13%.[34] This difference came from their study on patients diagnosed with DR-TB at entry. Their studies yielded SL-LPA, and MGIT to injectable anti-TB agents however WHO recommended drug groups for the treatment of RR/MDR-TB and all-oral regimens based on the susceptible TB and their benefit and harm.[35]

Proportions of DR-TB in a study in Ethiopia were higher than this study at 66%, 16%, and 17.9% for pan-susceptible, mono-resistance, and poly-resistance; respectively for newly diagnosed due to high incidence in this country. Nguyen et al. reported that central Vietnam (29.2%) was resistant to both antibiotics for phenotypically INH-resistant isolates at 46.3% had the Ser315Thr mutation. There were 8 different *rpo*B mutations in 22 (68.8%) of the RIF-resistant isolates with resazurin microtiter assay and polymerase chain reaction TaqMan.[17]

In this study, there were 18 (5.9%) MDR-TB including 1 (0.3%) pan-resistance. Of 20 MDR/RR-TB subjected to SC MGIT DST, only one quarter detected EDT-resistant TB while 100% were sensitive to AMK, LVX, and LZD. Neither pre-XDR-TB nor XDR-DR was detected due to LVX/LZD resistant-TB not being detected. In 2022, Wrohan *et al*. reported 88% MDR-TB, 8.2% as pre-XDR-TB, and 3.8% as XDR-TB in Ha Noi and Thanh Hoa, Northern, Vietnam performed by Xpert MTB/RIF, LPA, and DST.[11] The difference in MDR-TB detected came from the MDR-TB treated population and the old definition of pre-XDR-TB, XDR-TB used in the 2014 to 2016 period of study while our study conducted on presumptive TB and resistance classification was in line with WHO updated definitions from 2021. Minsk and Copenhagen had higher rates at 26.7% and 10%, 16.7% and 30.0%, 16.7% and 13.3% for MDR-TB, pre-XDR-TB, and XDR-TB detected among Belarusian HIV-positive patients.[36] However, the updated definition of pre-XDR-TB, and XDR-TB requires more studies of drugs for the TB-treating group A and B.[18]

Limitation

In this study, indirect LPA was only performed from inoculum culture of sputum, direct LPA test had not been studied from sputum sediment. Moreover, some DST discordant samples between LPA and MGIT should be detected by target next-generation sequencing. This study has not screened on previously treated TB patients yet.

Conclusion

Based on the gold standard of updated phenotypic DST, FL-LPA performance was compared in detecting RR-TB, HR-TB, and MDR-TB perfectly. Pre-XDR-TB and XDR-TB had not been found. We would keep practicing phenotypic culture-based DST with a diversity of recommended anti-TB agents and reaffirmed critical concentrations of INH, RIF and LVX of INH, RIF in the first line and LFV in the second line in screen DR-TB.

Ethical approval

This study was approved by the Ethics Committee in Biomedical Research of Cho Ray Hospital, Vietnam (No. 34-25/CN-HDDD).

Funding

None

Conflict of interest

None

CRedit authorship contribution statement

Phu Thien Truong: conceptualization, editing, validation; **Tran Ngoc Minh Le**: methodology, writing-original draft preparation; **Van Thi Hue Tran**: visualization, investigation, supervision; **Tung Thanh Phan**: data curation and software.

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