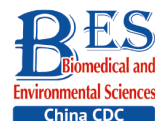


Original Article



Evaluation of Multidrug Resistant Loop-mediated Isothermal Amplification Assay for Detecting the Drug Resistance of *Mycobacterium tuberculosis**

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Abstract

Objective To evaluate multidrug resistant loop-mediated isothermal amplification (MDR-LAMP) assay for the early diagnosis of multidrug-resistant tuberculosis and to compare the mutation patterns associated with the *rpoB*, *katG*, and *inhA* genes at the Chinese Center for Disease Control and Prevention.

Methods MDR-LAMP assay was evaluated using 100 *Mycobacterium tuberculosis* (*Mtb*) isolates obtained from the National Reference Laboratory for Tuberculosis in China. Phenotypic resistance to isoniazid and rifampicin and whole-genome sequencing served as reference standards.

Results The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of MDR-LAMP were 85.5%, 93.6%, 96.7%, and 74.4% for the detection of resistance to isoniazid and rifampicin, respectively, and 80.5%, 92.3%, 98.6%, and 41.4% for the detection of *Mtb* cultured from smear-positive sputum samples, respectively. When DNA sequencing was used as the reference standard, the sensitivity, specificity, PPV, and NPV of MDR-LAMP were 93.1%, 92.3%, 97.2%, and 82.8% for the detection of *katG* and *inhA* gene mutations, respectively, and 89.1%, 88.9%, 93.4%, and 81.1% for the detection of *rpoB* gene mutation, respectively.

Conclusion MDR-LAMP is a rapid and accessible assay for the laboratory identification of rifampicin and isoniazid resistance of *Mtb* isolates.

Key words: MDR-LAMP; *Mycobacterium tuberculosis*; Diagnostic method

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INTRODUCTION

Drug-resistant tuberculosis is a major public health problem that poses a threat to global tuberculosis control programs.

The emergence of multidrug-resistant tuberculosis (MDR-TB) has severely hampered control because it is resistant to the two key drugs, isoniazid (INH) and rifampicin (RIF), in tuberculosis treatment. In the 2019 World Health Organization (WHO) Global

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Tuberculosis Report, 10 million new cases of tuberculosis were recorded worldwide in 2018, and the incidence of MDR-TB was approximately 3.4% of new cases and 18% of previously treated cases. In 2018, the number of patients with MDR-TB in the world was approximately 484,000, resulting in 214,000 deaths^[1]. Although the proportion of MDR-TB is lower in the total number of tuberculosis cases, the response to anti-tuberculosis treatment in clinical practice is poor. MDR is difficult to cure, causing grievous harm to patients and increasing transmission.

Definitive diagnosis of MDR-TB by conventional culture followed by drug susceptibility testing (DST) based on phenotypic tests is time-consuming and tedious. Rapid and accurate diagnosis followed by prompt treatment and completed therapy is essential for the effective control of MDR-TB. The conventional DST cannot provide the appropriate and timely diagnosis required for proper patient management. During the past decades, efficient molecular detection techniques that have been used for DST include Xpert MTB/RIF, GenoType MTBDRplus, and Abbott RealTime MTB RIF/INH Resistance^[2-4]. Loop-mediated isothermal amplification (LAMP), which was first reported in 2004, is a nucleic acid amplification technology used to detect *Mycobacterium tuberculosis* (*Mtb*)^[5,6]. MDR-LAMP assay is a new manual mutation site detection method based on the LAMP platform from Eiken Chemical Company in Japan. The probes (MDR-LAMP) detect mutations associated with the *rpoB* gene for RIF resistance, *katG* genes for high-level INH resistance, and the *inhA* regulatory region gene for low-level INH resistance.

This study aimed to evaluate the mutation detection accuracy of MDR-LAMP in the National Reference Laboratory for Tuberculosis in China.

METHODS

Mycobacterial Strain Culture

Mycobacterial strains were obtained from the National Reference Laboratory for Tuberculosis in China. We selected 65 MDR isolates, 22 INH mono-resistant isolates, 4 RIF mono-resistant isolates, and 9 INH- and RIF-sensitive isolates on the basis of the drug sensitivity test results. A total of 100 strains were re-cultured on Löwenstein-Jensen medium and incubated in 37 °C for 3 weeks.

DNA Extraction

For each sample, one loop of cultures was

suspended in 400 µL of TE buffer, boiled at 95 °C for 30 min, and then placed in an ice bath for 5 min. After cooling, the suspension was sonicated for 5 min and then centrifuged at 12,000 ×g for 5 min. Finally, 200 µL of the supernatant containing DNA was transferred into a new microcentrifuge tube.

Mycobacterium Species Identification by MALDI-TOF-MS

Measurements were performed with a Microflex LT (Bruker Daltonics) mass spectrometer laser. Spectra were recorded in the positive linear mode [delay: 170 ns; ion source 1 (IS1) voltage: 20 kV; ion source 2 (IS2) voltage: 16.65 kV; lens voltage: 7.20 kV; mass range: 2–20 kD]. Each spectrum was obtained after 6 × 200 shots (1200 shots) in automatic mode at a variable laser power, and the acquisition time ranged from 60 s to 120 s per spot. All signals with resolution ≥ 400 were automatically acquired using MALDI Biotyper RTC in FlexControl software version 3.4, and the identifications were obtained by MALDI Biotyper OC version 4.0 with the Mycobacteria Library v3.0 (2017) database, which contains 987 main spectrum profiles representing 173 species. An identification score was given using Biotyper software version 4.0 (Bruker Daltonics).

Drug Susceptibility Test by Broth Microdilution MIC

DSTs were performed at the National Reference Laboratory for Tuberculosis in China by using the broth microdilution MIC method. A panel of 13 drugs (including INH and RPF) using the Sensititre® MYCOTBI plates for *Mtb* (TREK Diagnostic Systems, Cleveland, USA) was used in the drug resistance test. During the experiments, 0.5 McFarland suspensions of bacteria were prepared by Ultrasonic Milling Instrument (TB Healthcare, China) from colonies grown on an L-J culture medium. Suspensions were diluted 100-fold with the addition of 100 µL of the 0.5 Mc suspensions to 10 mL of Mueller-Hinton broth with/without OADC. Aliquots of 100 µL of the standard 1.5×10^5 CFU/mL inoculum were distributed to each well by using the semi-automated Sensititre™ Auto-inoculator (Thermo Fisher, Scientific Inc., USA). The plates were sealed with the adhesive membranes accurately and adequately and incubated at the recommended temperature to prevent evaporation and skipped wells during incubation. MIC is defined as the lowest concentration without obvious visible bacterial growth compared with positive controls, which was measured by two readers, aided by Vizion™ Digital viewing system. *Mtb* H37Rv (ATCC 27294) was used

as quality control in every batch of drug susceptibility testing. The breakpoints based on CLSI M62 to INH and RPF were 0.25 and 2.

MDR-LAMP Assay

MDR-LAMP assay was performed using a detection kit (supplied by Eiken, Japan). The extracted DNA samples were denatured at 90 °C for 5 min, and 5 µL was added to each reaction tube that dispensed Reagent A, B, or C. When the measurement with the LAMP reaction step, denaturing step, and thermal dissociation step was finished, results were checked as follows: Reagent A: FAM curve: Presence of peaks over 50 °C indicates that the sample has a mutation on the *katG* legion (S315T, S315N); presence of peaks under 50 °C indicates that the sample has a mutation on the *inhA* Promotor-15. Reagent B: FAM curve: The presence of some peaks indicates that the sample has a mutation on the *rpoB* legion (S531L or L533P); Cy5 curve: Presence of some peaks indicates that the sample has a mutation on the *rpoB* legion (D516V or S531L). Reagent C: FAM curve: The presence of some peaks indicates that the sample has a mutation on the *rpoB* legion (H526Y or H526D). Other conditions were used to check correct amplification.

WGS for *katG*, *inhA*, and *rpoB* Mutation Identification

Genomic DNA of the INH and/or RIF-resistant mutants was subjected to WGS using the Illumina HiSeq 2500 system with the paired-end strategy as described previously^[7]. A previously validated pipeline was used for mapping the short sequencing reads to the reference genome. Mutations were called when they were present in more than two reads in the forward and reverse directions, with *Mtb* H37Rv strain (NC_000962.3) as a reference. Mutations in genes of the proline–glutamic acid (PE)/proline–proline–glutamic acid (PPE) family and in regions with repetitive sequences were excluded from the analysis.

Statistical Analysis

Data were analyzed using VassarStats (<http://vassarstats.net/index.html>), a website for statistical computation. Sensitivity, specificity, and positive and negative predictive values (PPV and NPV) were calculated of MDR-LAMP. The percentages of specificity and sensitivity were calculated using the formulae shown below: Sensitivity = true positives / (true positives + false

negatives) × 100 and Specificity = true negatives / (true negatives + false positives) × 100. The PPV was calculated by true positives × 100 / (true positives + false positives). The NPV was calculated by true negatives × 100 / (true negatives + false negatives). The concordance between the two tests was calculated using χ^2 and McNemar tests and expressed as a *P* value.

RESULTS

Phenotypic DST was performed on 100 *Mtb* strains using the CLSI-recommended broth microdilution MIC method. The phenotypic DST results of the 100 *Mtb* isolates selected in this study are listed in Supplementary Table S1, available in www.besjournal.com. Of the 100 *Mtb* isolates, 65 (65%) were MDR strains, 22 (22%) were mono-INH-resistant strains, 4 (4%) were mono-RIF-resistant strains, and 9 (9%) were INH- and rifampin-sensitive strains. The results for each probe detected by MDR-LAMP are shown in Figure 1. The performance of MDR-LAMP is summarized in Table 1. The sensitivity and specificity of MDR-LAMP reagents for detecting RIF resistance were 85.5% and 93.5%, respectively. The PPV and NPV were 96.7% and 74.4%, respectively. The sensitivity and specificity of detecting INH resistance were 80.5% and 92.3%, respectively, and the PPV and NPV were 98.6% and 41.4%, respectively. Considering that the *P* values are both lesser than 0.01, we investigated the reason why the coverage of the resistant genes tested or the accuracy of the MDR-LAMP reagents affected the consistency between MDR-LAMP and phenotypic DST results. As shown in Table 2, the sensitivity of the MDR-LAMP detection of INH (*katG* and *inhA*) resistance gene mutations was 93.2%, and the specificity was 92.3%. The PPV and NPV were 97.2% and 82.8% compared with whole-genome sequencing (WGS). The sensitivity and specificity of RIF (*rpoB*) resistance gene mutations were 89.1% and 88.9%, respectively, and the PPV and NPV were 93.4% and 82.1%, respectively. The results showed that the accuracy of the MDR-LAMP reagents to detect *rpoB* gene mutations must improve. We calculated the detection sensitivity, specificity, PPV, and NPV of each probe compared with the mutation results obtained by WGS analysis to evaluate the efficiency of each probe for detecting *rpoB*. The consistency rate of each probe for detecting *rpoB* S531L/L533P and *rpoB* H526Y/H526D mutations presented a significant

difference to WGS ($P < 0.05$). The specificity of the probe for detecting *rpoB* H526Y/H526D mutation was 93.7%, whereas that of the other probes was 100% (Table 3). As shown in Table 4, all the 11 *rpoB* S531L mutations detected by the FAM filter in *rpoB* S531L mutations were detected by the Cy5 filter,

whereas the sensitivity of the probe *rpoB* S531L/L533P was 29.7%. In conclusion, the number of resistance-related mutation sites should be increased in the MDR-LAMP assay to cover the phenotypic resistance of INH and RIF. In addition, the probes for detecting gene mutations should be

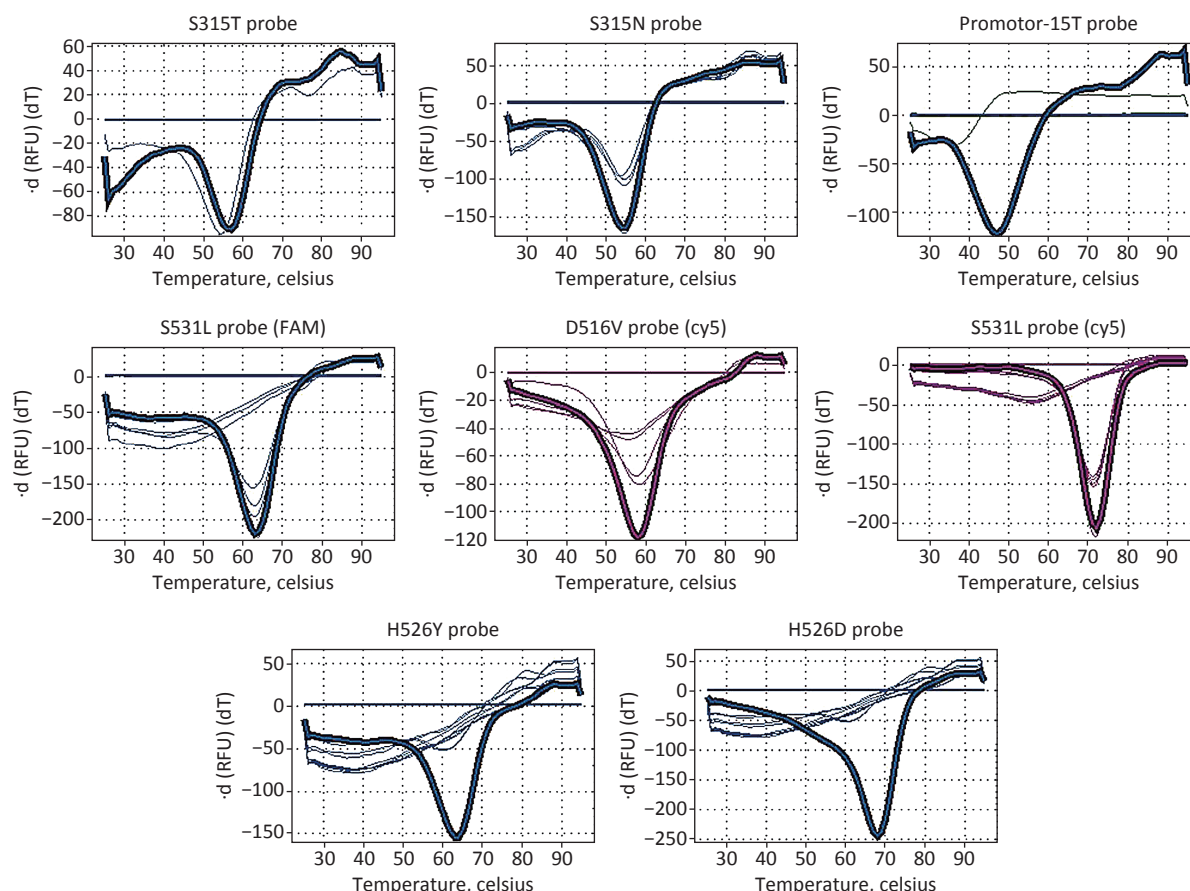


Figure 1. Degree Celsius distribution of each probe by MDR-LAMP reagents.

Table 1. Efficacy of MDR-LAMP test compared with the broth microdilution MIC method

Drugs	MIC	MDR-LAMP		Sub-total	Sensitivity (95% CI) ^a	Specificity (95% CI) ^a	PPV (95% CI) ^a	NPV (95% CI) ^a	χ^2 ^b	P^c
		Resistance	Sensitive							
RIF	Resistance	59	10	69	85.5% (74.5–92.5)	93.6% (77.2–98.9)	96.7% (87.6–99.4)	74.4% (57.6–86.4)	10.73 ^d	0.039
	Sensitive	2	29	31						
	Sub-total	61	39	100						
INH	Resistance	70	17	87	80.5% (70.3–87.9)	92.3% (62.1–99.6)	98.6% (91.4–99.9)	41.4% (24.1–60.9)	17.64 ^e	< 0.01
	Sensitive	1	12	13						
	Sub-total	71	29	100						

Note. ^aWilson 95% confidence interval: including continuity correction; ^b $\nu = 1$ the chi-square value reported is the Yates chi-square, corrected for continuity (^d $\nu = 1$, $P = 0.0011$, ^e $\nu = 1$, $P < 0.0001$); ^cMcNemar's test, two-tail.

optimized to improve accuracy, especially for the *rpoB* gene.

DISCUSSION

In our study, we evaluated an in vitro LAMP assay for the detection of RIF and INH resistance-associated mutations in *Mtb* complex samples. A nucleic acid amplification method relies on auto-

cycling strand displacement DNA synthesis^[5,8,9]. The targets are diverse and include the *rpoB*, *katG*, and *inhA* promoter genes. Compared with the phenotypic method, which is the gold standard for *Mtb* drug susceptibility testing, we obtained sensitivity and specificity of 85.5% and 93.5% for RIF resistance, respectively, and 80.5% and 92.3% for INH resistance, respectively. Compared with WGS, which has been proposed as the ultimate molecular

Table 2. Comparison of gene mutation identified by WGS and MDR-LAMP assay

Drugs (genes)	WGS	MDR-LAMP		Sub-total	Sensitivity (95% CI) ^a	Specificity (95% CI) ^a	PPV (95% CI) ^a	NPV (95% CI) ^a	χ^2 ^b	P ^c
		Mutation	Wild							
INH (<i>katG</i> , <i>inhA</i>)	Mutation	69	5	74	93.2% (84.3–97.5)	92.3% (73.4–98.7)	97.2% (89.3–99.5)	82.8% (63.5–93.5)	5.33 ^d	0.453
	Wild	2	24	26						
	Sub-total	71	29	100						
RIF (<i>rpoB</i>)	Mutation	57	7	64	89.1% (78.2–95.1)	88.9% (73.0–96.4)	93.4% (83.3–97.9)	82.1% (65.9–91.9)	9.62 ^e	0.549
	Wild	4	32	36						
	Sub-total	61	39	100						

Note. ^aWilson 95% confidence interval: including continuity correction; ^b $\nu = 1$ the chi-square value reported is the Yates chi-square, corrected for continuity (^d $\nu = 1$, $P = 0.021$, ^e $\nu = 1$, $P = 0.0019$); ^cMcNemar's test, two-tail.

Table 3. Evolution efficiency of each probe of MDR-LAMP

Reagent	Probes	WGS	MDR-LAMP		Sub-total	Sensitivity (95% CI) ^a	Specificity (95% CI) ^a	PPV (95% CI) ^a	NPV (95% CI) ^a	χ^2 ^b	P ^c
			Mutation	Wild							
Reagent B	FAM: <i>rpoB</i> S531L/L533P	Mutation	11	26	37	29.7% (16.4–47.2)	100% (92.8–100)	100% (67.9–100)	70.8% (60.1–79.7)	27.6 ^d	< 0.01
		Wild	0	63	63						
		Sub-total	11	89	100						
Reagent B	Cy5: <i>rpoB</i> D516V/S531L	Mutation	37	5	42	88.1% (73.6–95.5)	100% (92.3–100)	100% (88.3–100)	92.1% (81.7–97.0)	3.28 ^e	0.063
		Wild	0	58	58						
		Sub-total	37	63	100						
Reagent C	FAM: <i>rpoB</i> H526Y/ H526D	Mutation	20	1	21	95.2% (74.1–99.8)	93.7% (85.2–97.7)	80% (58.7–92.4)	98.7% (91.8–99.9)	4.3 ^f	0.219
		Wild	5	74	79						
		Sub-total	25	75	100						
Reagent A	FAM: <i>katG</i> S315N/S315T	Mutation	68	3	71	95.8% (87.3–98.9)	100% (85.4–100)	100% (93.3–100)	90.6% (73.8–97.6)	1.35 ^g	0.25
		Wild	0	29	29						
		Sub-total	68	32	100						
Reagent A	FAM: <i>inhA Promotor-15</i>	Mutation	1	2	3	33.3% (1.8–87.5)	100% (95.3–100)	100% (5.5–100)	98% (92.2–99.7)	0.51 ^h	0.5
		Wild	0	97	97						
		Sub-total	1	99	100						

Note. ^aWilson 95% confidence interval: including continuity correction; ^b $\nu = 1$ the chi-square value reported is the Yates chi-square, corrected for continuity (^d $\nu = 1$, $P < 0.0001$, ^e $\nu = 1$, $P = 0.0701$, ^f $\nu = 1$, $P = 0.0381$, ^g $\nu = 1$, $P = 0.2453$, ^h $\nu = 1$, $P = 0.4751$); ^cMcNemar's test, two-tail.

diagnostic test for detecting all molecular determinants for resistance^[10-12], sensitivity and specificity of 92.2% and 94.4% for RIF resistance gene mutations, respectively, and 95.9% and 100% for INH resistance gene mutations, respectively, were reported.

Drug-resistant tuberculosis is a permanent threat to the global control of the disease. The emergence of MDR-TB is increasing in some regions of the world. During the past decades, the methods currently available are based on the evidence that resistance-associated mutations occur in specific regions of the genes whose products are targeted by these antibiotics^[13]. MDR-LAMP assay is a molecular diagnostic test that detects INH and RIF resistance-associated mutations. The most frequent INH resistance-conferring mutations have been indicated in the *katG* and *inhA* gene regions^[14]. The *katG* S315I, S315N, S315T, and combined frameshifts and premature stop codons are high-confidence markers of INH resistance, and the *inhA* promoter-15 mutation is a moderate-confidence resistance mutation^[15]. The mutations in an 81-base pair region of the *rpoB* gene are most commonly associated with RIF resistance^[16]. The *rpoB* D516V, H526D, H526Y, and S531L are high-confidence markers, while L533P is a moderate-confidence marker of RIF resistance^[10,17]. MDR-LAMP assay covers the overlapping RIF resistance determining region of the *rpoB*, *katG*, and *inhA* genes and above mutations of the *rpoB* gene, mutations at codon 315 of the *katG* gene, and mutations in the promoter-15 of the *inhA* gene.

The TB-LAMP method was endorsed by the WHO in 2016 for the diagnosis of pulmonary tuberculosis

in adults, which has been a primary diagnostic test for pulmonary tuberculosis in low-resource settings^[13]. MDR-LAMP assay gains similar sensitivity and specificity to other genotypic assays used to screen drug resistance-associated mutations in *Mtb*^[18,19]. In conclusion, MDR-LAMP assay is a simple, rapid, specific, and cost-effective method that needs less sophisticated infrastructures and minimal biosafety requirements. The number of resistance-related mutation sites should be increased to cover the phenotypic resistance of INH and RIF and improve the accuracy of the MDR-LAMP assay. In addition, the probes for detecting *rpoB* should be optimized to improve accuracy.

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DECLARATION OF INTERESTS

The authors declare no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR STATEMENT

SONG Yi Meng: conceptualization, methodology, software. LIU Chun Fa: data curation, writing-original draft preparation. HE Ping: visualization, investigation. HE Wen Cong: supervision. LIU Dong Xin: software, validation. LI Yan Ming: supervision. ZHAO Yan Lin: writing-reviewing and editing.

Table 4. Degree Celsius distribution of each probe by MDR-LAMP reagents

Drugs (genes)	Mutation site	Number of strains	Predict degree Celsius	Actual degree Celsius (Number)
INH (<i>katG</i> , <i>inhA</i>)	<i>katG</i> S315T	68	FAM:56 °C	54 °C (25), 55 °C (32), 56 °C (9), -(2)
	<i>katG</i> S315N	3	FAM:54 °C	54 °C (2), -(1)
	<i>inhA</i> Promotor-15T	3	FAM:46 °C	46 °C (2), 48 °C (1)
RIF (<i>rpoB</i>)	<i>rpoB</i> S531L	36	FAM:63 °C and / or Cy5:73 °C	FAM:62 °C and Cy5:71 °C (2), FAM:62 °C and Cy5:72 °C (4), FAM:63 °C and Cy5:72 °C (5), Cy5:67 °C (1), Cy5:71 °C (5), Cy5:72 °C (14), Cy5:73 °C (1), -(4)
	<i>rpoB</i> D516V	6	Cy5:61 °C	60 °C (5), -(1)
	<i>rpoB</i> L533P	0	-	-
	<i>rpoB</i> H526Y	17	FAM:67 °C	62 °C (3), 64 °C (2), 65 °C (8), 66 °C (3), -(1)
	<i>rpoB</i> H526D	4	FAM: 69 °C	67 °C (3), 68 °C (1)

Note. “-” indicates “not detected.”

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Supplementary Table S1. The phenotypic DST results of the 100 Mtb isolates

Strain No.	LAMP MDR assay															
	MIC Cutoff-value		DST (S:Sensitivity, R:Resistance)		WGS Gene: (W:Wild, M:Mutant)					LAMP MDR (+:Detect, -:Not Detect)						
	INH: > 0.2	RIF: > 1	INH	RIF	rpoB	site	katG	site	inhA-Promotor-15	Reagent A	T	Reagent B	T	Reagent C	T	
031024	2	> 16	R	R	M	S531L	M	S315T	W	+	55 °C	+	Cy5:73 °C	-		
033059	4	> 16	R	R	W	-	W	-	M	-		-		+	64 °C	
033061	2	> 16	R	R	M	H526Y	M	S315T	W	+	54 °C	-		+	65 °C	
033062	2	> 16	R	R	M	H526Y	M	S315T	W	+	54 °C	-		+	65 °C	
033063	2	> 16	R	R	M	H526Y	M	S315T	W	+	56 °C	-		+	65 °C	
033064	2	> 16	R	R	M	H526Y	M	S315T	W	+	56 °C	-		+	65 °C	
033065	2	> 16	R	R	M	H526Y	M	S315T	W	+	54 °C	-		+	62 °C	
033066	2	> 16	R	R	M	H526Y	M	S315T	W	+	54 °C	-		+	65 °C	
033067	2	> 16	R	R	M	H526Y	M	S315T	W	+	54 °C	-		+	65 °C	
033068	2	> 16	R	R	M	H526Y	M	S315T	W	+	54 °C	-		+	65 °C	
033070	2	> 16	R	R	M	H526Y	M	S315T	W	+	54 °C	-		+	65 °C	
033071	2	> 16	R	R	M	H526Y	M	S315T	W	+	54 °C	-		+	64 °C	
033102	1	> 16	R	R	M	S531L	W	-	W	-		+	Cy5:71 °C	-		
033106	1	> 16	R	R	M	S531L	W	-	W	-		+	Cy5:72 °C	-		
033109	1	> 16	R	R	M	S531L	W	-	W	-		+	Cy5:72 °C	-		
033127	2	> 16	R	R	M	S531L	M	S315T	W	+	55 °C	-		+	62 °C	
033129	1	> 16	R	R	M	H526Y	M	S315T	W	+	55 °C	-		+	66 °C	
033159	4	> 16	R	R	M	S531L	M	S315T	W	+	55 °C	+	Cy5:72 °C	-		
033214	4	> 16	R	R	M	S531L	M	S315T	W	+	55 °C	+	Cy5:72 °C	-		
061021	0.5	> 16	R	R	M	S531L	W	-	W	-		+	Cy5:71 °C	-		
061026	4	> 16	R	R	W	-	M	S315T	W	+	54 °C	-		+	61 °C	
061045	4	≤ 0.12	R	S	W	-	M	S315T	W	+	54 °C	-		-		
061046	4	> 16	R	R	W	-	M	S315T	W	+	54 °C	-		-		
061102	0.06	> 16	S	R	M	H526Y	W	-	W	-		-		+	62 °C	
061105	4	≤ 0.12	R	S	W	-	M	S315T	W	+	55 °C	-		-		
061260	0.06	> 16	S	R	M	S531L	W	-	W	-		-		+	68 °C	
061300	4	≤ 0.12	R	S	W	-	M	S315T	W	+	55 °C	-		-		
061301	4	≤ 0.12	R	S	W	-	M	S315T	W	+	55 °C	-		-		
061305	4	> 16	R	R	W	-	M	S315T	W	+	55 °C	-		-		
061315	> 4	1	R	S	W	-	W	-	M	-		-		-		
071014	4	16	R	R	M	S531L	M	S315T	W	-		-		-		
071023	4	0.12	R	S	W	-	M	S315T	W	+	55 °C	-		-		
071028	4	0.5	R	S	W	-	M	S315T	W	+	55 °C	-		-		
071031	4	0.25	R	S	W	-	M	S315T	W	+	55 °C	-		-		
071040	2	16	R	R	M	H526Y	M	S315T	W	+	54 °C	-		+	62 °C	
071061	4	16	R	R	M	S531L	M	S315T	W	+	54 °C	+	FAM:63 °C, Cy5:72 °C	+	64 °C	

Continued

Strain No.	LAMP MDR assay														
	MIC Cutoff-value		DST (S:Sensitivity, R:Resistance)		WGS Gene: (W:Wild, M:Mutant)					LAMP MDR (+:Detect, -:Not Detect)					
	INH: > 0.2	RIF: > 1	INH	RIF	rpoB	site	katG	site	inhA-Promotor-15	Reagent A	T	Reagent B	T	Reagent C	T
071070	2	16	R	R	M	H526D	W	-	W	-	-	-	-	+	67 °C
071075	4	0.12	R	S	W	-	M	S315T	W	+	55 °C	-	-	-	-
071097	4	16	R	R	W	-	M	S315T	W	+	54 °C	-	-	-	-
071107	4	16	R	R	W	-	M	S315T	W	+	55 °C	-	-	-	-
071121	4	0.12	R	S	W	-	M	S315T	W	+	55 °C	-	-	-	-
071126	4	0.12	R	S	W	-	M	S315T	W	+	55 °C	-	-	-	-
071127	4	16	R	R	W	-	M	S315T	W	+	55 °C	-	-	-	-
071140	4	1	R	S	W	-	M	S315T	W	+	55 °C	-	-	-	-
081005	2	> 16	R	R	M	H526D	M	S315T	W	+	54 °C	-	-	+	68 °C
081044	2	> 16	R	R	M	S531L	M	S315T	W	+	54 °C	+	FAM:63 °C, Cy5:72 °C	-	-
081051	2	> 16	R	R	M	S531L	M	S315T	W	+	54 °C	+	FAM:63 °C, Cy5:72 °C	-	-
081056	2	> 16	R	R	M	S531L	M	S315T	W	+	54 °C	+	FAM:63 °C, Cy5:72 °C	-	-
081067	4	> 16	R	R	M	S531L	M	S315T	W	+	54 °C	+	FAM:63 °C, Cy5:72 °C	-	-
081161	0.06	> 16	S	R	M	S531L	W	-	W	-	-	+	Cy5:72 °C	-	-
081217	0.12	> 16	S	R	M	S531L	W	-	W	-	-	+	Cy5:71 °C	-	-
082248	4	16	R	R	M	S531L	M	S315T	W	+	54 °C	+	Cy5:71 °C	-	-
082308	0.25	> 16	R	R	M	S531L	W	-	W	-	-	+	Cy5:72 °C	-	-
082312	4	> 16	R	R	M	L533P	W	-	W	+	48 °C	-	-	-	-
091079	4	0.25	R	S	W	-	M	S315N	W	+	54 °C	-	-	-	-
091104	4	0.25	R	S	W	-	M	S315T	W	+	55 °C	-	-	-	-
091119	4	0.25	R	S	W	-	M	S315T	W	+	55 °C	-	-	-	-
102238	4	16	R	R	M	S531L	M	S315T	W	+	54 °C	+	FAM:62 °C, Cy5:72 °C	-	-
104186	0.03	0.12	S	S	W	-	W	-	W	-	-	-	-	-	-
104191	0.03	0.25	S	S	W	-	W	-	W	-	-	-	-	-	-
104200	0.06	0.5	S	S	W	-	W	-	W	-	-	-	-	-	-
104218	0.03	0.12	S	S	W	-	W	-	W	-	-	-	-	-	-
104229	0.03	0.12	S	S	W	-	W	-	W	-	-	-	-	-	-
151022	2	16	R	R	M	H526D	M	S315T	W	+	54 °C	-	-	+	67 °C
153058	4	16	R	R	M	H526Y	M	S315T	W	+	55 °C	-	-	-	-
154089	2	16	R	R	M	H526Y	M	S315T	W	+	54 °C	-	-	+	66 °C
154214	4	0.25	R	S	W	-	M	S315T	W	+	55 °C	-	-	-	-
154285	4	0.12	R	S	W	-	M	S315T	W	+	55 °C	-	-	-	-
154286	2	16	R	R	M	H526Y	M	S315T	W	+	55 °C	-	-	+	66 °C
171019	2	16	R	R	M	D516V	W	-	W	-	-	+	Cy5:60 °C	-	-
171026	0.5	> 16	R	R	M	S531L	W	-	W	-	-	+	FAM:62 °C, Cy5:72 °C	-	-

Continued

Strain No.	LAMP MDR assay														
	MIC Cutoff-value		DST (S:Sensitivity, R:Resistance)		WGS Gene: (W:Wild, M:Mutant)					LAMP MDR (+:Detect, -:Not Detect)					
	INH: > 0.2	RIF: > 1	INH	RIF	rpoB	site	katG	site	inhA-Promotor-15	Reagent A	T	Reagent B	T	Reagent C	T
171052	> 4	> 16	R	R	M	S531L	W	-	W	-		+	FAM:62 °C, Cy5:72 °C	-	
171064	> 4	> 16	R	R	M	H526D	M	S315T	W	+	54 °C	-		+	67 °C
171078	> 4	16	R	R	M	D516V	M	S315T	W	+	54 °C	-		-	
171079	> 4	16	R	R	M	D516V	W	-	W	-		+	Cy5:60 °C	-	
171292	2	> 16	R	R	M	H526Y	M	S315T	W	+	56 °C	-		+	64 °C
171607	4	> 16	R	R	M	S531L	M	S315T	W	+	56 °C	+	Cy5:72 °C	-	
172096	0.06	≤ 0.12	S	S	M	S531L	M	S315T	W	+	56 °C	+	Cy5:72 °C	-	
172300	4	> 16	R	R	M	D516V	M	S315T	W	+	56 °C	+	Cy5:60 °C	-	
172314	> 4	> 16	R	R	M	S531L	M	S315T	W	+	56 °C	+	Cy5:72 °C	-	
172573	2	> 16	R	R	M	S531L	M	S315N	W	+	54 °C	+	Cy5:72 °C	-	
173001	0.25	> 16	R	R	M	S531L	W	-	W	+	46 °C	+	FAM:62 °C, Cy5:71 °C	-	
173093	> 4	> 16	R	R	M	S531L	W	-	W	-		+	FAM:62 °C, Cy5:72 °C	-	
173422	0.5	≤ 0.12	R	S	W	-	W	-	W	-		-		-	
174067	0.06	0.25	S	S	W	-	M	S315T	W	-		-		-	
184403	4	16	R	R	M	S531L	M	S315T	W	+	55 °C	+	Cy5:71 °C	-	
185209	4	0.5	R	S	W	-	M	S315T	W	+	55 °C	-		-	
185544	4	16	R	R	M	S531L	M	S315T	W	+	56 °C	+	Cy5:67 °C	-	
185551	4	0.25	R	S	W	-	M	S315T	W	+	55 °C	-		-	
19200003	4	0.25	R	S	W	-	M	S315T	W	+	55 °C	-		-	
19200399	4	16	R	R	M	S531L	M	S315T	W	+	55 °C	+	Cy5:72 °C	-	
221211	2	1	R	S	M	D516V	M	S315N	W	-		+	Cy5:60 °C	-	
221227	0.25	16	R	R	M	S531L	W	-	W	-		+	FAM:62 °C, Cy5:71 °C	-	
221437	2	16	R	R	M	S531L	M	S315T	W	+	56 °C	+	Cy5:72 °C	-	
221518	4	16	R	R	M	S531L	W	-	M	+	48 °C	+	Cy5:72 °C	-	
221571	4	16	R	R	M	S531L	M	S315T	W	+	55 °C	+	Cy5:72 °C	-	
222280	4	16	R	R	M	D516V	M	S315T	W	+	55 °C	+	Cy5:60 °C	-	
233077	0.06	0.25	S	S	W	-	W	-	W	-		-		-	
235254	0.06	0.12	S	S	W	-	W	-	W	-		-		-	
320005	2	2	R	R	M	S531L	M	S315T	W	+	55 °C	-		-	