

# Diagnostic Plausibility of MTBDRplus and MTBDRsl Line Probe Assays for Rapid Drug Susceptibility Testing of Drug Resistant *Mycobacterium tuberculosis* Strains in Pakistan

Mona Javaid,<sup>1</sup> Altaf Ahmed,<sup>1\*</sup> Sunil Asif,<sup>1</sup> and Afsheen Raza<sup>1</sup>

<sup>1</sup>The Indus Hospital, Karachi, Pakistan

\*Corresponding author: Altaf Ahmed, The Indus Hospital, Korangi Crossing, Karachi, Pakistan. Tel: +92-2135112709, Fax: +92-2135112718, E-mail: altaf.ahmed@tih.org.pk

Received 2015 December 08; Revised 2015 December 27; Accepted 2015 December 27.

## Abstract

**Background:** World health organization (WHO) recommends the use of line probe assays (LiPAs) for rapid drug susceptibility testing (DST). However, only a limited number of studies from Pakistan have documented the performance characteristics of line probe assays in testing multi-drug resistant (MDR) strains of *Mycobacterium tuberculosis* (MTB).

**Objectives:** The objective of this work is to evaluate the diagnostic plausibility of the LiPA tests MTBDRplus and MTBDRsl on MDR MTB isolates from Pakistan.

**Patients and Methods:** This was a cross-sectional study conducted at the Indus hospital, Karachi. LiPA testing was performed on 196 smear-positive samples using BACTEC MGIT 960 as a gold standard.

**Results:** The sensitivity of MTBDRplus for isoniazid and rifampicin was found to be 88.8% and 90.2%, respectively, while sensitivity of MTBDRsl for fluoroquinolones, amikacin/capreomycin, and ethambutol was found to be 72.9%, 81.8%, and 56.6%, respectively.

**Conclusions:** The MTBDRplus and MTBDRsl genotypic testing can serve as useful additional tools for DST in a high-burden country like Pakistan provided it is used in combination with phenotypic testing.

**Keywords:** Line Probe Assays, Phenotypic Assays, Rare Mutation, Pakistan

## 1. Background

Every year, approximately nine million incident cases of infection with *Mycobacterium tuberculosis* (MTB) are reported globally. Approximately 1.5 million deaths per year due to tuberculosis (TB) infection, with an estimated 95% occurring in low- to middle-income countries, have been documented (1).

Pakistan ranks fifth among TB high-burden countries worldwide with approximately 420,000 new cases emerging annually. Pakistan is also estimated to have the fourth highest prevalence of multidrug-resistant TB (MDR-TB: *M. tuberculosis* resistant to at least isoniazid and rifampin) globally (2). Furthermore, reports of extensively drug resistant TB (XDR-TB: *M. tuberculosis* resistant to isoniazid and rifampin, with additional resistance to any of the fluoroquinolones and any one of the second-line injectable agents, i.e., kanamycin, capreomycin, or amikacin) are consistently being documented with increasing frequency (1.5% in 2006 to 4.5% in 2009) (3, 4). Considering the high rates of drug resistance in Pakistan, rapid and accurate methods for drug susceptibility testing (DST) become a significant priority with respect to timely initiation of therapy appropriate to the drug-resistance pattern as well as

facilitation of infection control.

World health organization (WHO) recommends various methods for detection of drug-resistant TB, including phenotypic and genotypic assays (5, 6). However, conventional phenotypic DST using the liquid medium BACTEC MGIT 960 system is considered the gold standard for TB diagnostics, as per WHO recommendation, while genotypic assays are in the evaluation phase.

However, the BACTEC MGIT 960 system is limited by its lengthy turnaround time of approximately 17 - 45 days, leading to delayed reporting. Compared to this, the genotypic method, molecular line probe assay (LiPA), permits rapid diagnosis of drug resistance as the methodology requires DNA isolation directly from the sample, amplification, and reverse hybridization onto nitrocellulose strips containing immobilized probes against mycobacterium-resistance-conferring genes. The strips are quickly interpreted using a template and reportable results are obtained within one to two days.

The recommendation by WHO on the use of commercial LiPAs was emphasized based on the need for evaluation of these assays in various epidemiological settings. Two commercially available LiPAs for detecting MTB-resistance-conferring genes against first- and second-

line drugs include GenoType MTBDRplus and MTBDRsl (Hain Lifesciences GmbH, Nehren, Germany). Briefly, MTBDRplus allows identification of isoniazid (INH) and rifampin (RIF) resistance by detecting mutations in the *rpoB*, *katG*, and *inhA* gene, while MTBDRsl detects mutations in the *gyrA*, *rrs*, and *embB* genes, i.e., resistance to fluoroquinolone (FQ), amikacin/capreomycin (AM/CM), and ethambutol (EMB).

The performance of MTBDRplus and MTBDRsl has been evaluated and documented in different countries with variable sensitivities and specificities (7-10). A number of studies report high resistance rates of TB by conventional DST in Pakistan (11); however, limited data on LiPA testing has been published from this region (12-15).

## 2. Objectives

Therefore, the main aim of this study is to evaluate the performance characteristics of the respective LiPAs in a setting with a high burden of MDR and XDR TB so that the effectiveness of these assays for rapid diagnostic use can be assessed.

## 3. Patients and Methods

### 3.1. Study Design and Settings

This was a cross-sectional study conducted at the Indus hospital (TIH), Karachi. TIH is a tertiary care hospital with extensive MDR TB clinics and directly observed treatment short-course (DOTS) setups conducted in collaboration with the National TB program (NTP), WHO, and global fund. An estimated 2382 patient specimens are processed on a monthly basis at the TIH TB lab followed by acid-fast bacilli (AFB) smear microscopy and inoculation into BACTEC MGIT 960 medium (*Mycobacterium* growth indicator tube, BD Diagnostics Systems, Sparks, MD).

The study was conducted from November 2010 to June 2011. A total of 196 pulmonary samples routinely submitted for AFB smear and culture were included in the study. The samples were randomly selected based on AFB smear positivity. Clinical information on cases was not an inclusion criterion and therefore was not analyzed for the study.

### 3.2. Ethics

The study was conducted after due approval was obtained from the Institutional Review Board of The Indus hospital. Informed consent was obtained from all enrolled patients.

### 3.3. Phenotypic Testing Microscopy

The samples were digested and decontaminated with 1% N-acetyl-L-cysteine-sodium hydroxide (NALC/NaOH) (16). After decontamination, specimens were neutralized and centrifuged at  $3000 \times g$  for 15 minutes. AFB smears were prepared from decontaminated sediment with auramine and a potassium permanganate counterstain and evaluated with a light-emitting diode (LED) objective ( $40\times$ ) for a minimum of 100 fields, as per the WHO/international union against tuberculosis and lung diseases (IUATLD) scale for fluorescent microscopy.

### 3.4. Culture and Drug Susceptibility Testing

Sediment was prepared for culture in mycobacterium growth indicator tubes (MGIT; Becton Dickinson) according to the manufacturer's instructions. In cultures with mycobacterium growth, further identification and differentiation of *M. tuberculosis* complex (MTBC) and other mycobacterium species was done using BD MGIT Tbc identification test according to the manufacturer's instructions.

Cultures positive for MTB were subjected to first-line drug susceptibility testing on BACTEC MGIT 960 using streptomycin (1.0  $\mu\text{g}/\text{mL}$ ), INH (0.1  $\mu\text{g}/\text{mL}$ ), RMP (1.0  $\mu\text{g}/\text{mL}$ ), ethambutol (5.0  $\mu\text{g}/\text{mL}$ ), and PZA (100  $\mu\text{g}/\text{mL}$ ) using the SIRE kit as per the manufacturer's recommendations. For second-line DST, the following concentrations were used: amikacin (1.0  $\mu\text{g}/\text{mL}$ ), ofloxacin (2.0  $\mu\text{g}/\text{mL}$ ), kanamycin (2.5  $\mu\text{g}/\text{mL}$ ), and ethionamide (5.0  $\mu\text{g}/\text{mL}$ ).

### 3.5. Genotypic Testing (Molecular Line Probe Assays)

#### 3.5.1. DNA Extraction

DNA was extracted using GenoLyse kits according to the manufacturer's protocol (Hain Lifescience, Germany). Briefly, 500  $\mu\text{L}$  of decontaminated sample material was transferred into a 1.5 mL screw-cap tube and centrifuged for 15 minutes at  $10,000 \times g$ . The pellet was resuspended in 100  $\mu\text{L}$  of lysis buffer and incubated for 5 minutes at  $95^\circ\text{C}$  in a water bath. Subsequently, 100  $\mu\text{L}$  of neutralization buffer was added to lysate, vortexed, and centrifuged for 5 minutes at full speed. Then, 5  $\mu\text{L}$  supernatant was directly used for polymerase chain reaction (PCR).

#### 3.5.2. Polymerase Chain Reaction

For PCR, ready-to-use amplification mix (primer-nucleotide) provided as part of the kit by Hain LifeSciences (Nehren, Germany) was used. Briefly, 5  $\mu\text{L}$  of extracted DNA was added to the PCR mix. The amplification was performed as follows: enzyme activation for 15 minutes at  $95^\circ\text{C}$ , 10 cycles of 30 seconds at  $95^\circ\text{C}$  and 2 minutes at  $58^\circ\text{C}$ ; and 30 cycles of 25 s at  $95^\circ\text{C}$ , 40 seconds at  $53^\circ\text{C}$ , and 40 seconds at  $70^\circ\text{C}$ . The final cycle consisted of 8 minutes at  $70^\circ\text{C}$ .

### 3.5.3. Hybridization

A 20  $\mu$ L aliquot of amplified product was hybridized to DNA probe-labeled strips provided in the GenoType MTBDRplus and MTBDRsl assay kits. Briefly, detection of resistance against first-line drugs was performed using the MTBDRplus kit. After detection, the specimens showing conclusive results for MDR TB were further analyzed using the MTBDRsl kit. All hybridization and detection steps were performed as per the manufacturer's instructions.

### 3.6. Statistical Analysis

Descriptive analysis was performed using SPSS version 19.0 for comparing MTBDRplus and MTBDRsl results with the gold standard BACTEC MGIT 960 liquid culture.

## 4. Results

As per recommendations, only smear-positive samples can be further analyzed using MGIT 960 and genotypic methods. Therefore, 196 smear-positive samples were selected for mycobacterium detection on MGIT culture. AFB smears were rated prior to culture. It was observed that 26/196 samples were scanty while the remaining 170 rated 1+ to 3+ (Table 1).

Of MGIT cultures, 74.5% (146/196) of the samples were positive while 13.2% (26/196) showed no growth. Contamination was observed in 9.7% (19/196) of the samples. Further analysis for identification of MTB revealed that 146 samples were *M. tuberculosis* while the remaining was identified as nontuberculosis mycobacterium (NTM).

Of the 146 MTB positive specimens, gold standard conventional DST was successfully performed. Of these, 15 and seven samples were INH and RMP mono-resistant while 76 samples were resistant for both RMP and INH, i.e., MDR strains. The approximate turnaround time for conventional DST was found to be 17 days. The samples were simultaneously tested on MTBDRplus and it was observed that 10 and 23 samples were INH and RMP mono-resistant while 87 samples tested positive for MDR TB.

Out of 146 samples tested for first-line drug susceptibility, 76 and 87 MDR samples were further analyzed by both MGIT 960 and MTBDRsl. Of these 76 and 87 MDR samples, a total of three and five samples were found to be XDR on MGIT and MTBDRsl, respectively. A total of five XDR samples were accurately tested on LiPAs.

Analysis for resistance patterns revealed that in *rpoB*-S531L, -S522L, -S522Q, in *katG*-S315T, in *inhA*-C15T, in *rrs*-A1401G, in *gyrA*-D94G, A90V, and in *embB*-M306V, -M3061 (2), and -M3061 (3) were the prevalent drug-specific resistances observed in the study isolates. Rare *rpoB* mutations

S522L and S522Q were found in 19/87 samples in the multidrug resistant strains. Detailed results on observed mutations are given in Table 2.

The percent discordance between MGIT 960 results and MTBDRplus and MTBDRsl results was found to be 36%. For discordant results, DST results were reconfirmed by repeat testing.

## 5. Discussion

The present study aimed to evaluate the performance characteristics of both MTBDRplus and MTBDRsl for efficiency in detecting first and second-line drug-resistance patterns in isolates from Pakistan.

The results from the study showed that sensitivity of MTBDRplus for INH and RMP resistance was 88.8% and 90.2%, respectively. Though, this is slightly higher for INH and lower for RMP than previously published data from Pakistan (12), the ranges for both INH and RMP are lower when compared to studies conducted worldwide (7, 8, 17). One possible reason for this discrepancy may be due to the TB strains isolated in this study cohort. Patient samples were taken irrespective of their treatment status, possibly leading to inclusion of patients who may be on treatment (appropriate or inappropriate) or who were non-compliant. In such strains, the possibility of accumulation of novel resistance-conferring mutations/deletions against drugs is common due to the natural selective pressure of antibiotic use (18). LiPA kits carry probes that cover common mutations reported worldwide and therefore it is possible that the sensitivities were compromised in this study. However, as sequencing was not performed in our study, the presence of novel mutant alleles is only speculated. Therefore, it is suggested that further studies validating the performance characteristics of MTBDRplus need to be conducted in Pakistan and compared with sequencing results so that novel mutation detection is evidenced. Such studies will not only help to highlight limitations and strengths of LiPA testing, but will also help to determine the diagnostic plausibility of introducing this test in hospital settings in Pakistan.

The sensitivity of MTBDRsl for fluoroquinolones (FLQ), amikacin/capreomycin (AM/CM), and ethambutol (EMB) was found to be 72.9%, 81.8%, and 56.6%, respectively. These sensitivity ranges are consistent with previously published data, wherein ranges of 70% - 90% for FLQ, 75% - 80% for AM/CM, and 30% - 70% for EMB have been reported (8-10, 19, 20). For FLQ, the sensitivity range is towards lower side and it is speculated that the included samples may be carrying mutations in the *gyrB* gene that are not detected by genotyping testing due to the lack of probes against *gyrB* in MTBDRsl. This may be considered a limitation of

**Table 1.** *Mycobacterium* Detection by AFB Smear and MGIT Culture

MGIT culture	AFB smear (N = 196)				
	Scanty	1+	2+	3+	Total
Positive	10	27	22	87	146
Negative	11	10	5	0	26
Contaminate	4	6	3	6	19
NTM	1	1	2	1	5

**Table 2.** Frequencies of Mutations Detected in Resistance-Confering Genes by Mtbdrplus and Mtbdrsl<sup>a</sup>

Resistance-Confering Gene	Mutation Detected	Number of Isolates with Specific Mutation
<b>rpoB</b>	S531L	36
	S522L, S522 Q	19
	D516Y.del 515	7
	D516V	2
	H526Y	4
	F505L, T508A, S509T	4
	510 - 513, 513 - 519	1
	513 - 519, 518 - 525	2
	526 - 529	2
	526 - 529 , 518 - 525	3
	S531P, S531Q*, S531W, L533P	3
	H526Y	4
	526 - 529 , 518 - 525	3
<b>katG</b>	S315T	65
<b>inhA</b>	C15T	4
<b>Rrs</b>	A1401G	10
	G1484T	1
<b>gyrA</b>	D94G	16
	A90V	10
	S91P	2
	D94Y	2
	D94A	1
	WT 2	1
<b>embB</b>	M306V	20
	M306I <sup>2</sup> )	16
	M306I <sup>3</sup> )	16

<sup>a</sup> Boldface represents change in amino acid.

the test, as significance of *gyrB* in FLQ resistance has been clearly documented in studies (21). Therefore, from this critical standpoint, inclusion of mutation probes against

*gyrB* gene may serve to increase the diagnostic plausibility of the test. However, due to the small sample size of our study, we recommend that further evaluation of MTB-

DRsl with MGIT should be conducted along with sequencing, so that evidence-based data can be generated. Furthermore, such large-scale evaluation would provide clear recommendations on MTBDRsl testing, thus helping clinicians to diagnose and choose appropriate FLQ therapy early in MDR treatment.

The detection rate of MTBDRsl for AM/CM mutation was found to be satisfactory, indicating that the test performs well in detecting the presence of mutations in the *rrs* gene. For EMB resistance, low detection rates, as detected globally, have been observed in our study isolates. In the same manner as for FLQ, this low sensitivity can be attributed to samples carrying mutations at codon positions not covered by the LiPA probes. However, for EMB, other factors, such as insufficient molecular understanding of resistance patterns, are important determinants of sensitivity. Studies have reported that codon 306 mutation (covered by MTBDRsl) has been isolated in both EMB-resistant and susceptible strains (19). It is possible that such a resistant/susceptible pattern occurs for other resistance-conferring genes as well. Therefore, diagnostic reliability on genotypic assay and MTBDRsl for EMB resistance requires further validation, especially before its use in a highly endemic country such as Pakistan.

Comparison of prevalent resistance patterns in *rpoB*, *katG*, *inhA*, *rrs*, and *gyrA* with previously reported data from Pakistan showed consistent results, i.e., there is a prevalence of S531L, S315T1, C15T, A1401G, and D94G, respectively (4, 12-14, 22). Interestingly, discrepancy in the second-most-prevalent mutation in *rpoB* was observed in our study. S522L, S522Q were found in considerable frequencies, i.e., 19 samples exhibited these mutations of which 14 were MDR strains. These mutations have not been reported in published literature from Pakistan. Studies from Australia and Kuwait and on cell lineages from East Asia have documented S522L and S522Q as less frequent/rare mutations with low fitness in culture as compared to wild types (23-29). However, though fitness-compromised, these mutations lie in the rifampicin resistance determining region (RRDR); therefore, a role in expression of RMP resistance is possible. Detection of these rare mutants with significant frequency in isolates from our cohort, specifically in MDR strains, indicates a pattern of evolutionary fitness of these mutants in response to extensive drug pressure in Pakistan. However, due to the limitation of this study on sequencing data, our findings are reported with caution. Furthermore, as suggested earlier, larger studies comparing LiPA results with sequencing data must be performed from other areas of Pakistan to authenticate the prevalence of such rare mutations.

INH monoresistance was detected in comparable frequencies by both gold standard and LiPA testing. This re-

sult is of significance as INH monoresistance detection has been documented as an important aspect in several studies (30-33). Studies indicate that early detection of INH monoresistance leads to better treatment outcomes via treatment modifications. Secondly, specific INH monoresistance detection is recommended instead of using an RMP surrogate. This is because INH susceptibility ranges from < 11, and > 40% of RMP-resistant isolates have been documented depending on the settings (34, 35). Keeping these recommendations in perspective, the INH monoresistance range in our study was determined and it was found to be 47% of the RMP-resistant isolates. This result supports recommendations of previous studies and also signifies caution for such diagnostic practice in Pakistan. In Pakistan, both INH and RMP are standard drugs used for patient management without considering the aspect of monoresistance. Clinicians are forced to utilize multidrug combinations due to high turnaround time for diagnostics (approximately 17-45 days in case of Bactec). The treatment regimen should be different (with different drug combinations instead of INH and RMP together) in case of detection of INH or RMP monoresistance and multidrug resistance. Both INH and RMP are important drugs and accumulation of high-level resistance to these drugs can be catastrophic for TB patient management. Keeping this aspect in perspective, utilization of LiPA testing can be very helpful in discriminating mono- and multidrug resistance in a timely manner, allowing clinicians to follow required treatment regimens as per the resistance patterns.

Analyzing the overall performance of MTBDRplus kits, the study results show that despite moderate sensitivities, the performance of this test for detection of INH and RMP monoresistance/MDR is satisfactory. Rapid detection of resistance against first-line drugs will provide useful and timely information for patient management and transmission control in a high-burden clinical setting. The performance of MTBDRsl requires caution for interpretation of XDR as the sensitivities for FQ and EMB were on the lower side. However, though the sensitivities may not be very high, in a country like Pakistan, where TB burden is high, the use of LiPA can still be a useful tool as it would help to prevent spread of MDR and XDR TB. Timely diagnostics play an important role in utilization of targeted therapeutics rather than irrational blind use of drugs. In Pakistan, patient management is started with first-line drugs when an AFB smear is reported. A patient may be harboring an XDR strain and will not respond to treatment, leading to loss of resources as well as time that is of the essence for such patients. Therefore, with LiPA testing in place, a large number of MDR and XDR cases will be diagnosed early in their disease progression and patient management with relevant drugs can be started accordingly.

In our study, discordance between MGIT 960 and LiPA test results was found to be 36%, which is within the range documented in studies from different geographical regions and settings (17, 36). The discordance has been attributed to various reasons. Firstly, the presence of DNA from dead bacteria, especially in treatment patients, poses a limitation for the LiPA test as these may be amplified and reverse hybridized on the probes, leading to false positive results. Secondly, expression of resistance is dependent on the ratio of resistant to susceptible bacilli. It has been reported that if the proportion of resistant cells in an isolate is less than 10% of mutant DNA, then sensitivity of molecular detection is lower as compared to culturing (37). Thirdly, occasionally slow resistance resulting from specific mutations is missed with MGIT (38). Therefore, we assume that similar reasons could be attributed to the discordance observed in this study.

The aim of this study was to highlight on the performance characteristics of LiPA tests in Pakistan. Several limitations of the LiPA test have been discussed. However, the main advantage of LiPA is its ability to detect common mutations with rapid turnaround time. In our study, the LiPA results were obtained in one day, while MGIT 960 results were obtained in 17 days. This time-saving advantage precludes the disadvantages associated with it, as timely and reliable DST, especially for first-line drugs, is crucial for prompt and effective treatment. In a highly endemic country such as Pakistan, where MDR and XDR rates are high, the introduction of LiPA tests as an additional diagnostic tool, along with gold standard MGIT, could be of value in order to reduce transmission and infection rates and guide appropriate treatment options to control amplification of drug resistance.

### 5.1. Conclusion

The present study has highlighted the limitations and advantages of using LiPA tests for the determination of MDR and XDR TB. Based on the results, it is concluded that MTBDRplus and MTBDRsl kits can serve as useful additional tools for rapid DST in a TB high-burden country such as Pakistan.

### Acknowledgments

We would like to thank TIH TB lab staff for their technical support.

### Footnotes

**Authors' Contribution:** Mona Javaid conceptualized, conducted and analyzed the data, Mona Javaid also wrote

the first draft of the manuscript. Altaf Ahmed designed the study, Sunil Asif performed the experiments and analyzed the data. Afsheen Raza reviewed and approved the final draft of the manuscript.

**Conflict of Interest:** The authors report no commercial relationship or potential conflict of interests.

**Funding/Support:** No funding from any sources was received for conducting this study.

### References

1. World Health Organization . Global Tuberculosis Report 2014 2014. Available from: [www.who.int/tb/publications/global\\_report/en](http://www.who.int/tb/publications/global_report/en).
2. World Health Organization . WHO EMRO Stop TB Programmes, Pakistan 2015. Available from: [www.emro.who.int/pak/programmes/stop-tuberculosis.html](http://www.emro.who.int/pak/programmes/stop-tuberculosis.html).
3. Ali A, Hasan Z, McNerney R, Mallard K, Hill-Cawthorne G, Coll F, et al. Whole genome sequencing based characterization of extensively drug-resistant Mycobacterium tuberculosis isolates from Pakistan. *PLoS One*. 2015;**10**(2):0117771. doi: [10.1371/journal.pone.0117771](https://doi.org/10.1371/journal.pone.0117771). [PubMed: [25719196](https://pubmed.ncbi.nlm.nih.gov/25719196/)].
4. Hasan R, Jabeen K, Ali A, Rafiq Y, Laiq R, Malik B, et al. Extensively drug-resistant tuberculosis, Pakistan. *Emerg Infect Dis*. 2010;**16**(9):1473-5. doi: [10.3201/eid1609.100280](https://doi.org/10.3201/eid1609.100280). [PubMed: [20735937](https://pubmed.ncbi.nlm.nih.gov/20735937/)].
5. World Health Organization . Molecular Line Probe Assays for rapid screening of patients at risk of multidrug-resistant tuberculosis (MDR-TB) 2008. Available from: [http://www.who.int/tb/features\\_archive/policy\\_statement.pdf](http://www.who.int/tb/features_archive/policy_statement.pdf).
6. World Health Organization . Use of liquid TB culture and drug susceptibility testing (DST) in low and medium income countries: Summary report of the expert group meeting on the use of liquid culture media: Summary report of the expert group meeting on the use of liquid culture media 2007. Available from: [http://www.who.int/tb/laboratory/use\\_of\\_liquid\\_tb\\_culture\\_summary\\_report.pdf](http://www.who.int/tb/laboratory/use_of_liquid_tb_culture_summary_report.pdf).
7. Anek-Vorapong R, Sinthuwattanawibool C, Podewils LJ, McCarthy K, Ngamlert K, Promsarin B, et al. Validation of the GenoType MTBDRplus assay for detection of MDR-TB in a public health laboratory in Thailand. *BMC Infect Dis*. 2010;**10**:123. doi: [10.1186/1471-2334-10-123](https://doi.org/10.1186/1471-2334-10-123). [PubMed: [20487550](https://pubmed.ncbi.nlm.nih.gov/20487550/)].
8. Felkel M, Exner R, Schleucher R, Lay H, Autenrieth IB, Kempf VA, et al. Evaluation of Mycobacterium tuberculosis drug susceptibility in clinical specimens from Nigeria using genotype MTBDRplus and MTBDRsl assays. *Eur J Microbiol Immunol (Bp)*. 2013;**3**(4):252-7. doi: [10.1556/EuJMI.3.2013.4.3](https://doi.org/10.1556/EuJMI.3.2013.4.3). [PubMed: [24294494](https://pubmed.ncbi.nlm.nih.gov/24294494/)].
9. Hillemann D, Rusch-Gerdes S, Richter E. Feasibility of the GenoType MTBDRsl assay for fluoroquinolone, amikacin-capreomycin, and ethambutol resistance testing of Mycobacterium tuberculosis strains and clinical specimens. *J Clin Microbiol*. 2009;**47**(6):1767-72. doi: [10.1128/JCM.00081-09](https://doi.org/10.1128/JCM.00081-09). [PubMed: [19386845](https://pubmed.ncbi.nlm.nih.gov/19386845/)].
10. Kiet VS, Lan NT, An DD, Dung NH, Hoa DV, van Vinh Chau N, et al. Evaluation of the MTBDRsl test for detection of second-line-drug resistance in Mycobacterium tuberculosis. *J Clin Microbiol*. 2010;**48**(8):2934-9. doi: [10.1128/JCM.00201-10](https://doi.org/10.1128/JCM.00201-10). [PubMed: [20573868](https://pubmed.ncbi.nlm.nih.gov/20573868/)].
11. Jabeen K, Shakoor S, Hasan R. Fluoroquinolone-resistant tuberculosis: implications in settings with weak healthcare systems. *Int J Infect Dis*. 2015;**32**:118-23. doi: [10.1016/j.ijid.2015.01.006](https://doi.org/10.1016/j.ijid.2015.01.006). [PubMed: [25809767](https://pubmed.ncbi.nlm.nih.gov/25809767/)].
12. Farooqi JQ, Khan E, Alam SM, Ali A, Hasan Z, Hasan R. Line probe assay for detection of rifampicin and isoniazid resistant tuberculosis in Pakistan. *J Pak Med Assoc*. 2012;**62**(8):767-72. [PubMed: [23862246](https://pubmed.ncbi.nlm.nih.gov/23862246/)].
13. Idrees F, Irfan M, Jabeen K, Hasan R. Genotype MTBDR plus Line Probe Assay as a rapid tool for the diagnosis of smear-negative tu-

- berculosis in bronchoalveolar lavage fluid in Pakistan. *Eur Respir J*. 2014;**44**(58):2693.
14. Irfan M, Jabeen K, Shahzad H, Zubairi ABS, Hasan R. Evaluation of sensitivity and specificity of GenoType MTBDRplus line probe assay in rapid diagnosis of tuberculous pleural effusion. *Int J Mycobacteriol*. 2015;**4**:111.
  15. Munir MK, Anwer N, Iqbal R, Nosheen S, Rehman S, Salam AA. Comparison of GenoType MTBDRplus Testing Kit with conventional method for Drug Susceptibility Testing of Isoniazid and Rifampicin in Tuberculosis Patients. *Pak J Med Sci*. 2014;**53**(2):25.
  16. Kent PKG. Public health mycobacteriology: a guide for the level III laboratory. Atlanta: Centers for Disease Control; 1985.
  17. Crudu V, Stratan E, Romancenco E, Allerheiligen V, Hillemann A, Moraru N. First evaluation of an improved assay for molecular genetic detection of tuberculosis as well as rifampin and isoniazid resistances. *J Clin Microbiol*. 2012;**50**(4):1264–9. doi: [10.1128/JCM.05903-11](https://doi.org/10.1128/JCM.05903-11). [PubMed: [223101019](https://pubmed.ncbi.nlm.nih.gov/223101019/)].
  18. Dheda K, Warren RM, Zumla A, Grobusch MP. Extensively drug-resistant tuberculosis: epidemiology and management challenges. *Infect Dis Clin North Am*. 2010;**24**(3):705–25. doi: [10.1016/j.idc.2010.05.001](https://doi.org/10.1016/j.idc.2010.05.001). [PubMed: [20674800](https://pubmed.ncbi.nlm.nih.gov/20674800/)].
  19. Brossier F, Veziris N, Aubry A, Jarlier V, Sougakoff W. Detection by GenoType MTBDRsl test of complex mechanisms of resistance to second-line drugs and ethambutol in multidrug-resistant Mycobacterium tuberculosis complex isolates. *J Clin Microbiol*. 2010;**48**(5):1683–9. doi: [10.1128/JCM.01947-09](https://doi.org/10.1128/JCM.01947-09). [PubMed: [20335420](https://pubmed.ncbi.nlm.nih.gov/20335420/)].
  20. Kontsevaya I, Mironova S, Nikolayevskyy V, Balabanova Y, Mitchell S, Drobniewski F. Evaluation of two molecular assays for rapid detection of mycobacterium tuberculosis resistance to fluoroquinolones in high-tuberculosis and -multidrug-resistance Settings. *J Clin Microbiol*. 2011;**49**(8):2832–7. doi: [10.1128/JCM.01889-10](https://doi.org/10.1128/JCM.01889-10). [PubMed: [21632897](https://pubmed.ncbi.nlm.nih.gov/21632897/)].
  21. Wang JY, Lee LN, Lai HC, Wang SK, Jan IS, Yu CJ, et al. Fluoroquinolone resistance in Mycobacterium tuberculosis isolates: associated genetic mutations and relationship to antimicrobial exposure. *J Antimicrob Chemother*. 2007;**59**(5):860–5. doi: [10.1093/jac/dkm061](https://doi.org/10.1093/jac/dkm061). [PubMed: [17412727](https://pubmed.ncbi.nlm.nih.gov/17412727/)].
  22. Qazi O, Rahman H, Tahir Z, Qasim M, Khan S, Ahmad Anjum A, et al. Mutation pattern in rifampicin resistance determining region of rpoB gene in multidrug-resistant Mycobacterium tuberculosis isolates from Pakistan. *Int J Mycobacteriol*. 2014;**3**(3):173–7. doi: [10.1016/j.ijmyco.2014.06.004](https://doi.org/10.1016/j.ijmyco.2014.06.004). [PubMed: [26786485](https://pubmed.ncbi.nlm.nih.gov/26786485/)].
  23. Ahmad S, Al-Mutairi NM, Mokaddas E. Variations in the occurrence of specific rpoB mutations in rifampicin-resistant Mycobacterium tuberculosis isolates from patients of different ethnic groups in Kuwait. *Indian J Med Res*. 2012;**135**(5):756–62. [PubMed: [22771609](https://pubmed.ncbi.nlm.nih.gov/22771609/)].
  24. Brown TJ, Herrera-Leon L, Anthony RM, Drobniewski FA. The use of microarrays for the identification of MDR Mycobacterium tuberculosis. *J Microbiol Methods*. 2006;**65**(2):294–300. doi: [10.1016/j.mimet.2005.08.002](https://doi.org/10.1016/j.mimet.2005.08.002). [PubMed: [16153726](https://pubmed.ncbi.nlm.nih.gov/16153726/)].
  25. Gagneux S. Fitness cost of drug resistance in Mycobacterium tuberculosis. *Clin Microbiol Infect*. 2009;**15** Suppl 1:66–8. doi: [10.1111/j.1469-0691.2008.02685.x](https://doi.org/10.1111/j.1469-0691.2008.02685.x). [PubMed: [19220360](https://pubmed.ncbi.nlm.nih.gov/19220360/)].
  26. Gagneux S, Burgos MV, DeRiemer K, Encisco A, Munoz S, Hopewell PC, et al. Impact of bacterial genetics on the transmission of isoniazid-resistant Mycobacterium tuberculosis. *PLoS Pathog*. 2006;**2**(6):ee61. doi: [10.1371/journal.ppat.0020061](https://doi.org/10.1371/journal.ppat.0020061). [PubMed: [16789833](https://pubmed.ncbi.nlm.nih.gov/16789833/)].
  27. Mariam DH, Mengistu Y, Hoffner SE, Andersson DI. Effect of rpoB mutations conferring rifampin resistance on fitness of Mycobacterium tuberculosis. *Antimicrob Agents Chemother*. 2004;**48**(4):1289–94. [PubMed: [15047531](https://pubmed.ncbi.nlm.nih.gov/15047531/)].
  28. Morlock GP, Plikaytis BB, Crawford JT. Characterization of spontaneous, In vitro-selected, rifampin-resistant mutants of Mycobacterium tuberculosis strain H37Rv. *Antimicrob Agents Chemother*. 2000;**44**(12):3298–301. [PubMed: [11083630](https://pubmed.ncbi.nlm.nih.gov/11083630/)].
  29. Yuen LK, Leslie D, Coloe PJ. Bacteriological and molecular analysis of rifampin-resistant Mycobacterium tuberculosis strains isolated in Australia. *J Clin Microbiol*. 1999;**37**(12):3844–50. [PubMed: [10565894](https://pubmed.ncbi.nlm.nih.gov/10565894/)].
  30. Bang D, Andersen PH, Andersen AB, Thomsen VO. Isoniazid-resistant tuberculosis in Denmark: mutations, transmission and treatment outcome. *J Infect*. 2010;**60**(6):452–7. doi: [10.1016/j.jinf.2010.03.017](https://doi.org/10.1016/j.jinf.2010.03.017). [PubMed: [20347869](https://pubmed.ncbi.nlm.nih.gov/20347869/)].
  31. Cattamanchi A, Dantes RB, Metcalfe JZ, Jarlsberg LG, Grinsdale J, Kawamura LM, et al. Clinical characteristics and treatment outcomes of patients with isoniazid-monoresistant tuberculosis. *Clin Infect Dis*. 2009;**48**(2):179–85. doi: [10.1086/595689](https://doi.org/10.1086/595689). [PubMed: [19086909](https://pubmed.ncbi.nlm.nih.gov/19086909/)].
  32. Jacobson KR, Theron D, Victor TC, Streicher EM, Warren RM, Murray MB. Treatment outcomes of isoniazid-resistant tuberculosis patients, Western Cape Province, South Africa. *Clin Infect Dis*. 2011;**53**(4):369–72. doi: [10.1093/cid/cir406](https://doi.org/10.1093/cid/cir406). [PubMed: [21810750](https://pubmed.ncbi.nlm.nih.gov/21810750/)].
  33. Menzies D, Benedetti A, Paydar A, Royce S, Madhukar P, Burman W, et al. Standardized treatment of active tuberculosis in patients with previous treatment and/or with mono-resistance to isoniazid: a systematic review and meta-analysis. *PLoS Med*. 2009;**6**(9):1000150. [PubMed: [20101802](https://pubmed.ncbi.nlm.nih.gov/20101802/)].
  34. Kurbatova EV, Cavanaugh JS, Shah NS, Wright A, Kim H, Metchock B, et al. Rifampicin-resistant Mycobacterium tuberculosis: susceptibility to isoniazid and other anti-tuberculosis drugs. *Int J Tuberc Lung Dis*. 2012;**16**(3):355–7. doi: [10.5588/ijtld.11.0542](https://doi.org/10.5588/ijtld.11.0542). [PubMed: [22640449](https://pubmed.ncbi.nlm.nih.gov/22640449/)].
  35. Smith SE, Kurbatova EV, Cavanaugh JS, Cegielski JP. Global isoniazid resistance patterns in rifampin-resistant and rifampin-susceptible tuberculosis. *Int J Tuberc Lung Dis*. 2012;**16**(2):203–5. doi: [10.5588/ijtld.11.0445](https://doi.org/10.5588/ijtld.11.0445). [PubMed: [22136739](https://pubmed.ncbi.nlm.nih.gov/22136739/)].
  36. Lacombe A, Garcia-Sierra N, Prat C, Maldonado J, Ruiz-Manzano J, Haba L, et al. GenoType MTBDRsl for molecular detection of second-line-drug and ethambutol resistance in Mycobacterium tuberculosis strains and clinical samples. *J Clin Microbiol*. 2012;**50**(1):30–6. doi: [10.1128/JCM.05274-11](https://doi.org/10.1128/JCM.05274-11). [PubMed: [22075597](https://pubmed.ncbi.nlm.nih.gov/22075597/)].
  37. Richter E, Rusch-Gerdes S, Hillemann D. Drug-susceptibility testing in TB: current status and future prospects. *Expert Rev Respir Med*. 2009;**3**(5):497–510. doi: [10.1586/ers.09.45](https://doi.org/10.1586/ers.09.45). [PubMed: [20477339](https://pubmed.ncbi.nlm.nih.gov/20477339/)].
  38. Rigouts L, Gumusboga M, de Rijk WB, Nduwamahoro E, Uwizweye C, de Jong B, et al. Rifampin resistance missed in automated liquid culture system for Mycobacterium tuberculosis isolates with specific rpoB mutations. *J Clin Microbiol*. 2013;**51**(8):2641–5. doi: [10.1128/JCM.02741-12](https://doi.org/10.1128/JCM.02741-12). [PubMed: [23761146](https://pubmed.ncbi.nlm.nih.gov/23761146/)].