Evaluation of Molecular Line Probe Assay for Rapid Detection of Mdr-Tb in A Tertiary Healthcare Setting in Mumbai, India

Nilma N Hirani , Ameeta A Joshi, Sarala Menon,Vaishali R Wabale, Pranali N Medhekar and Abhay S Chowdhary

Department of Microbiology, Grant Government Medical College & Sir J.J. Group of Hospitals, Mumbai, Maharashtra, India

*Corresponding Author: Dr Nilma Hirani Assistant Professor, Department of Microbiology, Grant Government Medical College & Sir J.J. Group of Hospitals, Mumbai, Maharashtra, India.

Abstract

Rationale: Rise in multi-drug resistant tuberculosis (MDR-TB), emergence of extensively drug resistant tuberculosis (XDR-TB) and high mortality of MDR-TB and XDR-TB patients stresses the need for rapid diagnostic tests to detect drug resistant tuberculosis. Molecular assays to detect gene mutations signifying drug resistance are recognised as most suitable for rapid diagnosis, amongst which line probe assays (LPA) have shown great promise.

Objectives: This study was designed to evaluate the utility of LPA for rapid detection of MDR-TB by comparing the results with conventional drug susceptibility testing (DST) by proportion method.

Methods: Study material included 544 samples (263 sputum specimens and 281 culture isolates), which were subjected to LPA using MTB-DR*plus* (Hain Lifescience, Germany). Conventional DST (1% proportion method) was also performed on 251 of these 544 strains. Comparative analysis of results was done using suitable statistical methods.

Measurements and Main Results: MDR-TB was detected in 368/544 (67.65%) samples within 48-72 hours using the LPA. Considering conventional DST as gold standard, LPA was found to be fairly sensitive (96.59%) and specific (89.42%), with 92.81% positive and 94.89% negative predictive value. Commonest mutation pattern in our region was presence of mutation 3 band (S531L) in rpoB gene locus for rifampicin and mutation 1 band (S315T1) in katG for isoniazid.

Conclusions: Line Probe Assay was found to be rapid, reliable and easy to perform diagnostic test. High sensitivity and specificity of LPA in detection of resistance to MDR strains complements its use for rapid diagnosis of MDR-TB.

Keywords: TB Mutations; Rifampicin resistance; Isoniazid resistance; MTBDRplus assay; MDR-TB

Introduction

Tuberculosis (TB) today is an important infectious disease associated with high morbidity and mortality especially in India. This is because of the rise in multi-drug resistant TB (MDR-TB) which is resistance to at least isoniazid (INH) and rifampicin (RIF) and emergence

Evaluation of Molecular Line Probe Assay for Rapid Detection of Mdr-Tb in A Tertiary Healthcare Setting in Mumbai, India

23

of extensively drug resistant TB (XDR-TB) which is resistance to first line drugs as well as fluoroquinolones and one of the injectable second line drugs (kanamycin, capreomycin, amikacin) [1]. The global burden for TB for the year 2014 is at 9.6 million cases [2], with having the highest incidence of TB in the world at 2.2 million cases, that constitutes approximately one-fifth of the global burden while global incidence of MDR-TB is at 480,000 cases with India constituting about 15% of the global burden with 71,000 cases, presently [3].

Though the conventional drug susceptibility testing (DST) is considered the 'Gold standard' for the detection of MDR and XDR-TB, it is time-consuming taking about 6-8 weeks. Hence, there is a need for the rapid diagnosis of MDR-TB to reduce the risk of treatment failure, control relapses and amplification of drug resistance as well as halt the spread of MDR-TB. Molecular assays to detect gene mutations that signal drug resistance such as the line probe assays are being widely recognised nowadays as best suited for rapid diagnosis for detection of both INH and RIF drug resistance [4]. The commercially available MTBDR*plus* assay (Hain lifescience, Nehren, Germany) can detect both, the presence of MTB complex as well as the presence of INH and RIF resistance and can be done directly on clinical samples (smear positive sputum) as well as culture isolates. In MTBDR*plus* assay, a multiplex PCR is followed by a reverse hybridization of the obtained DNA amplicons to membrane-bound probes. Rifampicin resistance is detected by the presence of mutations in the 81-bp hotspot region of the rpoB gene while INH resistance is detected by presence of mutations in 315 codon region (high level) and mutations in the inhA promoter region (low level) [5-10]. The present study aims to evaluate the utility of MTBDRplus assay for detection of TB and determine the mutation patterns in the various genomic regions. As such information is scarce; this study will give information which would be of epidemiological value for the country as Sir J.J. Hospital is one of the biggest tertiary health care centres in Western Maharashtra in India.

Methods

Setting

This study was carried out in the TB culture and DST Laboratory of the Department of Microbiology, Grant Government Medical College and Sir J. J. Group of Hospitals which is a tertiary care Hospital in the city of Mumbai, India with necessary ethical permissions. The Laboratory caters to a huge workload of approximately 5000 samples annually for TB culture which includes the outdoor as well as indoor patients of Sir J. J. Group of Hospitals. The study included 544 samples which comprised of 263 sputum samples and 281 culture isolates from patients attending the OPD and IPD of Sir J. J. Group of Hospitals between January, 2011 and June, 2012.

Sputum Specimens

Each patient was asked to submit one early morning and one spot sample as per RNTCP guidelines [1]. The sputum specimens were subjected to direct smears using Ziehl Neelsen staining followed by concentration and decontamination techniques using N-acetyl L-cysteine (NALC) sodium hydroxide (NaOH) [11]. Smears prepared were viewed and graded as per the RNTCP guidelines [12] and all smear positive (1+ or more) samples were then subjected to line probe assay using the Genotype MTBDR*plus* assay (version 1) as well as solid culture on in-house prepared Lowenstein-Jensen medium (LJ medium). Growth on LJ medium was observed on a weekly basis for a maximum of eight weeks and 89 of the 263 grown cultures after confirmation, were subjected to phenotypic solid drug susceptibility testing (DST) using economic variant of 1% proportion method using the following drugs from Sigma- Aldrich: Isoniazid (0.2 μg/ml), Rifampicin (40 μg/ml), streptomycin (4 μg/ml) and Ethambutol (2 μg/ml) [11]. The results of MTBDRplus assay were compared with that of phenotypic susceptibility testing.

Culture isolates

A total of 281 culture isolates which included 275 sputum smear negative culture positive and 6 extra-pulmonary samples, were obtained from conventional culture using solid LJ media and confirmed by the conventional biochemical methods [13]. These were then subjected to MTBDR*plus* assay and 162 of the 281 isolates were also processed for phenotypic drug susceptibility testing and the results were compared for INH and RIF susceptibility.

Evaluation of Molecular Line Probe Assay for Rapid Detection of Mdr-Tb in A Tertiary Healthcare Setting in Mumbai, India

24

Line probe assay

The MTBDR*plus* assay was performed as per manufacturer's instructions [14]. The important steps included: DNA extraction from smear-positive decontaminated sputum specimens/culture isolates, followed by amplification using a thermal cycler (Applied Biosystems) and hybridization using a pre-programmed Twincubator and the GT Blot-48 (Hain Lifescience) which is an automated hybridization machine that caters to a larger sample load [15]. The run was considered valid if the negative controls (molecular biology grade water used in Master-mix and DNA extraction areas) showed the presence of conjugate control (CC) and amplification control (AC) bands only. The presence of TUB band signifies that the organism belongs to *M. tuberculosis* complex. The rpoB, katG and inhA gene loci each has a control band the presence of which is mandatory for interpretation of results. The presence of rpoB gene locus predicts RIF resistance, while katG predicts high level and inhA low level INH resistance. Absence of wild type and/or presence of mutant band signify resistance to a particular drug. The product insert was further referred to for interpretation of banding patterns and troubleshooting.

Results

The study analysed a total of 544 LPA results (263 sputum and 281 culture isolates). Table 1 shows the detailed analysis of the same. Out of 544, 368 (67.65%) were found to be MDR. Remaining 176 (32.35%) were found to be sensitive to one/both drugs. The LPA results were further analyzed considering the proportion method as the 'gold standard'. Statistical calculations showed that LPA has 96.59% sensitivity and 89.42% specificity for MDR detection. The test also showed fair sensitivity and specificity for RIF and INH resistance detection (Table 2). Another interesting analysis was when LPA was performed on smear-positive sputum specimens and culture isolates of these same specimens, it exhibited 100% concordance. Additional information yielded by LPA was different mutation patterns. Out of 544 strains processed for LPA, 424 strains showed mutations, of which 11 (2.59%) strains showed unknown mutations. In all, there were 42 different banding patterns observed, with the most common banding pattern found in 227 (53.54%) of the 544 strains, that is, absence of WT8 band and presence of MUT3 band coding for S531L codon in the rpoB gene locus and absence of WT band and presence of MUT1 band coding for S315T1 codon in the katG gene locus. 2 (0.47%) of the 424 strains gave an unusual presentation with complete inhA gene deletion (Table 3) (figure 1). Table 4 shows the distribution of mutation in various gene loci. In the rpoB gene locus, the most common mutation was found to be the presence of MUT3 band coding for S531L codon amounting to 334 (84.99%) of the 393 strains. The katG gene locus showed maximum mutations in the MUT1 band region coding for S315T1 codon amounting to 362 (95.01%) of the 381 strains, while in the inhA gene locus maximum mutations were seen in the MUT1 band region coding for C15T codon amounting to 67 (75.29%) of the 89 strains.

MDR	368 (67.65%)
MONO RIFr	22 (4.04%)
MONO INHr	34 (6.25%)
RIF+INH SENSITIVE	120 (22.06%)

Table 1: *LPA Result Analysis (N = 5 44).*

	MDR	RIF	INH
Sensitivity	96.59%	96.15%	96.53%
Specificity	89.42%	88.42%	92.31%
Positive Predictive Value (PPV)	92.81%	93.17%	96.53%
Negative Predictive Value (NPV)	94.89%	93.33%	92.31%

Table 2: *Comparison of LPA with proportion method for detection of MDR strains, RIF and INH resistance.*

Evaluation of Molecular Line Probe Assay for Rapid Detection of Mdr-Tb in A Tertiary Healthcare Setting in Mumbai, India

25

rpoB Mutations	katG Mutations	inhA Mutations	Frequency (%)
D516V (MUT1)	S315T1 (MUT1)	---------	4 (0.94%)
D516V (MUT1)	------------------	C15T (MUT1)	1 (0.24%) *
D516V (MUT1) H526Y (MUT 2A)	S315T1 (MUT1)	Unknown	1 (0.24%)
S531L (MUT3) *	------------------	--------------------	1 (0.24%)
H526Y(MUT 2A)	S315T1 (MUT1)	---------	4 (0.94%)
H526Y(MUT 2A)	S315T1 (MUT1)	Unknown	2 (0.47%)
H526Y(MUT 2A)	Unknown	------------------	1 (0.24%)
H526Y(MUT 2A)	S315T1 (MUT1)	T8C(MUT3A)	1 (0.24%)
H526D (MUT2B)	S315T1 (MUT1)	---------	10 (2.36%)
H526D (MUT2B)	S315T1 (MUT1) *	C15T(MUT1)	1 (0.24%)
S531L (MUT3) *	S315T1 (MUT1)	-------------	1 (0.24%)
S531L (MUT3)	S315T1 (MUT1)	---------	227(63.64%)
S531L (MUT3)	S315T1 (MUT1)	-----------	1 (0.24%) *
S531L (MUT3) *	S315T1 (MUT1)	-----------	5 (1.18%)
S531L (MUT3)	S315T1 (MUT1) *	-----------	4 (0.94%)
S531L (MUT3)	S315T1 (MUT1)	C15T(MUT1)	39 (8.96%)
S531L (MUT3)	S315T1 (MUT1) *	C15T(MUT1)	2 (0.47%)
S531L (MUT3) *	S315T1 (MUT1)	C15T(MUT1)	1 (0.24%)
S531L (MUT3)	S315T2 (MUT2)	---------	10 (2.36%)
S531L (MUT3)	S315T1 (MUT1)	A16G(MUT2)	2 (0.47%)
S531L (MUT3)	S315T2 (MUT2)	T8C(MUT3A)	1 (0.24%)
S531L (MUT3)	S315T1(MUT1)	Complete gene deletion	2 (0. 47%)
S531L (MUT3)	S315T1 (MUT1)	Unknown	6 (1.42%)
S531L (MUT3)	S315T1 (MUT1) S315T2 (MUT2) *	----------------	1 (0.24%)
S531L (MUT3) *	S315T1 (MUT1) S315T2 (MUT2)	----------------	1 (0.24%)
S531L (MUT3)	S315T1 (MUT1)	T8C(MUT3A)	1 (0.24%)
S531L (MUT3)	Unknown	---------------------	1 (0.24%)
S531L (MUT3) *	Unknown	---------------------	1 (0.24%)
S531L (MUT3)	---------	C15T (MUT1)	9 (2.12%)
S531L (MUT3)	---------	A16G (MUT2)	2 (0.47%)
S531L (MUT3)	---------	Unknown	1 (0.24%)
S531L (MUT3)	--------	---------	13 (3.07%)
S531L (MUT3) *	--------	---------	2 (0.47%)
Unknown	S315T1 (MUT1)	----------------	16 (3.77%)
Unknown	S315T1 (MUT1)	C15T (MUT1) *	1 (0.24%)
Unknown	S315T1 (MUT1)	C15T (MUT1)	4 (0.94%)
Unknown	--------------------	C15T (MUT1)	3 (0.71%)

Evaluation of Molecular Line Probe Assay for Rapid Detection of Mdr-Tb in A Tertiary Healthcare Setting in Mumbai, India

26

---------	S315T1 (MUT1) *	C15T (MUT1) *	1 (0.24%)
----------	S315T1 (MUT1)	----------	23 (5.42%)
------------	S315T1 (MUT1)	Unknown	1 (0.24%)
----------	----------	C15T (MUT1)	5 (1.18)
Total number of strains with unknown mutations			11 (2.59%)
Total no. of strains with any mutations			424 (100%)

Table 3: *Mutation Patterns: LPA.*
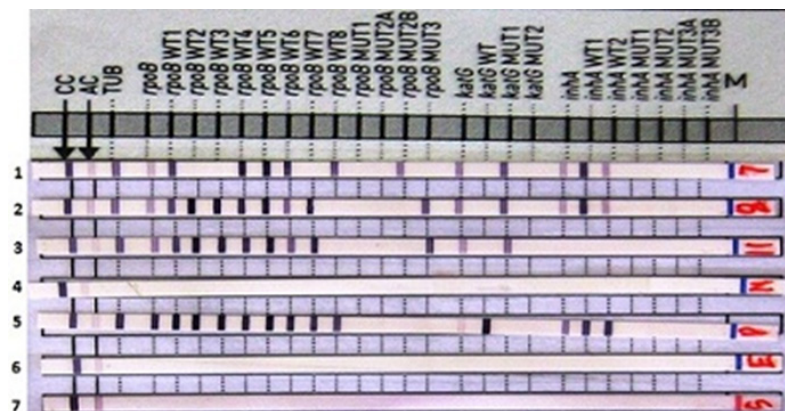
Figure 1: *Banding patterns on LPA.*

Strip 1 shows a MDR-TB strain with uncommon mutations and a double mutation in the rpoB gene locus (Absence of WT2 and WT3 with no corresponding MUT band + H526D codon) and a katG (S315T1 codon) mutation.

Strip 2 also shows a MDR-TB strain with a common mutation pattern in the rpoB (S531L codon) and katG (S315T1 codon) gene loci.

Strip 3 also exhibits a MDR-TB strain with common mutation pattern in the rpoB (S531L codon) and katG(S315T1 codon) gene loci along with complete inhA gene deletion.

Strip 4 shows the master mix negative control.

Strip 5 shows the positive control (H37Rv strain).

Strip 6 shows the DNA extraction negative control.

Strip 7 exhibits the swipe test (a negative control) mandatory for all molecular assays as per the ISO 15189 guidelines.

A total of 251 out of 544 strains processed for LPA were subjected to phenotypic solid DST using Lowenstein Jensen media. Comparison of results of both methods exhibited a concordance of 90.84% (228/251) and a discordance of 9.16% (23/251).

Discussion

Substantial progress has been made in our understanding of the molecular basis of *Mycobacterium tuberculosis* drug resistance in recent years. Molecular based assays are the most rapid and sensitive methods for the detection of drug resistance in TB. These assays detect all commonly occurring mutations. These techniques include direct sequencing of PCR products, SSCP analysis, heteroduplex analysis, dideoxy fingerprinting, an RNA/RNA duplex, base-pair mismatch assay, luciferase mycobacteriophage strategy, an rRNA/DNA-bioluminescence-labelled probe method, a reverse hybridization –based line probe assay, and other strategies [16]. The World Health Organisation (WHO) endorsed the use of molecular line probe assays for screening of MDR-TB in June, 2008 [17], following which the Genotype MTBDR*plus* assay is being used routinely in different countries [6-10, 17].

Evaluation of Molecular Line Probe Assay for Rapid Detection of Mdr-Tb in A Tertiary Healthcare Setting in Mumbai, India

27

This study evaluated the performance of GenoType MTBDR*plus* (Line probe assay) which detects resistance to INH and Rif in clinical isolates and sputum positive samples based on the detection of the most common mutations in rpoB, katG and inhA genes. It uses PCR and reverse-hybridization to probes immobilized on a plastic strip and improves a previous version of the same test, MTBDR that detected mutations in rpoB and katG genes [18]. The turnaround time for this assay is between 48-72 hours, which is extremely rapid, compared to the phenotypic conventional solid DST requiring 12-14 weeks.

The present study has found LPA to be rapid, reliable and easy to perform for the detection of MDR-TB and this could be substantiated by the fact that on analysis of our results of LPA performed on smear-positive sputum specimens and culture isolates of these same specimens, exhibited 100% concordance. The performance of this assay when co-related with the conventional proportion method for DST has given a sensitivity of 96.59%, 96.15 % and 96.53 % and specificity of 89.42 %, 88.42 % and 92.31 % for detection of MDR, rifampicin and isoniazid resistance, respectively. Similar studies conducted by Barnard., *et al.* 2008 [19] on 536 smear positive specimens in Cape Town, South Africa and Raveendran., *et al.* 2012 [20] from India using the same assay for smear positive pulmonary specimens and culture isolates, have concluded that this assay has good concordance with phenotypic drug susceptibility results.

High sensitivity of rifampicin resistance found in our study is in accordance with various other studies conducted across the world, such as Vietnam, South Africa, Pakistan, Mongolia and India ranging from 92-100 %. [19-23]. High sensitivity of rifampicin resistance found in our study could be attributed to the majority of mutations, that is 362 (92.11%) of the 393 strains in the 81 bp-hotspot region of the rpoB gene (Table 4). Among all the gene mutations conferring to Rifampicin resistance, codon S531L was found to be the most frequently encountered mutation (84.99%) which is comparable to the Indian study by Raveendran., *et al.* in 2012 (84.6%) [20], but higher than that reported in various other studies across the world (South Africa, Vietnam and Mongolia) [19,21,23]. The presence of mutations in codon 526 were at 5.34% which is lower than the study conducted in South Africa (8.6%), Europe (15%), India (19%), Pakistan (22.5%) and Iran (45.6%) [19, 24-27], but higher than the study conducted in Vietnam (1.8%) [21]. Mutations in the rpoB gene at the D516V codon were at 1.78% in our study which is very low compared to the studies from South Africa (9.6%) and Europe (44%) (19,24). We also saw a large number of unknown mutations, 7.89%, in our study which could be attributed to mutations in other regions of the rpoB gene locus or mutations other than those on the strip.

Locus	Mutant	Codon	No. of Strains
rpoB –WT1 –WT8	Mut 1	D516V	07 (1.78%)
	Mut 2A	H526Y	09 (2.29%)
	Mut 2B	H526D	12 (3.05%)
	Mut 3	S531L	334 (84.99%)
	Unknown		31 (7.89%)
		Total	393
katG – WT	Mut 1	S315T1	362 (95.01%)
	Mut 2	S315T2	13 (3.42%)
	Unknown		6 (1.57%)
		Total	381
inhA – WT1 – WT2	Mut 1	C15T	67 (75.29%)
	Mut 2	A16G	04 (4.49%)
	Mut 3A	T8C	03 (3.37%)
	Mut 3B	T8A	00 (0%)

Evaluation of Molecular Line Probe Assay for Rapid Detection of Mdr-Tb in A Tertiary Healthcare Setting in Mumbai, India

28

	Unknown		15 (16.85%)
		Total	89

Table 4: *Distribution of Mutations.*

Mutations in the katG gene locus coding for MDR-TB & high-level INH mono-resistance cases (73.53%) was found to be maximum in the S315T1 codon region amounting to 95.01%. This is in accordance with Huyen., *et al.* study from Vietnam & Van Rie., *et al.* study from South Africa [21-28]. A high prevalence of katG mutations has been reported in high TB- prevalent countries to attribute for INH resistance & much lower prevalence in low TB-prevalent countries. This could be attributed to the on-going transmission of the strains in the high- burden settings [29].

The presence of mutations in the inhA region accounts for 89 (20.99%) of the 422 strains showing mutations, with maximum mutations in the C15T (MUT1) region amounting to 67 (75.29%) of the 89 strains. Mutations accounting for 4.34% (16/368 strains) in MDRTB cases and 20.58% (7/34 strains) in INH mono-resistant cases in the inhA gene region were seen in our study, which is lower compared to the results seen in the study by Barnard., *et al.* at 27% of MDR strains and 54% of INH mono-resistant strains [19]. The presence of mutations in inhA gene region vary in different geographical locations from 18% in the study from Vietnam by Huyen., *et al.* [21] to 67.6% in Mongolia by Buyankhishig [23], to 24% from KwaZulu-Natal [30], while van Rie & colleagues reported mutations in the inhA gene region in only 2% isolates in Western Cape Province of South Africa [28], while Raveendran., *et al.* from India reported a prevalence of 5.4% in the inhA gene region in 2012 [20].

Two of the 424 strains showing mutations exhibited complete inhA gene deletion, which is a very rare finding and the probable explanation for the absence of all inhA bands including the inhA locus control band, is a mutation in the primer annealing sequence, which results in missing of the specific amplification product. Both of these strains were MDRTB with katG mutations as well, hence, this presentation would not have any implications on patient treatment.

Out of 544, 23 (4.22%) strains showed low level INH resistance, of which 16 (2.9%) were MDRTB strains and 7 (1.2%) were INH mono-resistant strains. These strains would not have been detected, if it was not for the MTBDR*plus* assay and this kit has increased the sensitivity of detection of INH resistance due to the increase in the number of probes bound onto the DNA strips coding for inhA gene region, which in turn could help the clinician in treatment modulation [5,8,31].

In our study, we had 24 cases (4.41%) of mixed infections which were detected by the MTBDR*plus* assay, which is similar to the findings by Huyen., *et al.* (3.6%) [21]. Of the 24 cases, mixed infections were seen in both, RIF & INH genomic loci in 2 cases (8.3%), while 22 cases (91.7%) showed mixtures either in the RIF or INH genomic loci. This assay can detect mixtures of drug resistant & susceptible bacterial population, though, it was not possible in our study to speciate these strains.

Of the total 251 strains subjected to both, LPA and conventional DST using solid LJ media, there was a 90.84% (228/251 strains) concordance seen in our study, while the 23 (9.16%) discrepant results showed false positive INH resistance in 6 strains by LPA, while being sensitive on conventional DST, and this could be because of a mutation, which might not be phenotypically expressed. Six strains of true positive INH resistance could not be detected on LPA and this could be because of the mutation being present in some other genomic region of INH, which is not incorporated on the DNA strip such as ahpC, kasA or ndh [20]. Hence, these were read as INH sensitive on LPA. False positive results for Rifampicin resistance were seen in 11/23 strains (these strains were Rifampicin sensitive on conventional DST). In this situation, performing a minimum inhibitory concentration (MIC) for these RIF isolates could be of value in confirming whether these strains could be picked up as resistant at a lower drug concentration on solid DST. Rifampicin resistance was not detected in 6 strains by LPA, but these strains were RIF resistant on conventional DST and this could be attributed to new mutations outside the 81 bp-hotspot region not probed on the strips, hence LPA could not detect them.

Evaluation of Molecular Line Probe Assay for Rapid Detection of Mdr-Tb in A Tertiary Healthcare Setting in Mumbai, India

29

To conclude, the MTBDR*plus* assay is a reliable, rapid & easy to perform technique for the simultaneous detection of Rifampicin & INH resistance in *M. tuberculosis*. The test has a high sensitivity & specificity for detection of Rifampicin resistance as well as MDR-TB, but, we also found a high sensitivity & specificity for INH resistance in our study, which suggests that the MTBDR*plus* assay is sensitive for detection of INH resistance. Detection of low-level INH resistance in our study by this MTBDR*plus* assay kit has been an added advantage as this would guide the clinician in recommending the treatment regime for patients with INH mono-resistance. But, on analysing the discordant results, it is finally the phenotypic solid DST which would be reliable & confirmatory in such cases, though, the LPA is rapid and has a turnaround time of around 48-72 hours, while in case of phenotypic DST, patients would have to wait for a period of 12-14 weeks. The commonest mutation detected was presence of S531L codon in the rpoB gene region and S315T1 codon in the katG gene region signifying RIF and INH resistance, respectively. This study may be of high epidemiological significance which analyses LPA with conventional 'gold standard' proportion method for DST.

Conflict of interest statement: Nil

Acknowledgement

References

1. Revised National Tuberculosis Control Programme: Guidelines on Programmatic Management of Drug Resistant TB (PMDT) in India May 2012.

2. WHO: Global Tuberculosis Report, 2015

3. TB India 2016: RNTCP Annual Status Report, Central TB Division. Available

4. Ling I., *et al*. "Rapid diagnosis of drug-resistant TB using line probe assays: from evidence to policy". *Expert Review of Respiratory Medicine* 2.5 (2008): 583-588.

5. Hilleman D., *et al*. "Application of Genotype MTBDR assay directly on sputum samples". *International Journal of Tuberculosis and Lung Disease* 10.9 (2006): 1057-1059.

6. Hilleman D., *et al*. "Use of the genotype MTBDR assay for rapid detection of rifampin and isoniazid resistance in Mycobacterium tuberculosis complex isolates". *Journal of Clinical Microbiology* 43.8 (2005): 3699-3703.

7. Cavusoglu C., *et al*. "Evaluation o the Genotype MTBDR assay for rapid detection of rifampin and isoniazid resistance in Mycobacterium tuberculosis isolates". *Journal of Clinical Microbiology* 44.7 (2006): 2338-2342.

8. Miotto P., *et al*. "Use of Genotype MTBDR assay for molecular detection of rifampicin and isonoazid resistance in Mycobacterium tuberculosis in clinical strains isolated in Italy". *Journal of Clinical Microbiology* 44.7 (2006): 2485-2491.

9. Makinen J., *et al*. "Comparison of two commercially available DNA line probe assays for detection of multidrug- resistant Mycobacterium tuberculosis". *Journal of Clinical Microbiology* 44.2 (2006): 350-352.

10. Brossier F., *et al*. "Performance of the Genotype MTBDR line probe assay for detection of resistance to rifampin and isoniazid in strains of Mycobacterium tuberculosis with low- and high-level resistance". *Journal of Clinical Microbiology* 44.10 (2006): 3659-3664.

11. Manual of Standard Operating Procedures (SOPs), Culture of Mycobacterium tuberculosis and Drug Susceptibility Testing on solid Medium (2009) Revised National Tuberculosis Control Programme ,Central TB Division, (Ministry of Health & Family Welfare) Version No. 01.01

Evaluation of Molecular Line Probe Assay for Rapid Detection of Mdr-Tb in A Tertiary Healthcare Setting in Mumbai, India

30

12. Module for Laboratory Technicians, October 2005, Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, Nirman Bhavan, New Delhi 110011 Second Edition.

13. Winn W., *et al*. 2006 Koneman's color atlas and textbook of diagnostic microbiology 6th ed, Lippincott Williams and Wilkins.

14. GenoType MTBDRplus,version 1.0 (product insert). (Internet) Feb 2007 (updated 2007 Apr 3; accessed 2007 May 9). Nehren, Germany; Hain Lifescience, GmbH. Available.

15. Somoskovi A,Gutierrez C, Paramasivan C. N., Sor E, Arora J, Reddy K. Practical course on the rapid molecular detection of M. Tuberculosis and its resistance to Isoniazid and Rifampicin by PCR based line probe assay.

16. Musser J. "Antimicrobial agent resistance in mycobacteria: molecular genetic insights". *Clin Microbiol Review* 8.4 (1995): 496-514.

17. WHO: Molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis (MDR-TB): Policy Statement, Geneva: World Health Organisation; 2008.

18. Hilleman D., *et al*. "Evaluation of the Genotype MTBDRplus Assay for Rifampicin and Isoniazid Susceptibility Testing of Mycobacterium tuberculosis strains and clinical specimens". *Journal of Clinical Microbiology* 45.8 (2007): 2635-2640.

19. Barnard M., *et al*. "Rapid Molecular Screening for Multi-drug Resistant Tuberculosis in a High Volume Public Health Laboratory in South Africa". *American Journal of Respiratory and Critical Care Medicine* 177.7 (2008): 787-792.

20. Raveendran R., *et al*. "Utility of Geno Type MTBDRplus assay in rapid diagnosis of multidrug resistant tuberculosis at a tertiary care centre in India". *Indian Journal of Medical Microbiology* 30.1 (2012): 58-63.

21. Huyen Mai NT., *et al*. "Validation of the GenoType® MTBDRplus assay for diagnosis of multidrug resistant tuberculosis in South Vietnam". *BMC Infectious Diseases* 10 (2010): 149-156.

22. Farooqui JQ., *et al*. "Line Probe Assay for detection of rifampicin & isoniazid resistant tuberculosis in Pakistan". *Journal of Pakistan Medical Association* 62.8 (2012): 767-772.

23. Buyankhishig BT., *et al*. "Rapid molecular testing for multi-resistant tuberculosis in Mongolia: A diagnostic study". *International Journal of Mycobacteriology* 1.1 (2012): 40-44.

24. Miotto P., *et al*. "Molecular detection of rifampicin and isoniazid resistance to guide chronic TB patient management in Burkina Faso". *BMC Infectious Diseases* 9.142 (2009):

25. Mani C., *et al.* "Mutations in the rpoB gene of multi-drug resistant Mycobacterium tuberculosis clinical isolates from India". *Journal of Clinical Microbiology* 39.8 (2001): 2987-90.

26. Ali A., *et al*. "M. tuberculosis Central Asian Strain 1 MDR isolates have more mutations in rpoB and katG genes compared with other genotypes". *Scandinavian Journal of Infectious Diseases* 41.1 (2009): 37-44.

27. Bahrmand AR., *et al*. "High-level rifampin resistance correlates with multiple mutations in the rpoB gene of pulmonary tuberculosis isolates from the Afganistan border if Iran". *Journal of Clinical Microbiology* 47.9 (2009): 2744-2750.

28. Van Rie A., *et al*. "Analysis for a limited number of gene codons can predict drug resistance of Mycobacteriun tuberculosis in a high incidence community". *Journal of Clinical Microbiology* 39.2 (2001): 636-641.

29. Mokrousov I., *et al*. "High prevalence of katG Ser315Thr substitution among isoniazid resistant Mycobacterium Tuberculosis clinical isolates from Northwestern Russia, 1996-2001". *Antimicrobial Agents and Chemotherapy* 46.5 (2002): 1417-1424.

30. Kiepiela P., *et al.* "Genomic mutations in the katG, inhA and ahpC are useful for the prediction of isoniazid resistance in M. Tuberculosis isolates from Kwa -Zulu Natal, South Africa". *Tubercle and lung disease* 80.1 (2000): 87-56.

31. Somoskovi A., *et al*. "Use of smear-positive samples to assess the PCR-based genotype MTBDR assay for rapid, direct detection of the Mycobacterium tuberculosis complex as well as its resistance to isoniazid and rifampicin". *Journal of Clinical Microbiology* 44.12 (2006): 4459-4463.