RAPID DETECTION OF MULTI-DRUG RESISTANT TUBERCULOSIS USING DIRECT DRUG SUSCEPTIBILITY TESTING AND LINE PROBE ASSAY

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ABSTRACT

Introduction: Tuberculosis, particularly multi-drug resistant tuberculosis (MDR TB), is a leading cause of morbidity and mortality in developing countries. Conventional methods of DST have a long turnaround time of around 2-3 months. Rapid detection of MDR TB can be achieved by direct tests, liquid based indirect susceptibility tests and molecular line probe assays. This study aims to evaluate the utility of Direct DST and line probe assay (LPA) for rapid detection of MDR TB.

Methodology: A total of 510 sputum concentrates (smear 1+/ more) were subjected to Direct DST and LPA. Conventional DST was put up from the primary cultures of all 510 strains using economic variant of 1% proportion method, considered the gold standard.

Results: Direct DST showed 100% specificity and sensitivity of 98.7%, 97.3% and 97.5% respectively for detection of Isoniazid (INH), Rifampicin (RIF) resistance and for MDR strains. LPA showed specificity of 98.4%, 96.8%, 97% and sensitivity of 97.5%, 98.9% and 98.9% for detection of INH, RIF resistance and MDR strains respectively. The commonest mutation pattern detected in our region was S531L (MUT3) for rpoB gene locus signifying RIF resistance + S315T1 (MUT 1) for katG gene locus signifying high level INH resistance.

Conclusion: Direct DST can be used as a good alternative for rapid detection of MDR-TB in resource poor settings. LPA is definitely a good tool, but requires requisite laboratory infrastructure and trained man-power. From our study, rifampicin resistance can be considered as a surrogate marker of MDR TB.

Key Words: MDR TB, Direct DST, LPA

INTRODUCTION

Tuberculosis (TB) continues to be a leading cause of morbidity and mortality in developing countries [1]. Global efforts for TB control are being challenged by the steady increase in drug resistant TB, particularly multidrug resistant tuberculosis (MDR TB), defined as resistance to rifampicin (RIF) and isoniazid (INH) [2]. As per the WHO Global TB Report 2015, there were an estimated 480 000 (range: 360 000-600 000) new cases of MDR-TB globally in 2014 and MDR TB has been reported in 3.3% new and 20% previously treated cases, respectively. [3]

MDR-TB requires 18-24 months of treatment with expensive second line drugs, some of which are parenteral drugs. The cure rate is much lower at about 60% than for drug susceptible TB [4]. Therefore, it is crucial that MDR TB should be detected as early as possible and measures implemented to effectively control its spread.

Detection of MDR-TB could be achieved by phenotypic and genotypic methods of drug sensitivity testing. The phenotypic methods include: a) Conventional methods of solid drug susceptibility testing [5] for detection of MDR TB which include 1% proportion method, resistance ratio and absolute concentration method. These methods require growth from

primary cultures of specimens on Löwenstein Jensen (LJ) medium. Hence, they have a long turnaround time of around 2-3 months. b) Direct tests for DST of mycobacteria in which decontaminated sputum specimens are directly inoculated on drug free and drug containing LJ media. This obviates the need to wait for growth on primary culture, thus, reducing the turnaround time by 4-6 weeks and can be applicable in a resource poor setting. Examples of direct tests are Direct DST [6] on drug containing media, Nitrate Reductase Assay [NRA] [7], Microscopic Observation Drug Susceptibility [MODS] [7, 8]. c) Liquid based indirect susceptibility tests such as MB/BACT, BACTEC and MGIT960 which have an improved turnaround time of about 25 -45 days, as compared to the solid conventional DST, but, these are costly and not available where the need is greatest[7,8]

The genotypic methods for rapid detection of MDR-TB include molecular line probe assays (LPA) which have a turnaround time of 48-72hrs. But, require specific laboratory infrastructure, technical expertise and are expensive. [9]

This study aims to evaluate the utility of DST and LPA for early detection of MDR-TB and also to evaluate rifampicin resistance as a surrogate marker for MDR-TB.

MATERIAL AND METHODS

This was a prospective study carried out in a tertiary care hospital. The study protocol was approved by institutional ethics committee.

Inclusion criteria: Clinically diagnosed pulmonary tuberculosis (PTB) cases with sputum smear 1+ and more

Exclusion criteria: 1. Sputum smear positive PTB cases less than 1+.

2. Smear negative PTB cases.

3. Extrapulmonary (+/-) specimens.

METHODOLOGY:

Each patient was asked to submit two sputum samples (one early morning and one spot sample) as per RNTCP Guidelines [11]. These were then subjected to direct smears by Ziehl Neelsen staining [12] and graded according to RNTCP guidelines [11]. The sputum samples were decontaminated and concentrated using the N-acetyl- L- Cysteine (NALC) – NaOH Concentration Method.[12]

Direct Drug Susceptibility Test (Direct DST) [6]

The sputum deposits obtained by NALC –NaOH method were inoculated with a 5 mm loop (27 SWG) onto a pair

of plain LJ and drug containing LJ media (INH 0.2 μg /ml; R-40 μg/ml) along with a Para nitro benzoic acid (PNB) slant to confirm the growth of *M.tuberculosis*. The slopes were incubated at 37°C and examined weekly for 8 weeks and the growth was recorded weekly.

The definition of resistance was based on the amount of growth seen on the drug-free medium. Thus, when the growth on the drug-free medium was 2+ or more, growth of 1+ or more on the drug containing medium was defined as resistance to the drug. When growth on the drug-free medium is 1+ or less (i.e. < 100 colonies), any growth on the drug-containing medium was considered to be an indication of resistance to that particular drug. For this purpose, the higher growth observed on the paired slopes were considered for interpretation.

Conventional DST by 1% proportion method (economic variant) [5]

Inoculum preparation : With a 3 mm loop, 4-5 mg (loopful) of primary culture was taken & inoculum was prepared in a sterile Bijou bottle containing 2 ml sterile distilled water & 6 sterile glass beads of diameter 3mm. Turbidity of the bacterial suspension was matched with McFarland standard no. 1 (10^7 to 10^8 cfu/ml) against a black background. This was the neat bacterial suspension standardized at 1 mg/ml. Further' 2 log dilutions were prepared. A loopful (using a 3 mm calibrated loop) of each dilution was inoculated on drug free & drug containing media [Isoniazid: 0.2μg/ml, Ethambutol: 2μg/ml, Streptomycin: 4μg/ml and Rifampicin: 40μg/ml.]

The slopes were incubated at 37°C. The growth was read at 28 days and again at 42 days.

(Growth recorded as - Confluent growth = 3+; More than 100 colonies = 2+; Record actual number of colonies = 1-100 cols.)

Interpretation
First reading was taken at 28th day after inoculation. The actual number of colonies (up to 100 colonies on the slope) on drug containing and drug-free slopes were counted.

Proportion of resistant bacilli existing in the strain was obtained by dividing the number of colonies obtained on drug containing slopes by that on drug free slope. Below a certain value – the critical proportion (1%)– the strain is classified as sensitive; above that value, it is classified as resistant. The proportions were reported as percentages. [5]

Internal Quality Control
Along with each batch of samples to be tested, a culture of H37Rv (a standard strain of *M.Tuberculosis*) or a known all

sensitive strain was also incorporated and the test was validated.

Line Probe Assay (LPA) [13]:

Line probe assay is a DNA strip technology using a cellulose acetate membrane strip for the detection of resistance to RIF and/or INH. The kit used at our center for LPA was GenoType MTBDR Plus version 1.0 manufactured by Hain Lifescience, Germany which detects resistance to RIF and/or INH in sputum samples (smear 1+ / more) and culture isolates.. The steps involved are DNA Extraction from sputum concentrates, Multiplex Amplification, reverse hybridization and interpretation of results.

DNA Extraction:

A 500 µl of the smear positive (1+ or more) decontaminated sputum specimen was used for the DNA extraction which involved centrifugation of the specimen at 10,000 g for 15 minutes and suspension of the pellet in 100 µl of molecular grade water. It was then subjected to heat killing at 95ºC for 20 minutes followed by sonication for 15 minutes and finally centrifugation at 10,000g for 5 minutes. 70-80 µl of the supernatant was taken and deep frozen at -20 ⁰C of which 5 µl was used for PCR.

Multiplex amplification:

The master mix was prepared in a DNA-free room and the DNA extract was added to the master mix in a PCR hood. Both these procedures were done in separate rooms following the restricted access and unidirectional work flow protocol. A master mix negative control was also included with every batch of master mix prepared.

The amplification was done using a thermo cycler, with a set programme for amplification i.e. different for direct smear positive sputum specimens and culture isolates

Reverse hybridization:

It involved the chemical denaturation of the amplicons, hybridization of the biotin-labeled amplicons to the single stranded membrane-bound probes on the cellulose acetate membrane strip. It was followed by stringent washing, addition of streptavidine-alkaline phosphate conjugate to the wells and an enzymatic reaction mediated by alkaline phosphatase was observed in the form of a coloured band following addition of substrate in those regions where the amplicon and probe had hybridized. After the final washing, the strips were removed from the wells, allowed to air dry and placed onto the sheet provided with the kit and interpreted.

Each run incorporated one positive control and two negative controls (1 extraction negative control + 1 master mix negative control).

Interpretation of Results

- Valid results can be interpreted by the presence of CC (conjugate control) and AC (amplification control) bands for each sample run. The presence of TUB band indicates that the organism belongs to *M.tuberculosis* complex.
- A mutation in the relevant gene (and resistance to the relevant drug) is signified by either an absence of wild type band and/or the presence of a mutant band for each gene. The rpoB, katG and inhA gene loci each have a control band which needs to be present in order to interpret the results.
- The presence of mutations in the rpoB gene locus predicts RIF resistance, in katG predicts high level INH resistance while in the inhA gene locus predicts low level INH resistance.
- For interpretation of results, the intensity of all the bands (except CC band) should be equal to or greater than the intensity of the AC band.
- In order for a batch of results to be valid, the negative control strip has to have a CC and AC band present, but no other bands.
- Presence of bands other than CC & AC bands in the NC indicates contamination & the run need to be repeated.
- The product insert was referred to for interpretation of banding patterns.

Statistical analysis:

Sensitivity, specificity, positive predictive value, negative predictive value of Direct DST & LPA was calculated considering 1% proportion method the gold standard.

RESULTS

A total of 531 smear positive (1+ and more) sputum specimens were collected from the patients attending the outpatient as well as inpatient departments of tertiary care hospital. Out of these 531 specimens, 9 specimens showed no growth and12 were contaminated on Direct DST, thus could not be processed further by 1% Proportion method. Hence these 21 specimens were excluded from data analysis, since the results of Direct DST and 1% Proportion method (gold standard test for comparison) were not available for them. Primary cultures of the remaining 510 specimens were then subjected to economic variant of 1% proportion method. Thus total 510 specimens (n=510) all of which were subjected to all 3 tests were included for analysis.

Table 1 shows the comparative evaluation of LPA, Direct DST % 1% Proportion method in terms of the number of MDR, RIF and INH monoresistant and pansensitive strains.

Table 2 shows the turn-around time by Direct DST. 74 out of 176 specimens with smear grading of 3+ gave results at

4th week, whereas for 172 out of 183 specimens with smear grading of 1+, results were obtained around 8th week; most of these being sensitive strains. Thus it was observed that higher the smear grading, we obtained the results earlier.

Tables 3A and 3B- Comparison of LPA with proportion method for detection of MDR strains, RIF and INH resistance. LPA has shown a sensitivity of 98.9%, 98.9 % and 97.5 % and specificity of 97 %, 96.8 % and 98.4 % for detection of MDR, rifampicin and isoniazid resistance, respectively.

Tables: 4A and 4B- Comparison of Direct DST with 1% Proportion method for detection of MDR strains, RIF and INH resistance. DST has shown a sensitivity of 97.5%, 97.3 % and 98.7 % and specificity of 100% for detection of MDR, rifampicin and isoniazid resistance, respectively.

Table 5 shows the banding patterns amongst resistant strains by LPA. There were 45 different patterns observed amongst the resistant strains. Commonest pattern (57.1%) seen was absence of wild type 8 band and simultaneous presence of mutation 3 band in the rpoB gene region coding for the S531L codon signifying RIF resistance + absence of wild type band and simultaneous presence of mutation 1 band in the katG gene region coding for S315T1 codon signifying high level INH resistance. 14 (2.7%) strains showed mixed infections and one strain showed complete inhA gene deletion. Low level INH resistance was seen in 13 (2.5%) of the 510 strains, of which 8 (1.5%) were MDR strains and 5 (0.9%) were INH mono-resistant strains.

Table 6 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 in 265(89.8%) of the 295 strains. The katG gene locus showed maximum mutations in the MUT1 band region coding for S315T1 codon amounting to 289(96.3%) of the 300 strains, while in the inhA gene locus maximum mutations were seen in the MUT1 band region coding for C15T codon amounting to 66(91.7%) of the 72 strains.

DISCUSSION

In the present study, we have performed the comparative evaluation of the results of drug susceptibility testing of total 510 sputum specimens by Direct DST, Line probe assay (LPA), and 1% Proportion method, considered the gold standard.

The performance of the Direct DST & the Genotype MT-BDR plus used for LPA test directly from smear positive (1+/ more) sputum specimens correlated very highly with conventional DST by 1% proportion method. (Table: 1)

The Direct DST described here would serve the purpose well in countries with limited resources. In our study, DST has shown a sensitivity of 97.5%, 97.3 % and 98.7 % and specificity of 100% for detection of MDR, rifampicin and isoniazid resistance, respectively. The concordance of the results of the 1% proportion method & Direct DST was found to be 97.6%. Thus, its performance characteristics suggest that the technique is equivalent to conventional 1% proportion method. The turn-around-time of the results obtained by Direct DST are shown in Table 2. Maximum results with smear grading of 3+ were obtained around 4th week, whereas for most of the specimens with smear grading of 1+ results were obtained around 8th week; most of these being sensitive strains. Thus it was observed that higher the smear grading, the results were obtained earlier.

The advantages of the Direct DST over the conventional 1% proportion method are that it gives sensitivity results at the same time as that of primary culture. This process not only reduces the turn-around-time by 4 weeks, but also contamination by eliminating the step of doing a subculture. Most importantly, the results of the Direct DST are more closely the representative of the bacterial population in the given sample unlike in the conventional test, which can suffer from errors of selection when drug susceptibility test is set up from a primary culture. In the Direct DST, resistance can be reported if adequate growth is seen on the drug slopes even when the plain medium is contaminated. Mathew *et al* also have reported the utility of Direct DST in MDR detection. Their results also indicate that resistance could be detected with growth as low as 10 colonies & in total agreement with the results of conventional test.[6] Hence, it is recommended that such results be accepted provisionally & confirmed with the conventional test on subculture.

The performance of Genotype MTBDRplus assay has correlated very well with the conventional 1% proportion method for DST with a sensitivity of 98.9%, 98.9 % and 97.5 % and specificity of 97 %, 96.8 % and 98.4 % for detection of MDR, rifampicin and isoniazid resistance, respectively. This test has the advantage of performing well and giving good results even in those specimens which are finally lost on culture.

Rifampicin resistance due to mutation in the rpoB gene was detected in 288 out of 291 phenotypically resistant RIF isolates. The resultant high sensitivity (98.9 %) for detection of rifampicin resistance found in our study could be attributed to all these mutations being present in the 81 bp- hotspot region of the rpoB gene (Table 6). This is in accordance with various other studies conducted across the world, such as S. Africa, Pakistan, Mongolia, India and Vietnam which have proven the mutations in the 81-bp region alone have got 98-100% sensitivity in detecting RIF resistance.[14-18]

Among all the gene mutations conferring to Rifampicin resistance, codon S531L was found to be the most frequently

encountered mutation in 265 out of 295 (89.8%) which is comparable to the Indian study by Raveendran et al in 2012 (84.6%) [17], but, higher than that reported in various other studies across the world [14,16,18].

Mutations at codon 526 in our study were less common (2.7%) than reported from the studies conducted in various other studies such as S. Africa (8.6%)[14], Europe (15 %) [19], India (19 %)[17], Pakistan (22.5%)[15] and Iran (45. 6%)[20], but higher than the study conducted in Vietnam (1.8%)[18].

Mutations in the rpoB gene at the D516V codon were at 1.4 % in our study which is very low compared to the studies from S. Africa (9.6%)[14] & Europe (44%)[19].

18 isolates (6.1%) showed unknown mutations with the absence of both wild type and mutation band. It could be attributed to a rare mutation in the corresponding genomic region which is not probed on the strip (e.g mutations in the 530-533 region of the rpoB gene other than S531L mutation leading to absence of both, wild type 8 and MUT 3 which codes S531L codon specifically) or it could be a silent mutation since this test only screens the nucleic acid sequence and not the amino acid sequence. Therefore it is possible that mutations that do not cause an amino acid change (silent mutations) will still produce the absence of one of the wild type probes. In such cases phenotypic resistance determination should be considered[13]. If deletion of wild type band is the only indication of RIF resistance and there is no mutation probe, it would be advisable to continue the patient on RIF therapy till phenotypic sensitivity report is available.[17]

Mutations in the katG gene locus were detected in 300 out of 313 (95.8%) INH resistant strains. These were found to be maximum in the S315T1 codon region of the katG gene amounting to 96.3%. This is in accordance with Huyen et al's study from Vietnam [18] & Van Rie et al's study from S, Africa [21]. A high prevalence of katG mutations has been reported in high TB prevalent countries which is attributed to INH resistance & much lower prevalence in low TB-prevalent countries. This could be due to the on-going transmission of the strains in the high- burden settings.[22]

The presence of mutations in the inhA region signifying low level INH resistance accounts for 72 (23%) of the 313 INH resistant strains, with maximum mutations in the C15T (MUT1) region amounting to 66 (91.7%) of the 72 strains. Mutations in the inhA gene region accounting for 23.5% (66/281 strains) in MDRTB cases and 18.8% (6/32 strains) in INH mono-resistant cases were seen in our study, which is similar for MDR strains but lower for INH resistant strains when compared to the study by Barnard et al at 27% of MDR strains and 54% of INH mono-resistant strains.[14] The presence of mutations in inhA gene region vary in different geographical locations from 2% isolates in Western

Cape Province of South Africa [21], 5.4% in the study by R. Raveendran et al[17] from India, 18% in the study from Vietnam by Huyen et al [18], 24% from KwaZulu-Natal [23] to 67.6% in Mongolia[16].

Out of the 327 strains showing mutations one strain 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. Since this strain was MDRTB with katG mutations as well, this presentation would not have any implication on patient treatment.

In our study, low level INH resistance alone was seen in 13 (2.5%) of the 510 strains, of which 8 (1.5%) were MDRTB strains and 5 (0.9%) were INH mono-resistant strains. These strains would not have been detected as MDR by the previous version of the assay (GenotypeMTBDR) which only tested for mutations in the katG gene.[24-26] The GenotypeMTBDRplus assay, with incorporation of additional probes to detect mutations in the inhA gene as well has a better sensitivity for detecting INH resistance.

In our study, 14 cases (2.7%) of mixed infections were detected by this assay showing presence of both the wild type as well as mutant band in the corresponding gene region. This is similar to the findings by Huyen et al[18] (3.6%). Of the 14 cases, mixed infections were seen in both, RIF & INH genomic loci in 5 (35.7%), while 9 (64.3%) out of 14 cases showed mixed infections either in the RIF or INH genomic loci.

In our study 21 discrepant results were obtained with LPA when compared to conventional DST, thus giving concordance of 95.9% (489/510 strains). 7/16 strains detected as RIF resistant by LPA were found to be sensitive on conventional DST. 5 out of these 7 false positive RIF resistant strains showed the presence of silent/unknown mutation which was not phenotypically expressed. In this situation results of phenotypic DST are confirmatory. For the rest of the false positive RIF resistant strains, performing phenotypic DST using minimum inhibitory concentration (MIC) would be confirmatory to check whether these isolates are showing resistance on a lower drug concentration. Rifampicin resistance was not detected in 3 strains by LPA which were RIF resistant on conventional DST. This could be attributed to other mutations outside the 81 bp-hotspot region which are not probed on the strip, hence LPA could not detect them. INH resistance was detected in 3 strains by LPA which were INH sensitive by conventional DST; 2 of which showed an unknown/ silent mutation which was not phenotypically expressed and the remaining 1 strain showed low level INH resistance on LPA. LPA could not detect INH resistance in 8 strains which were resistant by conventional DST. This could be because

of the mutation being present in other genomic loci such as kasA, intergenic region of oxyR-ahpC complex which are not probed on the DNA strip.[27,28]

This study also evaluated detection of monoresistance to INH & RIF. This approach was adopted because these 2 drugs are the key elements in short course TB chemotherapy & provide the most robust drug susceptibility results. Moreover, the only susceptibility data that are likely to be translated into clinical action (in terms of a change in drug regimen) in a programmatic setting would be identification of MDR TB. This is also seen with other well standardized methods, as resistance results for ETH and STR are less reliable than for INH and RIF. We found a higher incidence of INH monoresistance as compared to RIF monoresistance which is in accordance with the other studies worldwide. [14,16,18]

Because RIF mono resistance is rare, resistance to RIF is considered a surrogate marker of MDR TB. [29] Our results (Table 1) show that RIF resistance can be considered as a surrogate marker for MDR by all the three tests. Patients infected with RIF resistant strain of MTB generally have a poor prognosis, particularly because RIF resistance is often associated with resistance to other first line drugs.

Hence to summarize, successful treatment of MDR TB relies on prompt laboratory detection of drug resistance. This study found that Direct DST is a good rapid technique in resource-constraint settings. LPA is also a good rapid technique for rapid detection of MDR TB, but, it needs specific laboratory infrastructure, trained personnel, is expensive and needs an external quality assurance system. Both the techniques were well comparable with the gold standard 1% proportion method. Therefore, development of efficient laboratory strategies for rapid and reliable drug susceptibility testing of *M tuberculosis* is of importance for proper management of patients, particularly those with multi-drug resistant tuberculosis.

CONCLUSIONS

The results of this study have led us to following conclusions.

1. Direct DST can be used as a good method, extremely feasible for rapid drug susceptibility testing of *M tuberculosis* in resource poor settings. Similarly, Direct DST can also compete with LPA in terms of turnaround time (as cheaper technique and less turnaround time compared to1% proportion method).
2. Line probe assay is definitely a good tool for rapid detection of MDR provided one has the requisite laboratory infrastructure and technical expertise.
3. 1% Proportion method still continues to remain the gold standard, when it comes to smear negative cases as well as to identify extensively drug resistant (XDR)

cases of tuberculosis.
4. Rifampicin resistance can be considered as a surrogate marker for MDR-TB in settings with high prevalence of drug resistant tuberculosis.

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Table 1: Comparison of LPA, DST & 1% Proportion method

	MDR	Mono INH Resistant	Mono RIF Resistant	Pansensitive
LPA	281 (55%)	32 (6.2%)	14 (2.7%)	183 (35.8%)
Direct DST	270 (52.9%)	44 (8.6%)	13 (2.5%)	183 (35.8%)
1% Proportion method	277 (54.3%)	41 (8.0%)	14 (2.7%)	178 (34.9%)

Table 2: Turn-around time by Direct DST

Smear Result	Weeks							Total
	2	3	4	5	6	7	8	
3+	-	23	74	52	19	-	8	176
2+	-	-	5	15	71	-	60	151
1+	-	-	-	3	7	1	172	183

Tables 3A and 3B: Comparison of LPA with proportion method for detection of MDR strains, RIF and INH resistance

Table 3A:

	LPA	PROPORTION METHOD	
		R	**S**
MDR	**R**	**274 (53.7%)**	**07 (1.4%)**
	S	03(0.6%)	226 (44.3%)
RIF	R	288 (56.5)	07 (1.4%)
	S	03 (0.6%)	212 (41.6%)
INH	R	310 (60.8%)	03 (0.6%)
	S	08 (1.6%)	189 (37.1%)

Table 3B:

	MDR	RIF	INH
SENSITIVITY	98.9%	98.9%	97.5%
SPECIFICITY	97%	96.8%	98.4%
POSITIVE PREDICTIVE VALUE	97.5%	97.6%	99.0%
NEGATIVE PREDICTIVE VALUE (NPV)	98.7%	98.6%	95.9%

Tables 4A and 4B: Comparison of Direct DST with 1% Proportion method for detection of MDR strains, RIF and INH resistance

Table 4A:

	Direct DST	PROPORTION METHOD	
		R	S
MDR	R	270 (52.9%)	00 (0%)
	S	07(1.4%)	233 (45.7%)
RIF	R	283 (55.5)	00 (0%)
	S	08 (1.6%)	219 (42.9%)
INH	R	314 (61.6%)	00 (0%)
	S	04 (0.8%)	192 (37.6%)

Table 4B:

	MDR	RIF	INH
SENSITIVITY	97.5%	97.3%	98.7%
SPECIFICITY	100%	100%	100%
POSITIVE PREDICTIVE VALUE	100%	100%	100%
NEGATIVE PREDICTIVE VALUE (NPV)	97.1%	96.5%	98%

Table 5: Banding patterns amongst resistant strains by LPA

Sr No	rpoB	katG	inhA	No.
1	WT8A M3P	WTA M1P		187 (57.1%)
2	WT8A M3P	WTA M1P	inhA gene deletion	1 (0.3%)
3	WT8A M3P	WTA M1P	WT1A M1P	51 (15.6%)
4	WT8A M3P		WT1P M1P (mixed infection, low level)	1 (0.3%)
5	WT8A M3P		WT1A M1P (low level)	3 (0.9%)
6	WT8A (silent)	WTA (silent)		1 (0.3%)
7	WT8A (silent)	WTA M1P		1 (0.3%)
8	WT8A M3P	WTA M2P		2 (0.6%)
9	WT8A M3P	WTP M1P (mixed infection)		1 (0.3%)
10	WT8A M3P	WTA (silent)		1 (0.3%)
11	WT8A M3P		WT1,2A (silent, low level)	1 (0.3%)
12	WT8A M3P	WTA M1P	WT1A (silent)	1 (0.3%)
13	WT8A M3P	WTA M1P	WT2AA M3AP	1 (0.3%)
14	WT8A M2AP	WTA M1P		1 (0.3%)
15	WT8A (silent)	WTA M1P	WT1A M1P	1 (0.3%)
16	WT7A M2AP	WTA M1P		3 (0.9%)
17	WT7A M2AP	WTA M1P	WT1A (silent)	1 (0.3%)
18	WT7A (silent)	WTA M1P		1 (0.3%)
19	WT7,8A M2AP		WT1A M1P (low level)	1 (0.3%)
20	WT7A M2BP	WTA M1P	WT1A M1P	1 (0.3%)
21	WT3,4A (silent)		WT1A M1P (low level)	1 (0.3%)
22	WT3,4A (silent)	WTA M1P		2 (0.6%)
23	WT3,4A M1P	WTA M1P		3 (0.9%)
24	WT3,4A (silent)	WTA (silent)		1 (0.3%)
25	WT3A (silent)	WTA (silent)		1 (0.3%)
26	WT3,4A M1P	WTA M2P		1 (0.3%)
27	WT2,3,4A (silent)	WTA M1P		1 (0.3%)
28	WT2A (silent)	WTA M1P		2 (0.6%)
29	WTP M3P (mixed infection)	WTP M2P (mixed infection)		1 (0.3%)
30	WTP M3P (mixed infection)	WTA M1P		1 (0.3%)
31	WTP M3P (mixed infection)	WTA M1P	WTP M3AP (mixed infection)	2 (0.6%)
32	WTP M3P (mixed infection)	WTP M1P (mixed infection)		2 (0.6%)
33	WTP (mixed infection)		WT1A M1P (low level)	1 (0.3%)
34	WT2,3A (silent)	WTA M1P		1 (0.3%)
35		WTA M1P		21 (6.4%)
36		WTA M1P	WT1AM1P	1 (0.3%)
37		WTPM1P (mixed infection)		2 (0.6%)
38		WTA (silent)		3 (0.9%)
39			WT1AM1P (low level)	5 (1.5%)
40	WT8AM3P			5 (1.5%)
41	WT8A (silent)			1 (0.3%)
42	WT7A (silent)			2 (0.6%)
43	WT7A M2AP			1 (0.3%)
44	WTPM3P (mixed infection)			3 (0.9%)
45	WT3,4A (silent)			2 (0.6%)

Table 6: Distribution of mutations

Locus	Mutant	Codon	No. of Strains
rpoB –WT1 –WT8	Mut 1	D516V	4 (1.4%)
	Mut 2A	H526Y	7 (2.4%)
	Mut 2B	H526D	1 (0.3%)
	Mut 3	S531L	265 (89.8%)
	Unknown		18 (6.1%)
		Total	295
katG – WT	Mut 1	S315T1	289 (96.3%)
	Mut 2	S315T2	4 (1.3%)
	Unknown		7 (2.3%)
		Total	300
inhA – WT1 – WT2	Mut 1	C15T	66 (91.7%)
	Mut 2	A16G	0
	Mut 3A	T8C	03 (4.1%)
	Mut 3B	T8A	0
	Unknown		3 (4.1%)
		Total	72