

Molecular Detection of Rifampicin and Isoniazid Resistant *Mycobacterium Tuberculosis* and Their Associated Mutation Pattern From Smear Positive Sputum Samples From a Tertiary Care Centre of West U.P.

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Abstract

Background: Delayed diagnosis and treatment done on its basis has been proven to be a factor causing increase in rate of MDR TB. So we used GenoType MTBDR plus assay® in order to determine rate of MDR-TB, Isoniazid and Rifampicin mono resistance and common mutation pattern associated with them from our area in order to provide better patient care and reduce rate of MDR-TB. **Subjects and Methods:** This was a cross sectional prospective study comprising of 150 smear positive sputum samples collected during period of 1½ years from January 2018 to April 2019. **Results:** Out of total 150 smear positive samples 97 were from male patients and 53 from female patients. Rate of MDR TB found was 18/150 (12%), rifampicin mono resistance and isoniazid mono resistance was 10/150 (6.6%) and 13/150 (8.6%) respectively. Highest percentage of MDR TB was seen among defaulter cases (27.3%) followed by failure cases (25%) and relapse cases (12.5%). Mutation pattern most commonly reported for rifampicin is S531L in *rpoB* gene at codon 530-533 and for isoniazid was S315T1 in *katG* gene at codon 315. **Conclusion:** Problem of MDR-TB is continuously rising, so rapid detection of TB along with drug sensitivity is the need of hour and should be given utmost priority for END TB strategy. The GenoType MTBDR plus (LPAs) assay approved by world health organization is highly specific (≥99%) and sensitive (≥97%) diagnostic test for the detection of first line drugs resistance and is a rapid and reliable diagnostic tool.

Keywords: MDR-TB, LPA, *rpoB*, *katG* gene.

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Introduction

Tuberculosis (TB) cases are on a continuous rise and create a major public health problem. India has the quarter burden of world's tuberculosis and has highest number of newly diagnosed cases annually recorded so, there is an urgent need for rapid detection of drug resistance among TB patients.^[1] Despite implementation of revised national tuberculosis control programme, free DOTS therapy and other meticulous effort by government TB control is still a challenge for all public sector officials and doctors associated with its control programme. Line Probe Assay (LPA) is a rapid technique which is based on combination of polymerase chain reaction (PCR) followed by the DNA reverse hybridization strip assay, that is used to detect *Mycobacterium tuberculosis* (MTB) complex as well as drug sensitivity to rifampicin and isoniazid in very short duration.^[2,3]

Multidrug resistance tuberculosis arises due to random mutations which is seen or found in *rpoB* and *katG* genes and early detection of these mutation patterns is very

helpful for diagnosis and giving the appropriate therapy to the patients. LPA technique does not require the organism growth, and can be performed directly on the clinical samples. Rifampicin and Isoniazid are the main drugs of first-line anti-TB therapy, and resistance to these drugs can result in treatment failure, increased treatment cost and poor prognosis. LPA technique, by targeting mutation at the 81-base pair core region of the *rpoB* gene for rifampicin resistant detection and additional for isoniazid resistant strains have mutations with *katG* gene at codon 315.^[4]

Aim and Objective: - In India various studies reported that, rate of MDR-TB is high and continuously rising and rapid detection can be a key to reduce this spread. So, we set out to determine rate of MDR-TB with their common mutation pattern and risk factors associated with drug resistant TB from this area using MTBDR plus GenoType assay.

Subjects and Methods

This was a cross sectional prospective study carried out in the Pulmonary Medicine Department and Molecular Section

of Microbiology Department, comprising of 150 smear positive sputum samples collected during period of 1½ years from January 2018 to April 2019.

Sample Collection- Sputum samples were collected from the patients in a sterile, clear universal container who were attending OPD of pulmonary medicine department of Teerthankar Mahaveer Hospital# and these samples were sent to molecular section of Microbiology department.

Case Selection- Patients included in the study were new cases having symptoms of TB or previously treated pulmonary TB cases suspected to be MDR-TB from all age groups. Previously treated patients were categorised into defaulter, relapse or failure cases. Defaulter cases were those who left treatment in between the course of DOTS therapy. Failure cases were those patients who were not cured by first line anti-tubercular drugs and Relapse cases were those patients who were cured completely but symptoms reoccurred in them.

Zeil-Neelson (ZN) staining was done on collected samples and only smear positive samples were further processed for decontamination.

Decontamination- was done by mixing N-acetyl-L-cysteine (NALC) and Sodium hydroxide (NaOH) with sample in equal volume. This mixture is used within 24 hours of preparation.^[5]

DNA extraction- 500µl of the decontaminated specimen is taken in a microcentrifuge tube and centrifuged at 13000rpm for 15 minutes then supernatant was discarded and pellet is re-suspended in 100µl of molecular grade water and incubated in a heating bath at 95°C for 20 minutes. After this, it was kept in sonicator for 15 minutes.^[5]

Mastermix Preparation- Master mix was prepared by combining the 5µl extracted DNA with the specific primers (AM-A and AM-B) as per instruction in kit manual and then this mixture was kept in thermal cycler for amplification.

Amplification- Amplification was done in Veriti® 96 well thermocycler by Thermofisher™. For amplification denaturation was done for 15 minutes at 95°C followed by annealing at 65°C for 2 minutes and then extension at 70°C for 30 seconds repeated for 20 times followed by a final extension at 70°C for 8 minutes.

Hybridization- Hybridization was performed using hybridization kit of HAIN Life sciences® VER. 2.0 in a Twincubater / shaking water bath which is a semiautomatic washing and shaking device. Hybridization steps were performed using reagents provided with the kit as per manufacturer's instruction.^[5]

Results

A total 150 sputum samples which were smear positive were included in this study, out of this 10 (6.0%) and 13 (8.6%) were rifampicin and isoniazid mono resistant respectively. 18 (12.0%) cases were detected as Multi drug resistant (MDR). In our study a higher rate of rifampicin and isoniazid mono-resistance and MDR rate was found

among male patients comparatively to female i.e. 7.2%, 9.2% and 11.3% respectively. [Table 1]

Table 1: Sex-wise distribution of MDR and Mono drug resistant TB.

Male	Female	Sensitivity
7 (7.2%)	3 (5.6%)	RIF mono resistant (n=10; 6.0%)
9 (9.2%)	4 (7.5%)	INH mono resistant (n=13; 8.6%)
11 (11.3%)	7 (13.2%)	MDR (n=18; 12.0%)
70 (72.3%)	39 (73.5%)	Sensitive to RIF & INH (n=109; 72.6%)
97	53	Total (n=150)

Table 2: Mono resistant and MDR status on the basis of treatment history.

	Failure	Relapse	Defaulter	New cases
RIF mono resistant (10)	3 (18.7%)	-	4 (12.1%)	3 (3.4%)
INH mono resistant (13)	1 (6.2%)	2 (14.3%)	6 (18.1%)	4 (4.6%)
MDR (18)	4 (25%)	2 (14.3%)	9 (27.3%)	3 (3.4%)
Sensitive to RIF & INH (109)	8 (50%)	10 (71.4%)	14 (42.4%)	77 (88.5%)
Total (150)	16 (10.6%)	14 (9.3%)	33 (22.0%)	87 (58.0%)

We also found in our study higher rate of rifampicin and isoniazid mono-resistant and MDR rate in defaulter category was 12.1%, 18.1% and 27.3% respectively followed by failure and relapse, So the chances of getting drug resistant TB is higher in defaulter cases. [Table 2]

Table 3: Age wise distribution.

Age	RIF mono resistant	INH mono resistant	MDR	Sensitive to RIF & INH
0-20	1	2	2	15
21-40	2	5	5	23
41-60	5	4	6	44
Above 60	1	2	4	29

In our study majority of patients were in age group of 41-60 years followed by above 60 years age group. [Table 3]

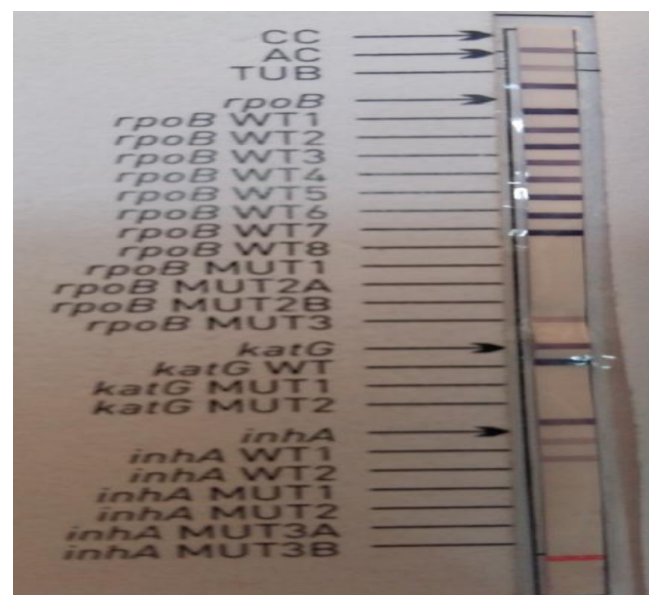


Figure 1: RIF mono resistant

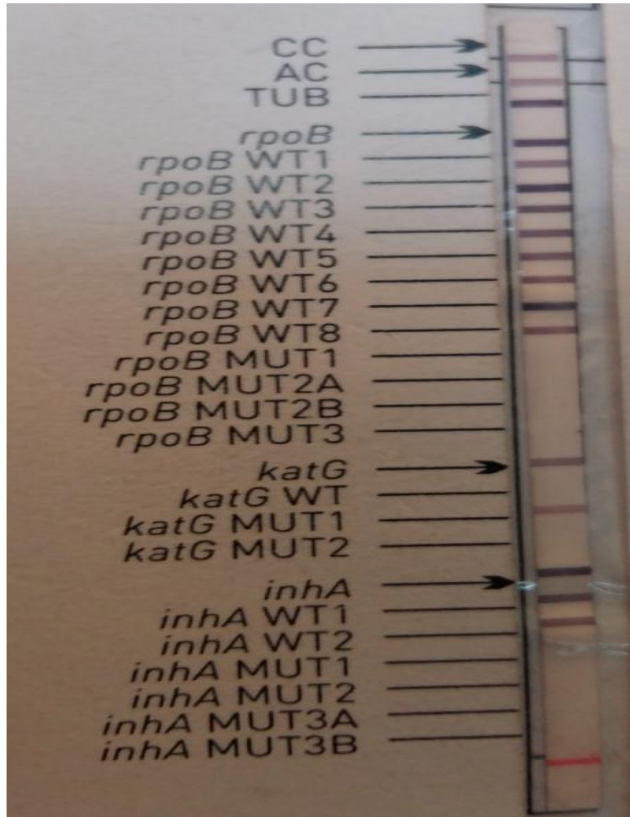


Figure 2: INH mono resistant

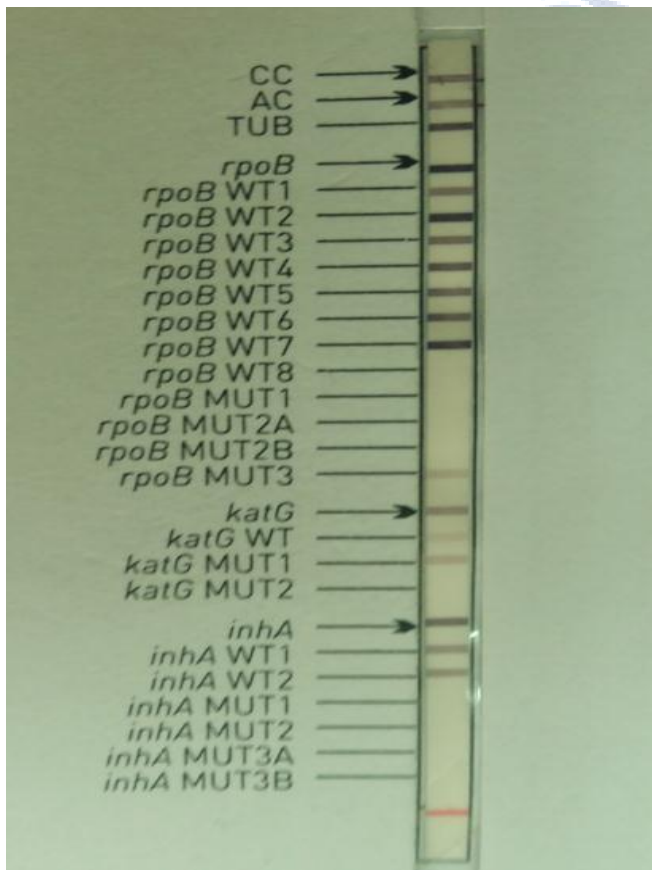


Figure 3: MDR

Mutation in *rpoB* gene: We found commonest mutation pattern causing rifampicin resistance was S531L in *rpoB* gene at codon 530-533 which was in found in more than 80% cases of rifampicin mono resistant and Multi drug resistant tuberculosis (MDR- TB).

Mutation in *katG* gene: Most common mutation pattern for isoniazid was S315T1 in *katG* gene at codon 315 which was found in more than 90% cases of isoniazid mono resistant and MDR-TB.

Discussion

In 2015-16, there is estimated 10.4 million new TB cases reported worldwide, of which 5.9 million were male and 3.5 million were female. One million Children were infected with TB. Smear microscopy is the major diagnostic tool for tuberculosis, especially in resource limited, high-TB burden developing countries such as India. However, the technique has low sensitivity and highly observer dependent, and is incapable in detecting drug resistance.^[15] So, there is need for rapid diagnostic tool, for detection of TB along with its resistance pattern. Here, we evaluated the utility of the latest LPA (version 2.0) for the rapid diagnosis of TB with their drug resistance pattern.^[6]

In this study, we observed that TB infection rate was found more in male than female and the ratio of male to female was 2:1. Similarly, high male to female ratio was reported by Jatav K et al.^[7] This could be due to males are more commonly exposed to risk factors like smoking. Rate of rifampicin and isoniazid mono resistant was found in this study was 6% and 8.6% respectively, which is similar as reported by Singhal R et al;^[8] in 2015 and Thakur C et al;^[9] in 2015 who reported, resistant to rifampicin is 4.6% and resistant to isoniazid 7.3% and rifampicin resistant rate of 6.1% and isoniazid resistant rate of 8.6% respectively. MDR TB rate found is 12% which is higher to study done by by Malhotra B et al;^[10] from Jaipur reported 4.5% and lower than the study done by Singhal S et al;^[11] in 2014 who reported MDR occurrence rate is 55%. Rate of MDR - TB is found high in defaulter cases than relapse and failure cases. This similar finding was reported by Ahmad S et al;^[12] in Aligarh. This finding indicates that defaulter cases are high risk to get mono resistant and MDR.

Most common mutation pattern found in this study for rifampicin was S531L in *rpoB* gene at codon 530-533, this similar finding is reported by Niudrat S et al;^[13] The second most common mutation pattern which is found in this study for isoniazid was S315T1 in *katG* gene at codon 315 and this finding is supported by Sharma BK. et al;^[14] in Nepal.

Conclusion

For sensitivity testing of both first line drugs LPA is the only method which is FDA approved and WHO endorsed rapid, sensitive and specific method for smear positive sputum samples. We found a high rate of MDR-TB from our area. This study highlights the need for quick identification and resistance pattern determination of

tuberculosis cases on routine basis in order to reduce the current rate of MDR-TB and improve patient care. We also found there are high rates of RIF and INH mono resistance in this area which should be kept in mind while treatment. We also found there is increased risk of MDR-TB in defaulter category of patient, so appropriate TB control guidelines can be implemented to focus on this category of patients.

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