IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
August 2022 Vol.:25, Issue:1

Rapid Molecular Detection of Drug Resistance Using Line Probe Assay in Pulmonary Tuberculosis Patients Attending Apollo General Hospital, Hyderabad

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ISSN 2349-7203
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S Manick Dass*[1], Raghavendra Rao M.V[2], M.S. Ratnamani[3], Gouri Priyanka[4], G Pavani[5], Dilip Mathai[6], M.A. Kareem[7]

1.Professor & HOD Dept. Of Microbiology Apollo institute of Medical sciences and Research, India. 2.Scientist-Emeritus and Director Research, Microbiology Apollo institute of Medical sciences and Research, India. 3. Asst Professor, Microbiology Apollo institute of Medical sciences and Research, India. 4. 2nd MBBS student Microbiology, Apollo institute of Medical sciences and Research, India. 5. Professor of Microbiology, Apollo institute of Medical sciences and Research, India. 6. Professor, Dean, Apollo institute of Medical sciences and Research, India. 7.Tutor Dept. Microbiology, Microbiology Apollo institute of Medical sciences and Research, India.

Submitted: 25 July 2022
Accepted: 31 July 2022
Published: 30 August 2022

Keywords: LPA (LINE PROBE ASSAY) test, Multi-drug-resistant tuberculosis (MDR-TB), Nucleic Acid Amplification Technologies (NAAT), multiplex PCR.

ABSTRACT

Infections are the leading cause of death worldwide; among all kinds of infections, bacterial infections are more common and frequent. *Mycobacterium tuberculosis*, primarily affects lungs, although it can attack other parts of the body Tuberculosis is the second biggest killer LPA (LINE PROBE ASSAY) test provides an early accurate diagnosis of drug-resistant tuberculosis among the culture positive pulmonary isolates by reverse hybridization DNA strip method. Molecular methods like LPA may detect silent mutations that do not confer phenotypic drug resistance, therefore presenting false resistant results. All mutations resulting in the drug resistance tuberculosis are not covered in commercial assay.

INTRODUCTION

Tuberculosis is a major public health problem, particularly in developing countries. The WHO estimates that one-third of the world's population is infected with *Mycobacterium tuberculosis*. Multi-drug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) are threats to the elimination of tuberculosis (TB) worldwide.[1]

In a high tuberculosis burden country like India, there is a need of rapid diagnostic test for early diagnosis and detection of drug resistance. The ability to rapidly and accurately detect drug resistance in *Mycobacterium tuberculosis* from clinical specimens is essential for appropriate treatment to be initiated in patients suffering from TB and for the prevention of further spread of drug-resistant strains. Line probe assay, endorsed by the WHO is a promising rapid genotypic tool for timely and appropriate treatment.[2]

The prevalence of multi-drug resistance TB (MDR-TB) is rising in several geographic regions.[3] In MDR-TB infectiousness is frequently prolonged, jeopardizing efforts to control TB. The conventional tuberculosis drug susceptibility tests are sensitive and specific, but they are not rapid. Nucleic Acid Amplification Technologies (NAAT) based on the amplification of specific fragments of nucleic acids (usually followed by hybridization to specific probes to ensure specificity) offer a rapid alternative to conventional bacteriological methods. Rapid identification is essential for effective treatment and control of MDR-TB.

OBJECTIVES

To perform Line probe assay on clinical isolates from pulmonary tuberculosis for the detection of resistance to first line and second-line anti-tubercular drugs.

MATERIAL AND METHODS

Study design: Cross-sectional study

Site: Apollo General Hospital, Hyderabad

Study Population: Cross-sectional study will be conducted in the Department of Medicine of Apollo Institute of Medical Sciences and Research, (AIMSR General Hospital), Hyderabad.

Duration of study: 2 months

Sample size: 30

Method:

Line Probe Assay [4-8] for the identification of *M. tuberculosis* and the detection of resistance to first and second-line ATT drugs.

Geno Type MTBDRsl (GTsl) - Hain Life Science

Samples would be subjected to Geno Type MTBDRsl, based on DNA reverse hybridization technology and allows for the molecular identification of the *Mycobacterium tuberculosis* complex and its associated genotypic susceptibilities to first and second-line ATT drugs. For each day the total number of positives and the negatives are noted. At the end the entire data is compiled and analyzed.

Procedure:

The molecular LPA can therefore be divided into three procedures.

- DNA extraction from cultured isolates (solid or liquid media)/smear-positive clinical isolates.
- Amplification of genes with biotinylated primers using multiplex PCR.
- Reverse hybridization, where probes (reaction zones or bands) on the strips are used to identify the *M. tuberculosis* target DNA associated with RMP and INH resistance.

OBSERVATION AND RESULTS

Total number of the positive and the negative samples for pulmonary tuberculosis were noted and LPA was performed from culture-positive isolates. At the end, the entire data was compiled and analyzed. The results were analyzed based on the drug resistance pattern.

Out of 32 pulmonary isolates 15 (46.87%) isolates were smear positive and all (100%) were culture positive. Valid results were obtained in LPA for all 32 culture isolates.

TOTAL NO OF ISOLATES	SMEAR POSITIVE	CULTURE POSITIVE
32	15	32

Total smear positive	Total smear negative	Total samples
15	17	32

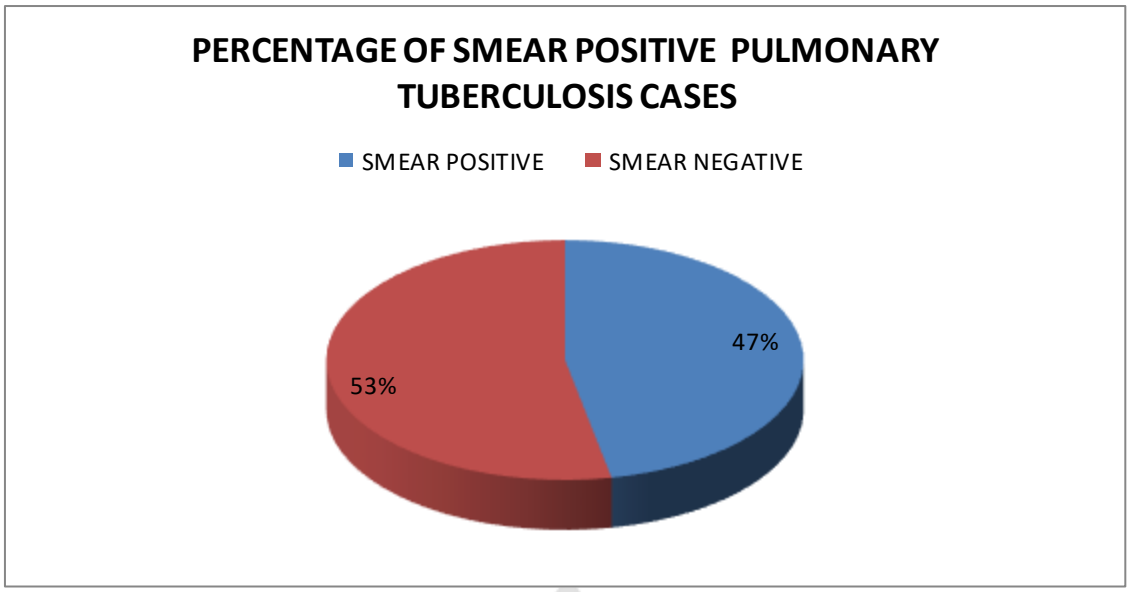

Fig 1: PERCENTAGE OF SMEAR POSITIVE PULMONARY TUBERCULOSIS CASES

Out of 32 pulmonary isolates 15 (46.87%) isolates were smear positive and 17 (53.13%) isolates were smeared negative. LiPA is reliable test for the detection of drug resistance from smear positive samples which saves time.

Table 1: Drug susceptibility patterns of MYCOBACTERIUM TUBERCULOSIS BY LPA

NO OF STRAINS	RIF	INH	EMB	FQ	AG
22	**S**	**S**	**S**	**S**	**S**
1	R	R	R	R	R
1	S	R	S	S	S
5	R	R	S	S	S
1	R	R	S	R	S
1	R	R	R	R	S
1	R	R	R	S	S

Out of 32 culture positive pulmonary isolates, 22 (68.75%) isolates were susceptible to both first line and second-line drugs. 10 (31.25%) were resistant to any of the first line and second-line drugs out of which 5 (50%) were resistant to both rifampicin and isoniazid (MDRTB); 1(10%) was resistant to isoniazid only (mono-resistant to INH); 1 (10%) was resistant to isoniazid, rifampicin and fluoroquinolones (MDRTB); 1(10%) was resistant to isoniazid, rifampicin and ethambutol (MDRTB); 1(10%) was resistant to isoniazid, rifampicin, fluoroquinolones and ethambutol (MDRTB), 1(10%) was resistant to isoniazid, rifampicin, fluoroquinolones, ethambutol and aminoglycosides (XDRTB to be confirmed by phenotypic methods).

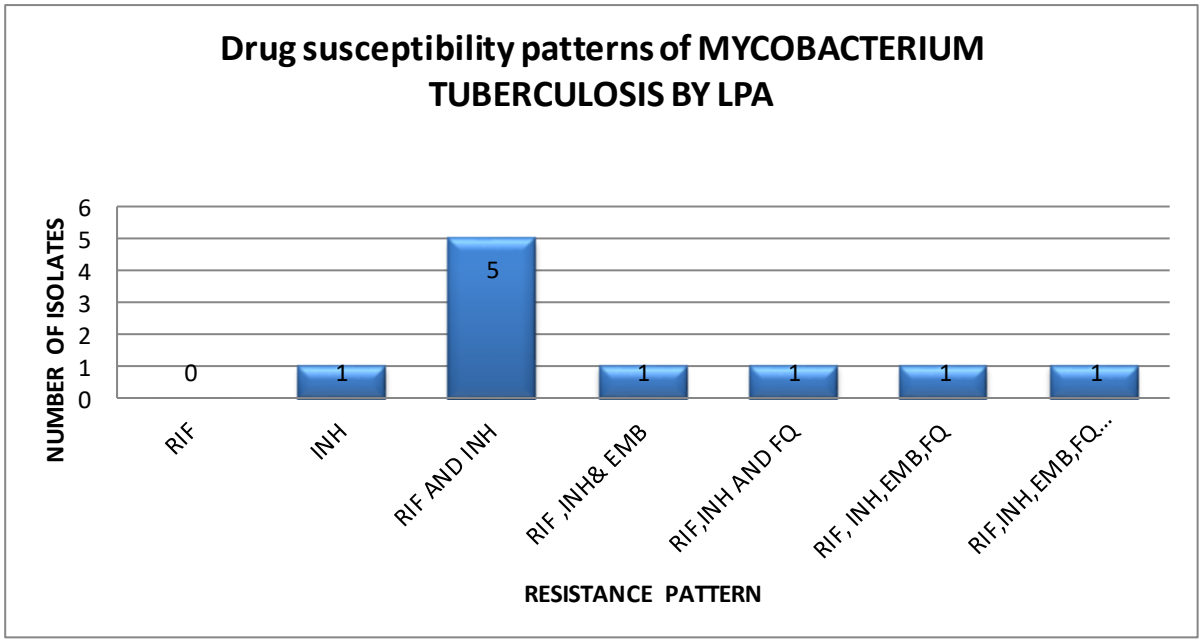

Fig 2: Drug susceptibility patterns of MYCOBACTERIUM TUBERCULOSIS BY LPA

- Resistant to both RIF and INH = 5
- Resistant to INH only = 1
- Resistant to INH, RIF and FQ = 1
- Resistant to RIF, INH, EMB = 1
- Resistant to RIF, INH, FQ and EMB = 1
- Resistant to RIF, INH, FQ, EMB and AG = 1

Table 2: Drug susceptibility patterns of MDR *M. tuberculosis*

NO OF STRAINS	RIF	INH	EMB	FQ	AG	INTERPRETATION
5	R	R	S	S	S	**MDRTB**
1	R	R	S	R	S	**MDRTB**
1	R	R	R	R	S	**MDRTB**
1	R	R	R	S	S	**MDRTB**
1	R	R	R	R	R	**XDRTB to be confirmed by phenotypic methods**

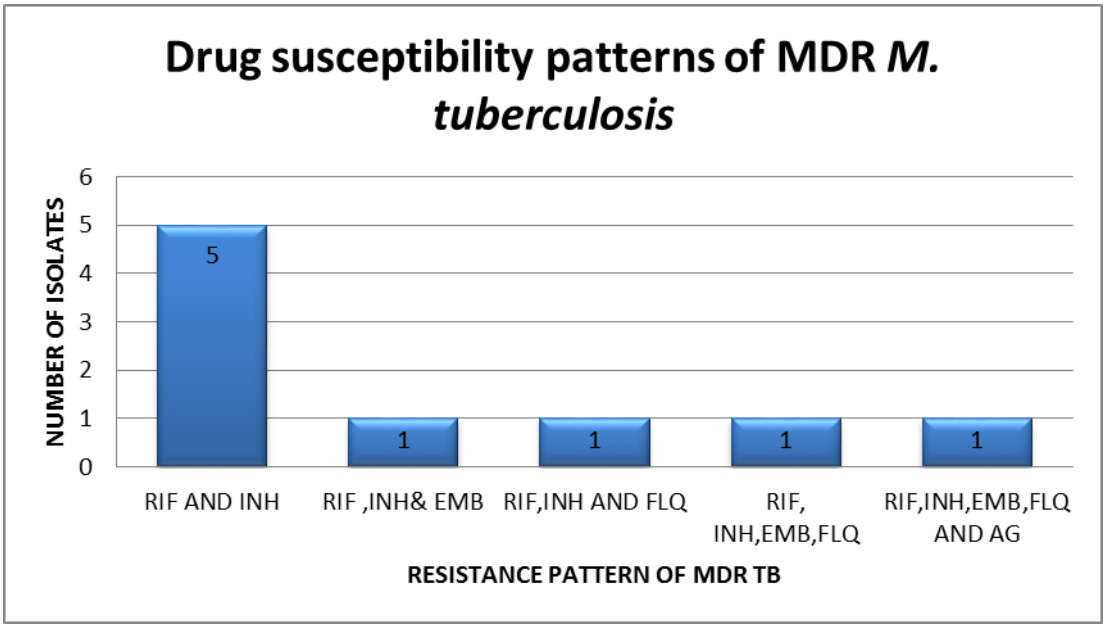

Fig 3: Drug susceptibility patterns of MDR *M. tuberculosis*

- Resistant to both RIF and INH = 5
- Resistant to INH, RIF and FQ = 1
- Resistant to RIF, INH, EMB = 1
- Resistant to RIF, INH, FQ and EMB = 1
- Resistant to RIF, INH, FQ, EMB and AG = 1

In our study of 32 isolates subjected to LiPA we found one case of XDRTB which was resistant to isoniazid, rifampicin, ethambutol, fluoroquinolones, and aminoglycosides but it is to be confirmed by phenotypic methods.

Table 3: GENES RESPONSIBLE FOR RESISTANCE TO FIRST LINE AND SECOND LINE DRUGS OF TUBERCULOSIS

DRUGS	GENES RESPONSIBLE FOR RESISTANCE	Number isolates
RIF	rpoB	9
INH	katG	9
	inhA	1
EMB	embB	3
FQ	gyrA	3
AG	rrs	1

==> Out of 10 isolates

- 9 (90%) were resistant to Rifampicin and the gene responsible for resistance is rpoB gene.
- 10 (100%) were resistant to Isoniazid out of which katG gene is responsible for resistancein 9 (90%) isolates and inhA gene is responsible for resistance in 1 (10%) isolate.
- 3 (30%) were resistant to ethambutol and the gene responsible for resistance is embB gene.
- 3 (30%) were resistant to fluoroquinolones and the gene responsible for resistance is gyrA gene.
- 1 (10%) was resistant to aminoglycosides and the gene responsible for resistance is rrs gene.

DISCUSSION

Line probe assay is a reverse hybridization molecular DNA assay that allows the identification of tuberculosis and also the most common genetic mutations which are responsible for resistance to first and second line tuberculosis drugs. This DNA reverse hybridization technology is based on the detection of species-specific sequences in the 23S

rRNA gene. Line probe assay can rapidly detect more than 90 % commonly encountered isolates of Mycobacteria from various clinical specimens.[16-18].

The LPA directly detect the organism and drug resistance pattern by analyzing the mutation in the rpoB region (confer resistance to rifampicin) , katG and inhA (confer resistance to Isoniazid), embB (confer resistance to ethambutol) , gyrA (confer resistance to fluoroquinolones), rrs (confer resistance to aminoglycosides). Missing of wild band along with presence or absence of band which got mutated on the specific region results in the identification of resistant genotype by line probe assay. Uncommon mutations resulting in occurrence of resistance can only be identified by performing the sequencing analysis. The Line Probe Assay was performed as per the standard protocol. The standard turnaround time for performing LPA is 2 – 3 days as per the guidelines given by WHO.

Out of 32 culture positive isolates subjected to LiPA, 22 (68.75%) isolates were susceptible to both first and second line drugs. 10 (31.25%) were resistant to any of the first line and second line drugs out which 5 (50%) were resistant to both rifampicin and isoniazid (MDRTB); 1(10%) was resistant to isoniazid only(mono-resistant to INH); 1 (10%) was resistant to isoniazid, rifampicin and fluoroquinolones(MDRTB); 1(10%) was resistant to isoniazid, rifampicin and ethambutol; 1(10%) was resistant to isoniazid, rifampicin, fluoroquinolones and ethambutol(MDRTB), 1(10%) was resistant to isoniazid, rifampicin, fluoroquinolones, ethambutol and aminoglycosides(XDRTB to be confirmed by phenotypic methods). rpoB gene codes for the enzyme RNA polymerase. Point mutation including insertions and deletions in rpoB gene region confer resistance to rifampicin. rpoBgene serves as the surrogate marker for detecting rifampicin resistance in drug-resistant tuberculosis.

Mutation in katG gene is commonly responsible for conferring high level resistance towards INH. In countries like India which are endemic to tuberculosis, INH resistance is a commonly seen phenomena and these isolates are not likely to be positive for rifampicin resistance. Low level of INH resistance is seen with mutation in inhA which results in approximately 15-20 percent of resistant cases.

Our study on INH resistance due to inhAgene (10%) is showing good agreement with other studies conducted by Brossier F et al and Albert H et al (5.4 – 21.1 %). The primary target of fluoroquinolones in gram-negative bacteria is DNA gyrase which has two sub-units gyrA and gyrB. gyrA gene is most commonly responsible for acquiring resistance to fluoroquinolones.

Structural mutations in embB gene are one of the factors responsible for ethambutol resistance. The gene responsible for resistance to aminoglycosides was found to be rrs.

Limitation of the study:

One limitation of the study was the inability to perform sequencing due to lack of this facility.

CONCLUSION

In our study the prevalence of MDRTB is 31.25%. LiPA is a reliable test for the detection of drug resistance from smear-positive samples which saves the time and helps in deciding treatment regime for patients suffering from multi-drug resistant tuberculosis.

LPA test provides an early accurate diagnosis of drug resistant tuberculosis among the culture positive pulmonary isolates by reverse hybridization DNA strip method. Based on these findings it is concluded that drug-resistant tuberculosis cases can be rapidly detected to prevent the further spread of multi drug resistant tuberculosis strains in the community especially in high Tuberculosis burden countries like India.

Molecular methods like LPA may detect silent mutations that do not confer phenotypic drug-resistance, therefore presenting false resistant results. All mutations resulting in the drug resistance tuberculosis are not covered in commercial assay.

SUMMARY

OBJECTIVES: To perform Line probe assay on clinical isolates from pulmonary tuberculosis for the detection of resistance to first line and second line drugs.

MATERIAL AND METHODS: A cross-sectional study was conducted for a period of two months for a sample size of 32 at Apollo general hospital, Hyderabad.

Line Probe Assay was done for the identification of *M. tuberculosis* and the detection of resistance to first and second line ATT drugs.

RESULT: Out of 32 culture-positive pulmonary isolates, 22 (68.75%) isolates were susceptible to both first line and second line drugs. 10 (31.25%) were resistant to any of the first line and second line drugs out of which 5 (50%) were resistant to both rifampicin and isoniazid; 1(10%) was resistant to isoniazid only; 1 (10%) was resistant to isoniazid,

rifampicin and fluoroquinolones; 1(10%) was resistant to isoniazid, rifampicin and ethambutol; 1(10%) was resistant to isoniazid, rifampicin, fluoroquinolones and ethambutol, 1(10%) was resistant to isoniazid, rifampicin, fluoroquinolones, ethambutol and aminoglycosides.

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