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Research Article

# MUTATIONS IN THE *RPOB* GENE OF *MYCOBACTERIUM TUBERCULOSIS* IDENTIFIED BY SEQUENCING METHOD

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**ABSTRACT**

**Objective:** To identify the mutation in the *rpoB* gene of *Mycobacterium tuberculosis* (MTB), using by sequencing method from pulmonary specimens of presumptive TB patients belonging to the districts of Tamil Nadu.

**Methods:** A total of 8697 clinical specimens of presumptive MTB patients were collected from various districts of Tamil Nadu. Smear microscopy was performed by light emitting diode fluorescent microscopy and all the smear positive samples were tested using line probe assay (LPA) to detect the percentage of drug resistance pattern and to identify the missing mutation in LPA by the sequencing method.

**Results:** Among 4897 smear positives subjected to LPA method; 407 (8.3%) MTB was not detected and 16 (0.3%) showed invalid result; 4473 (91.4%) strains showed MTB positive; 3695 (82.6%) were sensitive for both rifampicin (RIF) and isoniazid (INH) drugs; 502 (11.2%) were resistance for INH; 73 (1.6%) resistant for RIF; 203 (4.5%) were resistance for RIF and INH. Totally, 52 (1.2%) strains results cannot be confirmed by LPA and reported as sensitive for RIF, because of the faint and the missing bands in both wild type and mutation. These strains were sequenced and 39 (75%) strains showed resistant to RIF.

**Conclusion:** Hence LPA may be the molecular technology for the rapid, feasible and reliable method for the detection of multidrug resistant mutation but few confusion bands cannot be reported as resistance, which should be confirmed by either conventional phenotypic drug susceptibility testing or by sequencing method.

**Keywords:** Line probe assay, Sequencing, Mutation, Multidrug resistant tuberculosis.

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## INTRODUCTION

In 2013, more than 9 million people fell ill with tuberculosis (TB) and 4,80,000 people developed multidrug-resistant TB (MDR-TB) globally and there were an estimated 2,10,000 deaths from MDR-TB [1]. In worldwide, the TB disease remains the most dangerous infectious diseases. Providing treatment and control of TB is more complicated due to emergence of drug resistant even MDR strains, MDR-TB can be defined as resistant to both isoniazid (INH) and rifampin (RIF) with or without other anti-TB drugs [2]. Around 500 new smear-positive MDR-TB cases were estimated to occur per year in the country [3].

Conventional culture and drug susceptibility testing (C & DST) by solid or liquid is a slow process which may take 2-4 months, during this time a patient is often treated according to the standard regimen for drug-susceptible TB. This results delay in proper treatment, adversely affect the outcome of the treatment and transmission of the drug resistant TB [4]. The financial, infrastructural and human resource requirements, widespread implementation of culture-based DST is challenging in such settings. The specimen transport and the specimen contamination issues may also present further challenges [5], hence early diagnosis of TB and rapid detection of RIF resistance is important for proper management of drug-resistant TB [6]. Even though major efforts are being taken and implemented by the government to increase case detection, one-third of new TB cases are still missed due to nonavailability of rapid, low-cost, and accurate diagnostic facilities in high-TB-burden countries like India. Over the last many years, efforts were taken to improve and develop rapid diagnostic tools for diagnosis and DST of TB. The World Health Organization (WHO) issued 10 policy statements for improving diagnosis of TB, including the use of commercial and noncommercial DST methods and implementation of molecular methods such as the line probe assay (LPA) and Xpert *Mycobacterium* TB (MTB)/RIF assay [7].

In INH-resistant strains, the most common mutations were occurred in either the gene (*katG*) encoding catalase-peroxidase, which is required for the activation of prodrug INH, or in the promoter region of the *inhA*, encoding enoyl-acyl reductase, which is involved in *Mycobacterium* cell wall biosynthesis [8,9]. RIF resistance strains were serve as a surrogate marker for MDR-TB detection because 96% RIF-resistant strains are also resistant to INH [10]. Resistance in RIF was attributed to mutations within an 81-bp RIF resistance-determining region (RRDR) of the *rpoB* gene, corresponding to codons 507-533 in 96% of RIF resistance strains [11,12]. Mutations outside of RRDR were also reported with a frequency of <2% [13]. More than 50 mutations were characterized within this region by deoxyribonucleic acid (DNA) sequencing but only point mutations at codons 526 or 531 are known to cause high levels of RIF resistance [14]. In contrast, mutations in codons 511, 516, 518, 522, and 533 cause low-level resistance to RIF. Mutations conferring RIF resistance occur rarely in other regions of the *rpoB* gene [15]. Of the two recently introduced molecular diagnostic methods for RIF resistance detection, LPA technology is based on reverse hybridization of DNA on the strip, when the Xpert MTB/RIF assay is based on real-time polymerase chain reaction (PCR). The strip-based DNA hybridization, i.e., the genotype MTBDR*plus* (Hain LifeScience, Nehren, Germany) referred as to LPA. Both LPA and Xpert MTB/RIF assays show good performance (98% sensitivity) for RIF resistance detection when compared with the gold standard phenotypic DST. As per the WHO guidelines, the standard turnaround time for reporting the LPA results is 2-3 days,