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Prevalence of mutations in genes associated with rifampicin and isoniazid resistance in *Mycobacterium tuberculosis* clinical isolates

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ABSTRACT

Purpose: To analyze prevalence of mutations in genes associated with rifampicin and isoniazid resistance in *Mycobacterium tuberculosis* clinical isolates from patients with possible MDR TB of Puducherry, South India and to explore the association of specific mutations conferring rifampicin (RIF) resistance.
Methods: We performed a commercial Genotype MDBDRplus V.2.0 assay for the rapid detection of rifampicin and isoniazid resistance directly on sputum specimens of patients with possible MDR TB.
Results: Totally 558 multidrug resistant, 293 RIF mono resistant and 923 INH mono resistant tuberculosis were detected from the 12,786 patients with possible MDR TB samples. The 50.5% mutations were observed in the region of S531L in MDR TB patients and 55.6% in rifampicin monoresistant cases. In total isoniazid monoresistant, 68.0% mutations were detected in *katG* gene, which is more prevalent in comparison to *inhA* gene 32.0%. There were about 57.9% and 32.2% MDR TB cases diagnosed in the age group of >15 to ≤ 45 years and >45 to ≤ 60 years respectively.
Conclusions: The rate of occurrences of mutations were found widely in the Rifampicin Resistant Determination Region (81 bp) of *rpoB* gene and the hypervariable region 530–533 codons of rpoB gene is alarming in the specification. The higher frequency of mutation in codons of *rpoB* (S531L) and *katG* (S315T) gene help to design simple, new and less expensive molecular techniques to use in peripheral laboratories.

Introduction

The emergence and spread of multi-drug resistant tuberculosis (MDR-TB) is menacing to global tuberculosis control. According to WHO, nearly 50% of the world's burden of MDR-TB cases is in India and China [33]. The prevalence of MDR-TB is increasing throughout the world both among new tuberculosis cases as well as among previously treated cases [34]. The World Health Organization has estimated that India accounted for 26% of the total number of TB cases worldwide in 2015, with 3.9% and 21% of the new and retreatment cases respectively being caused by multi drug resistant strains [16].

In the majority of drug-resistant *M. tuberculosis* clinical isolates, drug resistance is due to mutations in genes or promoters region of genes activating the drug or encoding the drug targets. Studies have pointed out that the *M. tuberculosis* becomes resistant to RIF due to the mutations in *rpoB*, INH due to *katG* and *inhA* [30]. Resistance to rifampicin in mycobacterium results from point mutations mainly located in the 507–533 region of the *rpoB* polypeptide [12,13,26]. The most common mutations observed in rifampicin resistant *M. tuberculosis* isolates are Ser531Leu, His526Asp or Tyr, and Asp516Val [37]. Rifampicin resistance in *M. tuberculosis* strains is conferred by a diverse group of mutations within a hypervariable region of the *rpoB* gene, which codes for a β-subunit of RNA polymerase [3,7]. More than 95% of rifampicin resistant isolates possess mutations within this hyper variable regions of the *rpoB* gene [10].Resistance to isoniazid is mostly associated with the amino acid substitution Ser315Thr in *katG* (in roughly 70% of INH-resistant strains) and the −15 C-to-T mutation in the *inhA* promoter (in 15–35% of INH-resistant strains) [8,18,25].

The rapid diagnosis of multidrug resistant tuberculosis patients, place them on treatment regimens is indispensable in controlling the MDR-TB in a community and limit the nosocomial spread of MDR-TB through proper infection control methods. The

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Table 1
MDR TB suspects on gender and age wise.

RNTCP criteria	Sex	Total no of MDR TB suspects received				Total MDR TB suspects received	Grand total
		≤15 years	>15 ≤ to 45 years	>45 to ≤60 years	> 60 years		
Failure	Male	2	251	164	51	468	557
	Female	1	71	13	4	89	
Re treatment case S+ at 4th month	Male	1	258	190	53	502	590
	Female	3	62	18	5	88	
Contact of known MDR TB case	Male	5	41	14	5	65	104
	Female	6	27	6	0	39	
S+ at diagnosis, re treatment case	Male	10	2600	1961	576	5147	6035
	Female	13	592	223	60	888	
Any follow up smear positive	Male	3	1125	836	247	2211	2702
	Female	7	326	120	38	491	
S - at diagnosis, re treatment case	Male	2	430	374	149	955	1266
	Female	5	186	92	28	311	
HIV TB case	Male	32	716	256	28	1032	1532
	Female	27	375	90	8	500	
Total	Male	55	5421	3795	1109	10380	12786
	Female	62	1639	562	143	2406	
Grand total		117	7060	4357	1252	12786	

World Health Organization (WHO) recommended Line-probe assays (LPAs), which can simultaneously identify the *M. tuberculosis* complex and detect genetic mutations in the *rpoB* gene region related to rifampicin resistance, *katG* and *inhA* gene regions related isoniazid resistance [24]. The main objective of this study the prevalence of mutations in genes associated with rifampicin and isoniazid resistance in *Mycobacterium tuberculosis* clinical isolates from patients with possible MDR TB of Puducherry, South India and to explore the association of specific mutations conferring rifampicin (RIF) resistance.

Materials and methods

Specimen collection and processing

The study was conducted retrospectively in the Intermediate Reference TB Laboratory at Government Hospital for Chest Diseases, Puducherry for a span of 42 months between July 2012 and December 2015. The two sputum samples were collected in 50 ml sterile falcon tubes for each patient and transported through cold chain mechanism from the nine districts (Villupuram, Tanjore, Nagapattinam, Thiruvarur, Cuddalore, Dindigul, Perambalore, and Trichy) of Tamil Nadu state in addition to Puducherry state as per the criterias of Revised National Tuberculosis Control Programme, India. Twelve thousands seven hundred and eight six (12,786) sputum samples were collected from various age groups for this study, which included ≤15 years (n-117), >15 to ≤45 years (n-7060), >45 to ≤60 years (n-4357) and >60 years (n-1252) as described in Table 1. The sputum samples received at Intermediate Reference TB Laboratory were assigned lab number and consecutively screened for acid fast bacilli (AFBs) using Fluorescence (FM) microscopy [32].The smear positive sputum samples in Fluorescence microscopy were directly processed by GenoType MTBDRplus V.2.0 assay (Hain Life Sciences). The smear negative samples were subjected to liquid culture using the BACTEC MGIT 960 system (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA) under stringent conditions. The culture positive samples from the MGIT system were in turn subjected to the GenoType MTBDRplus V.2.0 assay (Fig. 1). All the laboratory bench works related with potentially infectious specimens were performed in a Class II biosafety cabinet placed at Bio Safety Level III facility. All processed specimens were stored at −20 °C for the duration of the study to allow for re-testing of specimens giving discrepant results.

GenoType MTBDRplus V.2.0 assay

The GenoType MTBDRplus V.2.0 assay was performed according to the manufacturer's protocol ([15]). The test is based on DNA strip technology and has three steps: DNA extraction, multiplex PCR amplification, and reverse hybridization. All three steps were performed as per the WHO recommendations [29,39].

BACTEC MGIT 960 culture

The smear negative and discrepant samples were processed in MGIT 960 culture tube. A 500 µl sample was taken out from decontaminated sample and inoculated in BACTEC MGIT 960 tube. After the culture flashed positive, MGIT tubes were confirmed for acid fast bacilli by ZN staining and further subjected to confirm as *M. tuberculosis* complex using Capilia TB Neo (TAUNS Corporation, Japan) and checked for contamination by growth on blood agar medium for 48 h at 37 °C ([6,11,21]). The confirmed positive MGIT tube was processed with the Genotype MTBDRplus V.2.0 assay (Hain Life Science, Nehren, Germany) as per the manufacturer's protocol.

Results

The sputum samples were collected in 50 ml sterile falcon tubes from each person with possible MDR TB mainly based on the criteria of Revised National Tuberculosis Control Programme and totally 12,786 person's with possible MDR TB sputum samples from different age groups (Table 1) were processed for the Auromine O phenol staining. Among them, (83.8%) sputum samples were smear positive,(16.2%) samples were smear negative. The Conventional BACTEC MGIT 960 procedure was performed for all smear negative TB person's with possible MDR TB samples and no results/invalid obtained from Genotype MTBDRplus V.2.0 assay. The 2% contamination samples are received on request for the further processing by the Genotype MTBDRplus V.2.0 assay. In total, 1774 (13.87%) drug resistant strains were identified by Genotype MTBDRplus assay V.2.0; among them 558 were multidrug resistant, 293 were RIF mono resistant and 923 were INH mono resistant from high-risk patients. The number of MDR TB cases diagnosed from each criteria were tabulated as shown in Table 2.

Overall, 83.8% smear positive specimens gave interpretable results within 2–3 days by Genotype MTBDRplus V.2.0 assay and only 16.2% of the samples gave smear negative results by Fluorescence Microscopy and those samples are further established with