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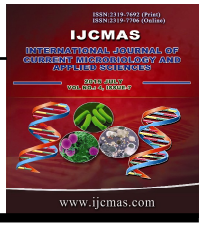

Original Research Article

Primary Multi Drug Resistance in New Pulmonary Tuberculosis Patients in Western Uttar Pradesh, India

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Keywords

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A B S T R A C T

Multidrug resistant tuberculosis (MDR-TB) has emerged as a significant global health concern, with an estimated 450000 incident cases in the world in 2012 (WHO report, 2013). Globally, an estimated 3.6% of new cases and 20.2% of previously treated cases have MDR-TB. We sought to ascertain the prevalence of MDR-TB among new cases of sputum-positive pulmonary TB. This study was conducted on sputum AFB positive samples, directly subjecting them to Line probe assay by GenoType MTBDR *plus*, which is designed to detect the mutations in the hotspot region of *rpo*B gene, *kat*G gene and regulatory region of *inh*A gene. MDR-TB was defined as infection caused by tubercle bacilli showing resistance to at least isoniazid and rifampicin. Line probe assay test of 110 specimens revealed drug resistance in 32(29.0%). Amongst the resistant isolates 8 (7.2%) were MDR-TB, 19 (17.3%) rifampicin (RIF) mono-resistant and 5 (4.5%) isoniazid (INH) mono-resistant. In MDR-TB isolates, RIF-associated mutations were *rpoB* Q513P and *rpoB* D516V in codon 516; *rpoB* H526Y in codon 526 and *rpoB* S531L in codon 531. Mutations associated with INH resistance were *katG* S315T1; *katG* S315T2 and *inhA* C15T. MDR-TB is being reported increasingly among new cases of sputum-positive pulmonary TB. Regular surveillance of MDR-TB using molecular line-probe assays, among newly diagnosed TB cases, will result in early diagnosis preventing its spread.

Introduction

Tuberculosis (TB) is the leading cause of death from a curable infectious disease. The introduction of multidrug therapy more than 50 years ago, to treat TB patients was largely the response to the emergence of drug resistance (Dye 2006).

Drug resistance continues to be a major global health problem. In 2012, there were 8.6 million new TB cases and 1.3 million TB deaths worldwide. This number of TB deaths is unacceptably large given that most are preventable. Though India is the second-most populous country in the world one fourth of the global incident TB cases occur in India annually. In the year 2012, out of global 8.6 million TB cases, about 2.3

million were estimated to have occurred in India (WHO, 2014).

There are alarming reports of increasing drug resistance from various parts of the globe which potentially threaten to disrupt the gains achieved in TB control over the last decade. Among the deaths there were an estimated 170000 from multi drug resistant tuberculosis (MDR-TB), which is a relatively high total compared with 450000 incident cases of MDR-TB. Globally, an estimated 3.6% of new cases and 20.2% of previously treated cases have MDR-TB (WHO, 2014).

The GenoTypeR MTBDR*plus* assay (Hain Lifescience, Germany) is a commercially available assay that combines detection of MTB complex with prediction of resistance to rifampicin (RIF) and isoniazid (INH). In the assay a multiplex PCR is followed by hybridization of the obtained DNA amplicons to membrane-bound probes.

It was found to have high sensitivity and high specificity for resistance in both the drugs and performs well when applied directly to AFB smear-positive sputum samples (Hillemann *et al.,* 2005). In June 2008, the World Health Organization (WHO) endorsed the use of molecular line-probe assays for MDR-TB screening (Cavusoglu *et al.,* 2006). The present study was therefore undertaken to find out the prevalence of MDR-TB among new cases of sputum-positive pulmonary TB.

Material and Methods

This study was conducted on 110 sputum samples which were obtained from clinically suspected pulmonary tuberculosis cases attending TB-Chest department from January 2013 to June 2014. Patients included 76 (69.1%) males and 34 (30.9%) females. All of them were between 18 and 80 years of age (weighted average, 43.4 years) and presented with fever, cough and weight loss, without prior history of any anti tubercular treatment. Standard definitions of new patients, pulmonary tuberculosis, mono resistance and multi drug resistance were used**.**

All individuals below 18 years of age, defaulters, HIV seropositives, relapses and sputum negative cases were excluded from this study.

In all 110 AFB positive, sputum samples were subjected to Line probe assay by GenoType MTBDR*plus*. This assay was performed as per the standard protocol provided by the manufacturer (HainLifescience, Germany) (Dooley *et al.,* 1992).

Results and Discussion

Total 110 non-repeat sputum samples included in this study were from equal number of patients. Smoking was leading associated predisposing factor found in 39(35.5%) cases, followed by steroid use 30(27.3%), hypertension 18(16.4%) and diabetes mellitus 21(19.1%).

All the samples were analyzed by using the genotype MTBDR *plus* assay. Seventy eight samples were found to be both RIF and INH sensitive (Fig. 1) while thirty two (29.0%) isolates were found to be resistant strains. Out of these 32 samples, 8 (7.2%) strains were found to be MDR-TB (Figs 2,3,4,5), 19 (17.3%) were RIF monoresistant (Fig. 6) and 5 (4.5%) were INH monoresistant (Table 1).

In the eight MDR isolates, RIF associated mutation *rpoB* Q513P was seen in codon 516 (5 of 8) and mutation *rpoB* S531L was seen in codon 531 (3 of 8). Mutations associated with INH resistance were seen as

katG S315T1 (4 of 8), *katG* S315T2 (1 of 8) and as *inhA* C15T (3 of 8).

In twenty seven RIF resistant isolates, 13 of 27 (48.3%) strains showed an absence of *rpoB* WT8 (*rpo*B wild type probe) and presence of *rpoB* MUT3 (*rpoB* mutation probe) hybridization band on a membrane strip, which represents a mutation associated with codon no. 531 of the *rpoB* gene (Fig. 6). Other mutations in the *rpoB* gene were associated with codon 516 in 8 of 27 (29.5%) and codon 526 in 6 of 27 (22.2%) (Table 2).

In thirteen INH resistant isolates, 6 of 13 (46.2%) strains showed an absence of *katG* WT (*katG* wild type probe) and presence of *katG* MUT1 (*katG* mutation probe 1). Two of thirteen (15.4%) showed absence of *katG* WT and presence of *katG* MUT2, while two of thirteen (15.4%) showed absence of *katG* WT as well as absence of any mutation band. Three of thirteen (23.0%) showed absence of *inhA* WT1 and presence of *inhA* MUT1.

MDR-TB is associated with high death rates of 50-80%, spanning a relatively short time from diagnosis to death. Drug resistance is a result of random genetic mutations in particular genes (Telenti *et al.,* 1993).

Resistance to RIF has been shown due to alteration of the beta subunit of the RNA polymerase encoded by the *rpoB* region. About 96% of RIF resistant isolates of MTB have point mutations in a 81-bp region of this gene, which are not present in susceptible isolates (Cockerill *et al.,* 1995). Resistance to INH is associated with a range of mutations affecting one or more genes such as those encoding catalase peroxadase *katG* (Banerjee *et al.,* 1994). The two gene operon (*inhA* locus) encoding the enoyl-acyl carrier protein reductase is involved in mycolic biosynthesis and mutations in this gene result in INH resistance (Drobniewski and Wilson, 1998).

Delay in the recognition of drug resistance results in a delay in initiation of treatment or effective chemotherapy, which is the major factor that contributes to MDR-TB outbreaks. Drug resistance can be assessed within 1 day by a molecular assay, genotype MTBDR*plus*. It is a rapid and reliable method for the specific detection of the most common and frequent mutation leading to RIF and INH resistance (Kapur *et al.,* 1994).

RIF-resistance mechanism involves missense mutations in the RRDR region of the *rpoB* gene. Extensive studies show that most of RIF-resistant strains harbor a mutation within the 81-bp region of the *rpoB* gene (Telenti *et al.,* 1997; De *et al.,* 2003). In our study, the S531L mutation was observed in 48.2% cases, followed by D516V in 29.6% cases and H526Y in 22.2% cases. A high frequency of the S531L mutation has also been reported in other studies performed in different countries (Hillemann *et al.,* 2005; Marttila *et al.,* 1998; Viader *et al.,* 2003; Rattan *et al.,* 1998).

In two specimens *rpoB* gene wild type as well as mutation was missing. These mutations might be different than provided in the assay strip. Such cases are considered as resistant (Figs 4 & 5).

Mutations in the *katG* and the *inhA* genes are associated with approximately 70% and 80% of INH-resistant MTB isolates (Hazbón *et al.,* 2006). Although INH resistance in MTB is more complex due to the implication of the number of genes, up to 95% of INH resistance may be due to mutations in *katG* (Kiepiela *et al.,* 2000).

Table.1 Rifampicin and Isoniazid susceptibility pattern (n=110)

Rifampicin / Isoniazid	Sensitive	Resistant	Total
Sensitive	78	19	97
Resistant	5	8	13
Total	83	27	110

Table.2 Mutations associated with Rifampicin and Isoniazid resistance detected by genotype MTBDR *plus* assay (n=110)

Gene	No. of isolates	Wild Type missing	Mutation present	Result
rpo B gene	83	NIL	NIL	RIF –S*
	13	WT 8	MUT 3	RIF –R*
	06	WT 3, 4	MUT 1	RIF –R
	04	WT 7	MUT 2B	RIF –R
	02	WT 7	MUT 2A	RIF –R
	01	WT 2	NIL	RIF –R
	01	WT 2, 3	NIL	RIF –R
kat G gene	100	NIL	NIL	INH-S*
	06	WT	MUT 1	INH-R*
	02	WT	MUT 2	INH-R
	02	WT	NIL	INH-R
inh A gene	107	NIL	NIL	INH-S
	03	WT 1	MUT 1	INH-R

(* RIF-S = Rifampicin sensitive, RIF-R = Rifampicin resistant, INH-S = INH sensitive, INH-R =INH resistant)

Figure.1 *Mycobacterium tuberculosis* susceptible to Rifampicin (RIF) and Isoniazid (INH)

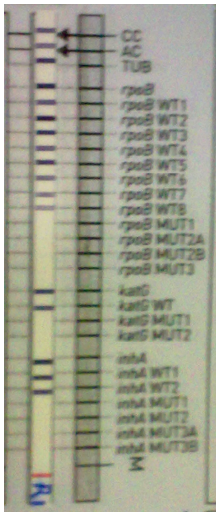


Figure.2 *Mycobacterium tuberculosis*, MDR-TB (Missing '*rpo*B WT 3,4' and '*kat*G WT' and presence of '*rpo*B MUT1')

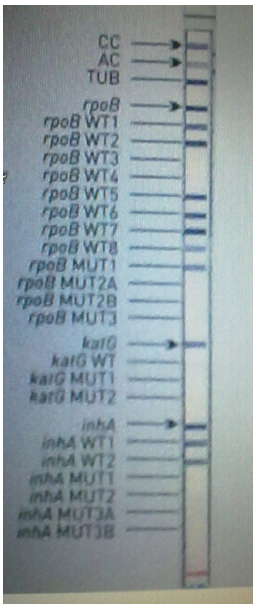


Figure.3 *Mycobacterium tuberculosis*, MDR-TB (Missing '*rpo*B WT 7' and '*kat*G WT' and presence of '*rpo*B MUT2B' and '*kat*G MUT1')

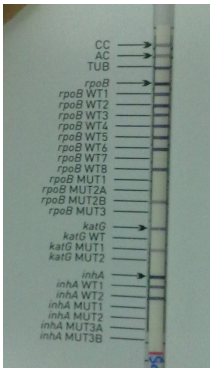


Figure.4 *Mycobacterium tuberculosis*, MDR-TB (Missing '*rpo*B WT2' and '*kat*G WT' and presence of '*kat*G MUT1')

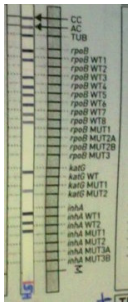


Figure.5 *Mycobacterium tuberculosis*, MDR-TB
(Missing '*rpo*B WT2'; '*rpo*B WT3' and '*inh*A WT1' and presence of '*inh*A MUT1')

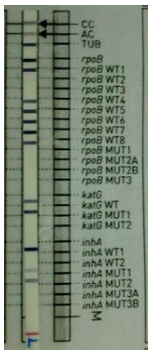
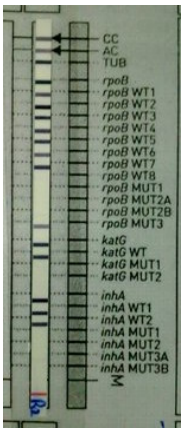


Figure.6 *Mycobacterium tuberculosis*, Rifampicin monoresistant
(Missing '*rpo*B WT8' and presence of '*rpo*B MUT3')



Ten of thirteen (77%) of the INH resistant isolates showed a mutation in the *katG* codon 315 and three (23%) of the INH resistant isolate showed a mutation in *inhA* gene. The prevalence of mutations in the *katG* and *inhA* genes seems to vary widely in different geographic locations.

Amongst INH resistant MTB isolates 72% to 97% mutations in *katG* and 2% to 24% mutations in *inhA* are reported from Africa (Van *et al.,* 2001, Baker *et al.*, 2005). Extensive studies from other countries have confirmed this variability in the contribution of different mutations to INH resistance (Mokrousov *et al.*, 2002, Sharma *et al.*, 2011).

In our study MDRTB was detected in 8(7.3%) isolates which is quite high percentage, considering that the study was conducted on primary cases only. This prevalence is higher compared to other studies in newly diagnosed patients: like, 1.1% (2 of 177) reported by Sharma *et al.* (2011), at Delhi and <1% (1 of 112) reported by Yimer *et al.* (2012), at Amhara, Ethiopia. This difference could be because both these studies used conventional drug susceptibility test while our study was by molecular method. Study based on a larger sample size is likely to give more accurate picture. However global prevalence of primary MDRTB in newly diagnosed cases is estimated to be about 3.6%, as per WHO

and it recommends line probe assay for MDRTB detection. Given high prevalence of MDRTB in India, all sputum positive cases may be screened for MDRTB using rapid diagnostic tests like line probe assays, facilitating early diagnosis and thereby reducing transmission of resistant strains in community.

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