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Research Article

Mycobacterium tuberculosis has affinity of frequent mutation in the codon sequence 530-533 of mutation type serine 531 leucine

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Abstract

As long as MDR-TB verified, use of inadequate and hence ineffective antibiotics may lead to further spread of resistant bacteria and amplification of resistance. Therefore, rapid diagnosis and identification of MDR-TB is a prerequisite for appropriate treatment. A total number of 400 sputum samples were processed by NALC - NaOH method. The sediment was used for preparation of smear, liquid culture and extraction of nucleic acid. Nucleic acid amplification and reverse hybridization were then performed according to guidelines of the manufacturer. The lack of hybridization on one or more of wild-type probes with or without hybridization on the mutation probes indicates presence of mutation. This study showed 105 (26.25%) RMP resistant isolates out of 400 samples. The codon 531 was affected in 82 (78.09%) isolates out of 105 RMP resistant isolates. Other mutations occurred 20 (19.04%) in MUT 1, 2 (1.9%) in MUT 2A and 1 (0.9%) in MUT 2B probes respectively. The most frequent type of mutation responsible for rifampicin resistance is Ser-531-Leu mutation of MUT 3 probe. In this study, it was observed that MTB has the affinity of mutation in the codon sequence 530-533 of mutation type Serine-531-Leucine for 'Rif' resistance which may be an important work to develop newer antibiotics for treating MDR-TB patients.

Keywords: Mycobacterium tuberculosis, Genotype MTBDR plus; RMP resistance; Serine531Leucine

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1. Introduction

Tuberculosis is a bacterial infectious disease passed on by droplet infection. In 2010, there were an estimated 8.8 million incident cases of TB globally, and an estimated 1.1 million deaths occurred. TB treatment requires a therapy over several months. Emergence and spread of Multi-Drug-Resistant tuberculosis (MDR-TB) is a major medical and public problem threatening global health. MDR-TB is defined as a TB that is resistant at least to Rifampicin (RMP) and Isoniazide (INH), the two most important first-line anti-TB drugs [3]. MDR-TB is a challenge to TB control due to its coplex, time-consuming diagnosis and obstacles in treatment. In 2010, there were an estimated 650,000 estimated cases MDR-TB among the world's 12 million prevalent cases [16].

As long as MDR-TB is not verified, use of inadequate and hence ineffective antibiotics may lead to further spread of resistant bacteria and amplification of resistance. Therefore, rapid diagnosis and identification of MDR-TB is a prerequisite for appropriate treatment [4].

The emergence and spread of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) are a major medical and public problem threatening the global health [5].

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MDR-TB is caused by mycobacteria which are at least resistant to the two most powerful first-line anti-TB drugs rifampicin and isoniazid. Conventional methods for mycobacteria culture and drug susceptibility testing are slow and elaborate, requiring sequential procedures for the diagnosis. During this time patients may be treated inappropriately, drug resistant strains may continue to spread, and amplification of resistance may occur. Therefore rapid diagnosis and identification of MDR-TB strains are prerequisites for the worldwide fight against TB [9].

The Genotype MTBDR *plus* enables a rapid result from pulmonary patient specimen and from culture material [11].

Also for diagnosing patients after treatment failure and relapse, with unknown anamnesis and originating from high prevalence areas of MDR-TB as well as for diagnosing patients in high prevalence TB countries and high burden MDR-TB regions the use of GENOTYPE MTBDR *plus* is reasonable [8]. Finally the test can also be applied for screening purposes to develop country-specific TB action plans.

2. Materials and methods

The study was conducted from July to September 2015. A total of 400 acid-alcohol-fast bacilli (AAFB) smear positive sputum samples were analyzed. The specimens were gone through Digestion-Decontamination (DD) process by N-acetyl L-cysteine-sodium hydroxide method. After centrifugation in cooling centrifuge (40C) at 3500 r.p.m. for 20 minutes, and discarding the supernatant, the pellet was resuspended in 1 ml of phosphate buffer [Na2HPO4(0.974%)+KH2PO4(0.907%)] and used for smear preparation by Ziehl-Neelsen's Method, liquid culture and extraction of nucleic acid.

2.1. DNA extraction

The Genotype MTBDR plus line probe assay (HAIN Lifescience GmbH, Nehren, Germany) was carried out according to the manufacturer's instructions. About 500 µL of the decontaminated and concentrated specimen was centrifuged @ 10000 g for 15 min, the supernatant was discarded. Then 100 µL of Lysis Buffer (A-LYS) was added to the pellet and mixed with the help of a vortex. The specimen was then heat killed at 95°C (hot oven) for 5 min. After that, 100 µL of Neutralisation Buffer (A-NB) was added and mixed again with vortex mixture. Then it was centrifuged @ 13000 g for 5 min. The supernatant which carries extracted DNA was used immediately for polymerase chain reaction (PCR) [12].

2.2. Amplification

Amplification was done according to the protocol for sputum material (PCR): denaturation at 95°C for 15 min; ten cycles of denaturation at 95°C for 30s and elongation at 58°C for 120s; an additional 30 cycles of denaturation at 95°C for 25s, annealing at 53°C for 40s, and elongation at 70°C for 40s; and a final extension step at 70°C for 8 min [13].

2.3. Hybridization

The biotin-labeled PCR product was denatured and hybridized to a strip with specific oligonucleotide probes. One probe is complementary with an *Mycobacterium tuberculosis complex* (MTBC) specific region of the 23S rRNA gene (TUB), and one probe is specific for the rpoB gene (beta-subunit of RNA polymerase), i.e. rpoB-Uni and should be always positive for all MTBC strains, while eight wild-type (WT) probes (WT1 to WT8) encompass the region of the rpoB gene encoding amino acids 505 to 533. Four other probes are specific for the most common mutations (MUT): D516V, H526Y, H526D, and S531L (probes rpoB MUT1, rpoB MUT2A, rpoB MUT2B, and rpoB MUT3, respectively). One probe detects a katG-specific gene (catalase-peroxidase) region and should always be positive for all MTBC strains. Three other probes are specific for the S315 region of katG. One is the wild-type probe (katG WT), while two others (katG MUT1 and MUT2) are designed to detect the AGC-to-ACC (S315T) and the AGC-to-ACA (S315T) mutations. Along with locus control for inhA gene (fatty-acid enoyl-acyl carrier protein reductase) there are two wild type probes and four mutation probes (inhA MUT1, inhA MUT2, inhA MUT3A and inhA MUT 3B) to detect mutations in inhA promoter region. rpoB predicts rifampicin (RIF) resistance, katG predicts high level isoniazid (INH) resistance and inhA predicts low level

INH resistance. After hybridization, membrane strips were attached to evaluation sheet, read and interpreted.

Mycobacterium tuberculosis H 37 RV was used as control. Both positive and negative controls were maintained for each run [14].

3. Result

This study showed 105 (26.25%) RMP resistant isolates out of 400 samples. The codon 531 was affected in 82 (78.09%) isolates Out of 105 RMP resistant isolates. Other mutations occurred 20 (19.04%) in MUT 1, 2 (1.9%) in MUT 2A and 1 (0.9%) in MUT 2B probes respectively [15].

4. Discussion

The most frequent type of mutation responsible for rifampicin resistance [6] is Ser-531-Leu mutation of MUT 3 probe as shown in Table1:

Mutation Probe	Codon Analysis	Type of Mutation	Number of MDR-TB Isolates
MUT 1	513-519	D516V	20
MUT 2A	526-529	H526Y	02
MUT 2B	526-529	H526D	01
MUT 3	530-533	S531L	82

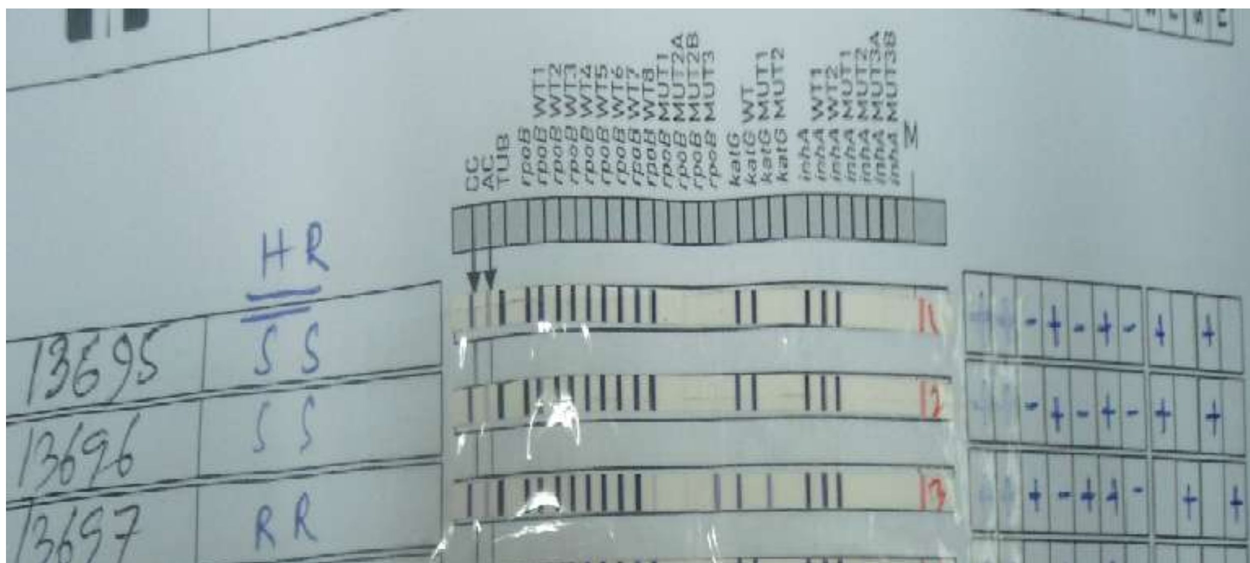


Fig No 01: 13697 shows rpoB MUT3 band on DNA strips indicating Serine-531-Leucine

In the current study we used the molecular assay (HAIN Lifescience GmbH Genotype MTBDRplus) for rapid detection of resistance to the most important anti-TB drugs (RIF and INH) on sputum samples [10] different districts in West Bengal.

It was observed that MTB has the affinity of mutation in the codon sequence 531 of mutation type Serine-531-Leucine for 'Rif' resistance.

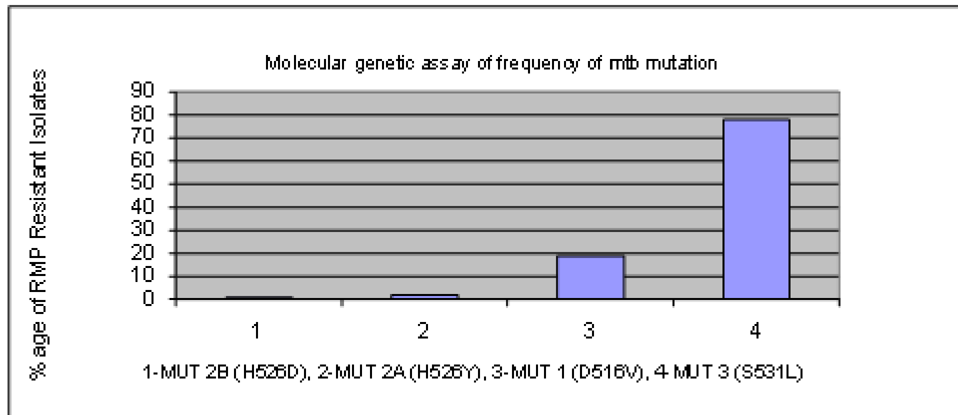
The above figure of the original work indicates mutation in β subunit of RNA Polymerase in sample no. IRL 13697 with a distinct band on DNA strip. This band represents codon 530-533 and mutation took place in the amino acid sequence serine to leucine.

The above table shows highest number (82) isolates, which are MDR by means of Rifampicin resistance and band came in rpoB MUT3 probe in each and every isolates, thus indicating the affinity of frequent mutations of *Mycobacterium tubeculosis* in that particular codon sequence which was our interest of study.

An attempt was made to record the mutation probe and type of mutation, which provides information on the mutations prevailing in this part of the country [9]. This may be an important work to develop newer antibiotics for treating MDR-TB patients by targeting that particular codon.

Conclusion

As Genotype MTBDR*plus* is a PCR based technique, this technique can easily detect very



low level of resistant bacteria, therefore, giving very less chance to miss any TB isolates. Hence, Genotype MTBDR *plus* can be a promising and suitable first line-screening test tool for TB diagnosis, treatment guidance, and surveillance [7].

Genotype MTBDR*plus* assay can be effectively used for rapid screening of drug resistant TB, and for improved sensitivity, additional probes can be integrated in the assay [2].

Most frequent mutation causing resistance is Ser-531-Lue that is very important to note and may be useful for epidemiological analysis [1].

In this study, it was observed that MTB has the affinity of mutation in the codon sequence 531 of mutation type Serine-531-Leucine for 'Rif' resistance, which may be an important work to develop newer antibiotics for treating MDR-TB patients by targeting that particular codon. Furthermore, this study can be used for molecular screening of MDR-TB in different districts of West Bengal, India.

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Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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