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Title: **"Molecular detection and identification of *Mycobacterium tuberculosis* from clinical samples and characterization of the probable gene(s) involved in fluoroquinolones & aminoglycosides resistance"**

## Abstract

Despite all advances made in the tuberculosis (TB) treatment and management, one third (32%) of the world population is infected with TB, a disease caused by *Mycobacterium tuberculosis* (*M.tuberculosis*). India is the highest TB burden country accounting for one fifth of the global incidence and 2/3rd of the cases in South East Asia. The main guideline of TB management programme involves rapid diagnosis, correct species characterization and providing effective drug regimen to the infected person. Keeping all these tasks in consideration, we designed this study in a manner that it would include evaluation of rapid diagnostic parameters, species identification and drug susceptibility testing for both first and second line anti-tubercular drugs. All the resistant isolates identified by this phenotypic testing were finally confirmed by genotyping.

In the first phase of our study, we evaluated the performance and reliability of BacT/ALERT for the isolation of *M. tuberculosis* and once all the subjects isolated in the study were characterized as *M.tuberculosis* by probe (accuprobe) based technique, we further screened susceptibility pattern of freshly grown isolates (log phase) against first line drugs i.e Streptomycin(STR), Isoniazid(INH), Rifampicin (RIF) and Ethambutol (ETM) by proportion method by both LJ and BacT/ALERT automation. The overall level of agreement between results from the BacT/ALERT system and LJ method was 96%. Concordance values for STR (100%), RIF (98.8%), and ETM (98.8%) were observed. The MDR-TB was found in 40.7% isolates in pulmonary isolates and 13.5% isolates in extra-pulmonary (EP) isolates. The reason behind this increased trend was because of CAT II (retreatment) cases which were predominantly taken in this study.

This study was based on the hypothesis that many alterations in the target genes would be acting simultaneously and contributing resistance towards major anti-tuberculous drugs. Also

changes in the functional activities due to mutations determined in the cell would be useful to find out the comprehensive effect of these on drug resistance. Structural genomic analysis of these gene helps in moving towards better understanding about the drug resistance mechanism. Thus, various target gene loci such as *rpo*B, *rps*L, *rrs*, *gyr*A and *gyr*B were sequenced in all the resistant subjects. The genotypic data generated was predominantly in compatibility with phenotypic susceptibility testing, therefore, suggesting that phenotypic methods still remain a vital screening complement to genetic characterization for drug susceptibility testing. The majority of mutations were found in previously treated cases (CAT II) suggesting that the environmental pressures posed by inappropriate designed drug regimen, poor compliance to treatment schedule and poor quality of drugs enhance evolutionary pressures resulting in selection of bacilli with spontaneous mutations leading to development of drug resistance.

We also identified novel positions in *gid*B region known to confer STR resistance. Mutations in *gid*B have been reported with high frequency, and this gene appears to be very polymorphic, with frame-shift and point mutations occurring in low MIC level STR resistant strains. We further screened these mutants with bioinformatics assay and observed that few mutants were in close proximity of active site pocket while other mutations being inside secondary structure units had overall effect on protein structure.

In this study, phenotypic results have shown seven (7/74) isolates (9.5%) which presented the definition of XDR isolates. Out of these, 06 were pulmonary strains and 01 was lymph node aspirate (EPTB). But, out of these seven, only five (6.8%) have shown genotypic evidence. The reason may be due to existence of some mechanism other than genetic mutations being involved in the resistance in the remaining two isolates. When this data is compared with nearly concurrent population based surveillance data, the proportion of resistance among new and previously treated TB cases were found to be significantly higher. This may be attributed to difference in sample size used in the present study.

In countries like India, most clinicians are not adhering to the antibiotics policy and are rampantly using second line drug treatment even in the absence of sensitivity reports. Such a malpractice culminates in the outbreak of MDR-TB and XDR-TB strain, beyond control.Therefore, it is highly recommended that the susceptibility pattern of the drugs to be used should be tested in order to provide patient with only effective antimicrobials.

Despite few limitations of this study, the existence of XDR-TB in India is well-understood. Its prevention calls for an urgent and rational use of second line anti-TB drugs and programmatic management of MDR-TB.