ABSTRACT

Title: Evaluation of Pyrosequencing assay for the rapid detection of resistance to Rifampicin and second-line drugs in *Mycobacterium tuberculosis* clinical isolates.

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Introduction
Drug resistant tuberculosis is a major global public health problem. Early diagnosis of drug-resistant tuberculosis (TB) is essential for minimizing the risk of *Mycobacterium tuberculosis* (MTB) transmission. The conventional culture based drug susceptibility testing (DST) methods for detection of drug-resistant *M. tuberculosis* are laborious and time consuming. Pyrosequencing (PSQ) assay has the ability to rapidly detect multiple mutations conferring resistance to many of the anti-tuberculosis drugs.

Objectives
To evaluate PSQ assay for the rapid detection of resistance to Rifampicin (RIF) Fluoroquinolones (FQ) and Aminoglycosides in MTB clinical isolates

Materials and methods
This is a prospective study for a period 1.5 years in which MTB clinical isolates were evaluated for the detection of mutations in *rpoB* gene (rifampicin), *gyrA* gene (fluoroquinolones), and *rrs* gene (aminoglycosides) by PSQ assay. DNA extraction from MTB clinical isolates was performed using Tris-HCl buffer and chloroform. The amplification of respective target genes was done followed by sequencing using the PyroMark Q24 ID system. The PSQ results were compared with the conventional drug susceptibility testing done in the laboratory.

Results and Discussion
Of the 57 isolates tested for RIF resistance, 40 were phenotypically resistant to Rifampicin. By PSQ the most predominant mutation observed in *rpoB* gene was TCG531TTG (82%). Twenty eight of the 50 isolates were phenotypically resistant to FQ. Of these, 20 isolates showed mutations in the 94^{th} codon of *gyrA* gene. The predominant mutation observed was GAC94GGC (55%). Of the 51 isolates tested for resistance to aminoglycosides 10 were phenotypically resistant to Capreomycin (CAP) and Kanamycin (KAN). Five of the 10 isolates had A1401G mutation. However, 4 of the resistant isolates had no mutation in the *rrs* gene segments evaluated suggesting that mutations exist in other sites of *rrs* gene. The sensitivity of PSQ assay for the detection of resistance to RIF, FQ, CAP and KAN was 100%, 100%, 40% and 50% respectively. The specificity of the PSQ assay was 100%.

Conclusion
The PSQ assay is a rapid and effective method for detecting and characterising drug resistance mutations from MTB clinical isolates.

Keywords

Pyrosequencing, Rifampicin, Fluoroquinolones, Aminoglycosides.