Challenge in Determination of Rifampin Resistant Mycobacterium tuberculosis by Real –time PCR Methods

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Background & Objectives: The emergence of drug-resistant strain of *M. tuberculosis* is one of the most critical issues facing TB researchers and clinicians. Rapid diagnosis of multidrug-resistant tuberculosis is essential for the prompt initiation of effective second-line therapy to improve treatment outcome and limit transmission of this obstinate disease.

Methods: This Methods will use to screen 50 *M. tuberculosis* clinical isolates, including 25 RIF-resistant strains and 25 susceptible strains based on the agar proportion Methods of drug susceptibility testing (DST). The rifampicin resistance determinant region (RRDR) of rpoB will target for the detection of rifampin (mono resistant to RIF is quite rare it has thus been proposed that resistance to RIF can be used as surrogate marker for mdr-mtb as nearly 90% of RIF resistant are also INH resistant). Additionally, this assay will multiplex to discriminate *Mycobacterium tuberculosis* complex (MTC) strains from nontuberculous Mycobacteria (NTM) strains by targeting the IS6110 insertion element.

Results: Real-time PCR is the most sensitive technique if purpose is to detect specific mutation in the amplified region. Different formats of labeled probes can be adapted to the detection of mutation(TaqMan,molecular beacons,and FERET). Our bioinformatics study and gene sequence around codon 531,526 lead to design LNA probe.

Conclusion: The current study describes the development of a unique real-time PCR assay for the detection of mutations conferring Rif resistance in *Mycobacterium tuberculosis*. Locked nucleic acid (LNA) probes will use to enhance the detection of strains containing specific single-nucleotide polymorphism (SNP).

Keywords: RIF-Resistant MTB; Realtime PCR; RRDR; LNA Probe