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Rapid detection of rifampin and isoniazid resistance in *Mycobacterium tuberculosis* isolates using high resolution melting curve analysis

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ABSTRACT

Aims and objectives: Early detection of multidrug-resistant tuberculosis (MDR-TB) is essential to prevent its transmission in the community and to initiate an effective anti-TB treatment regimen. The objective of this study is to evaluate molecular technique high resolution melting curve (HRM) assay to rapidly detect resistance-conferring mutations in *rpoB* and *katG* genes.

Methods: HRM analysis was used to screen 95 *Mycobacterium tuberculosis* (MTB) clinical isolates including 20 rifampin resistant (RIF-R), 21 isoniazid resistant (INH-R), and 54 fully susceptible (S) isolates determined by proportion method of drug susceptibility testing. 19 MTB isolates with known drug susceptibility genotypes were used as control strains for initial validation of the assay. Polymerase chain reaction (PCR) amplicons from *rpoB* and *katG* genes were sequenced to investigate the frequency and type of mutations and to confirm HRM results.

Results: All RIF-S and INH-S isolates generated wild-type HRM curves and were accurately identified as susceptible by this method. Similarly 19 out of 20 RIF-R and 18 out of 21 INH-R isolates correctly exhibited mutant-type HRM curves. These results were similar to those obtained by direct sequencing. However, 1 RIF-R and 3 INH-R isolates were falsely identified as susceptible by HRM assay. These strains were confirmed as having no mutation in their target regions by sequencing. The main mutations involved in RIF and INH resistance were found at codons *rpoB531* (60% of RIF-R isolates) and *katG315* (85.7% of INH-R isolates), respectively.

Conclusions: HRM was found to be a reliable, rapid and low-cost method to characterize drug susceptibility of clinical TB isolates in resource-limited settings.

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