



105/A

IFS - Pub-42

Identification of mutations associated with drug resistant *Mycobacterium tuberculosis* strains by molecular methods

S Maheswaran¹, D N Magana-Arachchi^{1*} and R M D Medagedara²

¹*Institute of Fundamental Studies, Hantana road, Kandy*

²*Teaching Hospital, Kandy*

Tuberculosis (TB) is one of the most common and deadly infectious diseases in the developing countries. With the increase in the number of tuberculosis patients worldwide, the emergence of the drug resistant TB is found. Multi-drug resistant strains which do not respond to rifampicin (RIF) or isoniazid (INH) have increased the demand for rapid and reliable drug susceptibility testing. Resistance to RIF is exclusively associated with mutations in the *rpoB* gene while the INH mutations have been found in two genes; the *katG* and the *mabA-inhA*. The objective of the study is to determine the pattern of drug resistance of *Mycobacterium tuberculosis* from tuberculosis patients attending the Central Chest Clinic, Kandy based on molecular techniques of PCR and DNA sequencing, targeting the mutations in *rpoB*, *inhA* and *katG* genes. A total of 100 acid fast bacilli positive sputum specimens were collected, treated and inoculated in L-Jensen medium. Antibiotic Sensitivity tests (ABST) for INH and RIF were carried out on MB 7H10 media for the 77 positive *M. tuberculosis* isolates which were confirmed by biochemical testing. DNA extractions were done using the standard CTAB method. PCR amplifications were carried out with gene specific primers, TR8 & TR9 for *rpoB* gene, Tb86 & Tb87 for *KatG* and Tb92 & Tb93 for *inhA* gene. PCR products were electrophoresed and DNA was eluted for sequencing.

In the total sputum specimens from ABST, 10.38% showed resistance to INH, 33.76% were resistant to RIF and 7.8% were multi drug resistant. These results tallied with molecular sequencing results. Twenty isolates showed mono resistance to RIF while being sensitive to INH. DNA sequence analysis of 19 of the 20 strains of *rpoB* gene and 5 of the 6 strains, of each *katG* and *inhA* genes revealed different kinds of mutations. In conclusion, INH-dissociated RIF resistance identified in the present study has rarely been reported before. A high rate of RIF resistance among the tuberculosis patients in Kandy emphasizes the need for a rapid and reliable method of diagnosing drug resistance. These findings will help in generating a novel and rapid drug resistance screening approach for treatment of MDR-TB.

Acknowledgements: Financial assistance by NRC grant number-07-47 is acknowledged.

nayomam@yahoo.com