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**IDENTIFICATION OF DRUG RESISTANT
MYCOBACTERIUM TUBERCULOSIS STRAINS
USING PCR & DNA SEQUENCING**

BY

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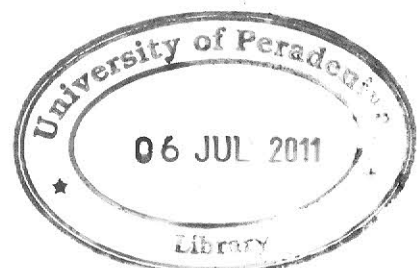
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Abstract

Drug resistant tuberculosis can be life-threatening and is a threat to tuberculosis control programmes in many countries. It substantially increases the cost and duration while decreasing the efficacy of treatment. Early detection of drug resistance is essential for the efficient treatment and control of drug resistant tuberculosis. Resistance to drugs is due to particular genomic mutations in specific genes of *Mycobacterium tuberculosis* (*M. tuberculosis*). The objective of this study was to determine the pattern of drug resistance of *M. tuberculosis* from tuberculosis patients attending Central Chest Clinic, Kandy and to investigate the type of mutations in *katG/ inhA* and *rpoB* genes of the identified isoniazid and rifampin resistant *M. tuberculosis* isolates respectively, using PCR and DNA sequencing methodology.

A total of 176 sputum specimens, positive for acid fast bacilli, from first visit patients and five from recurrent patients with tuberculosis attending the Chest Clinic/Hospital, Kandy were cultured. Antibiotic susceptibility tests for isoniazid and rifampin were carried out on Lowenstein-Jensen/Middlebrook 7H10 medium, using the proportion method. DNA extraction from the culture isolates (using CTAB), and directly from the sputum specimen (Boom's method) was carried out. PCR conditions were optimized for *inhA*, *katG*, and *rpoB* genes. Direct automated sequencing of *inhA*, *katG*, and *rpoB* genes from three, nine and eight *M. tuberculosis* culture isolates respectively and *rpoB* gene from three Mycobacteria other than tuberculosis (MOTT) culture isolates were carried out.

Of the 172 first visit tuberculosis patients analysed, 143 (83.2%) were within the economically productive age group of 21 to 60 years. The loss of such potential wage-earners would definitely place a high economic burden on the affected family, society and the country in addition to causing other social problems.

Of the 78 mycobacterial isolates from the first visit patient, 76 (97.4%) were biochemically identified as either *M. tuberculosis*, or *M. tuberculosis* complex and two (2.6%) as Mycobacteria other than tuberculosis (MOTT). Among those *M. tuberculosis*/*M. tuberculosis* complex isolates, 1.4% (1/72) showed isoniazid resistance and multi-drug resistance was observed in one of the 71 (1.4%) isolates. A high rate, 23.3% (17/73), of rifampin resistance detected among the tuberculosis patients in Kandy emphasizes the need for a rapid and reliable method of diagnosing drug resistance in this country. Isoniazid-dissociated rifampin resistance detected among sixteen (22.5 %) of the 71 *M. tuberculosis*/*M. tuberculosis* complex isolates in the present study has rarely been reported before. It raises doubts about the possibility of using rifampin resistance as a surrogate marker for the estimation of multi-drug resistance that was suggested by various researchers in other countries. The MOTT isolate grown from the recurrent tuberculosis patient was resistant to rifampin while being sensitive to isoniazid.

All the susceptible strains tested for DNA sequence possessed wild-type sequences whereas all the resistant strains tested had novel mutations. Successful amplification of

mycobacterial DNA from the sputum specimens and the DNA sequencing results of the resistant strains obtained in this study demonstrate the future possibility of using this PCR based assay and DNA sequencing for the rapid detection of isoniazid and rifampin resistance.

