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### **Detection of Non-Tuberculosis Mycobacteria with molecular typing**

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Non-Tuberculous Mycobacteria (NTM), Mycobacteria Other Than Tuberculosis (MOTT) or atypical mycobacteria include those species not belonging to the *Mycobacterium tuberculosis* complex. Many species of these mycobacteria are recognized human pathogens. Chronic pulmonary disease resembling tuberculosis is the most common clinical presentation associated with NTM. Besides an overlap of disease spectrum with *M. tuberculosis* infection with NTM may give rise to positive Acid-Fast Bacilli (AFB) smears and histopathology findings similar to those found in tuberculosis. This study was performed to develop a simple assay based on Polymerase Chain Reaction (PCR) methodology for definitive diagnosis of mycobacterial infection. Bronchoscopy specimens (n = 202) were collected from patients attending the Teaching Hospital Kandy, who had pulmonary symptoms, nodular or cavitory opacities on chest radiograph or an HRCT scan that showed multi-focal bronchiectasis with multiple small nodules. DNA extracted from the AFB positive colonies originating from clinical samples (n = 46) were amplified with PCR using mycobacterial specific Sp1 and Sp2 primers designed from the 16S-23S rDNA intergenic spacer (ISR) region.

Of the 46 samples, four gave a unique ~280-320 bp DNA fragment of rapidly growing mycobacteria. Twenty one gave a unique ~200-220 bp amplification indicating the presence of slow growing mycobacteria, while 15 had both bands indicating the presence of both slow and rapidly growing mycobacteria. For six cultures, no bands were present. DNA sequence analysis was performed on 21 fragments and the results revealed the presence of *Nocardia*, *M. intracellulare* and *M. phocaicum*. Six isolates were identified as rapidly growing *Mycobacterium sp.* Seven were identified as *M. tuberculosis*. Molecular analysis confirmed the presence of NTM in bronchoscopy specimens. Around 11% of the study population was suffering from non-tuberculosis mycobacterial diseases. Similar to countries in Africa, only microscopic examination is usually available to confirm the diagnosis of tuberculosis in Sri Lanka. The involvement of acid-fast NTM in tuberculosis like syndromes might result in the misdiagnosis of tuberculosis.

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