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**Title:** Detection of non-tuberculosis mycobacteria (NTM) in bronchoscopy specimens using molecular techniques

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**Body:** Background NTM include mycobacteria not belonging to the Mycobacterium tuberculosis complex. This study was performed to optimize a simple assay based on Polymerase Chain Reaction (PCR) and restriction enzyme digestion for diagnosis of mycobacterial infection. Methods Bronchoscopy specimens (n=202) were collected from patients attending the Teaching Hospital Kandy. DNA extracted from the AFB positive colonies (n=46) were amplified using known mycobacterial specific Sp1 and Sp2 primers. DNA sequencing was performed and the sequenced strains were digested with Hae III and Cfo I. Results Four isolates were rapid growers (~280-320 bp), 21 were slow growers (~200-220 bp) and 15 cultures had both rapid and slow growing mycobacteria. Sequence analysis revealed the presence of Nocardia, M. intracellulare and M. phocaicum. M. tuberculosis and Mycobacterium sp. Hae III digestion of H37Rv and M. bovis yielded 50 bp and 110 bp DNA fragments while M. phocaicum had 120 bp and 200 bp bands, Nocardia yielded 150 bp and 160 bp DNA fragments and M. intracellulare a 100 bp DNA fragment. Cfo I cleavage sites were not present in H37Rv, M. bovis, Nocardia and M. intracellulare hence remained undigested while M. phocaicum yielded 80 bp and 230 bp DNA fragments. Hae III along with only Cfo I was inadequate to differentiate M. tuberculosis complex isolates from M. intracellulare. PCR was able to differentiate the slow and rapid growers. Conclusion Molecular analysis confirmed the presence of NTM in bronchoscopy specimens and 11% of the study population was suffering from NTM diseases. The involvement of acid-fast NTM in tuberculosis like syndromes might result in the misdiagnosis of tuberculosis.