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Analysis of *Mycobacterium tuberculosis* and Nontuberculous Mycobacteria by Smart Cycler II Real Time PCR

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It is important to differentiate between *Mycobacterium tuberculosis* (MTB) and Non Tuberculous Mycobacteria (NTM) during theearly stages of diagnosis, as susceptibilityto anti-tuberculosis treatment varies with NTM co-infections.

Samples of viscous and non-viscous aspirations, urine, blood, bone marrow and fresh tissue fromsuspected tuberculous (TB) patients referred to the Nawaloka Metropolis Molecular Biology and Genetics Research Laboratory from January 2012 to March 2014 were simultaneously screened for MTB and NTM. A commercial MTB/NTM diagnostic kit (Seegene, USA) was used with Real Time PCR using Smart Cycler II (Cepheid, USA) technology for this analysis. Bacterial DNA was extracted from TB suspected samples (n = 656) and amplified for targeted genes IS6110 and MPB64 for MTB and 16S rRNA for NTM, respectively. Type of specimen, patient's age and gender were recorded. Of all samples tested, 396 (60.36%) consisted of pulmonary samples while the rest was extra pulmonary in nature. Of the 656 samples screened, 6.61% (n = 43) testedpositive for MTB, confirming pulmonary TB in 24 and extra-pulmonary TB in 19 cases. The composition of pulmonary TB confirmed samples consisted of 20 bronchial washings (41.86%), a single bronchial alveolar lung aspiration and 3 pleural aspirations. TB suspected samples of cerebrospinal fluid (n = 9), body fluids (n = 3; 2 of ascetic fluid and 1 lymph node aspiration), soft tissues (n = 2; 1 epididymal soft tissue, 1para spinal soft tissue), bone marrow aspirations (n = 4) and urine (n = 1), tested positive for extra pulmonary TB. A total of 16 samples (2.46%) tested positive for NTM. Bronchial washings (n = 9, 64.28%) yielded the highest number of NTM positives while the rest consisted of sputum (n = 4) and urine (n = 3) samples. Clinical significance of NTM positivity should be considered by the treating clinician. A higher number of males (n = 12) were diagnosed than their female counterparts (n = 2) of the 40-60 year age group that recorded the highest number of TB cases. A male predominance was also noted for NTM infections where patients above 60 years showed the highest number of confirmed infections, with 5 males and 2 females. Only a limited number of samples (n = 4, 0.609%) tested as co-infections of MTB and NTM. This is the first report on simultaneous screening for MTB and NTM using Real Time PCR in Sri Lanka.

Keywords: *Mycobacterium tuberculosis* (MTB), nontuberculous mycobacteria (NTM), Real Time PCR, Smart Cycler II, co-infections