SPECIES AND GENOTYPIC DIVERSITY OF NON-TUBERCULOUS MYCOBACTERIA ISOLATED FROM CHILDREN INVESTIGATED FOR PULMONARY TUBERCULOSIS AT THE IGANGA/MAYUGE DEMOGRAPHIC SURVEILLANCE SITE

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ABSTRACT

Detection of non-tuberculous acid-fast bacilli in respiratory secretions by direct smear microscopy might be misinterpreted as pulmonary tuberculosis (PTB) unless mycobacterial species identification is available. In Uganda, the relative prevalence of *M. tuberculosis* and non-tuberculous mycobacteria (NTM) in respiratory secretions, and therefore the magnitude of this diagnostic dilemma in the diagnosis of childhood pulmonary tuberculosis, remains unknown. This study aimed at identifying the species and genotypes of NTM isolated from Children investigated for symptoms suggestive of PTB in the Iganga/Mayuge Demographic Surveillance Site, a rural Uganda Community.

A total of 127 NTM isolates obtained from culture of 2500 infant induced sputum samples and 5000 adolescent sputum samples by the MGIT culture system were identified at the genus level by the mycobacteria specific 16S r DNA PCR. Genotype CM/AS DNA strip assay was used for the detection and identification to the species level. MIRU-VNTR genotyping specific for *Mycobacterium avium complex* species was performed by using six loci 292, X3, 25, 47, 7, and 10 that were previously identified as polymorphic for *M. avium* subsp. *paratuberculosis* strain K10. Additionally, Enterobacterial Repetitive Intergenic Consensus (ERIC) PCR was done on all strains and the PCR pictures were imported from the bio imager to the BioNumerics software version 5.0 (Applied Math's, Kortrijk, Belgium) for analysis to investigate possibility of clonality and transmission in the community.

Of the 127 isolates, 103 were confirmed positive for the genus specific 16S r DNA. Of the 103 isolates, Genotype CM/AS yielded 63 identifiable NTM; 32 non-identifiable NTM and eight strains belonging to the *M. tuberculosis* complex. The 63 NTM comprised of seven different species: *M. fortuitum* (63.5%), *M. szulgai* (14.3%), *M. gordonae* (9.5%), *M. intracellulare* (4.8%), *M. scrofulaceum* (3.2%), *M. lentiflavum* (3.2%), and *M. peregrinum* (1.6%). MIRU-VNTR genotyping of three *M. intracellulare* isolates showed three distinct strains and results were corroborated by ERIC-PCR genotyping on the same strains. Additionally, ERIC-PCR yielded distinct fingerprint patterns for *M. gordonae* and *M. szulgai*. However, the 40 *M. fortuitum* species generated 35 distinct patterns and two clusters with three and two isolates each, suggesting transmission. Socio-demographic characteristics of the subjects could not support the

possibility of transmission; hence clustering may be probably due to a common source.

The current study showed *M. fortuitum* to be the most predominant species among the children investigated for symptoms suggestive of PTB in this community. Furthermore, clustering was not supported by socio-demographics, suggesting no actual transmission between individuals. Similar studies should be done in different parts of the country, in both humans and the environment, so as to know the major species causing NTM infection in Uganda.