TITLE: METHODS FOR IDENTIFICATION OF NON-TUBERCULOUS MYCOBACTERIA: EVALUATION IN A REFERENCE LABORATORY.

AUTHORS: PEDACE, C.P.; SOUZA, A.R.; ALMEIDA, A.R.B.; SIMEÃO, F.C.S.; GALLO, J.F.; CHIMARA, E.

INSTITUTION: TUBERCULOSIS AND MYCOBACTERIOSIS LABORATORY, INSTITUTO ADOLFO LUTZ, SÃO PAULO, SP (AVENIDA DR. ARNALDO, 355, 9º ANDAR, CEP 01246-902, SÃO PAULO – SP, BRAZIL)

ABSTRACT:

The incidence of diseases caused by non-tuberculous mycobacteria (NTM) is increasing annually worldwide. The increase in the frequency of mycobacterial species isolation can be explained by several factors, such as the increase in immunocompromised individuals, innovation in isolation and identification techniques, increased research on NTM and the improvement of public health services to care tuberculosis cases. Accurate identification of NTM species assists in clinical management and is essential to evaluate species distribution in a given epidemiological setting. Most identification methods are now based on nucleic acid techniques. This study analyzed different methods to identify a set of clinical NTM isolates, obtained by diverse clinical materials, from the diagnostic routine of the Tuberculosis and Mycobacteriosis Laboratory at Institute Adolfo Lutz, Brazil, from September 2019 to May 2020. For this purpose, two commercial tests were evaluated, Speed-Oligo Mycobacteria SOM (Vircell, Granada, Spain) and Genotype Mycobacterium CM (Hain Lifescience, Germany), and an *in house* method, *hsp*65 gene restriction analysis (PRA). *hsp*65 gene sequencing was used as gold standard. The PRA method correctly identified species in 164/258 (63.6%), genus in 5/258 (1.9%), identified incorrectly 17/258 (6.6%) and did not identify 72/258 (27.9%) of the isolates. The SOM method correctly identified the species of only 108/258 (42.2%), genus in 43/258 (16.7%), identified incorrectly 49/258 (19%) and was not able to identify 58/258 (22.5%) of the isolates. The CM method, despite having correctly identified 157/258 (60.8%), genus in 41/258 (15.9%) and identified incorrectly 27/258 (10.5%), did not identify only 33/258 (12.8%) of the isolates. Three isolates had contamination could only be identified by the CM method. The evaluation showed the different potentials of the methods. While the PRA can identify a greater number of species, as it is an *in house* technique, it has limitations in detecting genetic material, resulting in the absence of an identification profile. On the other hand, commercial techniques are able to identify isolates even if they are contaminated. Thus, it is important for the clinical laboratory to evaluate the best method, or a combination of methods, considering the population that will be served and to stablish algorithms so that the correct identification of the species is made to assist the clinician in the proper therapeutic conduct.

Keywords: molecular diagnosis, nontuberculous mycobacteria, species identification.