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Evaluation of filter paper to transport inactivated Nontuberculous Mycobacteria for identification using the MALDI-TOF MS system

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Introduction

The identification of Nontuberculous Mycobacteria (NTM) is troublesome due to the complexity involved in the sample preparation process and to the time needed to obtain satisfactory results. Usually, the differentiation of species belonging to the *M. tuberculosis* complex from Non-Tuberculous Mycobacteria (NTM) is carried out at the State Center for Health Surveillance in Rio Grande do Sul state. However, the identification of NTM to species-level is only performed in the institution Professor Hélio Fraga in Rio de Janeiro state. Therefore, alternatives are needed to improve secure identification of the NTM regionally. The use of sterile filter paper previously impregnated with an inactivated bacterial biomass can be an option to transport microorganisms between laboratories (1). In addition, identification by MALDI-TOF MS would significantly reduce the time to obtain result (2). This study aimed to evaluate the use of filter paper to transport NTM for identification using MALDI-TOF MS.

Experimental section

A total of 60 isolates of NTM (41 *M. abscessus* complex, 11 *M. fortuitum* complex, 3 *M. avium* complex, 2 *M. smegmatis*, 2 *M. immunogenum*, 1 *M. intracellulare/M. chimaera*) identified by a reference method (PRA*hsp*65) were evaluated in this study. MALDI-TOF MS identification of each isolate was performed as follows: a) directly from original bacterial colony in solid media; b) after the extraction procedure recommended by Bruker®; and c) in filter paper after procedures of inactivation, impregnation and extraction (the paper filter was kept for 2 days in room temperature to simulate transport time). The score values were interpreted as follow: 2.00-3.00 - high-confidence identification (+++); 1.70-1.99 - low-confidence identification (+); 0.00-1.69 - no organism identification possible (-).

Results and Discussion

A total of 24 isolates were identified as (+++), 33 isolates as (+) and 3 isolates were not identified (-) directly from the original bacterial colony. Thirty-eight isolates were identified as (+++), 13 isolates as (+) and 9 isolates were not identified (-) after the extraction procedure. After filter paper disk transportation, a total of 49 isolates were identified as (+++), 10 isolates as (+) and 1 isolate were not identified (-). Considering the reliability of direct identification and after the extraction process, we obtained more high-confidence (+++) isolates after the extraction process, although 9 isolates were not identified after the extraction process, while only 3 isolates were not directly identified from original bacterial colony.

Conclusions

The mycobacterial cell wall, rich in mycolic acids, can make it difficult to directly identify these microorganisms by MALDI-TOF, so the manufacturer recommends the use of an extraction process to improve the sensitivity of the test. MNT present biohazard associated to their transport, as it has the ability to form biofilms and a safe method for identification is essential. Therefore, the inactivation of these microorganisms followed by impregnation in filter paper would make it possible for a large portion of MNT to be identified locally through reference laboratories that provide identification service using the MALDI-TOF MS. Moreover, inactivated MNT in filter paper does not present biohazard for transportation. Therefore, filter paper as a means to transport and storage of inactivated MNT can be considered a potential tool for faster, more accurate, biosafe and less expensive identification.

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