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P2405 Improved MALDI-TOF mass-spectrometry identification of non-tuberculous Mycobacteria isolates from MGITs using sonication

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Background: The growing number of NTM species described makes it necessary to apply an accurate diagnostic tool for the correct identification at the species level of Non-Tuberculous Mycobacteria (NTM) isolates from clinical specimens. This is a requirement to establish their clinical significance. MALDI-TOF MS has so far demonstrated a high correlation with other molecular diagnostic methods for the correct identification of NTM.

In this study, NTM identification of isolates from liquid medium was performed using MALDI-TOF MS in order to decrease the turnaround time until the final identification. An improved sample processing method that included a sonication step was evaluated.

Materials/methods: Sixty NTM isolates from 18 NTM species previously identified by DNA sequencing analysis were experimentally inoculated into a BACTEC Mycobacteria Growth Indicator Tubes (MGIT, Becton Dickinson) at a concentration of 0.5 MacFarland. Once the tubes flagged positive, 1 ml of culture medium from the bottom of the tube was collected, centrifuged, resuspended in 300 □l of HPLC-grade water and inactivated for 30 min at 95ºC. Tubes were cooled down and divided into two: 150 □l were submitted to sonication for 15 min and, afterwards, both tubes were mechanically disrupted using glass beads and protein extraction was performed according to the manufacturer's instructions. One microliter of supernatant was placed on the MALDI target plate in triplicates and protein spectra were acquired using the improved Mycobacteria method and identified with the Mycobacteria Library 4.0. The identification provided by MALDI-TOF MS as well as the score values obtained were compared between both groups.

Results: The implementation of the sonication step allowed the correct species-level identification of 58/60 of the analyzed NTM isolates, 53 of them with score values ≥1.7. On the other hand, the standard protocol yielded 51/60 correct species assignment, although 7 of them were unreliable, with score values <1.5.

Conclusions: The identification of NTM isolates from MGITs can be rapid and reliably performed using MALDI-TOF MS by including a sonication step in the sample processing method. However, further studies applying this processing to clinical NTM isolates are required in order to assess the performance of MALDI-TOF MS in routine samples.