INTRODUCTION

- Identification of moulds using growth morphology and microscopic morphology is subjective, time consuming and has variable performance.
- MALDI-TOF MS provides a rapid and accurate alternative to traditional mold identification.
- This study evaluated the performance of MALDI-TOF identification using two extraction methods, and the three major fungal databases currently available: Bruker MBT filamentous fungi library 3.0, the National Institute of Health (NIH) and Mass Spectrometry Identification (MSI).

METHODS

- 141 fungal clinical isolates were tested, including 28 dermatophytes, and 113 non-dermatophytes.
- Isolates were pre-characterized to the genus (141) or species/complex level (80) by colony morphology, microscopy or ITS sequencing.
- Dermatophytes were grown on sabouraud with chloramphenicol (SAB), dermatophyte test medium (DTM) and potato dextrose agar (PDA). Non-dermatophytes were grown on SAB, PDA and brain heart infusion with chloramphenicol (BHI).
- For the MSI method, 2 minutes centrifugation achieved same results as 10 minutes centrifugation. This modification was used in this study.
- Optimization of growth conditions: overall, scores were highest on the first day of visible colony growth. Examinations from young colonies were used in this study.
- Isolates were extracted using the Bruker extended direct transfer method and the modified MSI tube extraction method. The spectra were run on all three databases.
- *Aspergillus ustus* (ATCC 1041) was used as a positive control in each extraction.
- Identifications were only considered to be correct if scores were above the database recommended thresholds:
  - Genus level: >1.7 (Bruker, NIH)
  - Species/complex level: >2.0 (Bruker, NIH) >20 (MSI)
- Resolution of discrepant results: colony morphology and microscopy, and ITS sequencing at a reference lab were conducted for isolates with discrepant results.

RESULTS

- The performance of the tube extraction method is presented in Table 1. The performance of the extended direct transfer method is presented in Table 2. The modified tube extraction method performed better than the extended direct transfer method. Individually, the MSI database had the highest identification rate. The best results were obtained when all databases were used sequentially.
- Identification of most clinically relevant organisms was good. Some notable exceptions were *Acremonium, Phialophora, Basidiomycota* and *Trichosporum spp.* (Table 3). The discrepant results are shown in Table 4.

CONCLUSION

- MALDI-TOF MS is valuable tool for filamentous fungal identification.
- Tube extraction performs better than extended direct transfer.
- A stepwise testing algorithm using a combination of databases is most suitable in a clinical microbiology laboratory.

**Table 1. Performance of MALDI-TOF identification using the modified MSI tube extraction method.**

**Table 2. Performance of MALDI-TOF identification using the Bruker extended direct transfer method.**

**Table 3. Identification rates for all clinically relevant organisms.**

**Table 4. Discrepant results and resolution.**

**Table 5. Positive predictive values for the Bruker and NIH databases.**

**Figure 1: Recommended algorithm for filamentous fungal identification using MALDI-TOF MS.**

**References:**

1. Bruker MBT Filamentous Fungi Library Brochure 03-2018

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