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Establish in Medellin (Colombia) an in-house library to identify clinically important filamentous fungi by MALDI-TOF MS

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Epidemiology of fungal infections has changed in an important manner in the last decades, however timely diagnosis and treatment are still a current challenge.

Accurate and swift identification of the agents that cause fungal infections are paramount, since sources of infection, differences between therapeutic regimes, and in-vitro susceptibility profiles may vary amongst species and strains.

The application of Matrix Assisted Laser Desorption/Ionization Time-Of-Flight (MALDI-TOF) mass spectrometry (MS) for the identification of fungal samples is currently well-established based on the remarkable reproducibility for the measurement of constantly expressed and highly abundant proteins, such as ribosomal proteins, that are used to generate a fingerprint profile. However, the use of this tool for filamentous fungi identification in routine clinical laboratories is still limited due to several reasons, such as, 1) the biological variations among fungal clinical isolates or 2) the lack of a spectrum of reference in commercial libraries for the identification of emerging, endemic and prevalent fungi in tropical regions. Consequently, the establishment of an in-house library that will contain spectra for prevalent fungi found in routine mycological diagnosis at a local level, and of those not included in commercial libraries, is a demand.

The aim of this research was to establish a library of spectra for the identification of clinically important filamentous fungi through MALDI-TOF MS in a mycological diagnosis laboratory.
In order to establish the in-house spectra library, 21 strains were used, including dermatophytes (*Trichophyton* (3), *Microsporum gypseum* (1) and *Microsporum canis* (1)), *Aspergillus* (7), *Fusarium* (4), *Neoscytalidium dimidiatum* (1) and *Sporothrix* (4). Afterwards, the spectra were validated with 17 strains and 35 clinical isolates identified by classic and molecular methods. In addition, 16 of 17 strains and 29 of 35 clinical isolates were subjected to identification in commercial filamentous fungi libraries and in BDAL (Bruker Daltonics, Germany). The results obtained from the three libraries were compared. For the acquisition of the reference spectra for each genus and for some species, it was necessary to standardize growth and assay conditions.

The in-house generated library identified clinical isolates/strains down to species in the proportion of 82.5%/93.0% of the cases, and down to genus in 88.6%/100.0%. In addition, 11.4%/0.0% of the fungi not being identified. With the filamentous fungi library, identification down to genus and species were of 62.0%/64.3% and 31.0%/42.8%, respectively for clinical isolates and strains, while 37.9%/35.0% proportion remained unidentified. With the BDAL library identification the proportion for clinical isolates/strains was of 42.3%/7.1% for genus and 3.4%/0.0% for species; identification was not achieved in 51.7%/92.8% of the cases. Optimal growth time required to obtain proteins varied among genera and among some species. With the in-house built library, it was possible to identify strains and clinical isolates, in some cases, more accurately than with commercial libraries. In addition, fungal spectra that are not included in commercial libraries were included; this foster the standardization of the growth conditions for the different strains, the protein extraction technique, as well as the definition of criteria for the acceptance of a spectrum of reference.