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Identification of dermatophytosis and onychomycosis etiologic agents by classic and by MALDI-TOF MS phenotypic methods

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Skin, nail and hair infections are a frequent cause of dermatological consultation in tropical countries. The fungi involved in these infections are usually dermatophytes, *Candida* spp and some non-dermatophyte filamentous fungi (NDFF). Within the NDFF group, *Neoscytalidium dimidiatum* has been increase as common agent of onychomycosis. The identification of these etiologic agents, in routine mycological diagnosis, is mainly based on macro- and micro-morphology characters. However, this approach is subjective and the identification could be not accurate. This is an obstacle for the implementation of appropriate therapy, since that antifungal susceptibility profiles may vary among species. Currently, more sensitive, specific, rapid and cost-effective techniques are being introduced for the identification of microorganisms, such as Matrix Assisted Laser Desorption/Ionization Time-Of-Flight (MALDI-TOF) mass spectrometry (MS) with the protein profile of each microorganism. The aim of our study was to compare the identification by classic and MALDI-TOF MS phenotypic methods fungal isolates collected from patients with a clinical diagnosis of onychomycosis or dermatophytosis. In total, 134 clinical isolates were included in the study and identified by classic macro- and micro-morphology characters from de cultures. In addition, the isolates were analysed by MALDI-TOF MS. An in house spectra library was establish in order to identify *N. dimidiatum*, because this species is not included in commercial libraries.

Identification by classic approach resulted in 76 dermatophytes with *T. rubrum* (41), *T. mentagrophytes* complex (15), *T. tonsurans* (6), *E. flocossum* (2), *M. gypseum* (1) and *M. canis* (11); 26 *Candida* with *C. albicans* and *C. tropicalis* representing 5 and 4 isolates, respectively; for NDFF the prevalent species was *N. dimidiatum* with 25 out of 31 isolates, follow *Fusarium* spp (4)*, Aspergilllus flavus* (1) and *Penicillium* sp (1). One *Trichosporon* sp was also identified.

For MALDI-TOF MS the dermatophytes isolates identified as *T. rubrum* and *M. canis* reduced to 16 and 4, respectively and the prevalent group became *Trichophyton* spp (37). For NDFF, MALDI-TOF MS confirmed the identification at species level of 19 *N. dimidiatum*, the isolate *A. flavus* and at genus level the 4 *Fusarium* spp. Within the NDFF isolates, the *Penicillium* sp isolate was correctly identified by MALDI-TOF MS as *A. versicolor*. The *Trichosporon* sp isolate was also confirmed. Finally, the identification

of *Candida* isolates by MALDI-TOF MS were resolved at species level with *C. parapsilosis* (6), *C. haemulonii* (1) and *C. guilliermondii* (1).

The inconsistencies observed in the results from the two approaches used with more evidence for filamentous fungi than for yeasts identifications can be due to: 1) the established score to define the identification up to genus or species level; 2), sample preparation and the quality of the extraction of proteins; and, 3) the lack of spectra or low number of species included in the commercial libraries could interfere in the final outputs. The results of this work clearly show that the classic approach is prone of errors, much more work is needed to make MALDI-TOF MS identification a routine in clinical laboratories and to clarify these results identification by molecular biology is advised.

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