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Identification of dermatophytes isolated from *tinea capitis* infections using a liquid media MALDI-TOF MS protocol

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Objectives: *Tinea capitis* is a common childhood superficial infection of the scalp caused by dermatophytes. Conventional methods based on morphological characteristics to identify theses fungi are time-consuming and complex, requiring expert mycological knowledge. Recently, MALDI-TOF MS has become a powerful tool in clinical microbiology for rapid identification of these microorganisms. In the present study, a liquid media MALDI-TOF MS protocol was developed and validated for the fast and accurate identification of dermatophytes species responsible for *tinea capitis* infection, using both dermatophytes from reference strains and clinical isolates. The main purpose was to work with primary isolates in order to avoid the culture step and lead to a faster diagnosis. Identification techniques (conventional identification and MALDI-TOF MS based on liquid and solid culture) were evaluated and compared for their rate of correct identification and turnaround time.for the fast and accurate identification of dermatophytes species responsible for *tinea capitis* infection, using both dermatophytes from reference strains and clinical isolates. The main purpose was to work with primary isolates in order to avoid the culture step and lead to a faster diagnosis. Identification techniques (conventional identification and MALDI-TOF MS based on liquid and solid culture) were evaluated and compared for their rate of correct identification and turnaround time.

Methods: The in-house BCCM/IHEM database made from references strains on solid culture was used. First, the liquid media MALDI-TOF MS protocol was validated using references strains. Secondly, the same protocol was applied on clinical isolates collected from Saint-Pierre University Hospital and paralleled with solid media MALDI-TOF MS protocol.

Results: The use of the liquid media MALDI-TOF MS protocol resulted in a rate of 100% of correct identification at the species level for reference strains and 78,8% for clinical isolates. The protocol was statistically significantly faster than the conventional method. The results of the different methods disagreed for 17 isolates. The complete extraction from the solid media MALDI-TOF MS gave the highest correct species identification with the highest mean of log-scores.

Conclusion: The liquid media MALDI-TOF MS technique is an accurate method for the correct identification of dermatophytes at the species level and is much faster than the conventional technique. The main advantage lies in not needing a culture step for primary isolates, thus leading to a faster diagnosis. For the identification of closely related species, the complete extraction from solid media MALDI-TOF MS protocol remains the most reliable technique.

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Dermatophytes' identification by Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) - the experience of a clinical laboratory

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Objectives: Dermatophytes are a challenging group of fungi that infect the keratinized tissues. The taxonomy of these fungi has changed recently with the reclassification of some species and description of new ones. However, many clinical laboratories still base the identification of dermatophytes on their phenotype. Since dermatophytes are very pleomorphic, macro and micromorphology are often insufficient to reach a correct classification and may lead to misidentifications. The identification based on MALDI-TOF relies on the protein profile of the microorganism. Thus, this study aims to summarize our current laboratorial experience of dermatophyte identification using MALDI-TOF MS.

Methods: From january to april 2018, 95 dermatophytes isolates, collected from human keratinized samples and also from quality control programs were characterized by phenotypic analysis, and by

VITEK MS V3.2 bioMerieux. Before identification procedure, isolates were inoculated on Sabouraud Dextrose agar plates and incubated at 27°C during 5 to 10 days. Species were identified taking into account clinical features, as well as cultural, microscopic and physiological characteristics. Prior to MALDI-TOF MS analysis, the samples were pre-treated according to the manufacturer's protocol for filamentous fungi. Molecular identification by sequencing of the internal transcribed spacer 1 (ITS1) was performed in 34 of those isolates

Results: Through phenotypic analysis eight different species were identified (54 Trichophyton rubrum; 4 T.soudanense; 22 T.interdigitale; 1 T.mentagrophytes; 3 T.tonsurans; 7 Microsporum canis; 3 M.audouinii; 1 Microsporum spp.- (non canis or audouinii). MALDI-TOF analysis showed an identification agreement in 80 cases (84,2%) with a confidence level of 99,9%. Eight isolates showed divergent identification results: three T.rubrum were identified as T.violaceum, three T.soudanense were identified as T.rubrum, one T.mentagrophytes was identified as T.interdigitale and one T.tonsurans was identified as T.rubrum. In four cases MALDI-TOF analysis did not get a profile. The ITS sequencing analysis of discrepant results corroborated the MALDI-TOF identification in five of them. On the other hand, T.soudanense was only identified by phenotypic analysis since MALDI-TOF and ITS sequencing result was T.rubrum. MALDITOF identification of T.violaceum was not confirmed by ITS sequencing that identified T. rubrum instead, in accordance with the phenotypic identification.

Conclusion: Correct identification of dermatophytes to species level requires sequencing of the ITS, LSU, and/or beta-tubulin regions. The implementation of this methodology in a clinical laboratory is expensive and time consuming. MALDI-TOF identification is a good option for dermatophytes' identification performed in laboratory routine, since costs of consumables as well as time of sample preparation are lower than for PCR analysis and doesn't require long training period as phenotypic identification does. In this study, however, both methods failed to identify some species variants like Trichophyton soudanense or T. violaceum. The combined use of both MALDI-TOF and phenotypic methods seems to be the better approach for dermatophytes' identification since some species show significant phenotypic and clinical differences.

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A retrospective study of dermatophyte infections caused by *Tricophyton rubrum* and *T. interdigitale* in the County of Stockholm, Sweden.

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Objectives: Dermatophytosis is a common fungal infection caused by keratinophilic fungi that are capable of invading nail, hair and superficial layers of the skin of humans and animals. The aim of this study was to determine the epidemiologic profile of the most frequent dermatophytosis such as *Tinea unguium* and *T. pedis* caused by *Tricophyton rubrum* or *T. interdigitale* in Stockholm, Sweden.

Methods: In 2015 we developed and validated a rapid and sensitive real-time PCR method for detection of the two most prevalent dermatophytes in Northern Europe. Fungal DNA was extracted directly from clinical samples (toe or finger nail and skin scrapings from the feet) by using proteinase K and heat as pre-lysis step, followed by automated DNA extraction on the PSH/MagNA Pure 96 Compact. Real-time PCR was performed using pan-dermatophyte primers for detection of all dermatophytes and specific primers for identification of *T. rubrum* and *T. interdigitale*. Specimens from finger nails were also cultured on Chrom agar plates for detection of onychomycosis caused by yeasts.

Results: Laboratory records comprising PCR results of 13,683 specimens (3,304 skin respective 10,379 nail fragments) collected from May 2015 through March 2019 were retrospectively analyzed. *Tricophyton rubrum* (42%) was the predominant pathogen identified from these cases, followed by *T. interdigitale* (4,9%). In contrast, 2% of the samples were positive for pan-Derm indicating the prevalence of infections caused by other pathogens distinct to *T. rubrum* or *T. interdigitale*. Sex distribution analysis of the patients showed that males (40%) were more susceptible to suffer dermatophyte infections compared to females (21,9%). (Furthermore, the prevalence of