

identification in 2 cases, but reported microorganism identification in 3 cases. 235 samples reported as positive by the screening device, led to significant bacterial growth in culture. 220 of them (93.6%) had bacterial counts (BC) >100,000 CFU/mL, 7 (3.0%) had BC between 50.000 and 100.000 CFU/mL, and 8 (3.4%) had BC <50,000 CFU/mL. In whole, MALDI-TOF MS identified correctly the microorganism involved in the UTI, directly from the urine sample, in 91.8% of cases at the species level, and at 92.7% at the genus level. Failures were observed in only 6.9% of samples. The most frequent microorganism isolated was *E. coli* (173 isolates). MALDI-TOF ME identified the microorganism in 163 cases (94.2%) (97.6% when BC was >100,000 CFU/mL).

**Conclusion:** MALDI-TOF MS gives a reliable identification of UTI pathogens, previously to culture, in >90% of positive samples. MALDI-TOF performance is optimal when there is a high BCs and Gram negatives are involved.

**P1795 MALDI-TOF ICMS: capability, potentiality and limits in the fast identification of *Trichophyton rubrum* from clinical cases occurrence in Portuguese health centres**

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**Objective:** *Trichophyton rubrum* is presently the most common worldwide pathogen causing dermatophytoses such as tinea corporis, tinea capitis, tinea pedis, and onychomycosis [1]. The main aim of the present work was assess MALDI-TOF ICMS as a fast and reliable technique in the identification of *T. rubrum* from clinical cases occurrence in the Portuguese health centres, and evaluates the potentialities and limits of this new microbial identification technique on the taxonomy of these infectious dermatophytes.

**Methods:** Fungi were grown for 10 days in solid medium (SDA, Sabouraud Dextrose Agar) and then the mycelia were direct transferred from the SDA plate to the MALDI stainless steel template and mixed with 1 ml MALDI matrix solution (75 mg/ml 2,5-dihydroxybenzoic acid in ethanol/water/acetonitrile [1:1:1] with 0.03% trifluoroacetic acid). The sample mixtures were air dried at room temperature. The analyses were performed in our laboratory on an Axima LNR system (Kratos Analytical, Shimadzu, Manchester, UK) equipped with a nitrogen laser (337 nm). The mass range from m/z = 2,000 to 20,000 Da was recorded. *Escherichia coli* strain DH5 $\alpha$  with known mass values of ribosomal proteins was used for external calibration. The fungi classification was performed on the SARAMIS software (AnagnosTec mbH, Potsdam-Golm, Germany). Molecular biology was used when appropriated with PCR based-technology. The presence of a 203-bp PCR product confirmed *T. rubrum* identification.

**Results:** All strains were accurately and consistently identified as *T. rubrum* by MALDI-TOF ICMS combined to SARAMIS database analysis. Spectral mass analysis proven to be a rapid method since the analysis took only a few minutes to perform with the benefit of any laborious sample preparation procedures or any expensive chemical reagent was needed.

**Conclusions:** The fungal spectral analysis by MALDI-TOF ICMS was as good as molecular biology in order to identify *T. rubrum* but much faster and cheaper.

**Reference(s)**

- [1] Degreef, H. (2008) Clinical Forms of Dermatophytosis (Ringworm Infection) *Mycopathologia* 166: 257–265.

**P1796 A MALDI-TOF assay for the rapid identification of *Aspergillus* and *Candida* sp. in clinical samples**

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**Objectives:** Invasive mould infections are becoming worldwide increasing, resulting in significant morbidity and mortality especially in paediatric populations, immunocompromised and transplanted patients.

Particularly the phyla Ascomycota, and Basidiomycota are involved in severe infections in children. The Ascomycota includes yeasts of the genera *Saccharomyces*, *Pichia*, and *Candida* and many filamentous fungi of the genera *Aspergillus*, *Penicillium* and *Fusarium*. The Basidiomycota *Cryptococcus neoformans* is an emerging opportunistic human pathogen. Identification of the increasing diversity of fungal pathogens by conventional methods (biochemical methods for yeast and phenotypic methods for mould) is often difficult, time-consuming and frequently, for unusual fungi, inconclusive.

In our study, we exploited the MALDI-ToF MS (Matrix-assisted laser desorption ionization-time of flight-mass spectrometry) Biotyper to for rapid and specific identification of fungal species from clinical specimens.

**Methods:** 270 yeast and 80 mould fungi strains were collected from diversified clinical samples in our microbiology unit and grown on Sabouraud solid medium. Protein profiles were provided by using MALDI-ToF MS Biotyper for both clinical and reference strains, the latest purchased from the Centraalbureau voor Schimmelcultures (CBS) culture collection. Generated spectra were acquired and processed to produce appropriate species identification.

The results were compared to conventional biochemical and phenotyping identifications in order to produce concordance data. When appropriate, MALDI-TOF-based results were compared to sequencing-based identification analysis (D2 LSU 28S rDNA).

**Results:** MALDI-ToF MS provided the correct yeast identification with a sensitivity of 95% and specificity of 94% compared to conventional methods. For mould identification the MALDI-ToF MS provided a 80% of sensitivity, due to the absence of appropriate reference species in the database. The MALDI-ToF-MS identifications provided 100% of concordance with the 28S rDNA D2-LSU-based sequencing analysis for all the analysed isolates.

**Conclusions:** This study provides a fast and reliable method, based on MALDI-TOF MS detection, for the identification of fungal species in clinical samples. This procedure might overcome current diagnostic tests and may represent a new frontier for the rapid and specific management of fungal infections in paediatric patients.

**P1797 Identification of clinical fungi by MALDI-TOF MS: how to deal with growth-dependent variability in peak patterns**

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The identification of microorganisms by MALDI-TOF MS is about to replace biochemical identification procedures for routine diagnostics. While the mass spectral identification of most bacteria is straightforward, the identification of fungi with whole cell MALDI-TOF MS is more challenging for several reasons. Most importantly, the peak pattern of an individual isolate can change dramatically in dependence of incubation time and medium composition. Especially the transition from non-sporulating to sporulating mycelium generally results in marked differences in mass fingerprints.

One option to overcome this difficulty is to strictly standardize the cultivation conditions of reference and sample isolates. This can, however, be rather impractical due to differences in growth behaviour, particular medium demands, and handling requirements of individual isolates. Another strategy is to obtain reference data from well characterized isolates for different growth conditions. The latter strategy is followed for the Spectral Archive and Microbial Identification System (SARAMIS) by the acquisition of whole cell mass spectra of reference isolates grown on a variety of solid media and at different incubation times. Generally, reference isolates are incubated on three different media and mycelium samples are taken after three different incubation times. By this 3 x 3 approach the variability of mass fingerprints of individual isolates is largely captured and the mass spectra are deposited in the reference database. When multiple isolates of a species are contained in the database, the corresponding data were used to compute SuperSpectra for fully automated identification.