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MALDI-TOF MS and LC MS/MS: New insights for clinical fungal ID

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Since the last two decades MALDI-TOF MS (Matrix Assisted Laser Desorption Ionization – Time Of Flight – Mass Spectrometry) has been applied to analyse the chemical cellular composition of microorganisms, providing rapid and discriminatory proteomic profiles for their identification and subtyping [1]. The application of this technique for the identification of clinical fungal sample is currently well-established. The remarkable reproducibility of this method is based on the measurement of constantly expressed and highly abundant proteins. The usually observable molecular mass range is between 2 and 20 kDa, where important conservative ribosomal proteins are used as markers to generate a cellular fingerprint.

In the clinical mycology field, MALDI-TOF MS has been used as either an important component in the polyphasic identification of yeasts and filamentous fungi, or as a rapid diagnostic tool for routine fungal identification. Nevertheless, there are some important limitations to the MALDI-TOF MS method particularly in relation to the identification of closely related fungal taxa. In addition, commercial databases (CDB) used to archive and compare MALDI TOF MS spectra have also some limitations in their taxa coverage and some of them are based on the different protocols used by the main MALDI-TOF MS manufacturers. In some cases, these protocols have been changed over the last decade when MALDI-TOF MS got its triumph in the clinical microbiology. In contrast, the evolution of sample preparation over the last years was not accomplished by a clean-up process or adaptation in some CDB.

In general, the protocols used in different laboratories are often not standardised. Currently, a huge number of publications with different protocols for protein extraction and matrices used for MALDI-TOF MS analysis are available in the scientific literature [1]. This, together with the use of different (a) growth conditions such as temperature, culture medium composition, time of incubation, and light/dark conditions, and (b) biological material (mycelium, spores or mixture of both), can strongly affect the final fungal identification and the concomitant clinical diagnostic.

In this work experimental data of MALDI-TOF MS for clinical yeasts and filamentous fungi obtained through an international collaboration project involving partners from Argentina, Brazil, Chile and Portugal will be presented. Results obtained show that MALDI-TOF MS sample preparation protocols and fungal growth conditions can strongly affect identification of clinical fungi. In addition, it was markedly truth when phylogenetic closely related fungal species were analysed. In order to overcome such limitation, mass spectrometry systems (e.g. LC MS/MS) that are able to generate online mass spectra of specific ions with further online sequencing of these ions will be herein discussed as an alternative.

[1] Santos, C., Francisco, Mazza, M., Padovan, A.C.B., Colombo, A., Lima, N. In: The Triumph of MALDI-TOF Mass Spectrometry and New Developments in Tandem Mass Spectrometry for Clinical Microbiology (Eds. Shah, H. N., Russell, J. E., and Gharbia, S. E.), Wiley, 2015, accepted for publication.