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Paper Poster Session

Fungal diagnosis: from culture to molecular techniques

Identification of *Pseudallescheria/Scedosporidium* species using matrix assisted laser desorption/ionization time-of-flight mass spectrometry

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Background: The filamentous fungi belonging to the *Pseudallescheria/Scedosporium* species are opportunistic pathogens affecting immunocompromised patients and those with an underlying lung disease. The timely identification of these moulds is crucial for the rapid implementation of the appropriate antifungal therapy due to their different susceptibility. In this study we evaluated MALDI-TOF MS for the rapid identification of *Pseudallescheria/Scedosporium* species from clinical samples.

Material/methods: Thirty two fungal isolates –ten *Scedosporim prolificans*, ten *Scedosporium apiospermum*, ten *Pseudallescheria boydii* and 2 *Scedosporium aurantiacum* previously identified to the species level by partially amplifying and sequencing the β -tubulin (*benA*) gene using primers Bt2a and Bt2b- were added to in-house database of reference spectra following the manufacturer instructions.

Using this updated database and the MBT Filamentous Fungi Library 1.0 containing 365 references (Bruker Daltonics GmbH, Bremen, Germany), 63 additional fungal isolates from clinical samples were identified using a simplified protocol: the surface of the colonies was scraped with a metallic, sterile loop and the fungal mass was mixed with 1.5 μ l of 100% formic acid previously spotted onto the polished steel MALDI target plate. Once the mix was air-dried, the mixture was covered with 1 μ l of matrix and analysed with a Microflex LT bench top mass spectrometer (Bruker Daltonics, Bremen, Germany) using standard settings.

Results: MALDI-TOF MS provided a correct identification of 61 out of the 63 fungal isolates included in this study (96.82%). The remaining isolates were two *S. apiospermum* that could not be reliably identified (Table 1). Besides, the accuracy of the identification correlated with the high score values recorded: 46 isolates (73.02%) were identified with score values ≥ 1.800 , indicating that the identification was reliable both at the genus and species level. This fact was especially outstanding in the case of *S. prolificans*, with 81.82% of the isolates identified with a score value ≥ 1.800 .

Conclusions: MALDI-TOF MS has shown to be highly useful in the microbiology laboratory for the accurate identification of filamentous fungi from the *Pseudallescheria/Scedosporium* species. The use of the in-house database and the improvement in sample preparation allows a rapid and robust identification of these fungi, avoiding laborious and subjective morphological identification used in the microbiology laboratory so far.

Species identified by <i>benA</i> gene sequencing	Number of isolates	MALDI-TOF ID (score \geq 1.800)	MALDI-TOF ID (score 1.799-1.500)	MALDI-TOF ID NOT RELIABLE
<i>Scedosporium prolificans</i>	22	18	4	-
<i>Scedosporium_ apiospermum</i>	17	11	4	2
<i>Pseudallescheria boydii</i>	23	17	6	
<i>Scedosporium aurantiacum</i>	1	-	1	-
TOTAL	63	46 (73.02%)	15 (23.81%)	2 (3.17%)