

Identification and typing of the emerging pathogen *Candida auris* by matrix-assisted laser desorption ionisation time of flight mass spectrometry

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Summary

Candida auris is an emerging antifungal resistant yeast species causing nosocomial and invasive infections, emphasising the need of improved diagnostics and epidemiological typing methods. We show that MALDI-TOF VITEK-MS followed by amplified length polymorphisms allows for accurate species identification and subsequent epidemiological characterisation of strains encountered during potential outbreaks.

Key words: *Candida auris,* matrix-assisted laser desorption ionisation-time of flight mass spectrometry, amplified fragment length polymorphism, MS VITEK.

Introduction

Candida auris is an emerging pathogen first described in 2009 as a species closely resembling C. haemulonii and whose involvement in nosocomial fungaemia and deep-seated infections have widely been reported subsequently in East Asia.¹⁻⁴ Notably, C. auris strains appear to be clonal in Indian hospitals and exhibit specific antifungal resistance.^{5,6} This underlines that obtaining an accurate species identification and reproducible antifungal susceptibility testing are crucial to guide adequate therapy.⁷ However, C. auris is still frequently misidentified as C. haemulonii based on current taxonomy⁶⁻¹¹ and use of presently available non-MALDI-TOF commercial systems for yeast identification. Conventional commercial yeast identification methods seem to have come of age in the present era of new diagnostic approaches.¹² The aim of this study

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was to determine if an accurate identification could be obtained using the MS-VITEK matrix-assisted laser desorption ionisation time of flight mass spectrometry (MALDI TOF MS or MALDI). Bruker MALDI has been used successfully for the identification of a variety of yeast species,^{13,14} including *C. auris.*^{9,10} The secondary aim of this study was to define if typing of the strains would be feasible using MS-VITEK MALDI.

Material and methods

Knowledge base creation

Twelve reference strains of *C. auris* were used for the VITEK MS knowledge base creation. Strains were inoculated on Sabouraud (SDA; bioMérieux, La Balme, France) agar plates and incubated at 30 °C for 18 to 24 h prior to spectra acquisition.

Knowledge base testing and typing

The test of the expanded VITEK MS knowledge base was then carried out on 50 additional independent *C. auris* isolates that were not used to create the initial database. All of the 50 tests *C. auris* isolates originated from clinical specimens from three different countries

(India n = 43, South Korea n = 2 and Brazil n = 5) and were cultured on SDA plates incubated at 30 °C for 48 h prior to spectra acquisition.

Amplified length polymorphisms (AFLP) typing

Amplified length polymorphisms was performed in parallel on 82 *C. auris* strains (India, n = 67; South Africa, n = 6; Brazil, n = 5; Japan, n = 2; South Korea n = 2) as described previously (6). Control isolates of *C. haemulonii* (n = 4), *C. duobushaemulonii* (n = 4) and *C. pseudohaemulonii* (n = 3), were included. AFLP data were interpreted using the Pearson and UPGMA algorithms.

MALDI

MALDI identification was performed using VITEK MS Plus system (bioMérieux, Marcy l'Etoile, France) V2.0 according to the manufacturer's instructions. Yeast cells were deposited directly on the sample plate, covered by 0.5 μ l of formic Acid (FA; bioMérieux) and air-dried. One microlitre of α -cyano-4-hydroxy-cinnamic acid matrix (CHCA; bioMérieux) was added to each spot and air-dried before analysis.

Data exploration

Peak lists $(2000-20\ 000\ m/z)$ were imported into the SARAMIS spectra base (bioMérieux) for further analyses. Clusters were made to compare *C. auris* spectra to those obtained for other common yeast species. Also, multidimensional scaling analysis (MDS) was carried out to evaluate the power of MALDI to discriminate *C. auris* strains. This technique consists of representing spectra as points in a usually two-dimensional space, such that the distances between the points match the observed dissimilarities as closely as possible. Dissimilarity matrix used in this study for MDS is based on Spearman correlation.

Results and discussion

We successfully introduced *C. auris* as a new *Candida* species in the VITEK MS clinical database. All isolates of *C. auris* used to evaluate the performance of the knowledge base were correctly identified to the species level except four strains that were also found to be discordant by 18S. Cluster analysis of the MALDI spectra, based on peak similarity, clearly discriminated *C. auris* from other clinically relevant *Candida* species,



Figure 1 Cluster based on the relative similarity of spectra from representative isolates for the most common yeast species present in the VITEK MS database showing clear distinction between *Candida* species. A similarity below 65% for two spectra means that the strains belong to different species.

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Figure 2 Dendrogram of amplified length polymorphisms analysis of *Candida auris* isolates from India, Japan, South Africa and South Korea and members of an additional three different closely related *Candida* spp. It was constructed using UPGMA in combination with the Pearson correlation coefficient and was restricted to fragments 60–400 base-pairs in length. The scale bar identifies the percentage similarity.

© 2016 Blackwell Verlag GmbH *Mycoses*, 2016, **59**, 535–538 including *C. haemulonii* (Fig. 1). Thus, VITEK MS MALDI could be used to efficiently identify this emerging yeast species. Bruker MALDI has also been shown recently to successfully identify this emerging species.^{9,10}

Further, VITEK MS MALDI in this study could differentiate *C. auris* strains using the direct smear protocol. MDS discriminated distinct groups of C. auris spectra depending on the geographic origin of the strains (data not shown). An overlap between Indian and South African strains was observed, whereas strains from Brazil, Korea and Japan made distinct subgroups. These observations are in conformity with previous studies using AFLP^{5,6,10} and the Bruker platform demonstrating that the C. auris isolates from India are clonal and distinct from Korean, Japanese and Brazilian isolates. AFLP performed on the same set of strains also identified geographic clusters of Indian and Brazilian strains, whereas South African strains were randomly distributed among Indian and Brazilian clusters. Our data (Fig. 2) verified that AFLP reliably renders information on geographic clustering and that C. auris is efficiently segregated from C. haemulonii, C. pseudohaemuloni and C. duobushaemulonii isolates.¹⁰

Candida auris continues to be a worrisome non-*albicans* species causing life threatening invasive diseases such as pericarditis⁷ and fungaemia especially in intensive care settings.^{5,6,8} We here show that VITEK MS MALDI combined with AFLP allows rapid and accurate identification and subsequent typing of this emerging pathogen. Given the specific clinical characteristics of *C. auris*, the application of one of these two technologies will improve patient management.

Potential conflict of interest

VG, SM, MC, CV, GD, AvB are employees of Biomerieux. ALC has received educational funds from Pfizer and Gilead Sciences, funding for research from Pfizer and United Medical and funds for advisory board membership from MSD and United Medical. JFM received grants from Astellas, Basilea and Merck. He has been a consultant to Astellas, Basilea and Merck and received speaker's fees from Merck, United Medical and Gilead Sciences. All other authors declare no conflict of interest.

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