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Proceeding Paper

Identification Procedures of Yeast Species Recovered from Portuguese Intensive Care Units †

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Abstract: Yeast species other than *Candida albicans* are becoming more clinically relevant in the Intensive Care Unit (ICU) landscape, making it important to identify them correctly. The aim of this study was to evaluate the concordance of the identification of clinical isolates from ICUs, at the species level, by conventional and molecular methods. All isolates (n = 371) underwent identification by cultural, MALDI-TOF MS and PCR. The direct concordance between conventional and molecular identification was 92% (341/371). These results allow us to conclude that culture-based methodologies are still useful to reliably identify the most frequent yeasts, but for rare, uncommon or cryptic species, technologies such as MALDI-TOF MS or PCR are needed.

Keywords: *Candida* spp.; MALDI-TOF MS; PCR; yeasts; identification

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1. Introduction

Invasive fungal infections are increasing, and *Candida* spp. is the main cause [1]. Yeast species other than *Candida albicans* are becoming more frequent, and some of them may have variable patterns of susceptibility to antifungal agents, making it important to identify them correctly [2]. Molecular amplification methods (polymerase chain reaction (PCR)) and proteomic analysis by Matrix-assisted Laser Desorption Ionization–Time-of-flight Mass Spectrometry (MALDI-TOF MS) techniques have emerged as alternative methods [3,4]. The aim of this study was to evaluate the concordance of the identification of ICUs isolates, at the species level, by culture methods based on standard morphological and biochemical criteria, MALDI-TOF MS and PCR.

2. Materials and Methods

During a two-year multicenter prospective observational study in ICU patients from two tertiary hospitals located in the Lisbon metropolitan area, 988 axillar/inguinal swabs were taken and identified at the species level to unveil the prevalence of *C. auris* at Portuguese ICUs. This investigation has been approved by the Institutional Ethical Board of all institutions enrolled.

All swabs' isolates (n = 371) were plated on Sabouraud Dextrose Agar and chromogenic agar (CHROMagar®). For *C. albicans* suspected colonies, a filamentation test was made.

API® Candida or API® 20 C AUX galleries (bioMérieux, Marcy l'Etoile, France) were used for identification according to the enzyme profile and sugar assimilation pattern. PCR assays were optimized for *C. auris* and *Candida* cryptic species identification [5,6]. MALDI-TOF MS methods analysis with Vitek–MS®(bioMérieux, Marcy l'Etoile, France) system was used for definitive identification.

The following species presumptively identified by phenotypic methods from ICU patient samples were studied: *C. albicans* complex (n = 183), *C. parapsilosis* complex (n = 115), *C. glabrata* complex (n = 39), *C. tropicalis* (n = 15), *C. guilliermondii* (n = 3), *C. famata* (n = 2), *C. kefyr* (n = 1), *Saccharomyces cerevisae* (n = 1), *Rhodotorula* sp. (n = 9) and *Trichosporon* sp. (n = 3). ATCC collection strains *C. parapsilosis* 22019, *C. glabrata* 15126, *C. albicans* 90028 and *C. auris* DSMZ 21087 were included in the study.

3. Results

Identification of the 371 isolates by MALDI-TOF MS included 355 *Candida* species isolates. Namely, *C. albicans* (n = 185), *C. parapsilosis* complex (n = 112) [*C. parapsilosis sensu* stricto (n = 109), *C. orthopsilosis* (n = 2), *C. metapsilosis* (n = 1)], *C. glabrata* (n = 36), *C. tropicalis* (n = 15), *C. lusitaniae* (n = 4) and *C. guilliermondii* (n = 3). Other yeast species included: *Rhodotorula rubra* (n = 9); *Trichosporon inkin* (n = 5); *Trichosporon asahii* (n = 1); and *S. cerevisae* (n = 1). No isolate of *C. auris* was retrieved within this cohort. The direct concordance between the conventional identification method and MALDI-TOF MS was 92% (341/371). Discrepancies were observed with the following species: *C. parapsilosis*; *C. glabrata*; *C. tropicalis*; *C. guilliermondii*; *C. famata*; and *C. kefyr*. In this work, MALDI-TOF MS allowed the correct identification of the yeasts *C. lusitaniae*, *C. guilliermondii*, *S. cerevisae*, *T. inkin* and *T. asahii* erroneously identified by conventional methods such as *C. parapsilosis*. There was a 100% correlation between MALDI-TOF MS and PCR assays for the identification of cryptic species of *C. albicans*, *C. parapsilosis*, *C. glabrata* complexes and *C. auris*.

4. Discussion

The accurate identification of *Candida* and other yeast species is extremely important, contributing to the increase of knowledge about the epidemiology of these microorganisms in the ICU setting. As already published by other authors, a limitation of conventional methods is the inability to identify cryptic species of *C. albicans*, *C. parapsilosis* and *C. glabrata* or new emerging species like *C. auris* [7–9].

These results allow us to conclude that conventional methodologies are still useful to reliably identify the most frequently isolated yeast species from clinical samples, but when dealing with rare, uncommon, or cryptic *Candida* species, it is important to confirm them using technologies such as MALDI-TOF MS. The PCR assays used allowed a reliable species identification and has the potential to be implemented in a cost-effective manner into epidemiological studies to broaden the limited knowledge of cryptic and rare species.

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Institutional Review Board Statement: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Ethical Committee of the Prof. Doutor Fernando Fonseca Hospital on 13/11/2019 (54/2019) and by the Ethical Committee of the Beatriz Ângelo Hospital on 21/07/2021 (3655/2021).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: MDPI Research Data Policies.

Conflicts of Interest: The authors declare no conflict of interest.

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