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ORIGINAL ARTICLE

Evaluation of an Automated Yeasts Identification System for Identification of Yeast Isolates

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SUMMARY

Background: **Invasive candidiasis is the most important health-care-associated fungal infection worldwide. In the last two decades, the cause of the increase of fungal infections is immunosuppression or serious underlying diseases. Additionally, *Rhodotorula* species, *Blastoschizomyces capitatus*, and *Trichosporon* species are emerging as important human pathogens in immunocompromised patients with hematological malignancy.**
Methods: **Between January 2012 and January 2018, a total of 603 fungal organisms were isolated from blood culture samples and included in the study. All of the isolates were identified by using standard mycological methods, MALDI TOF MS system, and the Phoenix system. Sequence analysis was performed on yeasts that could not be definitively identified by using SMM and incompatible according to the results with Phoenix and MALDI-TOF MS analysis.**
Results: **603 fungal isolates including 594 *Candida* spp. and 9 other yeasts like species were analyzed. *C. albicans* was the most frequently isolated species. The results of identification by conventional methods and MALDI TOF MS were compared to the results of the Phoenix system. The observed concordance was 99.2%. The compatibility with other systems of the Phoenix system was 100%, 100%, 97.3%, 100%, and 96.9% for *C. albicans*, *C. parapsilosis*, *C. tropicalis, C. glabrata,* and *C. krusei*, respectively.**
Conclusions: **The BD Phoenix system was found to be a simple, reliable, and effective method to identify the main species of the genus *Candida* in our study.**
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KEY WORDS

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INTRODUCTION

Invasive candidiasis is the most important health-care-associated fungal infection, worldwide [1]. Although *C. albicans* is still the most common fungal pathogen responsible for bloodstream infections, this has changed considerably in recent years with increasing occurrence of infections by non-albicans *Candida* species [2]. It is important because susceptibility to antifungal agents varies among non-albicans *Candida* species. In addi-

tion, infections with non-albicans *Candida* species have also been associated with high mortality [3]. The incidence of non-albicans *Candida* species is increasing compared to that of *C. albicans*, and several species, such as *C. glabrata* and *C. krusei* may be resistant to azole antifungal therapy [4].

In the last two decades, the cause of the increase of fungal infections is immunosuppression or serious underlying diseases [5]. Additionally, *Rhodotorula* species, *Blastoschizomyces capitatus*, and *Trichosporon* species are emerging as important human pathogens in immunocompromised patients with hematological malignancy. Disseminated fungal infections due to *Trichosporon* spp. have increased in the recent years. The most common cause of deep tissue tricosporonosis and disseminated infections, especially when the host's immune system is suppressed, is *T. asahii*. *Trichosporon* has been reported to be the most common cause of noncandidal yeast infection in patients with hematological malignancies, and infections carry a mortality rate in excess of 80% [6].

Identification of active species in fungal infections is very important. For the initiation of the empiric treatment, rapid identification of *Candida* spp. bloodstream infections, which are isolated from isolates with fast, reliable, and accurate identification, is critical. Traditionally, the identification of fungi has been based on conventional tube-based biochemical reactions, with results compared to historical charts of expected biochemical reactions [7]. Genotypic methods and phenotypic methods that are based on morphology and biochemical analysis are used more widely in routine laboratories in the identification of *Candida* spp. and yeast-like organisms [8].

The identification of rare species of *Candida* is very important and attention should be paid to the definitions that are made with automated systems. Automated systems that are used in routine laboratories, especially Phoenix (Becton Dickinson Diagnostics, Sparks, MD, USA) and MALDI TOF MS systems (Bruker Daltonik GmbH, Bremen, Germany). However, *C. auris*, one of the rare *Candida* sp., is misidentified with some automated systems. *C. auris* is defined as *C. catenulata* and *C. haemulonii* with Phoenix system [9].

The aim of this study is to compare the Phoenix system and MALDI TOF MS system with conventional methods in common and rare isolates of yeast and yeast-like organisms.

MATERIALS AND METHODS

Between January 2012 and January 2018, a total of 603 fungal organisms isolated from blood culture samples from 572 patients with blood stream infections were included in the study. All of the isolates were identified by using standard mycological methods (SMM), MALDI TOF MS system, and the Phoenix system. Sequence analysis was performed on yeasts that could not be definitively identified by using SMM and incompatible according to the results with Phoenix and MALDI-TOF MS analysis. All isolates were tested using the BD Phoenix Yeast ID panel by the Phoenix system in accordance with the manufacturers' recommendations. The results of the BD Phoenix system, were compared to those of SMM and the MALDI TOF MS system. In order to identify the mismatched isolates, sequencing method which defines internal transcribed spacer 1 (ITS1) and ITS2 regions of ribosomal DNA was used by Instituto de Salud Carlos III in Spain.

At MALDI-TOF MS, sample and target plate preparation and formic acid-ethanol protein extraction was performed according to the recommendations of Bruker Daltonik GmbH.

Measurements were performed with a Bruker Microflex instrument, Biotyper software version 3.0, and database version 3.1.66 (Bruker Daltonik, Germany). Each run included a negative extraction control, *C. parapsilosis* ATCC 22019, and *C. albicans* ATCC 10231 quality control organisms.

The identification data generated by MALDI-TOF MS were classified following the manufacturer's instructions: a log-score value ≥ 2.0 indicated species identification, a score of 1.7 - 1.99 indicated identification at the genus level, and a score < 1.7 indicated non-reliable identification (NRI).

RESULTS

A total of 603 fungal organisms were isolated from blood culture samples. 603 fungal isolates including 594 *Candida* spp. and 9 other yeasts like species were analyzed. *C. albicans* was the most frequently isolated species (n = 246) (40.8%), followed by *C. parapsilosis* (n = 135) (22.4%), *C. tropicalis* (n = 73) (12.1%), *C. glabrata* (n = 56) (9.3%), *C. krusei* (n = 33) (5.5%), *C. kefyr* (n = 22) (3.6%), *C. lusitaniae* (n = 8) (1.3%), *C. dubliniensis* (n = 7) (1.2%), *C. guilliermondii* (n = 4) (0.7%), *C. pelliculosa* (n = 4) (0.7%), *Magnusiomyces capitatus* (n = 4) (0.7%), *Rhodotorula musiloginosa* (n = 3) (0.5%), *C. inconspicua* (n = 3) (0.5%), *Trichosporon asahii* (n = 2) (0.3%), *C. norvegensis* (n = 1) (0.2%), *C. rugosa* (n = 1) (0.2%), *C. catenulata* (n = 1) (0.2%).

The results of identification by conventional methods and MALDI TOF MS were compared to the results of Phoenix system. These results are shown in Table 1. The observed concordance was 99.2%. The compatibility with other systems of the phoenix system was 100%, 100%, 97.3%, 100% and 96.9%, for *C. albicans*, *C. parapsilosis*, *C. tropicalis, C. glabrata* and *C. krusei*, respectively. One *C. tropicalis* strain was misidentified as *C. glabrata*, one *C. krusei* strain was misidentified as *C. apicola* and one *C. guilliermondii* strain was misidentified as *C. pulcherrima* by the BD Phoenix system. Three strains (0.5%) were misidentified and two strains (0.3%) were unidentified by the BD Phoenix system.

Table 1. Identification by SMM and MALDI TOF MS were compared with the results of the Phoenix system.

Species No. of isolates (n)		Phoenix System and MALDI TOF MS/Standard mycological methods (SMM) No. of isolates					
		Correct identification		Misidentification		Unidentified	
		Phoenix	MALDI/SMM *	Phoenix	MALDI/SMM *	Phoenix	MALDI/SMM *
Common Candida species	C. albicans (246)	246	246				
	C. parapsilosis (135)	135	135				
	C. tropicalis (73)	71	73	1		1	
	C. glabrata (56)	56	56				
	C. krusei (33)	32	32	1	1		
Uncommon Candida species	C. kefyr (22)	22	22				
	C. lusitaniae (8)	8	8				
	C. dubliniensis (7)	7	7				
	C. guilliermondii (4)	3	4	1			
	C. pelliculosa (4)	4	4				
	C. insconspicua (3)	3	3				
	C. norvegensis (1)	1	1				
	C. rugosa (1)	1	1				
	C. catenulata (1)	1	1				
Other Yeast like species	Magnusiomyces capitatus (4)	3	4			1	
	Rhodotorula mucilaginosa (3)	3	3				
	Trichosporon asahii (2)	2	2				
Total	603 (100%)	598 (99.2%)		3 (0.5%)		2 (0.3%)	

DISCUSSION

C. albicans is still the most common fungal pathogen responsible for bloodstream infection, but isolation of non-Candida albicans species has increased. *C. albicans*, *C. parapsilosis, C. glabrata, C. tropicalis* and *C. krusei* account for more than 90% of the isolates. *C. albicans* (n = 246) (40.8 %) was the most common species in our study. Accurate and rapid identification for the treatment of *Candida* infection is particularly important in immunosuppressed patients. Due to the need for faster, simpler methods, manual biochemical-based testing kits and instrument-based semiautomated or automated methods have been developed for identification of organisms. Posteraro B. et al. used the BD Phoenix systems for identification of clinical yeast species in their study. They found the rate as 96.3%. [10].

In a study by Ener et al., according to the morphological identification of *Candida* sp., Phoenix Yeast ID Panel has been reported to have an accuracy of 92.5% [8]. In our study, the observed concordance rate was 99.2%. Only five strains (0.8%) were misidentified or unidentified by the BD Phoenix system. The concordance rate was 99.8% by MALDI-TOF MS. The identification by the SMM and MALDI TOF systems of *C. albicans, C. parapsilosis, C. glabrata, C. tropicalis,* and *C. krusei* with the Phoenix system was 100%, 100%, 100%, 97.3%, and 96.9%, respectively. We can conclude that

the BD Phoenix Yeast ID panel system is a reliable, convenient, and fast method for routine identification of the most commonly isolated yeast species from clinical samples. But the BD Phoenix system cannot distinguish *C. parapsilosis* complex as *Candida orthopsilosis* and *C. metapsilosis*. Because, it is a method, that defines microorganisms according to the phenotypic properties of yeasts. MALDI-TOF MS can distinguish these two types. In our study, two *C. parapsilosis* complex isolates were identified by MALDI-TOF MS as *C. orthopsilosis* and *C. metapsilosis.*

The patterns of growth on cornmeal agar are helpful in making a presumptive identification. Morphological evaluation of Corn Meal Tween-80 agar is considered the gold standard method for the identification because of production chlamydospore by *C. albicans.* It does not have significant characteristics of many *Candida* spp. Carbohydrate assimilation studies or the results derived from one of the commercial yeast identification systems are necessary before a definitive identification can be reported [11].

The difference between the sparse species and the common species was found to be significantly higher. Commercial microbial identification systems do not correctly identify uncommon species of candidiasis such as *C. norvegensis, C. krusei, C. insconpicua, C. famata, C. guilliermondii, C. auris.* On the other hand, *C. auris* is important because it shows multiple drug resistance and it has become an emerging global health threat. *C. auris* can be misidentified as a number of different organisms (*C. haemulonii, C. catenulata*) when using traditional phenotypic methods for the BD Phoenix yeast identification system [12]. Nowadays it is stated that *C. auris* can be reliably identified by MALDI-TOF MS systems with a research-use-only (RUO) library with *C. auris* entries [9].

Ener et al. found the concordance rate as 81.7% in identification of frequently isolated species (*C. albicans, C. tropicalis, C. parapsilosis, C. glabrata, C. kefyr*); however, it was 38.7% for the rarely isolated ones (*C. krusei, C. lusitaniae, C. inconspicua/C. norvagensis, C. catenulata*) [8]. Marucco et al. compared the identification results obtained for different species of the genus *Candida* with either the BD Phoenix Yeast ID automated method or the MALDI-TOF MS method. Between 2009 and 2014, 192 strains of the genus *Candida* were analyzed. Both systems were difficult for defining rare species while identifying common species well. One of the *C. krusei* isolates (n = 33) was incorrectly identified as *C. apicola* with the Phoenix System and one *C. krusei* isolate was identified as *C. insconspicua* by MALDI-TOF MS [13].

A recent study on validating the identification of *C. auris* with 4 biochemical identification platforms found that all *C. auris* isolates were misidentified as *R. glutinis* by API-20C AUX, as *C. haemulonii* (except 1, as *C. catenulata*) by Phoenix, as *C. haemulonii* by VITEK and as *C. famata, C. lusitaniae, C. guilliermondii* or *C. parapsilosis* by MicroScan (Beckman Coulter, Pasadena, CA) [14]. In the study of Erdem et al., four *C. krusei* isolates reported that two of them were wrong with Phoenix and one with MALDI TOF MS [15]. In our study, one of the two *C. krusei* isolates was wrong with Phoenix and one with MALDI TOF MS.

MALDI-TOF MS has emerged as one of the most reliable tools for fast and easy identification, differentiation and classification of microorganisms. But, the MALDI TOF MS system is very expensive, so this system cannot be used in most routine laboratories.

CONCLUSION

In our study, the BD Phoenix system was found to be a simple, reliable, and effective method to identify the main species of the genus *Candida*.

We believe that these studies are important in order to identify the species that have rare clinical features with resistance characteristics and to enrich the libraries of automated systems.

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Declaration of Interest:
All authors declare that they have no conflict of interest.

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