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Bloodstream Yeast Species and In Vitro Antifungal Susceptibility Profiles

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BACKGROUND/AIMS
Bloodstream yeast infections have increased substantially in many countries worldwide and pose a significant threat. This study investigated the distribution of bloodstream yeast species and determined the antifungal susceptibility profiles.

MATERIAL and METHODS
A total of 52 yeast isolates were collected from the microbiology laboratory of the University Hospital. These isolates were collected from blood specimens that were submitted for culture test from in-patients, who have high body temperature. Of these isolates, 41 isolates of *Candida* spp. were identified using Brilliance™ *Candida* Agar, germ tube test, and tested for the chitin synthase 1 (*CHS1*) gene. Selected isolates were tested against seven antifungal agents by disk diffusion method, and the minimum inhibitory concentration (MIC) was determined by MIC Test Strips for the mutidrug resistant isolates. The study protocol was reviewed and approved by the Institutional Review Board.

RESULTS
Candida albicans accounted for 52%, *C. glabrata* (26.9%), *C. krusei* (9.6%), and *C. tropicalis* (6.7%). The newly emerging yeasts included *C. parapsilosis*, *C. zeylanoides*, *C. apicola*, *Blastoschizomyces capitatus*, *Cryptococcus neoformans*, *Kluyveromyces*, and *Rhodotorula mucilaginosa*. Most Candida species were highly susceptible to amphotericin B and caspofungin. Between 38 and 69% of *C. albicans*, isolates were resistant to the azoles. The non-*Candida albicans* (NCA) species showed different susceptibility patterns. Anidulafungin had the lowest MIC_{90} at 0.047 µg/mL. The CHS1 gene was detected in *C. albicans* and in three NCA spp.

CONCLUSION
Identifying the isolated species and determining their susceptibility pattern are essential to alert physicians to infections with rare and uncommon fungal species to optimize the antifungal therapy.

Keywords: Bloodstream infections, *Candida* spp., chitin synthase 1 (*CHS1*) gene, *Cryptococcus* species, newly emerging yeasts

INTRODUCTION

Many *Candida* species pose no threat to humans as they live as harmless commensals in the gut flora, on the human skin and mucosa, and they are controlled by the host innate immune responses. However, *Candida* species can invade and cause disease when the immune system is compromised or mucosal barriers are disrupted.[1] In addition, overgrowths of some species have transformed these commensals into medically important agents causing infections in hospitals.[2]

There has been a steady increase in the incidence of invasive candidiasis since 1980s, which is largely due to the increasing population of immunocompromised individuals,[3] with *Candida albicans* as one of the most common opportunistic pathogens. It colonizes and invades various tissues and organs causing systemic fungal infections (eg, hematogenous disseminated candidiasis). The non-*Candida albicans* (NCA) isolates such as *C. krusei*, *C. tropicalis*, and *C. parapsilosis* also have emerged as clinically significant opportunistic pathogens.[4]

The intrinsic resistance to antifungal therapy seen in some *Candida* species, and the development of acquired resistance during treatment is a major challenge for clinicians.[5]

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Chitin is an integral structural polysaccharide in fungal cell wall that is required for cell shape and morphogenesis. The hyphal cell walls of *C. albicans* have been reported to have higher chitin content than other yeasts,[6] and the hyphae chitin synthase activity has been estimated to be twice that of yeast cells. Although chitin synthase 2 (CaChs2p) is the most abundant protein among the three *C. albicans* chitin synthases, it is not necessary for hyphal growth, viability, or virulence, whereas CaChsI is expressed in both yeast and hyphae at a lower level[7] and maintains the integrity of the lateral cell wall. Chitin has also recently emerged as a significant player in the activation and attenuation of immune responses to fungi and other chitin-containing parasites.[8] Therefore, the study of chitin synthase is important to the understanding of fungal growth and its potential as a target for the development of antifungal drugs. In addition, identifying the species of the isolates and determining the susceptibility pattern are essential for clinical management.

To the best of our knowledge, this is the first study to investigate the species distribution and antifungal susceptibility profile of bloodstream yeast infections in Jordan.

The aim of this study was to identify yeast species prevalent in bloodstream infections (also known as candidemia) and to determine their antifungal susceptibility profiles.

MATERIALS and METHODS

This retrospective study was ethically approved by Jordan University of Science and Technology Internal Review Board (IRB) for research on human specimens.

A total of 52 yeast nonduplicate isolates that were submitted to the microbiology laboratory for blood culture between July 2013 and August 2014 were collected, each recovered from one patient with bloodstream infection. Blood cultures were routinely done from in-patients, who have high body temperature. By definition, all positive blood cultures for *Candida* species were considered infections.

Isolates were inoculated onto Sabouraud dextrose agar (SDA) (Oxoid, Hampshire, UK) plates to obtain pure cultures. Gram-stained smears were prepared from overnight cultures and were viewed under the microscope for morphology. For the rapid presumptive identification of *Candida* species, single colonies were inoculated on Brilliance™ *Candida* agar (Oxoid, Hampshire, UK) supplemented with chloramphenicol. All plates were incubated at 30°C for 24-48 hours. The color and morphology of colonies were recorded, and isolates were stored in Sabouraud dextrose broth at –80°C until tested.

Main Points

- Understanding the role of dominant *Candida* species in BSI and their antifungal susceptibility are crucial to optimize therapeutic and prophylaxis measures.
- *C. albicans* was the predominant species identified in 63.4% of the cases, and *C. tropicalis* was predominant among non-*albicans* species (12.2%). Most *Candida* species were susceptible to amphotericin B, most *C. albicans* isolates were resistant to posaconazole and voriconazole (69% each), and 61.5% were resistant to fluconazole.
- The identification of newly emerging bloodstream fungal pathogens such as *Cryptococcus neoformans*, *Rhodotorula mucilaginosa*, *Blastoschizomyces capitatus*, and *Kluyveromyces* spp. should alert the clinical laboratories and increase the awareness of physicians and to their potential in similar cases.

The Germ tube test was used to differentiate *C. albicans* from other yeasts. A well isolated colony from overnight SDA culture was suspended into 2 mL of freshly pooled human sera, incubated at 35°C for 2-4 hours, and a drop of the suspension was microscopically examined for the formation of germ tubes. *C. albicans* grew as a yeast form at 30°C and the mycelial form at 35-42°C.[9]

Speciation of the *Candida* isolates was done using the Remel RapID™ YEAST PLUS System (Thermo Fisher Scientific, Lenexa, KS, USA) following the manufacturer instructions. This system is based on enzyme technology that employs conventional and chromogenic substrates to identify yeasts and yeast-like organisms. The interpretation of reactions was done according to ERIC® electronic code compendium (http://www.remel.com/eric/) designed for Remel RapID Systems.

The antifungal susceptibility testing of selected isolates was done by disk diffusion method on Mueller–Hinton agar supplemented with 2% glucose and methylene blue dye according to Clinical Laboratory Standard Institute, 2009.[10] Isolates were tested in triplicate against seven antifungal agents, namely, amphotericin B, caspofungin, and five azoles, which are fluconazole, itraconazole, ketoconazole, posaconazole, and voriconazole (Liofilchem®, Italy). The minimum inhibitory concentration (MIC) was determined for 24 isolates (19 *C. albicans* isolates and five *C. tropicalis* isolates) that showed resistance to two or more antifungal agents by the disk diffusion. MIC was determined using the MIC Test Strips (Liofilchem, Italy) on RMPI 1640 medium supplemented with 0.2% glucose and 1.5% agar. The 0.5 McFarland inoculum was swabbed in three directions on the entire RPMI-agar plate, and the MIC Test Strips were applied. The MIC was read after 24-48 hours, where the border of the elliptical inhibition zone edge intersected the scale on the strips (at the point of approximately 80% growth inhibition). The MIC50 and MIC90 values are the lowest concentration of the antibiotic at which 50% and 90% of the tested isolates were inhibited, respectively.

Control Strains

The quality of test performance was controlled by including the reference strains *C. albicans* (ATCC 10231), *C. parapsilosis* (ATCC 22019), and *C. krusei* (ATCC 6258) in the antifungal susceptibility testing and in the MIC test.

Detection of *CHS*I Gene by PCR

This virulence gene is present in four *Candida* species (*C. albicans*, *C. tropicalis*, *C. parapsilosis*, and *C. glabrata*).[11] DNA was extracted from the isolates and controls using Gentra Puregene Yeast/Bact Kit (Qiagen, USA) according to the manufacturer's instructions, and DNA was stored at –20°C until tested.

The PCR master mixture contained 1 µL of the prepared DNA and GoTaq® Green Master Mix (Promega, USA). This premixed ready-to-use solution contains bacterial-derived *Taq* DNA polymerase, $MgCl_2$, dNTPs, and reaction buffers at optimal concentrations for efficient amplification of DNA templates; it

also contains blue and yellow dyes for monitoring the progress during electrophoresis. The sequences of primers used in PCR-targeted sequences within the *CHSI* gene of *C. albicans* were primer 1, 5′-CGCCTCTGA TGGTGATGAT-3′, and primer 2, 5′-TCCGGTATCACCTGGCTC-3′. Primers, PCR mix ,and amplification conditions are those described by Jordan.[11] *C. parapsilosis* (ATCC 22019) was used as positive control, and the negative control tube containing the mixture with no DNA template was used in every PCR run. The PCR thermal cycler (Bio-Rad, USA) was used in all amplifications.

The amplicon size for each of the four *Candida* species is *C. albicans* (122 bp), *C. parapsilosis* (311 bp), *C. tropicalis* (519 bp), and *C. glabrata* (535 bp). Five microliters of the PCR products was electrophoresed along with 100 DNA ladder (Promega, USA) in 2% agarose gel stained with ethidium bromide. The DNA bands were visualized with UV light in a gel documentation system (Bio-Rad, USA), and images were taken.

Statistical Analysis

Descriptive statistics were used to describe the isolates and the antifungal susceptibility testing of selected isolates. Categorical data are expressed as numbers and percentages.

RESULTS

A single species was identified in each sample. The Remel RapID YEAST PLUS System identified 41 isolates as *Candida* spp., except one isolate that could not be identified by this system (Table 1).

The colonies of the isolates exhibited different colors based on the chromogenic color reactions on Brilliance *Candida* agar. All *C. albicans* and *C. tropicalis* species produced green and blue colonies, respectively. *C. krusei* produced light pink and beige brown colonies, while *C. glabrata* produced white or beige brown/yellow colonies.

The distribution of the identified yeasts is shown in Table 1. Seven different *Candida* species were identified in the 41 *Candida* isolates, and *C. albicans* (63.4%) was the predominate species. Ten NCA isolates were identified, and *C. tropicalis* (12.2%) was the most common followed by *C. glabrata* (9.6%). The isolates also included *C. parapsilosis* species complex, *C. zeylanoides*, *C. apicola*, and *C. krusei*.

TABLE 1. Distribution of Yeast Genera as Identified by Remel RapID YEAST PLUS System

Identified yeasts		Total (%)
	Candida spp.*	41 (78.8)
	C. albicans	26 (63.4)
	C. tropicalis	5 (12.2)
	C. glabrata	4 (9.8)
	C. parapsilosis	2 (4.9)
	C. zeylanoides	2 (4.9)
	C. apicola	1 (2.4)
	C. krusei	1 (2.4)
Cryptococcus neoformans		3 (5.7)†
Rhodotorula mucilaginosa		5 (9.6)
Blastoschizomyces capitatus		1 (1.9)
Kluyveromyces spp.		1 (1.9)
Not identified		1 (1.9)
Total		52 (100)

*Percentage of *Candida* species calculated out of 41 isolates.
†Percentage of non-*Candida* species calculated out of 52 isolates.

TABLE 2. Diameter of Zone of Inhibition in Millimeters for One *Candida apicola* (N = 1) and Two *Candida zeylanoides* isolates

Antifungals	*C. apicola*	*C. zeylanoides*
Amphotericin B	7	7, 20
Caspofungin	7	21, 15
Fluconazole	0	33, 41
Itraconazole	0	32, 32
Ketoconazole	27	34, 48
Posaconazole	17	12, 25
Voriconazole	0	33, 40

The presumptive color identification of the isolates on the Brilliance *Candida* agar media was confirmed by the Remel RapID YEAST PLUS System. In addition, 10 isolates were identified as yeast genera other than *Candida* (Table 1).

The diameter of zone of inhibition for susceptibility was interpreted (Table 2) as described using the CLSI[10] document for caspofungin, fluconazole, and voriconazole. It varied between the antifungal agents and was as follows: *C. albicans* and *C. parapsilosis* (≥19), *C. tropicalis* (≥25), and *C. krusei* (≥16). The criteria used for amphotericin B and posaconazole were those proposed by Nguyen et al.[12] and Carrillo-Munoz et al.,[13] respectively. As for itraconazole and ketoconazole, we followed the criteria by Rosco diagnostics (www.rosco.dk/gfx/pdf/yeasts.pdf).

The diameter of inhibition zone for *C. apicola* and *C. zeylanoides* is presented in Table 2 as there were no standard interpretive criteria for these species. *Candida* species were highly susceptible to amphotericin B and caspofungin (Table 3). *C. albicans* resistance to the azole group ranged from 38 to 69%.

All five *Candida tropicalis* isolates were susceptible to amphotericin B, caspofungin, 4 (80%), were susceptible to itraconazole, and isolates showed variable resistance to the remaining azoles (60% to ketoconazole and 100% to fluconazole).

The two *C. parapsilosis* isolates were susceptible to all the azoles. All *C. glabrata* isolates were susceptible to most of the azoles; however, they showed less susceptibility to posaconazole and voriconazole (Table 3). *C. krusei* is known to have intrinsic resistance to fluconazole, and the one isolate in this study was only resistant to posaconazole.

The MIC test was performed for 24 isolates (19 *C. albicans* and five *C. tropicalis*) that were resistant to more than one antifungal agent by the disk diffusion method. After 24 hours of incubation, a symmetrical inhibition ellipse centered along the strip was formed. The MIC was read directly from the scale on each strip in μg/mL, at the point where the edge of the inhibition ellipse intersects with the MIC Test Strip. The macrocolonies in the ellipse were read at 80% inhibition, and the partial inhibition of growth giving trailing microcolonies of decreasing size end points was ignored.

The supplementation of RPMI agar with additional glucose (2% final concentration) provided optimal growth of these species

TABLE 3. Susceptibility Pattern of *Candida* Species Isolated from Bloodstream Infections

	Number of isolates of each species				
Antifungal agents	***C. albicans* (N = 26)**	***C. glabrata* (N = 4)**	***C. krusei* (N = 1)**	***C. parapsilois* (N = 2)**	***C. tropicalis* (N = 5)**
Amphotericin B (20 µg)					
S	23 (88.5)	4 (100)	1 (100)	2 (100)	5 (100)
DD/I	1 (3.8)	0 (0)	0 (0)	0 (0)	0 (0)
R	2 (7.7)	0 (0)	0 (0)	0 (0)	0 (0)
Caspofungin (5 µg)					
S	23 (88.5)	4 (100)	1 (100)	1 (50)	5 (100)
DD/I	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)
R	3 (11.5)	0 (0)	0 (0)	0 (0)	0 (0)
Fluconazole (100 µg)					
S	10 (38.5)	4 (100)	NA	2 (100)	0 (0)
D/I	0 (0)	0 (0)	NA	0 (0)	0 (0)
R	16 (61.5)	0 (0)	NA	0 (0)	5 (100)
Itraconazole (50 µg)					
S	9 (34.6)	4 (100)	1 (100)	2 (100)	4 (80)
DD/I	1 (3.8)	0 (0)	0 (0)	0 (0)	0 (0)
R	16 (61.5)	0 (0)	0 (0)	0 (0)	1 (20)
Ketoconazole (15 µg)					
S	5 (19.2)	3 (75)	1 (100)	2 (100)	1 (20)
DD/I	11 (42.3)	1 (25)	0 (0)	0 (0)	1 (20)
R	10 (38)	0 (0)	0 (0)	0 (0)	3 (60)
Posaconazole (5 µg)					
S	6 (23.1)	1 (25)	0 (0)	2 (100)	1 (20)
DD/I	2 (7.7)	1 (25)	0 (0)	0 (0)	0 (0)
R	18 (69.2)	2 (50)	1 (100)	0(0)	4 (80)
Voriconazole (1 µg)					
S	8 (30.8)	3 (75)	1 (100)	2 (100)	1 (20)
DD/I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
R	18 (69.2)	1 (25)	0 (0)	0 (0)	4 (80)

S, susceptible; R, resistant; DD/I, dose dependent/intermediate; NA, not applicable; percentages are shown in parentheses.

and minimized the problem of trailing endpoints due to partial inhibition of growth by azoles.[14]

The MIC breakpoints for anidulafungin, fluconazole, voriconazole, and caspofungin were carefully interpreted according to CLSI[15] (Table 4). However, the criteria used for amphotericin B and posaconazole were as previously proposed.[12,13]

There was no current break point for itraconazole, and six *C. albicans* isolates and three *C. tropicalis* did not have zone of inhibition.

The lowest MIC_{50} of the tested isolates was recorded for anidulafungin (0.012 µg/mL) followed by itraconazole with an MIC_{50} at 0.016, and amphotericin B and voriconazole had low MIC_{90} (1.5 µg/mL); however, no clinical breakpoint is available at present (Table 4).

Amplicons of the *CHSI* gene (122 bp) were observed in *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, and the positive control (*C. parapsilosis* ATCC 22019), and not in the negative control (*C. krusei* ATCC 6258).

DISCUSSION

The results of this study report on the species distribution and newly emerging and rare yeast species isolated from bloodstream infections. *C. albicans* was the predominant species identified as 63.4%, and *C. tropicalis* was predominant among NCA isolates (12.2%), followed by *C. parapsilosis* species complex and *C. zeylanoides* each (4.9%).

TABLE 4. MIC_{50} and MIC_{90} Range and the Breakpoints for the Multidrug Resistant Isolates

Antifungal agents	**No of *C. albicans* isolates**	**No of *C. tropicalis* isolates**	**MIC (µg/mL)**			**Breakpoint (µg/mL)**
			Range	**MIC_{50}**	**MIC_{90}**	
Amphotericin B	18	5	0.23-1.5	1	1.5	≥1
Anidulafungin	18	5	0.002-0.12	0.012	0.047	≥1
Caspofungin	17	5	0.125-12	1	3	≥1
Fluconazole	12	3	0.012-192	1	192	≥8
Itraconazole	13	2	0.002-2	0.016	0.25	NA
Posaconazole	14	3	0.064-8	0.19	15	≥4
Voriconazole	13	3	0.008-4	0.047	1.5	≥1

MIC, minimum inhibitor concentration; NA, not applicable.

Candida species rank fourth in the United States and seventh in Europe among the causative agents of BSI in hospitals.[16,17] Although *C. albicans* remains the most common isolate obtained from invasive fungal infections and ICU patients, *C. glabrata* has emerged as the second most common cause of invasive candidiasis in the United States.[18] Despite the reported shift of species distribution, the results of our study showed that *C. albicans* is still the dominant species in bloodstream infections. The higher incidence of *C. albicans* infection can be largely explained by the presence of this yeast among the normal mucosal flora of most humans. These results are in agreement with earlier studies that recovered *C. albicans* in 47.5% of the BSI.[19] The bloodstream NCA identified in our study is in accordance with those reported earlier in candidemia cases (35-65%) in the general patient population.[20] However, another study reported greater frequency of the NCA species than that of *C. albicans* with *C. parapsilosis* as the most frequent in BSI.[21] Unlike other systemic mycoses, invasive *Candida* infections in immune compromised patients originate most frequently from endogenous reservoirs; however, exogenous infections are frequently reported in hospitalized patients.[22]

Our antifungal susceptibility testing results showed that all *Candida* species were susceptible to amphotericin B, with the exception of two *C. albicans* isolates that were resistant. The MIC_{50} and MIC_{90} for most species fall within what is considered a resistant range. The resistance of *C. albicans* isolates to the azoles group was 38-69%. The azole antifungals are the most frequent class used to treat *Candida* infections. Recent exposure to azoles and antibacterial drugs were reported as important risk factors for infection with fluconazole-resistant *Candida* spp.[23]

C. parapsilosis species complex in our study was susceptible to all tested antifungal agents (Table 3). Most of the *C. albicans* isolates were resistant to posaconazole and voriconazole (69% each), and 61.5% were resistant to fluconazole. The least resistance was observed to caspofungin (11.5%) and ketoconazole (38%). All *Candida* strains were reported earlier to be susceptible to amphotericin B and caspofungin, while 10.8% of the isolates were resistant to fluconazole.[24] Our four *C. glabrata* isolates were susceptible to several azoles, but they were less susceptible to posaconazole (Table 3). The one single *C. krusei* isolate in this study was only resistant to posaconazole.

A retrospective, case-comparative study in the United States reported that *C. albicans*, *C. tropicalis*, and *C. parapsilosis* that are generally considered to be susceptible to fluconazole accounted for 36% of isolates with a reduced susceptibility and 48% of resistant isolates.[25] One mechanism of resistance identified in *C. albicans* is the presence of point mutations in *ERG11*.[26]

Cross-resistance between the azoles among *Candida* species to multiple antifungal agents is also an important concern. It has been demonstrated for *C. glabrata*, *C. albicans*, *C. tropicalis*, and *C. parapsilosis*.[3]

Acquired resistance is generally less common than intrinsic resistance; however, data from various studies suggest that it is beginning to emerge in some countries. There are intrinsically resistant strains of *C. albicans* that can be part of a commensal growth or can be acquired from the environment or other individuals. Resistant strains of *C. albicans* can occur as a result of the normal distribution of MICs that all species exhibit, or they can develop resistance through several mechanisms.[27] There is the possibility that a strain can be induced to be resistant by exposure to the drug over long periods. In these cases, the drug itself does not cause resistance but, rather, selects for the growth of the more resistant cells in the population. In addition, a large population of yeast cells under selective drug pressure, specific random mutations that render the cell slightly more resistant, will eventually become the dominant strain in the population. Antifungal drug resistance can lead to the development of infections in high-risk patients receiving antifungal prophylaxis.

Primary resistance among certain fungi without prior exposure to the drug, eg, resistance of *C. krusei* to fluconazole and *Cryptococcus neoformans* to echinocandins, emphasizes the importance of the identification of fungal species in clinical specimens. However, secondary resistance develops among previously susceptible strains after exposure to the antifungal agent such as strains resistance of *C. albicans* and *C. neoformans* to fluconazole, which is usually dependent on altered gene expression.[28]

The *CHS1* gene was selected for PCR amplification because this virulence gene can identify four medically important *Candida* species, namely, *C. albicans*, *C. tropicalis*, *C. parapsilosis*, and *C. glabrata*, and it was detected in the four species of our isolates. This method allows the relatively rapid identification of *Candida* species in conjunction with morphologically and physiologically based identification procedures.

It is worth mentioning the identification of rare and newly emerging bloodstream fungal pathogens in this study by Remel RapID YEAST PLUS System (Table 1). They were three *C. neoformans*, five *Rhodotorula mucilaginosa*, one *Blastoschizomyces capitatus*, and one *Kluyveromyces* spp. These rare invasive yeast pathogens were defined by the European Society of Clinical Microbiology and Infectious Diseases, Fungal Infection Study Group, and the European Confederation of Medical Mycology.[29]

C. neoformans was first reported in dialysis-associated bacteremia in a patient with renal failure, who developed fungemia during the treatment, where this yeast species grew in the catheter tip and blood culture.[30] Cryptococci generally are found in soil contaminated with pigeon feces and are transmitted to humans primarily through inhalation. *C. neoformans* is one of the most important agents of the opportunistic mycoses.[3] *Cryptococcus* invasive infections are commonly associated with immunosuppressed individuals, those with liver cirrhosis, diabetes mellitus, or other medical conditions,[31] while being extremely rare in healthy individuals.[32]

Rhodotorula species were considered as nonvirulent saprophytes and common contaminant organisms. However, they have emerged as opportunistic pathogens, particularly in immunocompromised patients. *Rhodotorula* infection was first reported in 1985 in the medical literature, and the number of human infections increased after that time,[33] most likely because of the wider use of immunosuppressant and/or the use of central venous catheters. Bloodstream infections with *Rhodotorula* species were reported in 43 cases at Duke University Medical Center, USA, between 1960 and 2000.[34]

B. capitatus (formerly known as *Trichosporon* spp. and *Geotrichum capitatum*) was identified in one bloodstream case in our study. Infection with this rare yeasty was first reported in Switzerland[35] in a leukemic patient. *Kluyveromyces* spp. was identified in one case in our study, and this uncommon yeast species (*Kluyveromyces marxianus*, anamorph *Candida kefyr*) was earlier isolated from a patient with fungemia.[36]

The limitations of our study are mainly related to its retrospective nature, and therefore, we were unable to include the initial treatment and the follow-up on whether there was a persistence of positive blood cultures after the initiation of antifungal therapy. In addition, the low number of patients has limited the statistical power of the study.

In conclusion, several newly emerging yeast pathogens are reported in this study. These results should alert the clinical laboratories to the potential of rare fungi in similar cases. Identifying the species of the isolates and determining their susceptibility pattern are essential to optimize the therapeutic decisions regarding rational antifungal therapy.

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