

Clinical significance of the isolation of *Candida* species from hospitalized patients

Yankee C. Magalhães¹, Maria Rosa Q. Bomfim², Luciane C. Melônio¹,
Patrícia C.S. Ribeiro^{1,2,3}, Lécia M. Cosme^{1,4}, Cristianne R. Rhoden¹,
Sirlei G. Marques^{1,2,3}

¹Laboratório Cedro, São Luis, MA, Brazil.

²Universidade CEUMA, São Luis, MA, Brazil.

³Hospital Universitário, Universidade Federal do Maranhão, São Luis, MA, Brazil.

⁴Laboratório Central de Saúde Pública do Maranhão, São Luis, MA, Brazil.

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Abstract

In this study, we isolated and phenotypically identified 108 yeast strains from various clinical specimens collected from 100 hospitalized patients at three tertiary hospitals in São Luís-Maranhão, Brazil, from July to December 2010. The isolates were analyzed for their susceptibility to four of the most widely used antifungal agents in the surveyed hospitals, amphotericin B, fluconazole, 5-flucytosine and voriconazole. The species identified were *Candida albicans* (41.4%), *Candida tropicalis* (30.1%), *C. glabrata* (7.4%), *Candida parapsilosis* (5.5%), *Candida krusei* (4.6%), *Cryptococcus neoformans* (4.6%), *Trichosporon* spp. (3.7%), *Candida norvegensis* (0.9%), *Rhodotorula glutinis* (0.9%) and *Pichia farinosa* (0.9%). A higher isolation rate was observed in the following clinical specimens: urine (54 isolates; 50%), respiratory tract samples (21 isolates; 19.4%) and blood (20 isolates; 18.6%). *Candida albicans* isolates were 100% sensitive to all antifungal agents tested, whereas *Candida krusei* and *Cryptococcus neoformans* displayed intermediate resistance to 5-flucytosine, with Minimal Inhibitory Concentration (MIC) values of 8 mg/mL and 16 mg/mL, respectively. Both strains were also S-DD to fluconazole with an MIC of 16 mg/mL. *C. tropicalis* was resistant to 5-flucytosine with an MIC of 32 µg/mL. This study demonstrates the importance of identifying the yeast species involved in community and nosocomial infections.

Key words: fungal infections, susceptibility profile, nosocomial infections.

Introduction

Candida and *Cryptococcus* fungi, which belong to the *Saccharomycetaceae* and *Tremellaceae* families, respectively are the most medically important yeast species (Hazen and Howell, 2007). There are over 200 known species of the genus *Candida*, but only a relatively small number of *Candida* are pathogenic to humans (Chander, 2009). A number of species have been isolated from humans, including *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. lusitanae*, *C. guilliermondii*, *C. dubliniensis*, *C. famata*, *C. utilis*, *C. lipolytica*, *C. norvegensis*, *C. inconspicua*, *C. kefyr*, *C. rugosa*, *C. haemulonii*, *C. viswanathii* and *C. albicans* var.

stellatoidea (Basetti *et al.*, 2007; Hattori *et al.*, 2009; Barbedo and Sgarbi, 2010).

The incidence of fungal infections due to *Candida* spp. has been increasing in recent years (Severo *et al.*, 2009; Binder and Lass-Flörl, 2011), profoundly affecting public health (Vandeputte *et al.*, 2012). Substantial morbidity and mortality are associated with fungal infections in various patient groups (Gudlaugsson *et al.*, 2003). Currently, *Candida* yeasts are the most common opportunistic pathogens in humans and are associated with almost 80% of all nosocomial fungal infections (Pappas *et al.*, 2004; Kim and Sudbery, 2011). However, cases of cryptococcosis have increased sharply during the past two decades as a result of the AIDS epidemic, cancer chemotherapy, and

immunosuppression in organ transplant recipients (Chaya-kulkeeree and Perfect, 2006; Pfaller *et al.*, 2007; Karkowska-Kuleta *et al.*, 2009; Kim and Sudbery, 2011). Cryptococcosis is a systemic mycosis caused by two species of the encapsulated basidiomycetes, *Cryptococcus neoformans* and *C. gattii*, which cause infection in immunocompromised individuals and in immunologically normal hosts, respectively (Sionov *et al.*, 2009).

Because most fungal infections are opportunistic, the frequency of these infections in different countries varies considerably (Xavier *et al.*, 2008). Moreover, factors inherent to the host can lead to the establishment of infection (Pappas *et al.*, 2004). In this context, opportunistic fungal pathogens, such as *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus*, have accounted for most of the mycotic infections in immunocompromised individuals, and these infections often become life-threatening (Pfaller *et al.*, 2007; Xavier *et al.*, 2008; Karkowska-Kuleta *et al.*, 2009).

Difficulties in diagnosing fungal infections early have resulted in the lack of timely and appropriate antifungal therapies to effectively treat fungal infections (Pappas *et al.*, 2004). The incidence of fungal infection has risen, especially in ill patients with the greatest risk, and the list of potential pathogens previously considered components of the human microbiota has expanded (Fridkin, 2005). Thus, it is extremely important to monitor fungal infections, especially in patients in intensive care units, patients with degenerative and neoplastic diseases, transplant recipients, premature newborns and patients with AIDS (Xavier *et al.*, 2008; Karkowska-Kuleta *et al.*, 2009; Kim and Sudbery 2011).

The aims of this study were to analyze the prevalence of yeast species isolated from various clinical materials taken from patients hospitalized in three hospitals from São Luis-MA and to determine the *in vitro* activity of four antifungal agents, amphotericin B (AMB), fluconazole (FCZ), flucytosine (5-FC) and voriconazole (VCZ).

Materials and Methods

Ethical aspects

This study was approved by the Research Ethics Committee, University Center of Maranhão (UNICEUMA), under Protocol No. 0014/10.

Clinical samples

In this study, we used 100 clinical samples collected from patients at one private hospital "A" (n = 37) and two public hospitals "B" (n = 31) and "C" (n = 32) in São Luís, Maranhão, Brazil, from July to December 2010. All patients were treated at tertiary hospitals. For the isolation, fungi were cultured in media containing mycobiotic agar and Sabouraud Dextrose Agar (Difco Laboratories, Detroit, MI, USA). The plates were incubated at 28 °C or 37 °C for

1 to 25 days and examined daily until fungal growth was observed.

An automated method (Vitek-2 Compact bioMérieux, Marcy-L'Etoile, France) was used to identify the fungal isolates. The inoculum was diluted with 3 mL of physiological saline 0.45% fungal colony. Samples with a McFarland standard number of two were used with the DensiChek-bioMerieux (Vitek). These standardized suspensions were applied to the identification cards, which were then sealed and subjected to biochemical reading by an optical sensor. We used the YST card (Yeast identification) (bioMérieux) to determine the gender and species of the yeast isolates. For the blood and cerebrospinal fluid (CSF) cultures, the BACT ALERT automated system was used (bioMérieux). Additionally, samples were identified using traditional phenotypic methods, such as zymography, auxanography, proof of chlamydospores and filamentation. For the *Cryptococcus* strains, we used a urease test, a thermotolerance test at 35 °C, a test for sensitivity to cycloheximide and staining of the capsule with India ink.

To investigate antifungal susceptibility, minimal inhibitory concentrations (MICs) were calculated using 180 µL of standardized inoculum diluted in a tube containing 3 mL of saline to 0.45%; the sample was then applied to the AST-YSO1 card (bioMérieux). We tested the following antifungal drugs: amphotericin B (AMB), fluconazole (FCZ), 5-flucytosine (5-FC) and voriconazole (VCZ). The analysis and interpretation of the data were performed according to the standards of the Clinical and Laboratory Standards Institute M27-A3 and M27-S3 (CLSI, 2008a; 2008b). Yeasts that belonged to repeated biological samples from the same patient were excluded. The following reference isolates from the American Type Culture Collection (ATCC) were used as controls: *C. neoformans* ATCC MYA-4566, *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019.

Statistical analysis

To compare the activity of the antifungal drugs against the fungi tested, we determined the maximum and minimum MIC values, the geometric mean (MedGeo) and the MIC values at which 50% (MIC50) and 90% (MIC90) of the isolate population was inhibited. The data were analyzed using Epi Info version 6.0 (Dean *et al.*, 1994).

Results

From the 100 different clinical specimens used in this study, we identified 2,004 microorganisms consisting of 1,896 (94.6%) bacterial strains and 108 (5.4%) yeast strains isolated from Hospital A [1,045 (52.1%) bacteria and 42 (2.1%) yeast], Hospital B [355 (17.7%) bacteria and 32 (1.6%) yeast] and Hospital C [496 (24.8%) bacteria and 34 (1.7%) yeast]. The majority of yeast species were isolated from female patients (56%; 44% from males), with the

most affected age group being over 60 years of age (51%). These patients were predominantly admitted to the Intensive Care Unit (66%).

Evaluation of the frequency of yeast infection in each hospital indicated that there was a large number of isolates at Hospital A (39%) compared with Hospitals B (29.6%) and C (31.4%); of these isolates, *C. albicans* was the most prevalent species. The number of non-*albicans Candida* strains evaluated from the three hospitals represented 48.1% of the total sample (Table 1).

Table 2 presents data showing that 89.8% of all the isolates (97 isolates) belonged to the *Candida* genus, whereas the others were *Cryptococcus*, *Pichia*, *Rhodotorula* and *Trichosporon species*, totaling 10.2% of the isolates. We identified the following species: 45 *C. albicans* (41.7%), 8 *C. glabrata* (7.5%), 1 *C. norvegensis* (0.9%), 5 *C. krusei* (4.6%), 6 *C. parapsilosis* (5.6%), 32 *C. tropicalis* (29.7%), 5 *Cryptococcus neoformans* (4.6%), 1 *Pichia farinosa* (0.9%), 1 *Rhodotorula glutinis* (0.9%) and 4 *Trichosporon spp.* (3.7%).

Yeasts were detected mainly from urine (54, 50%), the respiratory tract (21, 19.4%), blood (20, 18.6%), the catheter tip (7, 6.4%) and CSF (2, 1.8%), and the remaining four specimens (3.7%) were from wound secretions (1, 0.9%), wound fragments (1, 0.9%), vaginal secretions (1, 0.9%) and stools (1, 0.9%). The most predominant species in the urine samples were *C. albicans* (24, 22.2%), *C. tropicalis* (20, 18.6%) and *Candida glabrata* (4, 3.7%). The most predominant clinical specimens from the respiratory tract were *C. albicans* (13, 12.1%) and *C. tropicalis* (4, 3.7%). *C. tropicalis* was the most prevalent species isolated from the blood (5, 4.6%), with the same isolation rate (3.7%) as *C. albicans* and *C. parapsilosis*. Notably, three *C. neoformans* strains were isolated from blood samples and two from the CSF (Table 2).

Table 3 shows the susceptibility of the fungal isolates to the antifungals tested in this study. All of the *C. albicans* strains were susceptible to all four antifungals, AMB, FCZ, 5-FC and VCZ. These yeast strains exhibited an MIC₅₀ and MIC₉₀ of 1 µg/mL for AMB, 5-FC and FCZ and lower MIC₅₀ and MIC₉₀ values (0.12 mg/mL) for VCZ. Of the five *C. krusei* strains isolated, two showed an MIC of 8 mg/mL and appeared to be susceptible dependent upon dose (S-DD) to FCZ at an MIC of 16 mg/mL. Of the 32 *C. tropicalis* strains, only two were resistant to 5-FC with a MIC of 32 mg/mL, corresponding to 6.25% of the isolates. All isolates exhibited smaller MIC₅₀ and MIC₉₀ values for VCZ (0.12 mg/mL), whereas these values were higher for FCZ (1 and 2 mg/mL, respectively) compared with VCZ (Table 3).

A *C. neoformans* strain isolated from cerebrospinal fluid and another from blood exhibited an MIC of 16 / mL for 5-FC and FCZ. This result was confirmed using an epsilometric E-test. Since no interpretive breakpoints have been determined for *C. neoformans* we used those considered in document M27-A3 CLSI (CLSI, 2008b) for *Candida*.

Discussion

Candida species are ubiquitous fungi that include the most common fungal pathogens that cause superficial and invasive diseases in humans (Chander *et al.*, 2009; Fisher *et al.*, 2011). Candiduria is rarely present in healthy individuals but is commonly found in hospitalized patients, especially those with multiple predisposing factors, including structural abnormalities of the kidney, diabetes mellitus, indwelling urinary catheters, immunosuppression and exposure to antimicrobials (Trofa *et al.*, 2008; Fisher *et al.*, 2011). In this study, 89.9% of the clinical isolates obtained from three different hospitals (A, B and C) were of the *Candida* genus. *C. albicans* was the most prevalent species

Table 1 - Frequency of yeast species isolated from clinical specimens taken from patients hospitalized in public and private hospitals in São Luís - MA from July to December 2010.

Species isolated	Hospital A	Hospital B	Hospital C	Total isolates
<i>Candida albicans</i>	17 (15.8)	16 (14.8)	12 (11.1)	45 (41.7)
<i>Candida glabrata</i>	2 (1.85)	4 (3.7)	2 (1.85)	8 (7.4)
<i>Candida krusei</i>	2 (1.85)	2 (1.85)	1 (0.9)	5 (4.6)
<i>Candida norvegensis</i>	0 (0.0)	1 (0.9)	0 (0.0)	1 (0.9)
<i>Candida parapsilosis</i>	2 (1.85)	0 (0.0)	4 (3.7)	6 (5.6)
<i>Candida tropicalis</i>	12 (11.1)	10 (9.3)	10 (9.3)	32 (29.7)
<i>Cryptococcus neoformans</i>	3 (2.8)	1 (0.9)	1 (0.9)	5 (4.6)
<i>Pichia farinose</i>	0 (0.0)	0 (0.0)	1 (0.9)	1 (0.9)
<i>Rhodotorula glutinis</i>	1 (0.9)	0 (0.0)	0 (0.0)	1 (0.9)
<i>Trichosporon spp.</i>	2 (1.85)	0 (0.0)	2 (1.85)	4 (3.7)
Total	41 (37.9)	34 (31.5)	33 (30.6)	108 (100%)

Table 2 - Distribution of 108 yeast isolates identified in cultures of different biological materials evaluated during July to December 2010.

Yeasts isolated	Clinical specimens						N° total isolates (N°. (%))
	Urine (N°. (%))	Blood (N°. (%))	R.T. (N°. (%))*	Catheter tip (N°. (%))	Liquor (N°. (%))	Others (N°. (%))**	
<i>Candida albicans</i>	24 (22.2)	4 (3.7)	13 (12.1)	2 (1.85)	0 (0.0)	2 (1.85)	45 (41.7)
<i>Candida glabrata</i>	4 (3.7)	2 (1.85)	1 (0.9)	1 (0.9)	0 (0.0)	0 (0.0)	08 (7.4)
<i>Candida norvegensis</i>	1 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	01 (0.9)
<i>Candida krusei</i>	2 (1.85)	1 (0.9)	1 (0.9)	1 (0.9)	0 (0.0)	0 (0.0)	05 (4.6)
<i>Candida parapsilosis</i>	1 (0.9)	4 (3.7)	0 (0.0)	1 (0.9)	0 (0.0)	0 (0.0)	06 (5.6)
<i>Candida tropicalis</i>	20 (18.6)	5 (4.6)	4 (3.7)	1 (0.9)	0 (0.0)	2 (1.85)	32 (29.7)
<i>Cryptococcus neoformans</i>	0 (0.0)	3 (2.8)	0 (0.0)	0 (0.0)	2 (1.85)	0 (0.0)	05 (4.6)
<i>Pichia farinosa</i>	0 (0.0)	1 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	01 (0.9)
<i>Rhodotorula glutinis</i>	0 (0.0)	0 (0.0)	1 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	01 (0.9)
<i>Trichosporon spp.</i>	2 (1.85)	0 (0.0)	1 (0.9)	1 (0.9)	0 (0.0)	0 (0.0)	04 (3.7)
Total	54 (50.0)	20 (18.6)	21 (19.4)	7 (6.4)	2 (1.85)	4 (3.7)	108 (100)

*Respiratory Tract.

**Others (Wound secretions, wound fragments, vaginal secretions, and stools of patients).

in the urinary tract, respiratory tract and catheter tip, followed by *C. tropicalis* and *C. glabrata*. These results are consistent with the literature showing that *C. albicans* is the yeast most commonly isolated from the urinary tract and is responsible for approximately 50% to 70% of cases of candiduria, followed by *C. glabrata* (5% to 33%) and other species of non-albicans yeasts (8% to 28%) (Kauffman, 2005; Trofa *et al.*, 2008; Moen *et al.*, 2009). Surveys conducted in Brazil also show that the three most prevalent species isolated from the urine of hospitalized patients were *C. albicans* (35.5% to 70%), *C. tropicalis* (4.6% to 52.5%) and *C. glabrata* (7% to 8.8%) (Binelli *et al.*, 2006; Furlaneto *et al.*, 2011).

As a common and widespread opportunistic yeast pathogen, *Candida* species account for 8-10% of all nosocomial bloodstream infections (Lundston and Sobel, 2001; Colombo *et al.*, 2002; França *et al.*, 2008). In this study, *C. tropicalis* was the most prevalent strain found in blood samples (4.6%), whereas *C. albicans* and *C. parapsilosis* were isolated from 3.7% of samples. These data are consistent with previously published results showing that *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and other non-albicans *Candida* (NAC) species have been recovered with increasing frequency (Krcmery and Barnes, 2002; Perea and Patterson, 2002). It is therefore interesting that *C. tropicalis* infections have increased since 2003, whereas infections due to all other non-albicans species have been decreasing (Yap *et al.*, 2009). In a study performed at the University Hospital of the Federal University of Mato Grosso do Sul (HU-UFMS), NAC samples were recovered more frequently from blood culture samples than *C. albicans* and *C. parapsilosis* (Xavier *et al.*, 2008). It was also demonstrated that 39% of surveyed blood cultures harbored NAC (Hanh *et al.*, 2008).

In this study, five cases of cryptococcosis were detected in three blood samples and in two cerebrospinal fluid samples from patients at three different hospitals in São Luis, Maranhão State, Brazil. We observed an incidence of 4.6% of cryptococcosis in immunocompromised patients without HIV infection. Our data are consistent with previous studies showing that this yeast is predominantly an opportunistic agent that causes infection in adults with acquired immunodeficiency syndrome (AIDS), although it has been identified in other types of immunocompromised conditions (Chayakulkeeree and Perfect, 2006; Kiertiburanakul *et al.*, 2006; Pfaller *et al.*, 2007). Notably, infections due to *Cryptococcus* in hospitalized patients have increased in recent years, and more than 80% of cryptococcosis cases worldwide are associated with HIV infection (Sionov *et al.*, 2009).

Most *Candida* species were sensitive to all four antifungals tested; however, two *C. tropicalis* isolates were resistant to 5-FC. In addition, two strains of *Candida krusei* exhibited an intermediate response to 5-FC and another strain was S-DD to FCZ. These data are consistent with a prospective surveillance study conducted in 16 U.S. hospitals for three years, in which *C. tropicalis*, *C. parapsilosis* and *C. krusei* together accounted for approximately 20% of opportunistic infections. *Candida tropicalis* showed the highest rate of in vitro resistance to 5-FC among *Candida* species (Diekema *et al.*, 2006). Epidemiological studies have identified risk factors associated with antifungal drug resistance (Canuto and Rodero, 2002). In the present study, we observed several resistant strains; only one *C. krusei* isolate demonstrated dose-dependent susceptibility to FCZ, but all *C. glabrata* strains were susceptible to this antifungal action.

Table 3 - In vitro antifungal susceptibility and the MIC50 and MIC90 of yeasts isolated from patients admitted to three hospitals in São Luís, MA, from July to December 2010.

Antifungal agents/yeasts isolated	Isolates (n°)	MIC (µg/mL)		
		Range	MIC ₅₀	MIC ₉₀
Anfotericina B				
<i>Candida albicans</i>	45	0.5 - 2	1	1
<i>Candida glabrata</i>	8	0.5 - 2	ND	ND
<i>Candida krusei</i>	5	0.5 - 1	ND	ND
<i>Candida norvegensis</i>	1	0.5	ND	ND
<i>Candida parapsilosis</i>	6	0.5	ND	ND
<i>Candida tropicalis</i>	32	0.25 - 0.5	0.5	0.5
<i>Cryptococcus neoformans</i>	5	0.5 - 1	ND	ND
<i>Pichia farinosa</i>	1	ND	ND	ND
<i>Rhodotorula glutinis</i>	1	ND	ND	ND
<i>Trichosporon</i> spp.	4	ND	ND	ND
Fluconazol				
<i>Candida albicans</i>	45	1	1	1
<i>Candida glabrata</i>	8	2 - 4	ND	ND
<i>Candida krusei</i>	5	8 - 16	ND	ND
<i>Candida norvegensis</i>	1	4	ND	ND
<i>Candida parapsilosis</i>	6	2	ND	ND
<i>Candida tropicalis</i>	32	1 - 4	1	2
<i>Cryptococcus neoformans</i>	5	1 - 16	ND	ND
<i>Pichia farinosa</i>	1	ND	ND	ND
<i>Rhodotorula glutinis</i>	1	ND	ND	ND
<i>Trichosporon</i> spp.	4	ND	ND	ND
5-Flucytosine				
<i>Candida albicans</i>	45	1	1	1
<i>Candida glabrata</i>	8	1	ND	ND
<i>Candida krusei</i>	5	4 - 8	ND	ND
<i>Candida norvegensis</i>	1	2	ND	ND
<i>Candida parapsilosis</i>	6	1	ND	ND
<i>Candida tropicalis</i>	32	1 - 32	1	1
<i>Cryptococcus neoformans</i>	5	1 - 16	ND	ND
<i>Pichia farinosa</i>	1	ND	ND	ND
<i>Rhodotorula glutinis</i>	1	ND	ND	ND
<i>Trichosporon</i> spp.	4	ND	ND	ND
Voriconazol				
<i>Candida albicans</i>	45	0.12	0.12	0.12
<i>Candida glabrata</i>	8	0.12 - 1	ND	ND
<i>Candida krusei</i>	5	0.12	ND	ND
<i>Candida norvegensis</i>	1	0.12	ND	ND
<i>Candida parapsilosis</i>	6	0.12	ND	ND
<i>Candida tropicalis</i>	32	0.12	0.12	0.12
<i>Cryptococcus neoformans</i>	5	ND	ND	ND
<i>Pichia farinosa</i>	1	ND	ND	ND
<i>Rhodotorula glutinis</i>	1	ND	ND	ND
<i>Trichosporon</i> spp.	4	ND	ND	ND

Legend: MIC = minimum inhibitory concentration, MIC90 = minimum inhibitory concentration for 90% of the isolates and MIC50 = minimum inhibitory concentration for 50% of the isolates, ND = Not determined.

Increased use of FCZ has led to the development of resistance in *Candida* spp., particularly *C. glabrata* and *C. krusei* (Colombo *et al.*, 2002). Some NAC species are inherently or secondarily resistant to fluconazole, including 75% of *C. krusei* isolates, 35% of *C. glabrata*, 10-25% of *C. tropicalis* and *C. lusitaneae* (Krcmery and Barnes, 2002; Lewis, 2009). Some studies have reported failures in prophylaxis and treatment of *C. glabrata*, *C. krusei* and *Trichosporon* spp. with fluconazole (Colombo *et al.*, 2002; Paul *et al.*, 2007).

A strain of *C. neoformans* isolated from CSF has a higher MIC value for FCZ (16 mg/mL). This patient had already been treated with FCZ for 30 days but was not responding to the established therapy and subsequently died. FCZ has been the drug of choice for treating fungal infections and has been widely used to manage cryptococcosis in AIDS patients, but its prolonged use has led to the emergence of FCZ-resistant strains (Warnock, 2007; Sobel *et al.*, 2011). According to Consensus in Cryptococcosis (2008), testing for antifungal susceptibility can be useful in the clinic, where these tests are performed only in cases of treatment failure or in patients with frequent relapses who received repeated cycles of antifungal treatment with azoles or amphotericin B.

In this study, all *Candida* strains were susceptible to amphotericin B. Resistance to AMB appeared to be uncommon among isolates of *C. albicans*, *C. tropicalis* and *C. parapsilosis*, but there are data suggesting that resistant strains of *C. glabrata* and *C. krusei* exist (Krcmery and Barnes, 2002; Antunes *et al.*, 2004). Randomized, double-blind studies with adults and/or children with a liposomal AMB demonstrated the efficacy of this drug in invasive fungal infections, including invasive candidiasis, candidemia, invasive fungal infection aspergillosis, histoplasmosis and cryptococcal meningitis (Moran *et al.*, 2011).

The antifungal agent voriconazole showed excellent activity against the yeast isolates evaluated in this study. Notably, VCZ has the potential to be effective against some strains that are resistant to fluconazole (Paul *et al.*, 2007).

Using the automated method (Vitek-2 Compact bio-Mérieux, Marcy-L'Etoile, France), the MIC results for the three antifungal agents were highly reproducible, even in comparison with results attained using the reference method for analyzing broth microdilutions that has been tested in three U.S. laboratories with 426 isolates of *Candida* spp. (Severo *et al.*, 2009). Our study showed a high level of reproducibility for the three antifungal agents: VCZ (98.1%), 5-FC (99.3%) and AMB (100%). The authors also suggest that, in addition to the high level of reproducibility, the automated system provides rapid results that represent a major step towards optimizing treatments for invasive *Candida* infections (Severo *et al.*, 2009).

The results of our study reinforce the importance of identifying *Candida* species, defining their antifungal sus-

ceptibility profiles and determining the prevalence of these infections in the hospital environment to improve treatment options for patients with invasive fungal infections. Therefore, the ability to accurately identify and periodically survey the susceptibility profile of yeasts to antifungal agents is crucial for monitoring the development of resistance and for assisting health professionals in delineating guidelines for appropriate use of these medicines.

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