Candidemia epidemiology and susceptibility profile in the largest Brazilian teaching hospital complex

ABSTRACT

Introduction: Although the spectrum of fungi causing bloodstream fungal infections continues to expand, *Candida* spp. remains responsible for the majority of these cases. **Objective**: The purpose of this study was to characterize the candidemia epidemiology, species distribution and antifungal susceptibility patterns at a Brazilian tertiary teaching public hospital with 2,500 beds. **Methods**: Records from the microbiology laboratory were used to identify patients with positive blood cultures during 2006. The *in vitro* activity of amphotericin B, caspofungin, itraconazole, fluconazole, voricanozole, and posaconazole were determined using the Etest method. **Results:** One hundred and thirty-six cases of candidemia were identified and 100 strains were available for antifungal susceptibility testing. The overall incidence of candidemia was 1.87 cases/1.000 admissions and 0.27 cases/1.000 patient-days. Among the patients, 58.1% were male, and the median age was 40 years old. *C. albicans* was the most common species (52.2%), followed by *C. parapsilosis* (22.1%), *C. tropicalis* (14.8%), and *C. glabrata* (6.6%). All strains were susceptible to amphotericin B with a MIC_{90} of 0.5 µg/mL. Overall susceptibility for voriconozole, fluconazole, and caspofungin was $\geq 97\%$ with a MIC_{90} of 0.064, 4.0 and 1.0 µg/mL, respectively. For itraconazole the susceptibility rate was 81% with a MIC_{90} of 0.5 µg/mL. Posaconazole also demonstrated good *in vitro* activity with a MIC_{90} of 0.25 µg/mL. **Conclusion:** This is the first antifungal susceptibility report in our institution.

Keywords: candidemia, *Candida* spp, susceptibility testing, *Candida* epidemiology, *Candida* Etest, antifungal susceptibility profile, *Candida* antifungal agents, azoles.

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INTRODUCTION

During the past decades, publications have documented an increase in health care associated fungal infections in different patient populations.[1] A recent study of the epidemiology of sepsis found that the annual number of cases caused by fungal organisms in the United States increased by 207% between 1979 and 2000.[2] Although the spectrum of fungi causing bloodstream infections has increased, *Candida* spp. remains the most common agent of such infections accounting for approximately 80% of cases.[3-6] As candidemia carries a high morbidity and mortality rate[1,4] various antifungal agents have been preemptively or prophylactically used in routine care of high-risk patient populations.[1,7] However, the widespread use of antifungal agents raises concerns about their potential ecological effects in selecting and spreading resistant fungal strains or species. There have been many reports of breakthrough fungal infections with strains showing intrinsic or acquired resistance to the exposed drug.[8-12] The antifungal susceptibility testing (AFST) helps clinicians in choosing the appropriate antifungal therapy. Currently, the AFST reference methodology for *Candida* spp. is a broth dilution proposed by the Clinical Laboratory Standards Institute (CLSI). Susceptibility breakpoints are available for fluconazole, itraconazole, voriconozole, and echinocandins.[13] This methodology is extremely time consuming and difficult to perform in routine practice at clinical laboratories.

AFST commercial systems like Etest strips (AB Biodisk, Sweden) have good categorical consistency with the reference method and is a validated alternative methodology to perform routine AFST.[14-20]

In Brazil candidemia data and antifungal susceptibility patterns of associated strains are still sparse. In our institution we record the occurrence of cases candidemia of since 1996 (data not shown). In 2006, as there was an increase in the occurrence of *Candida* from blood cultures, we then started a project to monitor the resistance patterns during this period. The aim of this study was to evaluate the epidemiology of candidemia, species distribution, and susceptibility profile over one year at our institution, the largest Brazilian tertiary teaching hospital.

METHODS

The study was conducted at the Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HC), a 2,500 bed tertiary teaching complex, located in the city of São Paulo, Brazil, that provides care for a varied population, including immunosuppressed patients. The HC Complex is mainly comprised of five hospitals attached to the Universidade de São Paulo Medical School, including a general hospital, and other facilities specialized in heart diseases, a pediatric hospital, an orthopedic and a psychiatric hospital, with overall 1,975 ward beds and 325 intensive care unit (ICU) beds.

Case Definition

Records from the microbiology laboratory were used to identify patients with positive peripheral blood cultures for *Candida* species from January 1st to December 31st, 2006. Candidemia was defined as the isolation of *Candida* spp. from blood culture (one isolate per patient). A new episode was considered as the identification of the same *Candida* species more than 30 days after the first positive blood culture or finding a new *Candida* species. Cases were reviewed using a standardized data extraction form observing demographic data, underlying diseases, and patient hospital location at the time of the first positive blood culture.

Organism identification

Blood cultures were monitored using an automated culture system (Bactec 9240 System, Becton, Dickinson, USA). Isolates were stored frozen in BHI with 20% glycerol and 0.2% agar at -70°C. Stored strains were subcultured onto potato dextrose agar (Remel, USA) and chromogenic media for *Candida* species (CHROMagar Microbiology, USA) to ensure purity. Germ tube test, growth at 42°C, assimilation test by API 20C AUX (bioMerieux, French) and microscopy features in cornmeal Tween 80 medium (Oxoid, USA) were used for specie identification confirmation.

Susceptibility testing

Antifungal susceptibility testing was performed using the Etest method (AB BIODISK, Sweden) according to the manufacturer's instructions. The following drugs were tested: amphotericin B, caspofungin, fluconazole, itraconazole, voriconazole, and posaconazole. A 150 mm commercial agar plate (Probac, Brazil) containing RPMI 1640 medium supplemented with 2% glucose buffered with MOPS in a pH of 7.0 was used. The *inocula* suspension was adjusted with a spectrophotometer to match the transmittance produced by a 0.5 McFarland density standard at a 530 nm wavelength. The agar surface was inoculated using a nontoxic swab according to the manufactures instructions. Etest strips were applied after excess moisture was absorbed and the agar surface was completely dry. The plates were incubated at 35°C and the MIC (µg/mL) was read after 24 hours. The MIC (µg/mL) was read as the lowest concentration at which the border of the elliptical zone of growth inhibition intersected the scale on the Etest strip. For the azoles and caspofungin, an 80% growth inhibition was used as the MIC endpoint, and for amphotericin B, nearly complete growth inhibition (100%) was used according to manufacture instructions. For MICs that yielded results falling in between conventional serial twofold dilution, the next highest dilution was assigned.

Category MIC breakpoints (BP)

Interpretations for fluconazole, itraconazole, voriconazole and caspofungin were according M27A3, 2008 CLSI document, 2008.[13] Interpretative breakpoints have not yet been established for amphotericin B, but breakpoints proposed by Nguyen *et al*. were used to classified strains.[21] For posaconazole there is not an established interpretative breakpoint yet.

Quality control

Quality control was assured by testing the strains recommended by CLSI *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 in each batch of MIC testing.[13]

Statistical analysis

The incidence rates were calculated as the number of candidemia episodes per 1,000 admissions and per 1,000 patient-days. Categorical data were analyzed using Chi-square and Fisher's exact tests, as appropriate. A p value ≤ 0.05 was considered statistically significant. Data analyses were performed by Epi-Info 6.04 software CDC (Centers for Disease Control and Prevention).

RESULTS

During 2006, the prevalence of fungi and *Candida* spp. in positive blood cultures were 4% and 3.5% respectively, being 86.1% of fungemia related to *Candida* species. One hundred and thirty six candidemia episodes were identified in 132 patients. The overall incidence of candidemia was 1.87 per 1,000 admissions and 0.27 per 1,000 patient days. There was a wide variation of incidence rates among hospitals from the HC complex ranging from 0.0 to 7.6 cases per 1,000 admissions (Table 1). Seventy seven patients (59.9%) were male,

and the median age was 40 years old (0-87 years). Fifty one patients were children, and 62.7% had less than one year old. Among adult patients (> 16 years old) the median age was 54 years, and 3 months old among children (0-16 years old); 91% of patients were hospitalized. Of these, 56.5% were in clinical wards, 26.3% in intensive care units, and 17.2% in surgical wards. The majority of patients had an underlying disease, being malignancy the most frequent one (32.5%) (Table 2). Among children younger than one year, prematurity was observed in 56.2% of candidemia cases.

Table 1. Candidemia: Incidence rates and species distribution in different hospitals from Hospital das Clínicas complex from January to December 2006

Candida spp	General hosp		Pediatric hosp		Cardiac hosp		Ortopedic hosp		Axiliary 1 hosp		Axiliary 2 hosp		Total	
	ADM	Pat/day	ADM	Pat/day	ADM	Pat/day	ADM	Pat/day	ADM	Pat/day	ADM	Pat/day	ADM	Pat/day
C. albicans (66)	1.01	0.15	3.63	0.55	0.91	0.31	0.38	0.05	0	0	2.37	0.03	1.0	0.17
C. parapsilosis (26)	0.27	0.05	2.90	0.44	0.30	0.10	0	0	0.39	0.03	0	0	0.37	0.05
C. tropicalis (18)	0.25	0.04	0.73	0.11	0.38	0.12	0	0	0.39	0.03	0	0	0.28	0.04
C. glabrata (9)	0.20	0.03	0	0	0.08	0.02	0	0	0	0	0	0	0.14	0.02
C. krusei (2)	0.03	0	0	0	0.08	0.02	0	0	0	0	0	0	0.03	0.004
C. guilliermondii (1)	0.03	0	0	0	0	0	0	0	0	0	0	0	0.02	0.002
C. lusitaneae (1)	0.03	0	0	0	0	0	0	0	0	0	0	0	0.02	0.002
C. pelliculosa (1)	0	0	0	0	0	0	0.18	0.03	0	0	0	0	0.02	0.002
C. utilis (1)	0.03	0	0	0	0	0	0	0	0	0	0	0	0.02	0.002
Total (125)	1.85	0.27	7.26	1.10	1.75	0.57	0.56	0.08	0.78	0.06	2.37	0.03	1.87	0.27
Admission (ADM)	39,397		2,755		13,113		5,313		2,563		422		65,276	
Patient days (pat/day)	265,774		1,8251		40,585		38,369		33,936		39,887		455,546	

Hosp, hospital.

Table 2. Candidemia in **Hospital das Clínicas** *complex – Underline disease and distribution among* **C. albicans** *and other* **Candida** *species*

Underline disease	*C. albicans* (71)	*non albicans C* (65)	Total	p-value
Solid Cancer	14	11	25	0.67
Prematurity	9	9	18	0.84
Hematologic malignancy	7	10	17	0.33
Cardiac disease	8	6	14	0.69
Neurologic disease	4	4	8	0.89
Organ transplant	4	3	7	0.79
Autoimmune disease	3	3	6	0.91
Gastrointestinal disease	4	2	6	0.47
Pulmonary disease	4	1	5	0.20
Vascular disease	1	3	4	0.27
HIV	2	2	4	0.93
Politrauma	3	1	4	0.35
Genetic – Hereditary disease	2	1	3	0.61
Skin and soft tissue disease	1	4	5	0.14
Renal disease	2	0	2	0.17
Tuberculosis	1	0	1	0.33
Diabetes Mellitus	1	0	1	0.33
Non evaluated	1	5	6	NA

The most frequent specie was *C. albicans* (52.2%), followed by *C. parapsilosis* (22.1%), *C. tropicalis* (14.7%), *C. glabrata* (6.6%), and *C. krusei* (1.6%). Table 3 summarizes the species' distribution. Differences in species distribution among non-*C. albicans* were seen in the facility type (Table 1), patient location, and age group (Table 4).

Antifungal susceptibility testing (AFST) was performed among 100 *Candida* spp. (56 *C. albicans*, 21 *C. parapsilosis*, 14 *C. tropicalis*, 4 *C. glabrata*, 2 *C. krusei*, and 1 isolate each of *C. guilliermondii*, *C. lusitaneae*, and *C. pelliculosa*) as 36 isolates lost viability. Table 5 summarizes *in vitro Candida* spp. susceptibilities rates, MIC, MIC_{50} and MIC_{90} ranges.

Overall susceptibility to amphotericin B was 100% (MIC_{50} 0.125 μg/mL / MIC_{90} 0.5 μg/mL), for voriconozole (MIC_{50} 0.008 μg/mL / MIC_{90} 0.064 μg/mL), fluconazole (MIC_{50} 0,25 μg/mL / MIC_{90} 4 μg/mL), and caspofungin (MIC_{50} 0.125 μg/mL / MIC_{90} 1 μg/mL) were ≥ 97% for itraconazole (MIC_{50} 0.032 μg/mL/MIC_{90} 0.5 μg/mL); the susceptibility rate was 81%. Decreased susceptibility to caspofungin was seen with 9.5% of *C. parapsilosis* (2/21 isolates with MICs 4 μg/mL). All *C. albicans*, *C. tropicalis* and *C. parapsilosis*. *C. glabrata* and *C. krusei* were susceptible to fluconazole, but one of each, had decreased susceptibility to fluconazole. Decreased susceptibility to itraconazole was seen in 12.5% of *C. albicans*; 23.8%, of *C. parapsilosis*; 21.4% of *C. tropicalis*, 75% of*C. glabrata* (3/4 isolates) and 100% of C. gulliermondii (1/1 isolate). Voriconazole had good *in vitro* activity, but a MIC of 4 μg/mL in one *C. glabrata* isolate was recorded. Posaconazole exhibited low MICs for all isolates, except one C. gulliermondii (32 μg/mL) and one *C. glabrata* (4 μg/mL).

Table 3. Distribution of Candida *species isolates from blood culture in Hospital das Clínicas complex from January to December 2006*

Candida spp	n	%
C. albicans	71	52.2%
C. parapsilosis	30	22.1%
C. tropicalis	20	14.8%
C. glabrata	9	6.6%
C. krusei	2	1.5%
C. guilliermondii	1	0.7%
C. lusitaneae	1	0.7%
C. pelliculosa	1	0.7%
C. utilis	1	0.7%
Total	136	100.0%

Table 4. Distribution of Candida *species and candidemia cases by age group in Hospital das Clínicas complex from January to December 2006*

Candida spp	< 1		> 1 to < 15		> 16 to < 64		> 65		Total	
	n	%	n	%	n	%	n	%	n	%
Distribution of candidemias by age groups	32	23.5%	19	14.0%	65	47.8%	20	14.7%	136	100.0%
C. albicans (71)	16	50.0%	9	47.4%	36	55.4%	10	50.0%	71	52.2%
C. parapsilosis (30)	15	46.9%	4	21.1%	9	13.9%	2	13.9%	30	22.1%
C. tropicalis (20)	0	0.0%	5	26.3%	10	15.4%	5	25.0%	20	14.7%
C. glabrata (9)	1	3.1%	0	0.0%	6	9.2%	2	10.0%	9	6.6%
C. krusei (2)	0	0.0%	0	0.0%	2	3.1%	0	0.0%	2	1.6%
C. guilliermondii (1)	0	0.0%	0	0.0%	0	0.0%	1	5.0%	1	0.7%
C. lusitaneae (1)	0	0.0%	1	5.2%	0	0.0%	0	0.0%	1	0.7%
C. pelliculosa (1)	0	0.0%	0	0.0%	1	1.5%	0	0.0%	1	0.7%
C. utilis (1)	0	0.0%	0	0.0%	1	1.5%	0	0.0%	1	0.7%
Total (136)	32	100.0%	19	100.0%	65	100.0%	20	100.0%	136	100.0%

Table 5. Antifungal susceptbility testing of 100 Candida *spp. isolated from blood culture in Hospital das Clínicas complex from January to December of 2006*

Specie (n) Drogas	S n (%)	SDD n (%)	R n (%)	MIC$_{90}$ µg/mL	MIC$_{50}$ µg/mL	Range µg/mL
C. albicans (56)						
Amphotericin B	56 (100)	0 (0)	0 (0)	0.5	0.125	0.016 - 1
Caspofungin	56 (100)	0 (0)	0 (0)	1	0.125	0.016 - 1
Fluconazole	56 (100)	0 (0)	0 (0)	0.5	0.125	0.125 - 4
Itraconazole	49 (87.5)	6 (10.7)	1 (1.8)	0.25	0.016	0.008 - 1
Voriconazole	56 (100)	0 (0)	0 (0)	0.064	0.008	0.008 - 0.125
Posaconazole	NC	NC	NC	0.064	0.008	0.008 - 0.064
C. parapsilosis (21)						
Amphotericin B	21 (100)	0 (0)	0 (0)	0.125	0.125	0.032 - 0.5
Caspofungin	19 (90.5)	0 (0)	2 (9.5)	2	1	0.064 - 4
Fluconazole	21 (100)	0 (0)	0 (0)	4	0.5	0.125 - 8
Itraconazole	16 (76.2)	3 (14.3)	2 (9.5)	0.5	0.032	0.008 - 1
Voriconazole	21 (100)	0 (0)	0 (0)	0.064	0.016	0.008 - 0.125
Posaconazole	NA	NA	NA	0.25	0.016	0.016 - 0.5
C. tropicalis (14)						
Amphotericin B	14 (100)	0 (0)	0 (0)	1	0.125	0.016-1
Caspofungin	14 (100)	0 (0)	0 (0)	1	0.125	0.064 - 2
Fluconazole	14 (100)	0(0)	0 (0)	1	0.25	0.125 - 1
Itraconazole	11 (78.6)	1 (7.1)	2 (14.3)	1	0.016	0.008 - 32
Voriconazole	14 (100)	0 (0)	0 (0)	0.032	0.016	0.008 - 0.032
Posaconazole	NA	NA	NA	0.25	0.064	0.008 - 0.125
C. glabrata (4)						
Amphotericin B	4 (100)	0 (0)	0 (0)	1	-	0.125 - 1
Caspofungin	4 (100)	0 (0)	0 (0)	1	-	0.125 - 1
Fluconazole	2 (50)	1 (25.0)	1 (25.0)	256	-	0.125 - 256
Itraconazole	1 (25.0)	0 (0)	3 (75.0)	32	-	0.016 - 32
Voriconazole	3 (75.0)	0 (0)	1 (25.0)	0.125	-	0.008 - 4
Posaconazole	NA	NA	NA	32	-	0.008 - 32
C. krusei (2)						
Amphotericin B	2 (100)	0 (0)	0 (0)	0.25	-	0.032 - 0.25
Caspofungin	2 (100)	0 (0)	0 (0)	0.5	-	0.064 - 0.5
Fluconazole	1 (50)	0 (0)	1 (50)	64	-	0.125 - 64
Itraconazole	2 (100)	0 (0)	0 (0)	0.125	-	0.016 - 0.125
Voriconazole	2 (100)	0 (0)	0 (0)	0.125	-	0.008 - 0.125
Posaconazole	NA	NA	NA	0.25	-	0.064 - 0.25
Others (3)						
Amphotericin B	3 (100)	0 (0)	0 (0)	0.5	-	0.016 - 0.5
Caspofungin	3 (100)	0 (0)	0 (0)	2	-	0.016 - 2
Fluconazole	3 (100)	0 (0)	0 (0)	4	-	0.032 - 4
Itraconazole	2 (66.7)	0 (0)	1 (33.3)	32	-	0.008 - 32
Voriconazole	3 (100)	0 (0)	0 (0)	0.064	-	0.008 - 0.064
Posaconazole	NA	NA	NA	4	-	0.008 - 4
Total (100)						
Amphotericin B	100 (100)	0 (0)	0 (0)	0.5	0.125	0.016 - 1
Caspofungin	98 (98.0)	0 (0)	2 (2.0)	1	0.125	0.016 - 4
Fluconazole	97 (97.0)	1 (1.0)	2 (2.0)	4	0.25	0.125 - 256
Itraconazole	81 (81.0)	10 (10)	9 (9.0)	0.5	0.032	0.008 - 32
Voriconazole	99 (99.0)	0 (0)	1 (1.0)	0.064	0.008	0.008 - 4
Posaconazole	NA	NA	NA	0.25	0.016	0.008 - 32

Note: S, suceptible; SDD, susceptible dose dependent; R, resistant. Breakpoints according to CLSI M27 A3.
NA, categorical results not applicable. Others *C guilliermondii; C. lusitaneae; C. pelliculosa.* MIC 90 was not calculated when the number of isolates tested was < 10.

DISCUSSION

Candidemia remains a persistent challenge in many hospitals worldwide leading to significant mortality and overload of healthcare costs. There is a wide variation in the incidence of candidemia reported in the literature underscoring the need for local and global epidemiological studies.

The prevalence of *Candida* spp. among blood cultures, in our study period, was 3.5%, being the ninth most common isolated microorganism from blood cultures, differing from other studies were this microorganism was listed as the 5th most frequent one.[1,4,6]

The overall incidence rate (1.87 cases/1,000 admissions) was similar to previous Brazilian reports with rates ranging from 1.66 to 2.49 cases per 1,000 admissions when comparing different hospitals from the HCFMUSP complex.[6,22] Important differences were seen among the hospitals, probably reflecting distinct patient populations and differences in risk factors. Reports from Canada, USA and Europe showed a much lower incidence in candidemia rates, ranging from 0.045 to 0.38 cases per 1,000 admissions.[23,27] The high rates of candidemia in Brazilian public hospitals were highlightened by Colombo *et al.* in a multicenter study and were multifactor related, such as difficulties in implementing hospital infection control measures, limited number of health care workers, and less aggressive antifungal prophylaxis practices.[6,22]

C. albicans was the specie most frequently isolated followed by *C. parapsilosis*, *C. tropicalis* and *C. glabrata*. This finding is consistent with national and Latin American studies.[6,22,28,29] However, important differences of species distribution have been documented when evaluating by age group and clinical wards (nurses or ICU). Of note, *C. parapsilosis* was the most prevalent non-*C. albicans* species, probably because a high proportion of children younger than 1 year old accounted for almost 25% of cases. *C. parapsilosis* is commonly linked to prematurity, use of central venous catheter, and parenteral nutrition.[1,30,32] Among patients with more than one year old *C. tropicalis* was the most common non-*C. albicans* species.

C. glabrata was the second non-*C. albicans* in ICU and deserves attention. *C. glabrata* and *C. krusei* have been an infrequent cause of candidemia in Brazil. Data from USA and European countries showed *C. glabrata* and *C. parapsilosis* as the most prevalent non-*C. albicans* species.[1,4,23,24] The reasons for this variation are still not clear, but exposure to azoles, subjacent diseases, age group, ward location, and use of medical devices could explain these differences.[1] Reports that evaluated the use of azoles as a risk factor associated with non-*C. albicans* candidemia that are intrinsically resistant or with reduced resistance to those drugs, such as *C. krusei* and *C. glabrata*, remain controversial.[1,3,24,33-34] Girão *et al.* evaluated candidemia rates from one ICU in our hospital over a seven year period and did not find an increase in *C. glabrata* and/or *C. krusei* [incidence], despite increasing fluconazole use during this time. On the other hand, Pasqualotto *et al.* demonstrated an association between fluconazole use and higher incidence rates of *C. glabrata* fungemia.[35]

Antifungal susceptibility testing as a routine procedure, is an important tool to guide therapeutical choices and monitor local resistance. AFST strips by Etest are a validated agar based methodology that may be an alternative, because it is relatively easy to perform, with drug choice flexibility, and have very good consistency with the reference methodology.[14-20] Occasionally category discordances between Etest and the microdilution method may occur due to the heteroresistance and trailing phenomenon, generating higher MICs. These factors were mainly observed with *C. tropicalis* for itraconazole and fluconazole, and with *C. glabrata* for fluconazole. Some authors demonstrated that heteroresistance detection was possible only with the use of Etest and that the reference methodology was unable to highlight this phenomenon.[14]

Similar to other national and international studies, antifungal resistance was a rare event. We did not find any *Candida* spp. with MICs >1 µg/mL for amphotericin B, and 98% were susceptible to caspofungin, with two *C. parapsilosis* isolates with a MIC of 4 µg/mL. Pfaller *et al.* evaluated 5,346 *Candida* spp. isolates and found a susceptibility rate of 99% to echinocandins. However, the MIC90 for *C. parapsilosis* was higher than other *Candida* species, and the reduced susceptibility mechanism is related to amino acid changes within the FKS1 in a hot spot region.[36,38]

Fluconazole resistant isolates were restricted to *C. glabrata* and *C. krusei*. The susceptibility profile to fluconazole was (97%), similar to other Brazilian and Latin American studies.[6,22,29,39-40,42] European and North American studies have showed lower sensitivity, rates ranging from 86.6 to 95.8%.[39,43] *C. krusei*, despite its known intrinsic resistance to fluconazole, may show false *in vitro* susceptibility and care should be taken to report those results.

For itraconazole, the percentage of non-susceptible isolates was 19% (10% SDD and 9% R). Higher itraconazole MICs may be associated to technical errors in the MIC readings due to the trailing phenomenon.[14] The trailing phenomenon is most often encountered with azoles and is characterized by incomplete growth inhibition. With appropriate training, reading errors should be minimal.

Voriconazole overall susceptibility was high and the only resistant strain was one *C. glabrata* (MIC of 4 µg/mL) that showed cross-resistance to all azoles.

There are no official breakpoints for posaconazole, but the MIC90 (0.25 µg/mL) was much lower than previously reported by other authors.[20,42,44] Of note, one isolate of *C. glabrata* and one of *C. guilliermondii* had a very high MIC (4 µg/mL).

Candidemia has a high incidence in our institution and despite low resistant rates it poses a therapeutic challenge, especially among non-*C. albicans* species. Knowledge of local epidemiology and AFST profile may help physicians in selecting the appropriate therapy. Etest offers a validated alternative methodology for routine use in clinical laboratories, especially in countries where commercial microdilution panels are not available. Continuous surveillance to monitor trends in incidence, species distribution, and antifungal drug susceptibility profiles, should be performed at a local and global level, since epidemiological studies continue to reveal variations in candidemia epidemiology.

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