A 16-Year Analysis of Antifungal Susceptibilities of Invasive *Candida* spp Tested in Our Daily Hospital Routine

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Abstract

Objective: Invasive *Candida* infections often cause high morbidity and mortality especially in the critically ill or immunosuppressive patients. We analyzed the species distribution and antifungal susceptibility data of 1371 invasive *Candida* strains isolated in Cerrahpasa Medical Faculty mycology laboratory over 16 years.

Methods: We performed susceptibility tests for the strains isolated from blood or deep sites and/or from patients unresponsive to the initial antifungal treatment, and all results were routinely reported to clinicians. The tests against amphotericin B (AMB) and azoles were performed using Clinical and Laboratory Standards Institute (CLSI) guidelines from 1998 to 2012 and using Etest from 2012 to 2014. The Sensititre YeastOne (SYO) colorimetric method was used to test *Candida* echinocandin susceptibility between 2012 and 2014. In this retrospective analysis, resistance or non-wild type (non-WT) phenotypes to systemic antifungals were determined by the previous and recently revised CLSI breakpoints (BPs) and by method-dependent species-specific epidemiological cutoff values (ECVs), respectively.

Results: Overall, *Candida albicans* was the most commonly isolated species (48%) followed by *C. parapsilosis* (20%), *C. glabrata* (12%), and *C. tropicalis* (12%). The new epidemiological BPs provided by CLSI changed the percentage of resistant *C. albicans*, *C. parapsilosis*, and particularly *C. tropicalis* isolates to fluconazole (FLZ). Using the ECVs, reduced susceptibility to FLZ was higher among *C. albicans* isolates (33.4%), whereas itraconazole (ITZ) was higher in *C. glabrata* (58.1%) than in all other species.

Conclusion: Antifungal susceptibility tests are a key component of the care of patients with invasive candidiasis. Knowledge of local prevalence of antifungal resistance and susceptibility patterns might affect clinical decision-making.

Keywords: Invasive *Candida* species, antifungal susceptibility, new breakpoints, method-dependent epidemiological cutoff values

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Onaltı yıl boyunca hastanemizde rutin olarak test edilmiş olan invaziv Candida spp antifungal duyarlılıklarının analizi

Öz

Amaç: İnvaziv *Candida* infeksiyonları kritik veya bağışıklığı baskılanmış hastalarda sıklıkla yüksek morbidite ve mortaliteye sebep olurlar. Biz Cerrahpaşa Tıp Fakültesi mikoloji laboratuvarında 16 yıl boyunca hasta materyallerinden ayrılan 1371 invaziv *Candida* kökeninin tür dağılımı ve antifungal duyarlılık verilerini analiz ettik.

Yöntemler: Duyarlılık testleri kandan veya derin vücut bölgelerinden ve/veya başlangıç antifungal tedaviye yanıtsız hastalardan ayrılan kökenlere yapıldı ve tüm sonuçlar rutin olarak klinisyenlere bildirildi. Amfoterisin B (AMB) ve azollere karşı testler 1998'den 2012'ye kadar Clinical and Laboratory Standards Institute (CLSI) rehberlerine göre ve 2012'den 2014'e kadar Etest kullanılarak yapıldı. *Candida*'ların ekinokandinlere duyarlılık testleri için 2012'den 2014'e kadar Sensititre YeastOne (SYO) yöntemi kullanıldı. Bu retrospektif analizde, sistemik antifungallere direnç önceki ve yeni gözden geçirilmiş CLSI direnç sınırları ile ve doğal olmayan fenotipler yönteme bağlı türe özgül epidemiyolojik eşik değerleri kullanılarak belirlendi.

Bulgular: En sıklıkla ayrılan tür *Candida albicans* (%48)'ı *C. parapislosis* (20%), *C. glabrata* ve *C. tropicalis* (ikisi de 12%) izledi. CLSI'ın yeni önerdiği sınır değerleri kullanıldığında *C. albicans*, *C. parapsilosis* ve özellikle *C. tropicalis*'in (FLZ)'e direnç yüzdeleri değişti. Epidemiyolojik eşik değerleri kullanıldığında bütün türler içerisinde FLZ'e azalmış duyarlılık *C. albicans* kökenlerinde daha yüksek (%33.4) bulunurken *C. glabrata* için itrakonazol (ITZ)'e azalmış duyarlılık daha yüksek (%58.1) olarak belirlendi.

Sonuç: Yerel antifungal direnç ve duyarlılık paternlerinin bilinmesi klinik karar vermeyi etkileyebilir.

Anahtar Sözcükler: İnvaziv *Candida* türleri, antifungal duyarlılık, yeni direnç sınır değerleri, yönteme bağlı epidemiyolojik eşik değerleri

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Candida species are the main cause of opportunistic fungal invasive infections with considerably increasing incidence in patients with underlying conditions. Isolation of *Candida* sp *in vitro* less susceptible to antifungals and recovery of increasingly resistant isolates during antifungal therapy are also growing problems [1]. It is important to determine the species distribution and resistance rates of invasive *Candida* isolates to develop proper treatment strategies in the medical centers.

Since 1997, susceptibility tests of *Candida* spp. to antifungal agents have been in use due to the standardization developed by Clinical and Laboratory Standards Institute (CLSI) (formerly National Committee for Clinical Laboratory Standards). Susceptibility testing of isolated clinically significant fungi has been performed in our laboratory as a part of routine procedure over 16 years. We performed susceptibility tests for the strains isolated from blood or deep sites and/or from patients unresponsive to the initial antifungal treatment. All results were reported to clinicians. In the meantime, developments in CLSI reference methods (M27-A–M27-A3 and supplements S3, S4) [2-6] improved the ability to detect emerging resistance patterns. The biggest new changes were (i) the change in BPs for fluconazole (FLZ), voriconazole (VRZ), and echinocandins from those previously established for all *Candida* species to species-specific breakpoints (BPs) for the five most common species, and (ii) the establishment of species-specific epidemiological cutoff values (ECVs) for the systemically active antifungal agents and *Candida* species to assess MICs when no BPs were established (Table 1). This value does not provide a categorical placement of an MIC as susceptible or resistant but differentiate between WT and non-WT strains to identify those strains with acquired or mutational resistance mechanisms.

BPs have not been established for AMB and any species of *Candida* and reference broth microdilution methods of *Candida* echinocandin susceptibility testing were limited by significant interlaboratory variability in caspofungin (CAS) MICs. Recently CLSI proposed ECVs for *C. albicans, C. glabrata, C. krusei, C. parapsilosis*, and *C. tropicalis* against AMB, anidulafungin (AND), ITZ, micafungin (MCF) [7]. Method-dependent ECVs for AMB and echinocandins by Etest and for echinocandins by SYO colorimetric assay were also proposed to identify *Candida* isolates with reduced susceptibility to these agents, but these ECVs will not categorize a *Candida* isolate as susceptible or resistant, as BS do [8-10].

This retrospective study aimed to analyze the species distribution and antifungal susceptibility of clinically significant *Candida* strains isolated and routinely tested in the Deep Mycosis Laboratory of a large university hospital over a 16-year period, using the old and new CLSI clinical BPs and method-dependent ECVs.

Material and Methods

Isolates

The clinical samples were sent from different wards. Each isolate was obtained from a different patient and was checked to ensure purity. All isolates were identified to the species level by classical mycological tests including germ tube formation; blastoconidia, pseudohyphae, true hyphae, and chlamydoconidia formation; urease activity; growth at various temperatures; and carbohydrate assimilation patterns [11-15]. The API 20C AUX system (Biomérieux, Marcy, l'Etoile, France) was also used to identify the isolates if needed.

Antifungal susceptibility tests

AMB (Bristol-Meyers Squibb, Wallingford, Conn.), FLZ (Pfizer, Istanbul, Turkey), ITZ (Janssen Pharmaceuticals, Beerse, Belgium), VRZ (Pfizer, Istanbul, Turkey), and posaconazole (PSZ, Schering-Plough, Istanbul, Turkey) were obtained as standard powders. Tests for AMB, FLZ, and ITZ were performed since 1998, for VRZ since 2005, and for PSZ since 2006 up to December 2012 using CLSI broth dilution methods, then using the E test strips (Biomérieux, France) up to December 2014. Echinocandins were tested for *Candida* blood and urine isolates of symptomatic patients using SYO assay in between 2012 and 2014. Broth dilution testing was performed according to the approved documents of the CLSI [2-6]. Antibiotic Medium 3 (Oxford, England) was used to test AMB. Drug-free and yeast-free controls were included in the studies. *Candida krusei* ATCC 6258 (American Type Culture Collection, Manassas, VA, USA), *C. parapsilosis* ATCC 22019 and reference strains, *C. albicans* ATCC 90028, and ATCC 90029, *C. glabrata* ATCC 90030, *C. parapsilosis* ATCC 90018 strains were included for quality control (QC) purposes in each testing run. Isolates were classified as resistant based on both the previous and the recently revised CLSI BPs. Differences in resistant rates between the previous and revised BPs were assessed for significance by Fisher's exact test, and a P value of ≤ 0.5 was considered significant. E test and SYO were used according to the manufacturers' instructions. Both tests were validated using CLSI QC and reference isolates.

Results

Species distribution

A total of 1371 *Candida* strains were isolated. Distribution of isolated *Candida* spp. by clinical specimens

Table 1. *In vitro* susceptibilities of 1173 invasive *Candida* spp isolates to five systemically active antifungal agents and interpretive breakpoints according to the CLSI documents

		MIC (µg/mL)			Previous MIC breakpoints				Revised breakpoints				ECV (CLSI)	No. of isolates according to ECVs (%)	
Species (No. of tested isolates)	**Antifungal agent**	**Range**	**50%**	**90%**	**BP**	**No. R/ NS (%)**	**No. S-DD or I* (%)**	**No. S (%)**	**BP**	**No. R (%)**	**No. S-DD or I* (%)**	**No. S (%)**		**≤ECV**	**>ECV**
C. albicans (545)	AMB	≤0.03–4	0.125	0.25									2	543 (99.6)	2 (0.4)
	FLZ	≤0.125–≥64	0.25	2	≥64	13(2.4)	9 (1,7)	523 (96,0)	≥8	24 (4.4)	7 (1.3)	514 (94.3)	0.5	363 (66.6)	182 (33.4)
	ITZ	≤0.03–2	0.06	1	≥1	55 (10.0)	61 (11.2)	429 (78.7)	-				-		
	VRZ	≤0.015–2	0.015	0.06	≥1	0 (0)	8 (1.5)	537 (98.5)	≥4	15 (2.8)	18 (3.3)	512 (93.9)	0.03	477 (87.5)	68 (12.5)
	PSZ	≤0.015–0.25	0.003	0.06									0.03	510 (93.6)	35 (6.4)
C. glabrata (148)	AMB	0.06–1	0.25	1									2	146 (98.6)	2 (1.4)
	FLZ	1–≥64	7(4.8)	7 (4.8)	≥64	134 (90.4)	88 (60.7)	46 (31.0)	≥64	14 (9.5)	134 (90.5)	-	8	134 (90.5)	14 (9.5)
	ITZ	≤0.03–4	0.25	1	≥1	23 (15.5)	65 (43.9)	60 (40.5)	-				4	62 (41.9)	86 (58.1)
	VRZ	0.03–2	0.125	0.25		0 (0)	3 (2.0)	145 (97.9)	≥4				0.5	142 (96.0)	6 (4.0)
	PSZ	≤0.015–1	0.06	0.125									1	148 (100.0)	0 (0)
C. guilliermondii (17)	AMB	0.125–2	0.25	1									2	17 (100.0)	0 (0)
	FLZ	0.06–16	1	4	≥64	0	1	16	-				8	16	1
	ITZ	0.06–2	0.5	2	≥1	7	7	3	-				-		

Table 1. *In vitro* susceptibilities of 1173 invasive *Candida* spp isolates to five systemically active antifungal agents and interpretive breakpoints according to the CLSI documents (Continue)

		MIC (µg/mL)			Previous MIC breakpoints				Revised breakpoints				ECV (CLSI)	No. of isolates according to ECVs (%)	
Species (No. of tested isolates)	**Antifungal agent**	**Range**	**50%**	**90%**	**BP**	**No. R/ NS (%)**	**No. S-DD or I* (%)**	**No. S (%)**	**BP**	**No. R (%)**	**No. S-DD or I* (%)**	**No. S (%)**		**≤ECV**	**>ECV**
	VRZ	≤0.015–1	0.03	0.25		0	0	17	≥4				0.12	15	2
	PSZ	≤0.015–0.5	0.015	0.25									1	17	0
C. kefyr (10)	AMB	0.25–4	0.5	1											
	FLZ	0.125–2	0.125	1	≥64	0	0	10	-				1		
	ITZ	0.06–1	0.125	0.5	≥1	6	3	1	-						
	VRZ	≤0.015–0.03	<0.015	0.03	≥2	0	0	10	≥4						
	PSZ	≤0.015–0.125	<0.015	0.03											
C. krusei (79)	AMB	0.125–≥16	0.5	2	-								2	72 (91.1)	7 (8.9)
	FLZ	8–≥64	16	32	-				-				32	75 (95.0)	4 (5.0)
	ITZ	0.125–2	0.25	1	-				-				1	75 (95.0)	4 (5.0)
	VRZ	0.03–0.5	0.03	0.25		79 (100)	0 (0)	0 (0)	≥4	79 (100)	0 (0)	0 (0)	0.5	79 (100)	0 (0)
	PSZ	<0.015–0.5	0.06	0.5									1	79 (100)	0 (0)
C. lusitaniae (7)	AMB	≤0.03–2	0.125	0.5									2	7	0
	FLZ	0.125–2	0.25	1	≥64	0	0	7	-				1	6	1
	ITZ	≤0.03–0.5	0.06	0.25	≥1	0	0	7					1	6	1
	VRZ	≤0.015–0.06	0.03	0.06		0	0	7	≥4				0.03	7	0
	PSZ	≤0.015–0.06	0.03	0.06		0	0	7					0.06	5	2

Table 1. *In vitro* susceptibilities of 1173 invasive *Candida* spp isolates to five systemically active antifungal agents and interpretive breakpoints according to the CLSI documents (Continue)

		MIC (µg/mL)			Previous MIC breakpoints				Revised breakpoints				ECV (CLSI)	No. of isolates according to ECVs (%)	
Species (No. of tested isolates)	Antifungal agent	Range	50%	90%	BP	No. R/ NS (%)	No. S-DD or I* (%)	No. S (%)	BP	No. R (%)	No. S-DD or I* (%)	No. S (%)		≤ECV	>ECV
C. parapsilosis (223)	AMB	≤0.03–1	0.125	0.5									2	223 (100)	0 (0)
	FLZ	0.06–16	0.25	1	≥64	2 (1.0)	2 (1.0)	194 (98.0)	≥8	4 (2.0)	1 (0.5)	193 (97.5)	1	204 (91.5)	19 (8.5)
	ITZ	0.03–1	0.125	0.5	≥1	3 (1.5)	96 (48.5)	99 (50)							
	VRZ	≤0.015–0.25	0.06	0.125	≥1	0 (0)	0 (0)	223 (100)	≥4	0 (0)	10 (4.5)	213 (95.5)	0.03	198 (88.8)	25 (11.2)
	PSZ	≤0.015–0.5	0.03	0.125									0.06	213 (100)	0 (0)
C. tropicalis (144)	AMB	≤0.03–1	0.125	0.5									2	144 (100)	0 (0)
	FLZ	0.125–≥64	0.25	8	≥64	0 (0)	4 (2.8)	140 (97.2)	≥8	16 (11.1)	0 (0)	128 (88.9)	1	131 (91.0)	13 (9.0)
	ITZ	≤0.03–1	0.125	0.5	≥1	7 (4.9)	60 (41.7)	77 (53.4)					0.5	137 (95.1)	7 (4.9)
	VRZ	≤0.015–0.25	0.03	0.125	≥1	0 (0)	0 (0)	144 (100)	≥4	0 (0)	12 (8.3)	132 (91.7)	0.06	129 (89.6)	15 (10.4)
	PSZ	≤0.015–0.5	0.06	0.125									012	137 (95.1)	7 (4.9)

*: 50% and 90%; BP: breakpoint; ECV: epidemiologic cutoff value; MIC encompassing 50% and 90% of isolates tested, respectively; R and S-DD: resistant and susceptible-dose dependent

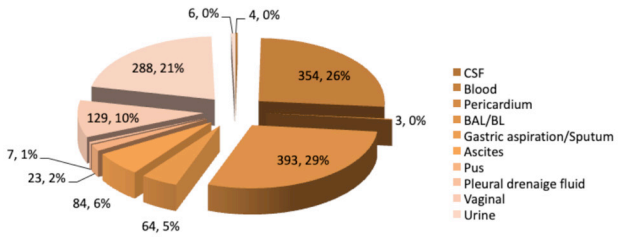

Figure 1. Totally 1371 clinically significant *Candida* strains isolated in between December 1998 and December 2014 by body site

CSF: cerebrospinal fluid; BAL: bronchoalveolar lavage fluid; BL: bronchial lavage fluid

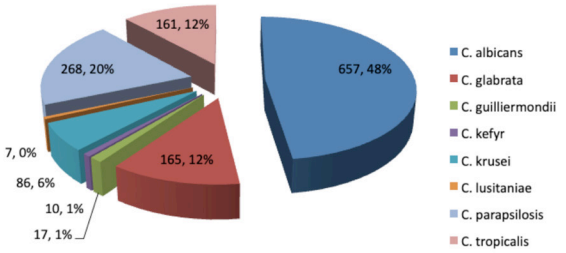

Figure 2. Distribution of *Candida* spp. isolated (between December 1998 and December 2014)

is shown in Figure 1. Overall, the leading species was *C. albicans* (48%), followed by *C. parapsilosis* (20%), *C. glabrata* (12%), *C. tropicalis* (12%), *C. krusei* (6%), *C. guilliermondii* (1%), *C. kefyr* (1%), and *C. lusitaniae* (0%), (Figure 2). Overall, the fraction of non-*albicans* species was near to *C. albicans* (52%). Of the significant non-*albicans* species, of 165 *C. glabrata* isolates, 51 were from blood; 44 from urine; 21 from vagina; 19 from lower respiratory tract; 11 from ascites; 9 from gastric aspirates; 4 from pleural drainage fluid; and 2 from each of cerebrospinal fluid, pus, and pericardium cultures, and of the 243 *C. parapsilosis* isolates, 142 were from blood; 78 from urine; 21 from each of lower respiratory tract and vagina; and 2 of each from ascites, articular fluid, and pus cultures. Among 354 (25.8%) blood isolates, *C. parapsilosis* (34%) was the leading species, followed by *C. albicans* (31%), *C. tropicalis* (15%), and *C. glabrata* (14.4%). Only seven isolates of *C. krusei* (1.1%) were from blood over the 16 years.

Antifungal susceptibilities

All the data for susceptibility testing using CLSI guidelines 1998–2012 are shown in Table 1. We found statistically significant differences when comparing the susceptibility of *C. albicans* and *C. tropicalis* to FLZ ($P < 0.05$). *Candida tropicalis* demonstrated the major percentage of resistant isolates to FLZ followed by *C. albicans* (11.1% and 4.4%, respectively) using the new BPs. Five (20.8%) of the FLZ resistant *C. albicans* isolates showed cross resistance to VRZ. The new BPs increased the absolute number of *C. albicans, C. glabrata, C. parapsilosis,* and *C. tropicalis* isolates that were resistant to FLZ. Regarding the new ECVs, 33.4% of *C. albicans* isolates were found non-WT against FLZ, and 12.5% of them were found non-WT against VRZ. All *C. lusitaniae, C. parapsilosis,* and *C. tropicalis* isolates studied showed WT phenotype to AMB, and *C. albicans* isolates showed very low non-WT phenotype (0.4%) to this agent. The MIC_{50} values of AMB for all the species were below or equal to 0.25 µg/mL except those of *C. krusei* isolates that were 0.5 µg/mL.

All the data for susceptibility testing of 198 *Candida* spp strains isolated from blood and urine cultures using Etest against AMB, FLZ, ITZ, VRZ, and PSZ and using SYO colorimetric test against echinocandins are summarized in Table 2 and 3, respectively. This group of *C. albicans* strains showed lower WT phenotypes (3.6%) against AMB and a lower percentage of WT phenotype (84.8%) against MCF than AND and CAS (92.9%). However, the MIC_{50} and MIC_{90} values of all were relatively low. Although the number of strains was small, all non-*albicans Candida* stains tested showed high number of WT phenotypes to echinocandin antifungals.

Discussion

Although *Candida albicans* was the most frequently isolated species as the causative agent of *Candida* infections, some variations in the species distribution and the susceptibility to antifungals have been shown to occur among institutions, localities, or countries. Knowledge of local susceptibility patterns is essential for clinical decision-making [1]. In our study, *C. albicans* was the most frequently isolated species followed by *C. parapsilosis*, but the overall percentage of non-*albicans* species (52%) was near to *C. albicans*.

The main role of species-specific BPs is to predict the clinical outcome of treatment with a given antifungal agent. The ECVs were established to differentiate WT (those without mutational or acquired resistance mechanisms) from non-WT strains (those having mutational or acquired resistance mechanisms) [16]. To date, *Candida* antifungal ECVs were reported largely from research or reference laboratories but long-term data on clinical isolates obtained by hospital laboratories were limited [17-19]. Herewith we present the long-term data about the susceptibility of invasive *Candida* isolates that were generated as part of routine patient care and reported to clinicians in a large university hospital. Using new CLSI BPs, some previously susceptible MICs were then classified as resistant (Table 1). It seemed that the new BSs are probably more sensitive in the detection of resistant isolates. Our ex-

Table 2. *In vitro* susceptibilities of 198 clinical *Candida* spp strains isolated from hemoculture (n=110) and urine (n=88) cultures of symptomatic patients against AMB and azole antifungals by the Etest method (December 2012–December 2014)

Species (No. of tested isolates)	Antifungal agent	MIC (µg/mL)			No. of isolates according to ECVs (%)	
		Range	50%	90%	≤ECV	>ECV
C. albicans (112)	AMB	≤0.03–2	0.06	0.5	108 (94.4)	4 (3.6)
	FLZ	0.125–≥64	0.25	1		
	ITZ	≤0.03–2	0.06	1		
	VRZ	≤0.015–1	0.03	0.125		
	PSZ	≤0.015–1	0.003	0.125		
C. glabrata (17)	AMB	0.06–2	0.5	1	17	0
	FLZ	4–≥64	16	32		
	ITZ	≤0.03–4	0.5	1		
	VRZ	0.03–8	0.03	0.25		
	PSZ	≤0.015–1	0.03	0.25		
C. krusei (7)	AMB	0.125–2	0.25	0.5	7	0
	FLZ	8–≥64	32	64		
	ITZ	0.25–2	0.25	1		
	VRZ	0.015–0.5	0.015	0.25		
	PSZ	<0.015–0.25	0.03	0.25		
C. parapsilosis (45)	AMB	≤0.03–1	0.06	0.5	45	0
	FLZ	0.125–8	0.25	1		
	ITZ	0.03–2	0.125	0.5		
	VRZ	≤0.015–0.125	0.06	0.125		
	PSZ	≤0.015–0.5	0.03	0.125		
C. tropicalis (17)	AMB	≤0.03–1	0.06	0.5	17	0
	FLZ	0.125–8	0.25	2		
	ITZ	≤0.03–2	0.125	0.5		
	VRZ	≤0.015–0.5	0.03	0.25		
	PSZ	≤0.015–0.125	0.03	0.06		

*: 50% and 90%; MIC: encompassing 50% and 90% of isolates tested, respectively

perience showed higher percentages of non-WT phenotypes among clinical *Candida* isolates, especially for *C. glabrata* strains, probably due to the strains obtained from patients who failed to respond to the initial empirical antifungal treatment. *Candida glabrata* isolates are intrinsically less susceptible to all antifungal agents. Acquired resistance to azoles was most often seen for FLZ. In comparison with *C. albicans, C. glabrata* develops FLZ resistance more easily following prolonged therapy due to its haploid state [20]. In our study, ITZ non-WT phenotype percentage was surprisingly higher (58.1%) among *C. glabrata* than that of FLZ (9.5%). AMB ECVs showed far fewer (1.4%) non-WT isolates, whereas PSZ had none. Because we analyzed retrospective data, unfortunately we have no chance to investigate the main mechanisms of resistance including alterations in the *C. glabrata ERG11* (*CgERG11*) gene that encodes the azole target enzyme or upregulation

Table 3. *In vitro* susceptibilities of 198 clinical *Candida* spp strains isolated from hemoculture (n=110) and urine (n=88) cultures of symptomatic patients against echinocandins by Sensititre YeastOne broth microdilution method and method-dependent ECVs (December 2012–December 2014)

Species (No. of tested isolates)	Antifungal agent	BP	MIC (µg/mL)			ECV (SYO)	No. of isolates according to ECVs (%)	
			Range	50%	90%		≤ECV	>ECV
C. albicans (112)	ANF	≥1	0.015–0.25	0.06	0.25	0.12	104 (92.9)	8 (7.1)
	CSF		0.125–1	0.25	0.25	0.25	104 (92.9)	8 (7.1)
	MCF		0.015–2	0.03	0.5		95 (84.8)	17 (15.2)
C. glabrata (17)	ANF	≥0.5	0.015–0125			0.12	17	0
	CSF		0.125–0.5			0.25	14	3
	MCF		0.015–0.06				16	1
C. krusei (7)	ANF	≥1	0.03–0.25			0.25	7	0
	CSF		0.25–1			1	7	0
	MCF		0.25–0.5				7	0
C. parapsilosis (45)	ANF	≥8	0.5–8			4	41	4
	CSF		1–8			2	40	5
	MCF		1–4				45	0
C. tropicalis (17)	ANF	≥1	0.125–0.5			0.5	17	0
	CSF		0.03–0.25			0.25	17	0
	MCF		0.03–0.5				16	1

*: 50% and 90%; BP: breakpoint; ECV: epidemiological cutoff value; MIC encompassing 50% and 90% of isolates tested, respectively

of the *CgCDR1* and *CgCDR2* genes, which encode efflux pumps to explain this phenomenon [20].

The echinocandins CAS and MCF were well-established first-line agents for the treatment of invasive candidiasis including candidemia [21-23]. The CLSI and European Committee on Antimicrobial Susceptibility Testing (EUCAST) have developed reference broth microdilution methods to test *Candida* spp and echinocandins, and *Candida* species-specific echinocandin BPs were designed to identify *fks* mutant isolates that were less likely to respond to treatment [9, 24]. However, both reference methods were limited by interlaboratory variability in CAS MICs against *C. albicans, C. glabrata, C. tropicalis*, and *C. krusei* [25]; and AND or MCF MICs were suggested as a surrogate marker to predict susceptibility and resistance to CAS [26, 27]. Agar diffusion based Etest assay has widely been used for determination of MIC to antifungal drugs of *Candida* species, particularly in clinical microbiology laboratories. Etest gave reliable MICs and excellent categorical agreement when compared with the results by CLSI and EUCAST methods with the exception of *C. krusei* and CAS [28]. Etest AMB ECVs were able to identify *Candida* isolates with reduced susceptibility to this agent; however, these ECVs would not categorize an isolate as susceptible or resistant, as BPs do. The SYO colorimetric method for susceptibility testing of *Candida* spp. and echinocandins did not pose a problem; and species-specific SYO ECVs for AND, CAS, and MCF correctly classified *Candida* isolates with *fks* mutations to identify non-WT isolates with reduced susceptibility to AND, MCF and especially to CAS [8]. Both commercial methods recommend the use of CLSI interpretive criteria of MIC results for *Candida* spp. [8, 29]. Method-dependent ECVs could aid the clinician and laboratory staff in identifying echinocandin and AMB potential resistance (non-WT isolates) instead of relying on CLSI interpretive BPs [8-10]. In our study, we used the species-specific BPs and method-dependent ECVs for the interpretation of Etest AMB and SYO echinocandin MICs.

To our knowledge, reported long-term hospital laboratory experiences with *Candida* polyene, azole, and echinocandin susceptibilities and comparison with the

old and new BPs and the percentage of WT phenotypes was limited [17, 18]. In this retrospective study, using previous and new criteria, we analyzed long-term data generated as part of routine patient care and reported to clinicians by a large university hospital mycology laboratory. This review could provide a useful insight into the real-world patterns of *Candida* susceptibility for clinical decision-making and the future research.

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