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Candida species distribution and antifungal susceptibility testing according to European Committee on Antimicrobial Susceptibility Testing and new vs. old Clinical and Laboratory Standards Institute clinical breakpoints: a 6-year prospective candidaemia survey from the fungal infection network of Switzerland

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Abstract

We analyzed the species distribution of *Candida* blood isolates (CBIs), prospectively collected between 2004 and 2009 within FUNGINOS, and compared their antifungal susceptibility according to clinical breakpoints defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) in 2013, and the Clinical and Laboratory Standards Institute (CLSI) in 2008 (old CLSI breakpoints) and 2012 (new CLSI breakpoints). CBIs were tested for susceptiblity to fluconazole, voriconazole and caspofungin by microtitre broth dilution (Sensititre® YeastOne™ test panel). Of 1090 CBIs, 675 (61.9%) were *C. albicans,* 191 (17.5%) *C. glabrata*, 64 (5.9%) *C. tropicalis*, 59 (5.4%) *C. parapsilosis*, 33 (3%) *C. dubliniensis*, 22 (2%) *C. krusei* and 46 (4.2%) rare *Candida* species. Independently of the breakpoints applied, *C. albicans* was almost uniformly (>98%) susceptible to all three antifungal agents. In contrast, the proportions of fluconazole- and voriconazole-susceptible *C. tropicalis* and F-susceptible *C. parapsilosis* were lower according to EUCAST/new CLSI breakpoints than to the old CLSI breakpoints. For caspofungin, non-susceptibility occurred mainly in *C. krusei* (63.3%) and *C. glabrata* (9.4%). Nine isolates (five *C. tropicalis*, three *C. albicans* and one *C. parapsilosis*) were cross-resistant to azoles according to EUCAST breakpoints, compared with three isolates (two *C. albicans* and one *C. tropicalis*) according to new and two (2 *C. albicans*) according to old CLSI breakpoints. Four species (*C. albicans, C. glabrata, C. tropicalis* and *C. parapsilosis*) represented >90% of all CBIs. *In vitro* resistance to fluconazole, voriconazole and caspofungin was rare among *C. albicans*, but an increase of non-susceptibile isolates was observed among *C. tropicalis*/*C. parapsilosis* for the azoles and *C. glabrata*/*C. krusei* for caspofungin according to EUCAST and new CLSI breakpoints compared with old CLSI breakpoints.

Keywords: Breakpoint, *Candida*, candidaemia, Clinical and Laboratory Standards Institute, European Committee on Antimicrobial Susceptibility Testing, resistance, species

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Introduction

Candida species are among the top ten pathogens causing bloodstream infections [1]. Candidaemia is an invasive fungal infection associated with substantial morbidity, mortality and

healthcare costs [2]. Changes in species distribution and a shift to more resistant isolates are increasingly described [3–5]. There have been significant differences in clinical breakpoint values defined by the Antifungal Susceptibility Testing Subcommittee of the Clinical and Laboratory Standards Institute (CLSI) in the USA and by the European Committee on Antimicrobial Susceptibility Testing (AFST-EUCAST) in Europe. In recent years a harmonization of these breakpoints as well as the definition of species-specific breakpoints has been achieved and the breakpoints have been lowered in order to better detect low level resistance [6–8].

The goal of our study was to analyse the species distribution of *Candida* blood isolates (CBIs) prospectively collected in the hospitals of the Fungal Infection Network of Switzerland (FUNGINOS) and to determine antifungal susceptibility to fluconazole, voriconazole and caspofungin according to the new species-specific clinical breakpoints defined by the EUCAST in Europe (in 2013) as well as by the CLSI (in 2008 [old CLSI breakpoints] and 2012 [new CLSI breakpoints]) in the USA. We also aimed to evaluate the frequency of cross- and multiresistant isolates.

Material and Methods

Participating hospitals and microbiology laboratories

All Swiss university hospitals (n = 7) and a representative sample of university-affiliated tertiary care centres (n = 10) of FUNGINOS prospectively collected CBIs between 1 January 2004 and 31 December 2009.

Sixteen microbiology laboratories were affiliated with the 17 participating hospitals. All laboratories used automated blood culture systems [11 Bactec (Becton Dickinson, Sparks, MD, USA) and five BacT/Alert (bioMérieux, Durham, NC, USA)]. The CBIs of each participating centre were sent to the FUNGINOS mycology reference laboratory in Lausanne.

Species identification, antifungal susceptibility testing and interpretation

In the mycology reference laboratory, the CBI were identified by recognized standard laboratory techniques [9] and antifungal susceptibility testing for fluconazole, voriconazole and caspofungin was performed using the microtitre broth dilution method with the Sensititre® YeastOne™ test panel (version 4.0 from 2004 to 2007; version 7.0 from 2007 to 2009).

Interpretation of susceptibility was performed by applying the clinical interpretive breakpoints defined by the CLSI in 2008 (≪old CLSI breakpoints≫) [10,11] and 2012 (≪new CLSI breakpoints≫) [12] as well as EUCAST in March 2013 (≪EUCAST breakpoints≫; http://www.eucast.org/clinical_breakpoints/; version 6.1). EUCAST has not yet defined clinical breakpoints for caspofungin.

The proportions of susceptible vs. non-susceptible or resistant CBIs were calculated and compared according to EUCAST and CLSI breakpoints.

Definitions

Susceptibility and non-susceptibility. A CBI was considered susceptible when the minimal inhibitory concentration (MIC) was at or below the breakpoint defined by EUCAST or CLSI. Non-susceptibility of a CBI was defined when its MIC was higher than the breakpoints defined by EUCAST/CLSI and includes both dose-dependent susceptible, intermediate and resistant isolates.

Cross-resistance. Cross- resistance was defined as resistance to two antifungals of the same drug class. We evaluated cross-resistance to azoles, defined as resistance to the two azoles tested (fluconazole and voriconazole).

Multi-resistance. Multi-resistance was defined as resistance to two antifungal drug classes, namely the azoles (fluconazole and voriconazole) and echinocandin tested (caspofungin).

Data collection and analysis

For data entry and analysis Microsoft Excel® (Microsoft Corporation, Redmond, WA, USA) and its tools were used.

Results

Species distribution

Within the 6 years of the study, a total of 1090 CBIs underwent central re-identification and susceptibility testing. The most frequently isolated species were *C. albicans* (675; 61.9%) followed by *C. glabrata* (191; 17.5%), *C. tropicalis* (64; 5.9%) and *C. parapsilosis* (59; 5.4%), accounting for 90.7% of the total number of isolates. The remaining 9.3% of the species consisted of *C. dubliniensis* (33; 3%), *C. krusei* (22; 2%), *C. lusitaniae* C (12; 1.1%), *C. guilliermondii* (9; 0.8%), *C. kefyr* (8; 0.7%), *C. pelliculosa* (6; 0.6%), *C. famata* (4; 0.4%), *C. norvegensis* (3; 0.3%), *C. inconspicua* (2; 0.2%) and *C. rugosa* (2; 0.2%).

Antifungal susceptibility

We applied interpretive breakpoints defined by EUCAST and CLSI [6–8] [http://www.eucast.org/clinical_breakpoints/; version 6.1], summarized in Table 1. The percentage of susceptibility of the different *Candida* species to fluconazole, voriconazole and caspofungin is shown in Fig. 1(a–c).

TABLE 1. Antifungal clinical breakpoints (in mg/L)

Fluconazole

	Old CLSI bp (2008)			New CLSI bp (2012)			EUCAST bp 2013	
***Candida* species**	**S**	**SDD**	**R**	**S**	**SDD**	**R**	**S**	**R**
C. albicans	≤8	16–32	≥64	≤2	4	≥8	≤2	>4
C. tropicalis	≤8	16–32	≥64	≤2	4	≥8	≤2	>4
C. parapsilosis	≤8	16–32	≥64	≤2	4	≥8	≤2	>4
C. glabrata	≤8	16–32	≥64		≤32	≥64	≤0.002	>32
C. krusei	R	R	R	R	R	R	–	–

Voriconazole

	Old CLSI bp (2008)			New CLSI bp (2012)			EUCAST bp 2013	
***Candida* species**	**S**	**SDD**	**R**	**S**	**I**	**R**	**S**	**R**
C. albicans	≤1	2	≥4	≤0.12	0.25–0.50	≥1	≤0.12	>0.12
C. tropicalis	≤1	2	≥4	≤0.12	0.25–0.50	≥1	≤0.12	>0.12
C. parapsilosis	≤1	2	≥4	≤0.12	0.25–0.50	≥1	≤0.12	>0.12
C. glabrata	≤1	2	≥4	IE	IE	IE	IE	IE
C. krusei	≤1	2	≥4	≤0.5	1	≥2	IE	IE

Caspofungin

	Old CLSI bp (2008)		New CLSI bp (2012)		
***Candida* species**	**S**	**R**	**S**	**I**	**R**
C. albicans	≤2	>2	≤0.25	0.5	≥1
C. tropicalis	≤2	>2	≤0.25	0.5	≥1
C. parapsilosis	≤2	>2	≤2	4	≥8
C. glabrata	≤2	>2	≤0.125	0.25	≥0.5
C. krusei	≤2	>2	≤0.25	0.5	≥1

bp, breakpoint; CLSI, Clinical and Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; I, intermediate; IE, insufficient evidence; R, resistant; SDD, dose-dependent susceptible; S, susceptible; –, antifungal susceptibility testing not recommended.

Fluconazole. Non-susceptibility was found in 13 (1.6%) vs. three (0.4%) of all *C. albicans* isolates when applying EUCAST/new CLSI breakpoints vs. the old CLSI breakpoint. Likewise, seven (11%) *C. tropicalis* isolates were non-susceptible when applying EUCAST/new CLSI breakpoints as opposed to two (3%) when applying the old CLSI breakpoints, and nine (15.3%) *C. parapsilosis* were non-susceptible according to EUCAST/new CLSI bp vs. two (3.4%) according to old CLSI breakpoints. Ninety-two (48.2%) of all *C. glabrata* isolates were non-susceptible when the old CLSI breakpoint was applied, mostly dose-dependent susceptible (76; 39.8%), whereas all 191 isolates were by definition non-susceptible according to the new CLSI breakpoint. EUCAST has not defined a breakpoint for *C. glabrata* because of insufficient evidence.

Voriconazole. Non-susceptibility was found in four (0.6%) vs. three (0.4%) of all *C. albicans* isolates according to the EUCAST/new CLSI breakpoints vs. the old CLSI breakpoint and in 14 (22%) vs. none of the *C. tropicalis* isolates. For *C. parapsilosis*, only one (1.7%) isolate was non-susceptible according to the EUCAST/new CLSI breakpoints vs. none according to the old CLSI breakpoint. Seven (3.7%) of all *C. glabrata* isolates tested non-susceptible according to the old CLSI breakpoint, whereas no breakpoint was defined by CLSI 2012 and EUCAST due to insufficient evidence. Four (18.2%) *C. krusei* isolates tested non-susceptible when applying the new CLSI breakpoint, while only one (4.5%) was non-susceptible when applying the old CLSI breakpoint. EUCAST has not defined a breakpoint for voriconazole in *C. krusei* because there is insufficient evidence that this species is a good target for therapy with this drug.

Caspofungin. Due to significant inter-laboratory variations in MIC ranges, EUCAST has not defined breakpoints for caspofungin yet, anidulafungin and micafungin being the echinocandins for which a breakpoint was recently established. According to the new CLSI breakpoint, one (0.1%) of all the *C. albicans* isolates was found non-susceptible vs. none when the old CLSI breakpoint was applied. Two (3%) vs. one (1.6%) of the *C. tropicalis* isolates and none vs. none of the *C. parapsilosis* isolates were non-susceptible according to the new CLSI vs. the old CLSI breakpoint, in contrast to 18 (9.4%) vs. none of all *C. glabrata* isolates. Fourteen (63.3%) *C. krusei* isolates tested non-susceptible when applying the new CLSI breakpoint vs. none when applying the old CLSI breakpoint.

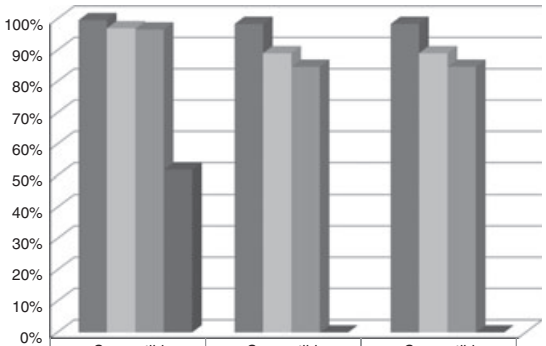
Cross-resistance to azoles. A resistance to the two azoles (fluconazole and voriconazole) tested was found in nine (0.8%) of all CBIs (five *C. tropicalis*, three *C. albicans* and one *C. parapsilosis*) when applying the EUCAST breakpoints vs. three isolates (two *C. albicans* and one *C. tropicalis*) according to new CLSI breakpoints. Only two isolates (two *C. albicans*) were cross-resistant according to the old CLSI breakpoints (Table 2).

Multi-resistance. One *C. tropicalis* isolate was resistant to fluconazole and voriconazole according to the EUCAST and new CLSI breakpoints and, despite the fact that a breakpoint for caspofungin has not yet been established by EUCAST, we considered this isolate with the very high MIC of 16 mg/L for caspofungin as resistant. No CBI was multiresistant when old CLSI breakpoints were applied (Table 2).

Discussion

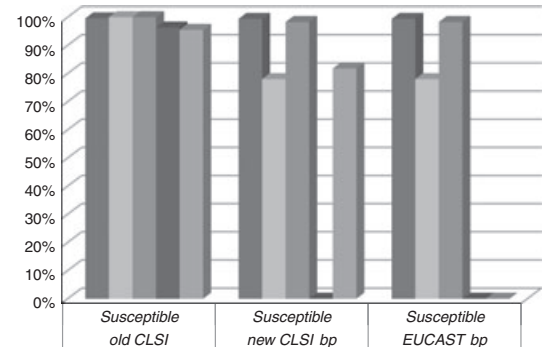
Candidaemia is one of the most common invasive fungal infections in the hospital setting and associated with a high attributable mortality [2,13]. An epidemiological shift from *C. albicans* to other, usually more resistant *Candida* species has been observed in the past years [14,15]. The *in vitro* activity of antifungal agents against different species of *Candida* is not

(a)



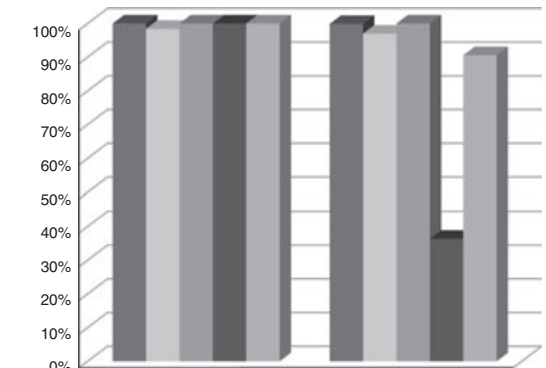
	Susceptible old CLSI bp	Susceptible new CLSI bp	Susceptible EUCAST bp
C. albicans (n = 675)	99.6%	98.4%	98.4%
C. tropicalis (n = 64)	97.0%	89.0%	89.0%
C. parapsilosis (n = 59)	96.6%	84.7%	84.7%
C. glabrata (n = 191)	51.8%	0.0%‡	0.0%*

(b)



	Susceptible old CLSI	Susceptible new CLSI bp	Susceptible EUCAST bp
C. albicans (n = 675)	99.6%	99.4%	99.4%
C. tropicalis (n = 64)	100.0%	78.0%	78.0%
C. parapsilosis (n = 59)	100.0%	98.3%	98.3%
C. glabrata (n = 191)	96.3%	0.0%·	0.0%·
C. krusei (n = 22)	95.5%	81.8%	0.0%·

(c)



	Susceptible old CLSI bp	Susceptible new CLSI bp
C. albicans (n = 675)	100.0%	99.9%
C. tropicalis (n = 64)	98.4%	97.0%
C. parapsilosis (n = 59)	100.0%	100.0%
C. krusei (n = 22)	100.0%	36.4%
C. glabrata (n = 191)	100.0%	90.6%

FIG. 1. (a) Susceptibility of *Candida* blood isolates to fluconazole according to breakpoints applied. *No susceptible isolates (MIC ≤0.002 mg/L); 175 isolates with an MIC of 0.25–32 mg/L; 16 resistant isolates (MIC >32 mg/L). ‡CLSI breakpoints define dose-dependent susceptibility (MIC ≤32 mg/L) and resistance (MIC ≥64 mg/L), thus per definition there are no susceptible isolates of *C. glabrata.* (b) Susceptibility of *Candida* blood isolates to voriconazole according to breakpoints applied. *No CLSI and EUCAST breakpoints due to insufficient evidence. (c) Susceptibility of *Candida* blood isolates to caspofungin according to breakpoints applied. bp, breakpoint; CLSI, Clinical and Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing.

TABLE 2. Cross- and multiresistant strains according to breakpoint applied

Strain code	Year	Species identification	Fluconazole MIC mg/L	Voriconazole MIC mg/L
Cross-resistance				
Old CLSI bp (n = 2)				
4	2004	*Candida albicans*	256	16
83	2006	*C. albicans*	256	8
New CLSI bp (n = 3)				
4	2004	*C. albicans*	256	16
83	2006	*C. albicans*	256	8
96	2006	*Candida tropicalis*	32	1
EUCAST bp (n = 9)				
4	2004	*C. albicans*	256	16
33	2006	*C. albicans*	32	0.5
83	2006	*C. albicans*	256	8
15	2007	*Candida parapsilosis*	32	0.25
19	2005	*C. tropicalis*	8	0.25
40	2005	*C. tropicalis*	8	0.25
52	2005	*C. tropicalis*	8	0.5
96	2006	*C. tropicalis*	32	1
23	2009	*C. tropicalis*	16	0.5
Multiresistance				
Old and new CLSI bp (n = 0)				
EUCAST bp (n = 1)[a]				
40	2005	*C. tropicalis*	8	0.25

bp, breakpoint; CLSI, Clinical and Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing.
[a]Caspofungin MIC: 16 mg/L (no EUCAST breakpoint).

uniform and each of them has a specific antifungal susceptibility profile. Both CLSI and EUCAST established clinical breakpoints for antifungals against *Candida* species, on the basis of MIC distributions, pharmacokinetics, pharmacodynamics, epidemiological cut-off values (ECOFF) and clinical outcomes depending on MIC values, for the five most common *Candida* species, *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis* and *C. krusei* [16–19]. In the past 3 years, CLSI adjusted their susceptibility breakpoints for fluconazole by lowering them to the same MIC values as EUCAST for *C. albicans*, *C. parapsilosis* and *C. tropicalis*. The objectives were to detect emerging resistance among the most common *Candida* species and to harmonize the breakpoints with those of EUCAST [6]. CLSI still defines breakpoints for fluconazole in *C. glabrata* as well as voriconazole in *C. krusei* [7,12] while EUCAST did not because of insufficient evidence. However, applying the CLSI breakpoints, there are by definition only dose-dependent susceptible/resistant and no susceptible *C. glabrata* isolates anymore. The single breakpoint for all three echinocandins and all *Candida* species proposed by the CLSI in 2008 (susceptible: $\leq$2 mg/L) has been revised and species-specific, lower breakpoints defined in order to identify isolates with resistance mechanisms, especially *Candida* strains with FKS mutations, possibly leading to treatment failures [8]. EUCAST recently defined clinical breakpoints for anidulafungin and micafungin (http://www.eucast.org/clinical_s/).

In Switzerland, the majority of CBIs collected between 2004 and 2009 were *C. albicans* (61.9%), followed by *C. glabrata* (17.5%). The proportion of *C. albicans* is comparable with data from Denmark [20], but is higher than in countries [21] such as Spain (49%) [22], the UK (52%) [23], South Korea (38%) [24], Mexico (32%) [25] and the USA (45%) [26]. A trend towards more non-albicans *Candida* species in Switzerland was observed transiently in 2006 but it did not persist in the following years. This is in sharp contrast to the shift towards more resistant species described in several European countries and in the USA [27,28]. Overall, *C. albicans* remained the most common cause of candidaemia in Switzerland and was almost uniformly (>98%) susceptible to all three antifungal agents tested, independently of the breakpoints applied. In contrast, the proportions of fluconazole-susceptible *C. tropicalis* and *C. parapsilosis* were lower according to the EUCAST and new CLSI vs. old CLSI breakpoints. A decrease in fluconazole-susceptibility in candidaemia isolates in general has been described in Denmark, independently of the breakpoints applied [28]. Yet, the proportion of fluconazole resistance among *C. tropicalis* and *C. parapsilosis* in the Danish study was lower than that in the present study (6.7% vs. 11% and 6% vs. 15%) when applying the EUCAST breakpoints. Differences in the use of fluconazole, especially as prophylaxis, between countries or institutions might account for these differences in susceptibility rates, as well as the possible spreading of resistant clones.

Regarding voriconazole, applying the EUCAST and new CLSI breakpoints did not change the proportions of non-susceptibility for *C. albicans*, which remained below 1% independently of the breakpoint applied. However, the application of the EUCAST and new CLSI breakpoints for voriconazole increased the proportion of non-susceptible *C. tropicalis* isolates (22%) vs. the old CLSI breakpoint (0%). This proportion of voriconazole susceptibility in Switzerland is comparable to that reported from the USA (<1% resistance) [26] when applying the old CLSI breakpoint and our level of non-susceptibility is lower compared with data from Austria and Germany (applying the new CLSI breakpoint) describing 38% of non-susceptibility to voriconazole [29]. However, compared with a Danish study reporting 6% of *C. tropicalis* isolates as non-susceptible to voriconazole, we observed a higher rate of non-susceptibility in *C. tropicalis* isolates when applying the EUCAST breakpoint [28]. Besides spreading of resistant clones, as mentioned above for fluconazole, differences in availability and utilization policies of voriconazole between different countries and institutions might explain this discrepancy. Compared with Spanish data applying the old CLSI breakpoints, we observed a similarly low proportion of *C. glabrata* isolates to be non-susceptible to voriconazole (4% vs. 1.2% in Spain), and the same was true for *C. krusei* (5% vs. 4%) [30]. However, the application of the new CLSI breakpoints significantly increased the proportion of non-suscepti-

bility for *C. krusei* from 5% to 36% (for *C. glabrata*, breakpoint definition was dropped by the new CLSI document due to insufficient evidence). The small number of *C. krusei* isolates ($n = 22$) and the fact that the majority (71%) of non-susceptible isolates had an intermediate susceptibility and not true resistance might lead to an overestimation of the proportions of non-susceptible isolates.

Regarding the echinocandins, most *Candida* isolates were susceptible to caspofungin when applying the new CLSI breakpoints, except for a rather high proportion of *C. krusei* and some *C. glabrata* with an *in vitro* non-susceptibility rate of 64% and 9%, respectively. Data published in 2010 analysing CBIs from all over the world and also applying the new CLSI breakpoints found lower proportions of non-susceptibility to caspofungin for *C. krusei* and *C. glabrata* (0–9%) [4]. This important difference in the rate of non-susceptibility to caspofungin is probably due to the fact that our study includes both truly resistant as well as intermediate isolates, when applicable, whereas the cited study considered only truly resistant isolates.

Regarding cross- and multiresistance, overall only nine (0.8%) isolates were cross-resistant to azoles according to EUCAST, compared with only three (0.3%) and two (0.2%) according to the new and old CLSI breakpoints, respectively. This difference is explained by the lower EUCAST breakpoint for voriconazole. Only one isolate of *C. albicans* was multiresistant according to the new CLSI breakpoints. The same isolate was also considered multiresistant according to EUCAST breakpoints (even if EUCAST has not yet defined caspofungin breakpoints due to significant inter-laboratory variations in MIC ranges) regarding the very high MIC of 16 mg/L for caspofungin. Although a limited number of antifungal agents were tested, our data confirm the scarcity of cross- and multiresistance within the CBIs of Switzerland.

The strengths of this FUNGINOS study are its prospective and multicentric design with collection of CBIs from a large number of patients with candidaemia, reflecting the nationwide epidemiology of this life-threatening complication. Furthermore, standardized identification and antifungal susceptibility testing was centralized in the FUNGINOS reference laboratory using international standards. A limitation of this study is that the clinical significance of the increased proportions of non-susceptible *C. glabrata* and *C. krusei* could not be analysed due to the lack of clinical data.

In conclusion, four species (*C. albicans*, *C. glabrata*, *C. tropicalis* and *C. parapsilosis*) represented more than 90% of all CBIs, with *C. albicans* remaining the predominant species in Swiss candidaemia over a 6-year period (2004–2009). *In vitro* resistance to fluconazole, voriconazole and caspofungin was rare among *C. albicans*, but an increase of non-susceptibility was observed among *C. tropicalis*/*C. parapsilosis* for voriconazole, among *C. parapsilosis* for fluconazole and among *C. glabrata*/*C. krusei* for caspofungin according to EUCAST and new CLSI breakpoints compared with old CLSI breakpoints. The recent modification of clinical breakpoints, especially EUCAST breakpoints, has already contributed to a change of treatment guidelines, in particular regarding *C. glabrata*.

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Transparency Declaration

TC has received honoraria for consultancy, board membership and speakers bureaus from Pfizer, for consultancy, development and educational presentations from Merck Sharp & Dohme, for consultancy from Novartis and Immunexpress, for speakers bureaus from Bio-Mérieux, as well as for development and educational presentations from Gilead. He also received grant support for travel and meeting expenses from Astellas, Pfizer and Merck Sharp & Dohme. JF has received honoraria for board membership from Merck Sharp & Dohme and travel grants from Merck Sharp & Dohme, Gilead, Janssen, Bristol-Myers Squibb, Roche, ViiV, Abbot and Boeringer Ingelheim. OM has received honoraria for board membership and consultancy from Gilead, Merck Sharp & Dohme, Novartis and Pfizer. He has received grant support from Associates of Cape Code, Bio-Mérieux and Bio-Rad. He has received

honoraria for lectures and speakers bureaus from Gilead, Merck Sharp & Dohme, Novartis, Pfizer and Roche Diagnostics. CO has received honoraria for board membership from Gilead and travel grants from Merck Sharp & Dohme, Gilead, Janssen, Bristol-Myers Squibb, Roche, ViiV, Abbot and Boeringer Ingelheim. All other authors declare no conflict of interest.

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References

1. Marchetti O, Bille J, Fluckiger U *et al.* Epidemiology of candidemia in Swiss tertiary care hospitals: secular trends, 1991–2000. *Clin Infect Dis* 2004; 38: 311–320.
2. Zaoutis TE, Argon J, Chu J, Berlin JA, Walsh TJ, Feudtner C. The epidemiology and attributable outcomes of candidemia in adults and children hospitalized in the United States: a propensity analysis. *Clin Infect Dis* 2005; 41: 1232–1239.
3. Pfaller MA, Diekema DJ, Gibbs DL *et al.* Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: a 10.5-year analysis of susceptibilities of Candida Species to fluconazole and voriconazole as determined by CLSI standardized disk diffusion. *J Clin Microbiol* 2010; 48: 1366–1377.
4. Pfaller MA, Moet GJ, Messer SA, Jones RN, Castanheira M. Candida bloodstream infections: comparison of species distributions and antifungal resistance patterns in community-onset and nosocomial isolates in the SENTRY Antimicrobial Surveillance Program, 2008–2009. *Antimicrob Agents Chemother* 2011; 55: 561–566.
5. Sampaio Camargo TZ, Marra AR, Silva CV *et al.* Secular trends of candidemia in a tertiary care hospital. *Am J Infect Control* 2010; 38: 546–551.
6. Pfaller MA, Andes D, Diekema DJ, Espinel-Ingroff A, Sheehan D. Wild-type MIC distributions, epidemiological cutoff values and species-specific clinical breakpoints for fluconazole and Candida: time for harmonization of CLSI and EUCAST broth microdilution methods. *Drug Resist Updat* 2010; 13: 180–195.
7. Pfaller MA, Andes D, Arendrup MC *et al.* Clinical breakpoints for voriconazole and Candida spp. revisited: review of microbiologic, molecular, pharmacodynamic, and clinical data as they pertain to the development of species-specific interpretive criteria. *Diagn Microbiol Infect Dis* 2011; 70: 330–343.
8. Pfaller MA, Diekema DJ, Andes D *et al.* Clinical breakpoints for the echinocandins and Candida revisited: integration of molecular, clinical, and microbiological data to arrive at species-specific interpretive criteria. *Drug Resist Updat* 2011; 14: 164–176.
9. Warren HBHK. Candida, Cryptococcus, and other yeasts of medical importance. In: Murray PR, ed. *Manual of Clinical Microbiology*. Washington DC: ASM Press, 2008; 1184–1199.
10. Clinical and Laboratory Standards Institute. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, in Approved standard M27-A3*. Wayne, PA: Clinical and Laboratory Standards Institute, 2008a.
11. Clinical and Laboratory Standards Institute. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, in 3rd informal Supplement, M27-A3*. Wayne, PA: Clinical and Laboratory Standards Institute, 2008b.
12. CLSI. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Fourth International Supplement. CLSI document M27-4*. Wayne, PA: Clinical and Laboratory Standards Institute, 2012; 32.
13. Gudlaugsson O, Gillespie S, Lee K *et al.* Attributable mortality of nosocomial candidemia, revisited. *Clin Infect Dis* 2003; 37: 1172–1177.
14. Hope W, Morton A, Eisen DP. Increase in prevalence of nosocomial non-*Candida albicans* candidaemia and the association of *Candida krusei* with fluconazole use. *J Hosp Infect* 2002; 50: 56–65.
15. Poikonen E, Lyytikainen O, Anttila VJ *et al.* Secular trend in candidemia and the use of fluconazole in Finland, 2004–2007. *BMC Infect Dis* 2010; 10: 312.

16. Cuesta I, Bielza C, Larranaga P *et al*. Data mining validation of fluconazole breakpoints established by the European Committee on Antimicrobial Susceptibility Testing. *Antimicrob Agents Chemother* 2009; 53: 2949–2954.
17. Pfaller MA, Diekema DJ, Sheehan DJ. Interpretive breakpoints for fluconazole and Candida revisited: a blueprint for the future of antifungal susceptibility testing. *Clin Microbiol Rev* 2006; 19: 435–447.
18. Rodriguez-Tudela JL, Almirante B, Rodriguez-Pardo D *et al*. Correlation of the MIC and dose/MIC ratio of fluconazole to the therapeutic response of patients with mucosal candidiasis and candidemia. *Antimicrob Agents Chemother* 2007; 51: 3599–3604.
19. Turnidge J, Paterson DL. Setting and revising antibacterial susceptibility breakpoints. *Clin Microbiol Rev* 2007; 20: 391–408, table of contents.
20. Arendrup MC, Fuursted K, Gahrn-Hansen B *et al*. Seminational surveillance of fungemia in Denmark: notably high rates of fungemia and numbers of isolates with reduced azole susceptibility. *J Clin Microbiol* 2005; 43: 4434–4440.
21. Pfaller MA, Messer SA, Moet GJ, Jones RN, Castanheira M. Candida bloodstream infections: comparison of species distribution and resistance to echinocandin and azole antifungal agents in Intensive Care Unit (ICU) and non-ICU settings in the SENTRY Antimicrobial Surveillance Program (2008–2009). *Int J Antimicrob Agents* 2011; 38: 65–69.
22. Rodriguez-Hernandez MJ, Ruiz-Perez de Pipaon M, Marquez-Solero M *et al*. [Candidemias: multicentre analysis in 16 hospitals in Andalusia (Spain)]. *Enferm Infecc Microbiol Clin* 2011; 29: 328–333.
23. Chalmers C, Gaur S, Chew J *et al*. Epidemiology and management of candidaemia–a retrospective, multicentre study in five hospitals in the UK. *Mycoses* 2011; 54: e795–e800.
24. Jung SI, Shin JH, Song JH *et al*. Multicenter surveillance of species distribution and antifungal susceptibilities of Candida bloodstream isolates in South Korea. *Med Mycol* 2010; 48: 669–674.
25. Gonzalez GM, Elizondo M, Ayala J. Trends in species distribution and susceptibility of bloodstream isolates of Candida collected in Monterrey, Mexico, to seven antifungal agents: results of a 3-year (2004 to 2007) surveillance study. *J Clin Microbiol* 2008; 46: 2902–2905.
26. Lyon GM, Karatela S, Sunay S, Adiri Y. Antifungal susceptibility testing of Candida isolates from the Candida surveillance study. *J Clin Microbiol* 2010; 48: 1270–1275.
27. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev* 2007; 20: 133–163.
28. Arendrup MC, Bruun B, Christensen JJ *et al*. National surveillance of fungemia in Denmark (2004 to 2009). *J Clin Microbiol* 2011; 49: 325–334.
29. Schmalreck AF, Willinger B, Haase G *et al*. Species and susceptibility distribution of 1062 clinical yeast isolates to azoles, echinocandins, flucytosine and amphotericin B from a multi-centre study. *Mycoses* 2012; 55: e124–e137.
30. Peman J, Canton E, Quindos G *et al*. Epidemiology, species distribution and in vitro antifungal susceptibility of fungaemia in a Spanish multicentre prospective survey. *J Antimicrob Chemother* 2012; 67: 1181–1187.