We analyzed the species distribution of Candida blood isolates (CBI), prospectively collected between 2004 and 2009 within FUNGINOS, and compared their antifungal susceptibility according to clinical breakpoints defined by: EUCAST in 2013, CLSI in 2008 (old CLSI breakpoints) and 2012 (new CLSI breakpoints). CBI were tested for susceptibility to fluconazole, voriconazole and caspofungin by microtitre broth dilution (Sensititre® YeastOne(TM) test panel). Of 1090 CBI, 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 3 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 (5 C. tropicalis, 3 C. albicans, 1 C. [..]
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

C. Orasch1, O. Marchetti1, J. Garbino2, J. Schrenzel3, S. Zimmerli4, K. Mühlethaler4, G. Pfyffer5, C. Ruef6, J. Fehr6, R. Zbinden7, T. Calandra1, J. Bille8 and the Fungal Infection Network of Switzerland (FUNGINOS)*

1) Infectious Diseases Service, Department of Medicine, Lausanne University Hospital, Lausanne, 2) Infectious Diseases Service, Department of Medicine, Geneva University Hospitals, Geneva, 3) Institute of Microbiology, Department of Laboratories, Geneva University Hospitals, Geneva, 4) Institute for Infectious Diseases, University of Bern, Bern, 5) Institute for Microbiology, Kantonsspital, Luzern, 6) Division of Infectious Diseases and Hospital Epidemiology, University Hospital, Zürich, 7) Institute for Medical Microbiology, University of Zurich, Zurich and 8) Institute of Microbiology, Department of Laboratories, Lausanne University Hospital, Lausanne, Switzerland

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 susceptibility 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-susceptible 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 microtiter 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).
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

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

<table>
<thead>
<tr>
<th>Candida species</th>
<th>Fluconazole</th>
<th>Voriconazole</th>
<th>Caspofungin</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>≤4 ≤0.12</td>
<td>≤2 ≤0.25</td>
<td>≤2 ≤0.25</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>≤2 ≤0.12</td>
<td>≤2 ≤0.25</td>
<td>≤2 ≤0.25</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>≤2 ≤0.12</td>
<td>≤2 ≤0.25</td>
<td>≤2 ≤0.25</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>≤2 ≤0.5</td>
<td>≤2 ≤0.25</td>
<td>≤2 ≤0.25</td>
</tr>
<tr>
<td>C. krusei</td>
<td>≤2 ≤0.5</td>
<td>≤2 ≤0.25</td>
<td>≤2 ≤0.25</td>
</tr>
<tr>
<td></td>
<td>Rp</td>
<td>Rp</td>
<td>Rp</td>
</tr>
</tbody>
</table>

Old CLSI bp: 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
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.
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: ≤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-

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

<table>
<thead>
<tr>
<th>Strain code</th>
<th>Species identification</th>
<th>Fluconazole MIC mg/L</th>
<th>Voriconazole MIC mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-resistance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Old CLSI bp (n = 2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2004</td>
<td>Candida albicans</td>
<td>256</td>
</tr>
<tr>
<td>83</td>
<td>2006</td>
<td>C. albicans</td>
<td>256</td>
</tr>
<tr>
<td>New CLSI bp (n = 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2004</td>
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</tr>
<tr>
<td>83</td>
<td>2006</td>
<td>C. albicans</td>
<td>256</td>
</tr>
<tr>
<td>96</td>
<td>2006</td>
<td>Candida tropicalis</td>
<td>32</td>
</tr>
<tr>
<td>EUCAST bp (n = 9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2004</td>
<td>C. albicans</td>
<td>256</td>
</tr>
<tr>
<td>33</td>
<td>2006</td>
<td>C. albicans</td>
<td>32</td>
</tr>
<tr>
<td>83</td>
<td>2006</td>
<td>C. albicans</td>
<td>256</td>
</tr>
<tr>
<td>15</td>
<td>2007</td>
<td>Candida parapsilosis</td>
<td>32</td>
</tr>
<tr>
<td>19</td>
<td>2005</td>
<td>C. tropicalis</td>
<td>8</td>
</tr>
<tr>
<td>40</td>
<td>2005</td>
<td>C. tropicalis</td>
<td>8</td>
</tr>
<tr>
<td>52</td>
<td>2005</td>
<td>C. tropicalis</td>
<td>8</td>
</tr>
<tr>
<td>96</td>
<td>2006</td>
<td>C. tropicalis</td>
<td>32</td>
</tr>
<tr>
<td>23</td>
<td>2009</td>
<td>C. tropicalis</td>
<td>16</td>
</tr>
</tbody>
</table>

| Multiresistance | | | |
| Old and new CLSI bp (n = 0) | | | |
| EUCAST bp (n = 1) | | | |
| 40 | 2005 | C. tropicalis | 8 | 0.25 |

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

Acknowledgements

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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, ViV, 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
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**Appendix**

Thomas Bregenzer, Anna Conen and Hans Fankhauser, Kantonsspital, Aarau, Switzerland.
Ursula Flückiger, Nina Khanna and Reno Frei, University Hospital Basel, Switzerland.
Ulrich Heininger and Roland Hertel, Universitätsskinderspital, Basel, Switzerland.
Mario Francioli, Ospedale San Giovanni, Bellinzona, Switzerland.
Marina Dolina, Istituto Cantonale di Microbiologia, Bellinzona, Switzerland.
Madeleine Rothen, Spitalzentrum, Biel, Switzerland.
Olivier Dubuis, Viollier Microbiology Laboratories, Bienne, Switzerland.
Philipp Tarr and Suzanne Graf, Kantonsspital, Bruderholz, Switzerland.
Felix Fleisch, Martin Risch and Eva Ritzler, Kantonsspital, Chur, Switzerland.
Christian Chuard, Véronique Erard and Dominique Fracheboud, Hôpital Cantonal, Fribourg, Switzerland.
Stéphane Emonet, Infectious Diseases Service, Geneva University Hospital, Geneva, Switzerland.
Daniel Genne and Reto Lienhardt, Hôpital Communal, La-Chaux-de-Fonds, Switzerland.
Jean-Philippe Chave, Corinne Andreutti-Zaugg and Alberto Gallusser, Cliniques Cécil et La Source, Lausanne, Switzerland.
Peter Graber and Suzanne Graf, Kantonsspital, Liestal, Switzerland.
Rita Monotti, Ospedale Regionale, Locarno, Switzerland.
Enos Bernasconi, Ospedale Civico, Lugano, Switzerland.
Martin Krause and Karin Herzog, Kantonsspital, Münsterlingen, Switzerland.
Rein-Jan Piso and Urs Schibli, Kantonsspital, Olten, Switzerland.
Frank Bally, Nicolas Troillet and Lysiane Tissière, Institut Central des Hôpitaux Vaudois, Sion, Switzerland.
Katja Boggian and Thomas Bruderer, Kantonsspital Sankt Gallen, Switzerland.
Jacques Gubler, Kantonsspital, Winterthur, Switzerland.
Gerhard Eich, Stadtpital Triemli, Zürich, Switzerland.
Christoph Berger, Universitätsskinderspital, Zürich, Switzerland.

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