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Antifungal Susceptibility Profiles of Bloodstream Yeast Isolates by Sensititre YeastOne over Nine Years at a Large Italian Teaching Hospital

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Sensititre YeastOne (SYO) is an affordable alternative to the Clinical and Laboratory Standards Institute (CLSI) reference method for antifungal susceptibility testing. In this study, the MICs of yeast isolates from 1,214 bloodstream infection episodes, generated by SYO during hospital laboratory activity (January 2005 to December 2013), were reanalyzed using current CLSI clinical breakpoints/epidemiological cutoff values to assign susceptibility (or the wild-type [WT] phenotype) to systemic antifungal agents. Excluding *Candida albicans* (57.4% of all isolates [$n = 1,250$]), the most predominant species were *Candida parapsilosis* complex (20.9%), *Candida tropicalis* (8.2%), *Candida glabrata* (6.4%), *Candida guilliermondii* (1.6%), and *Candida krusei* (1.3%). Among the non-*Candida* species (1.9%), 7 were *Cryptococcus neoformans* and 17 were other species, mainly *Rhodotorula* species. Over 97% of *Candida* isolates were susceptible (WT phenotype) to amphotericin B and flucytosine. Rates of susceptibility (WT phenotype) to fluconazole, itraconazole, and voriconazole were 98.7% in *C. albicans*, 92.3% in the *C. parapsilosis* complex, 96.1% in *C. tropicalis*, 92.5% in *C. glabrata*, 100% in *C. guilliermondii*, and 100% (excluding fluconazole) in *C. krusei*. The fluconazole-resistant isolates consisted of 6 *C. parapsilosis* complex isolates, 3 *C. glabrata* isolates, 2 *C. albicans* isolates, 2 *C. tropicalis* isolates, and 1 *Candida lusitaniae* isolate. Of the non-*Candida* isolates, 2 *C. neoformans* isolates had the non-WT phenotype for susceptibility to fluconazole, whereas *Rhodotorula* isolates had elevated azole MICs. Overall, 99.7% to 99.8% of *Candida* isolates were susceptible (WT phenotype) to echinocandins, but 3 isolates were nonsusceptible (either intermediate or resistant) to caspofungin (*C. albicans*, *C. guilliermondii*, and *C. krusei*), anidulafungin (*C. albicans* and *C. guilliermondii*), and micafungin (*C. albicans*). However, when the intrinsically resistant non-*Candida* isolates were included, the rate of echinocandin nonsusceptibility reached 1.8%. In summary, the SYO method proved to be able to detect yeast species showing antifungal resistance or reduced susceptibility.

Almost all of the classes of antifungal agents available to date, such as polyenes, azoles, flucytosine, and echinocandins, are systemically active against *Candida* or non-*Candida* yeasts causing bloodstream infections (BSIs) (1–4). Nevertheless, the expanding use of newer (e.g., caspofungin or posaconazole) and older (e.g., fluconazole) antifungal agents for prophylactic or empirical purposes (5, 6) has led to and in part has driven the changing epidemiology of fungemia (7–10) and the emergence of fungal pathogens with decreased susceptibility or resistance to currently prescribed antifungals (11, 12). It is noteworthy that while *Candida albicans* is the most frequently encountered species in most hospital settings worldwide (13), non-*albicans Candida* species (i.e., *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, *Candida krusei*, etc.) were recently shown to be the cause of two-thirds of all cases of candidemia in a population-based laboratory study (14). Additionally, more than half of *Candida* isolates found to be resistant to one of two antifungal classes (i.e., azoles and echinocandins) were *C. glabrata*, with 8 of 9 isolates being resistant to both an echinocandin and fluconazole (14). It is also notable that in about 62% of candidemia episodes studied over a 10-year period at Duke University Hospital, patients who failed to respond or responded only initially to an echinocandin therapy were infected with *C. glabrata* isolates for which the MICs indicated echinocandin resistance and which harbored *FKS* mutations (15).

In keeping with the need for reproducible and clinically relevant fungal susceptibility testing, the Sensititre YeastOne (SYO; Thermo Fisher Scientific, MA) colorimetric plate was marketed to provide an easy and affordable alternative to the Clinical and Laboratory Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standard broth microdilution methods (16, 17). It now represents, to our knowledge, a suitable method for the routine testing of the susceptibilities of clinical *Candida* isolates to amphotericin B, flucytosine, fluconazole, itraconazole, posaconazole, voriconazole, and the three echinocandins, particularly when it is used on a large

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scale (18; see also reference 19). Using 24-h MIC results obtained by SYO, Huang et al. assessed the *in vitro* antifungal susceptibility profiles of 474 blood *Candida* isolates by applying the newly revised CLSI clinical breakpoints (CBPs) or, in the absence of CBPs, epidemiological cutoff values (ECVs) for nine antifungal agents (20). Based on data from a prospective candidemia study, van Hal et al. were able to support the revised fluconazole CBP for *C. albicans* by use of the MICs that were obtained using the SYO method (21).

In the present study, we carried out a retrospective analysis of antifungal MIC data generated by the SYO system during a 9-year hospital laboratory activity with regard to fungal BSIs. Thus, the original MICs of 1,250 isolates of *Candida* and non-*Candida* species from 1,214 infectious episodes were reanalyzed by adopting the current interpretive criteria to determine the rates of antifungal resistance and to detect emerging resistance among the isolates. Furthermore, isolates of *Candida* species showing elevated echinocandin MICs were molecularly characterized to define the mechanisms of echinocandin resistance.

MATERIALS AND METHODS

Data collection. A total of 1,214 BSI episodes due to *Candida* or non-*Candida* species were diagnosed in 1,214 patients during the years from 2005 to 2013 and identified through a search of the clinical microbiology laboratory information system at the Università Cattolica del Sacro Cuore (UCSC), a large institution comprising a 1,200-bed tertiary-level hospital in Rome, Italy. Episodes in which more than one fungal species were detected were considered polyfungal BSIs, whereas episodes occurring in patients whose blood samples for culture for analysis of the incident episode (i.e., the first blood culture positive for a fungal species) were collected >48 h after hospital admission were considered hospital-onset BSIs (HO-BSIs). Outpatient-acquired BSIs were episodes detected ≤48 h after hospital admission. As no multiple episodes of fungemia in the same patient (defined as episodes due to the same fungal species that occurred at least >21 days after the incident episode) were diagnosed, all the first episodes of fungemia diagnosed during the study period were included in the study. Data were reported into a customized database created for the inclusion of patient identifiers, hospital wards or outpatient services/departments, dates of BSI onset, and the species and antifungal susceptibility patterns of the yeast isolates from the BSI patients (n = 1,250 isolates, including those recovered from episodes with a single [n = 1,214] or mixed [n = 36] fungal etiology). Additionally, data concerning the dosage and duration of any antifungal treatment, primary disease, source of fungal infection, and clinical outcome were retrieved from the patients' hospital charts (only for patients infected with isolates nonsusceptible [including susceptible dose dependent/intermediate and resistant] to antifungals), whereas data on hospital antifungal consumption (in defined daily doses [DDDs] per year) were available from the UCSC pharmacy database. The study did not require oversight by the institutional ethics committee because of its descriptive nature.

Species identification and antifungal susceptibility testing. Yeast organisms were isolated, after growth on Difco *Candida* bromcresol green (BCG) agar plates, from cultures of patient blood, which was collected as part of normal clinical practice and processed using a Bactec (BD Diagnostic Systems, Sparks, MD) or BacT/Alert (bioMérieux, Marcy l'Etoile, France) system. Isolates were identified to the species level by standard methods, such as morphology on cornmeal-Tween 80 agar, growth at 45°C (for *C. albicans*/*C. dubliniensis*), and/or yeast assimilation/enzymatic tests using Vitek 2 and RapID Yeast Plus identification systems (22) or, since 2010, by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (23), supplemented by molecular identification, as needed (24). This was the case for isolates yielding inconclusive phenotypic profiles or insufficient mass spectra. Antifungal susceptibility testing was performed as part of routine patient care, and colorimetric MIC endpoints were determined visually, after 24 of incubation at 35°C in a non-CO_2 atmosphere, using the SYO panel (progressively upgraded until it included all 10 antifungal agents available in 2009 [the SYO-10 version]) for a total of 1,250 (100% tested with amphotericin B, flucytosine, fluconazole, itraconazole, and voriconazole), 1,059 (84.7% tested with caspofungin), 908 (72.6% tested with posaconazole), and 740 (59.2% tested with anidulafungin and micafungin) isolates, according to the manufacturer's instructions. In cases in which a prolonged incubation of SYO plates was required (e.g., for cryptococcal isolates), visual readings of MICs was performed regardless of colorimetric changes. The concentrations of the antifungals in version SYO-10 ranged from 0.12 to 8 μg/ml for amphotericin B, 0.06 to 64 μg/ml for flucytosine, 0.015 to 8 μg/ml for anidulafungin, 0.008 to 8 μg/ml for caspofungin, micafungin, voriconazole, and posaconazole, 0.12 to 256 μg/ml for fluconazole, and 0.015 to 16 μg/ml for itraconazole. As the ranges for amphotericin B, flucytosine, fluconazole, and itraconazole were different from those for the previous SYO versions (SYO-06, SYO-07, SYO-8) used throughout the study period (see Table S1 in the supplemental material), MIC values of 0.008 to 0.12 μg/ml for amphotericin B and of 0.03 to 0.12 μg/ml for fluconazole were reported as ≤0.12 μg/ml, MIC values of 0.03 to 0.06 μg/ml for flucytosine were reported as ≤0.06 μg/ml, and MIC values of 0.008 to 0.015 μg/ml for itraconazole were reported as ≤0.015 μg/ml.

Data analysis. The interpretive antifungal MIC breakpoints were the species-specific CBPs of fluconazole, voriconazole, and echinocandins (25–27), which were recently revised by the CLSI (28) to identify resistant strains of the 5 most common species of *Candida* (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*); exceptions were the species *C. krusei*, for which all isolates are defined to be intrinsically resistant to fluconazole, and the voriconazole and *C. glabrata* combination, for which no CBPs were assigned by the CLSI (26, 29). The CLSI resistance breakpoint for fluconazole was defined as an MIC of >4 μg/ml against *C. albicans*, *C. parapsilosis*, and *C. tropicalis* and an MIC of >32 μg/ml against *C. glabrata*; the CLSI resistance breakpoint for voriconazole was defined as an MIC of >0.5 μg/ml against *C. albicans*, *C. parapsilosis*, and *C. tropicalis* and an MIC of >1 μg/ml against *C. krusei*. The CLSI resistance breakpoint for anidulafungin, caspofungin, and micafungin was defined as an MIC of >0.5 μg/ml against *C. albicans*, *C. tropicalis*, and *C. krusei* and an MIC of >4 μg/ml against *C. parapsilosis*; the CLSI resistance breakpoint both for anidulafungin and caspofungin and for micafungin was defined as an MIC of >0.25 μg/ml and >0.12 μg/ml, respectively, against *C. glabrata*. In lieu of CBPs, the ECV of >0.5 μg/ml was used to identify isolates of *C. glabrata* nonsusceptible (i.e., isolates with the non-wild-type [non-WT] phenotype) to voriconazole; ECVs of >0.06 μg/ml, >0.25 μg/ml, >0.12 μg/ml, >2 μg/ml, and >0.5 μg/ml were used to identify isolates of *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, and *C. krusei*, respectively, nonsusceptible (non-WT phenotype) to posaconazole (29). ECVs were also used to identify isolates of *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, and *C. krusei* nonsusceptible (non-WT phenotype) to amphotericin B (>2 μg/ml for all) and flucytosine (>0.5 μg/ml for of *C. albicans*, *C. parapsilosis*, *C. tropicalis*, and *C. glabrata* and >32 μg/ml for *C. krusei*), as well as those of other *Candida* species, such as *Candida guilliermondii* (>2 μg/ml and >1 μg/ml) and *Candida lusitaniae* (>2 μg/ml and 0.5 μg/ml) (29). For *C. guilliermondii* and the echinocandins, the CLSI resistance breakpoint of >4 μg/ml (28, 29) was used. Also, ECVs for triazoles and echinocandins were used to identify nonsusceptible (non-WT phenotype) isolates of *C. guilliermondii* (only for triazoles), *C. lusitaniae*, and other *Candida* species, such as *Candida dubliniensis*, *Candida kefyr*, and *Candida pelliculosa* (29). Among non-*Candida* yeasts, we used ECVs only for *Cryptococcus neoformans* and fluconazole (16 μg/ml), itraconazole (1 μg/ml), posaconazole (0.5 μg/ml), and voriconazole (0.25 μg/ml), as reported elsewhere (30); *Rhodotorula* species, *C. neoformans*, and *Trichosporon asahii* were considered intrinsically resistant to echinocandins. Rates of resistance were not calculated for the remaining species and antifungal compound combinations.

All *Candida* isolates with MICs for anidulafungin, caspofungin,

and/or micafungin greater than the CBPs or ECVs were investigated for the presence or absence of a mutation in the hot spot (HS) regions of the *FKS* gene, as previously described (see reference 31 and references therein). This gene encodes the target enzyme (glycan synthase) for echinocandins (32).

Statistics. All incidence rates were calculated using as the denominator the summed numbers of inpatient days of the UCSC hospital during the study period and are presented per 1,000 inpatient days (33). Categorical variables were analyzed using the chi-square test or Fisher's exact test, and continuous variables were analyzed by the Mann-Whitney U test. Significance was set as a P value of <0.05 (two-tailed). All analyses were done using STATA software (version 11.1; StataCorp, College Station, TX).

RESULTS AND DISCUSSION

Table 1 shows the distribution of species for the BSI episodes caused by 1,250 yeasts during the study period (January 2005 to December 2013). Among the isolates, 1,226 were *Candida* species and 24 were non-*Candida* species (7 *C. neoformans* isolates and 17 isolates of other species). As expected, *Candida* species accounted for 98.1% of the BSI isolates and *C. albicans* was the predominant species ($n = 718$ isolates, 57.4%), followed by the *C. parapsilosis* complex ($n = 262$, 20.9%), *C. tropicalis* ($n = 102$, 8.2%), *C. glabrata* ($n = 80$, 6.4%), *C. guilliermondii* ($n = 20$, 1.6%), and *C. krusei* ($n = 16$, 1.3%); miscellaneous species of *Candida* ($n = 28$, 2.2%) included *C. lusitaniae* ($n = 9$, 0.7%) and 10 other infrequent species ($n = 19$, 1.5%). Non-*Candida* yeasts accounted for 1.9% of all BSI isolates, and these were dominated by *Rhodotorula* species (*Rhodotorula mucilaginosa*, *Rhodotorula glutinis*, and *Rhodotorula dairenensis*; 9 isolates) and *C. neoformans*, which together accounted for 1.3% of all BSI isolates and 66.6% of all non-*Candida* yeasts. Overall, we recorded 1,214 first episodes of BSI, among which 1,183 were diagnosed in patients admitted to medical wards ($n = 580$, 47.8%), surgical wards ($n = 335$, 27.6%), the intensive care unit (ICU; $n = 166$, 13.7%), and oncology or hematology ward ($n = 102$, 8.4%) at the time of blood sample collection; the remaining 31 (2.5%) BSI episodes were acquired when the patients were outpatients (Table 1). Compared with the other *Candida* species, *C. albicans* and the *C. parapsilosis* complex were more likely to infect patients with hematological diseases and/or malignancies ($P < 0.001$), whereas *C. albicans*, the *C. parapsilosis* complex, and *C. guilliermondii* were more likely to infect ICU patients ($P = 0.024$, $P = 0.004$, and $P = 0.014$, respectively). As calculated from the total number of inpatient days ($n = 3{,}574{,}148$), the overall incidence rate was 0.33/1,000 inpatient days; the highest incidence was observed in ICU patients (0.61/1,000 inpatient days), followed by medical patients (0.42/1,000 inpatient days), malignancy patients (0.29/1,000 inpatient days), and surgical patients (0.21/1,000 inpatient days). Also, the overall incidence rates per 1,000 inpatient days were calculated for *C. albicans*, the *C. parapsilosis* complex, *C. tropicalis*, *C. glabrata*, *C. guilliermondii*, and *C. krusei* (Table 1).

Among the 1,183 HO-BSI patients, the median time from the time of admission to the time of detection of the first positive blood culture was 25 days (interquartile range [IQR], 11 to 42 days), with *C. krusei* BSIs being diagnosed the earliest (9 days; IQR, 4 to 20 days; $P < 0.001$) and *C. albicans* or *C. tropicalis* BSIs being diagnosed the latest (28 days [IQR, 16 to 45 days; $P < 0.001$] and 20 days [IQR, 9 to 38 days; $P = 0.02$], respectively) (Table 1). The number of total BSIs averaged ~135 per year, with no discernible trends in either the number of infections or the species distribution per year being found ($P > 0.05$). The median age of all BSI patients (72 years) did not differ significantly with respect to whether the causative species was *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, or *C. krusei*, with the exception of patients infected with *C. guilliermondii*, who were aged 48 years ($P < 0.001$) (Table 1). Polyfungal BSIs occurred in 35 patients (2.8%), of which 34 were infected by 2 species and 1 was infected by 3 species (*C. glabrata*, a *C. parapsilosis* complex isolate, and *C. tropicalis*) (Table 1). In 26 (74.3%) of these patients, *C. albicans* was isolated in combination with another yeast, among which *C. glabrata* and the *C. parapsilosis* complex accounted for 9 episodes each. Other mixed BSIs involved species like *Blastoschizomyces capitatus*, *Candida famata*, *C. pelliculosa*, *Candida rugosa*, and *R. mucilaginosa*, which are not commonly isolated worldwide (34, 35), although these species must be regarded as emerging causes of fungemia (36). It is noteworthy that *C. parapsilosis* was isolated together with *C. famata* in one case and with *C. guilliermondii* in another case. In fact, less prevalent *Candida* species are difficult to differentiate from each another with many identification systems that are currently used in clinical laboratories (37), except for the newly introduced MALDI-TOF mass spectrometry (23), and polyfungal fungemias also fail to be detected using a combination of conventional identification methods, like the ID 32C system plus CHROMagar (38).

Excluding *C. albicans*, the rank order of the six most frequently encountered *Candida* species in the present study was *C. parapsilosis* complex > *C. tropicalis* > *C. glabrata* > *C. guilliermondii* > *C. krusei* > *C. lusitaniae* (frequency range, 20.9 to 0.7%). As in other European countries (38), the *C. parapsilosis* complex was the most common of the non-*albicans Candida* species, but this is in apparent contrast to the findings of fungemia surveillances recently conducted in the United States (35, 39). In one of these studies, *C. parapsilosis* was found to be the most prevalent species in 4 of 24 medical centers surveyed, whereas *C. krusei* ranked second or third in prevalence in seven of these centers (39). Thus, it is not surprising that *C. guilliermondii* (accounting for 18 single-species infections and 2 mixed infections) was fourth in rank order among the non-*albicans Candida* species in our study. Likewise, the *C. parapsilosis* complex, *C. tropicalis*, and *C. glabrata* were the first three species to be identified as causes of invasive candidiasis among 1,072 isolates from a 3-year national surveillance in China (40).

Table 2 shows the results of testing of the *in vitro* susceptibilities of BSI isolates to nine antifungal agents, as routinely performed using the SYO method. Although such testing was done by common laboratory personnel, quality control procedures were performed each time that a new SYO panel batch was used during the study period, and the MICs for control strains (*C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019) were within the acceptable range for the antifungals tested in each run (data not shown). As shown in Table 2, the MICs for the 1,250 yeast isolates were not always determined for all antifungals, since the number of antifungals in the SYO panels increased over time, i.e., from 6 in 2005 (version SYO-06) to 10 in 2009 (version SYO-10). Although ketoconazole has been available since the SYO-06 version, the MICs of this nonsystemic antifungal agent were disregarded in the present analysis.

Among 1,209 isolates of common and less common *Candida* species (including 9 isolates of *C. lusitaniae* and 2 isolates of *C. dubliniensis*), over 97% were of the WT phenotype for amphoter-

TABLE 1 Characteristics of BSI episodes by causative organism over a 9-year study period[a]

Characteristic	Result for the following yeast species (no. of episodes):								
	All BSIs (n = 1,214)	*C. albicans* (n = 692)	*C. parapsilosis* (n = 248)	*C. tropicalis* (n = 92)	*C. glabrata* (n = 69)	*C. guilliermondii* (n = 18)	*C. krusei* (n = 13)	Other[b] (n = 47)	Multiple species[c] (n = 35)
Median (IQR[d]) age (yr)	72 (56–82)	74 (59.5–82)	65.5 (49–81)	75 (61–83.5)	71 (61–79)	48 (4–71)	67 (54–78)	59 (41–79)	76 (68–86)
No. (%) of male patients	661 (54.4)	364 (52.6)	132 (53.2)	54 (58.7)	38 (55.1)	11 (61.1)	8 (61.5)	32 (68.1)	22 (31.0)
No. (%) of patients with BSI in the following yr:									
2005	94 (7.7)	41 (43.6)	28 (29.8)	9 (9.6)	6 (6.4)	1 (1.1)	3 (3.2)	3 (3.2)	2 (2.1)
2006	115 (9.5)	70 (60.9)	17 (14.8)	9 (7.8)	12 (10.4)	0 (0.0)	2 (1.7)	3 (2.6)	3 (2.6)
2007	118 (9.7)	71 (60.2)	26 (22.0)	5 (4.2)	5 (4.2)	1 (0.8)	1 (0.8)	7 (5.9)	2 (1.7)
2008	129 (10.6)	71 (55.0)	19 (14.7)	7 (5.4)	9 (7.0)	6 (4.6)	2 (1.5)	6 (4.6)	9 (7.0)
2009	153 (12.6)	89 (58.2)	28 (18.3)	16 (10.4)	8 (5.2)	3 (2.0)	0 (0.0)	1 (0.6)	8 (5.2)
2010	145 (11.9)	87 (60.0)	31 (21.4)	8 (5.5)	7 (4.8)	2 (1.4)	1 (0.7)	4 (2.7)	5 (3.4)
2011	155 (12.7)	90 (58.1)	30 (19.3)	13 (8.4)	4 (2.6)	3 (1.9)	1 (0.6)	11 (7.1)	3 (1.9)
2012	175 (14.4)	111 (63.4)	36 (20.6)	11 (6.3)	10 (5.7)	0 (0.0)	0 (0.0)	7 (4.0)	0 (0.0)
2013	130 (10.7)	62 (47.7)	33 (25.4)	14 (10.8)	8 (6.1)	2 (1.5)	3 (2.3)	5 (3.8)	3 (2.3)
Incidence rate of BSI[e]	0.33	0.23	0.08	0.03	0.025	0.007	0.005	ND	ND
No. (%) of patients in the following category[f]:									
Medical	580 (47.8)	329 (56.7)	123 (21.2)	47 (8.1)	30 (5.2)	7 (1.2)	4 (0.7)	22 (3.8)	18 (3.1)
Surgical	335 (27.6)	205 (61.2)	63 (18.8)	23 (6.9)	21 (6.3)	3 (0.9)	1 (0.3)	10 (3.0)	9 (2.7)
ICU	166 (13.7)	108 (65.1)	20 (12.0)	12 (7.2)	8 (4.8)	6 (3.6)	2 (1.2)	4 (2.4)	6 (3.6)
Oncology-hematology	102 (8.4)	34 (33.3)	37 (36.3)	7 (6.9)	9 (8.8)	1 (1.0)	5 (4.9)	8 (7.8)	1 (1.0)
Outpatient setting[g]	31 (2.5)	16 (51.6)	5 (31.2)	3 (9.7)	1 (3.2)	1 (3.2)	1 (3.2)	3 (9.7)	1 (3.2)
Median (IQR) time (days) to BSI onset[h]	25 (11–42)	28 (16–45)	25.5 (14–42.5)	20 (9–38)	24 (10–40)	22.5 (13–31)	9 (4–20)	21 (9–30)	26 (11–44)

[a] From a total of 1,214 episodes of bloodstream infection (BSI) identified between January 2005 and December 2013, 35 episodes had a polyfungal etiology (see footnote *c* of Table 1 for details about the infecting species).

[b] Other *Candida* and non-*Candida* species included isolates of *C. lusitaniae* (n = 9), *Cryptococcus neoformans* (n = 7), *Blastoschizomyces capitatus* (n = 5), *Rhodotorula mucilaginosa* (n = 6), *C. famata* (n = 3), *C. dubliniensis* (n = 2), *C. lipolytica* (n = 2), *C. rugosa* (n = 2), *Candida utilis* (n = 2), and 1 isolate each of *Candida intermedia*, *C. kefyr*, *C. lambica*, *C. norvegensis*, *C. pelliculosa*, *Rhodotorula dairenensis*, *Rhodotorula glutinis*, *Saccharomyces cerevisiae*, and *Trichosporon asahii*.

[c] Multiple species included 71 isolates of *C. albicans* (n = 9) plus *C. glabrata* (n = 9), *C. albicans* (n = 9) plus *C. parapsilosis* (n = 9), *C. albicans* (n = 5) plus *C. tropicalis* (n = 5), *C. albicans* (n = 2) plus *C. krusei* (n = 2), *C. albicans* (n = 1) plus *C. guilliermondii* (n = 1), *B. capitatus* (n = 1) plus *C. tropicalis* (n = 1), *C. famata* (n = 1) plus *C. parapsilosis* (n = 1), *C. glabrata* (n = 1) plus *C. parapsilosis* (n = 1), *C. glabrata* (n = 1) plus *C. parapsilosis* (n = 1) plus *C. tropicalis* (n = 1), *C. guilliermondii* (n = 1) plus *C. parapsilosis* (n = 1), *C. krusei* (n = 1) plus *C. tropicalis* (n = 1), *C. pelliculosa* (n = 1) plus *C. tropicalis* (n = 1), *C. rugosa* (n = 1) plus *C. tropicalis* (n = 1), and *R. mucilaginosa* (n = 1) plus *C. parapsilosis* (n = 1).

[d] IQR, interquartile range.

[e] The incidence rate was calculated for unique BSI isolates of yeast species and is presented per 1,000 inpatient days. ND, not determined.

[f] ICU, intensive care unit. The oncology-hematology category includes patients hospitalized in the oncology or hematology ward.

[g] This also includes patients who visited the emergency department during the study period.

[h] The time indicates the interval elapsing from the day of the patient's admission to the day that the first blood culture was found to be positive for that patient.

TABLE 2 *In vitro* susceptibilities of yeast BSI isolates tested against nine antifungals by SYO method

Species	Antifungal agent	No. of isolates tested	MIC (μg/ml)[a]			No. (%) of isolates in the indicated susceptibility category by CBP[b]				No. (%) of isolates by ECV[b]	
			Range	50%	90%	S	S-DD	I	R	Wild type	Non-wild type
C. albicans	Amphotericin B	718	≤0.12 to 1	0.12	0.5					718 (100)	0 (0.0)
	Flucytosine	718	≤0.06 to 8	≤0.06	0.12					714 (99.4)	4 (0.6)
	Fluconazole	718	≤0.12 to 16	0.25	0.5	716 (99.7)	0 (0.0)		2 (0.3)		
	Itraconazole	718	≤0.015 to 1	0.03	0.06					713 (99.3)	5 (0.7)
	Voriconazole	718	≤0.008 to 0.5	0.008	0.008	716 (99.7)	2 (0.3)		0 (0.0)		
	Posaconazole	530	≤0.008 to 1	0.008	0.03					526 (99.2)	4 (0.8)
	Caspofungin	619	≤0.008 to 4	0.03	0.06	618 (99.8)		0 (0.0)	1 (0.2)		
	Anidulafungin	431	≤0.015 to 1	≤0.015	0.03	430 (99.8)		0 (0.0)	1 (0.2)		
	Micafungin	431	≤0.008 to 1	≤0.008	0.015	430 (99.8)		0 (0.0)	1 (0.2)		
C. parapsilosis complex[c]	Amphotericin B	262	≤0.12 to 2	0.25	0.5					262 (100)	0 (0.0)
	Flucytosine	262	≤0.06 to ≥64	0.06	0.12					257 (98.1)	5 (1.9)
	Fluconazole	262	≤0.12 to 16	0.5	2	244 (93.1)	12 (4.6)		6 (2.3)		
	Itraconazole	262	≤0.015 to 0.5	0.03	0.12					262 (100)	0 (0.0)
	Voriconazole	262	≤0.008 to 0.25	0.015	0.03	260 (99.2)	2 (0.8)		0 (0.0)		
	Posaconazole	186	≤0.008 to 0.25	0.03	0.06					186 (100)	0 (0.0)
	Caspofungin	218	0.03 to 2	0.25	0.5	218 (100)		0 (0.0)	0 (0.0)		
	Anidulafungin	156	≤0.015 to 2	0.5	1	156 (100)		0 (0.0)	0 (0.0)		
	Micafungin	156	0.03 to 2	0.5	1	156 (100)		0 (0.0)	0 (0.0)		
C. tropicalis	Amphotericin B	102	≤0.12 to 1	0.25	0.5					102 (100)	0 (0.0)
	Flucytosine	102	≤0.06 to ≥64	0.06	32					83 (81.4)	19 (18.6)
	Fluconazole	102	≤0.12 to 16	0.25	0.5	99 (97.0)	1 (1.0)		2 (2.0)		
	Itraconazole	102	≤0.015 to 0.5	0.12	0.25					102 (100)	0 (0.0)
	Voriconazole	102	≤0.008 to 0.25	0.03	0.06	101 (99.0)	1 (1.0)		0 (0.0)		
	Posaconazole	75	≤0.008 to 0.5	0.06	0.25					63 (84.0)	12 (16.0)
	Caspofungin	84	0.015 to 0.12	0.03	0.06	84 (100)		0 (0.0)	0 (0.0)		
	Anidulafungin	64	≤0.015 to 0.12	0.015	0.03	64 (100)		0 (0.0)	0 (0.0)		
	Micafungin	64	0.015 to 0.06	0.03	0.03	64 (100)		0 (0.0)	0 (0.0)		
C. glabrata	Amphotericin B	80	≤0.12 to 1	≤0.12	0.5					80 (100)	0 (0.0)
	Flucytosine	80	≤0.06	≤0.06	≤0.06					80 (100)	0 (0.0)
	Fluconazole	80	0.25 to ≥256	8	16		77 (96.3)		3 (3.7)		
	Itraconazole	80	≤0.015 to ≥16	0.5	1					77 (96.3)	3 (3.7)
	Voriconazole	80	≤0.008 to ≥8	0.12	0.5					77 (96.3)	3 (3.7)
	Posaconazole	53	0.25 to ≥8	0.5	2					50 (94.3)	3 (5.7)
	Caspofungin	62	0.03 to 0.25	0.06	0.12	62 (100)		0 (0.0)	0 (0.0)		
	Anidulafungin	42	≤0.015 to 0.12	≤0.015	0.03	42 (100)		0 (0.0)	0 (0.0)		
	Micafungin	42	≤0.008 to 0.03	0.015	0.015	42 (100)		0 (0.0)	0 (0.0)		
C. guilliermondii	Amphotericin B	20	≤0.12 to 0.5	0.12	0.25					20 (100)	0 (0.0)
	Flucytosine	20	≤0.06 to ≥64	0.06	64					14 (70.0)	6 (30.0)
	Fluconazole	20	0.5 to 8	2	8					20 (100)	0 (0.0)
	Itraconazole	20	0.03 to 0.5	0.12	0.5					20 (100)	0 (0.0)
	Voriconazole	20	≤0.008 to 0.12	0.03	0.12					20 (100)	0 (0.0)
	Posaconazole	19	0.03 to 0.5	0.12	0.25					19 (100)	0 (0.0)
	Caspofungin	19	0.06 to 4	0.25	1	18 (94.7)		1 (5.3)	0 (0.0)		
	Anidulafungin	13	0.12 to 1	0.5	1	13 (100)		0 (0.0)	0 (0.0)		
	Micafungin	13	0.06 to 0.5	0.25	0.5	13 (100)		0 (0.0)	0 (0.0)		
C. krusei	Amphotericin B	16	≤0.12 to 1	0.03	0.5					16 (100)	0 (0.0)
	Flucytosine	16	1 to 16	4	16					16 (100)	0 (0.0)
	Fluconazole	16	16 to 64	64	64					16 (100)	0 (0.0)
	Itraconazole	16	0.06 to 0.5	0.25	0.5					16 (100)	0 (0.0)
	Voriconazole	16	0.03 to 0.5	0.12	0.5	16 (100)	0 (0.0)		0 (0.0)		
	Posaconazole	10	0.06 to 0.25	0.25	0.25					10 (100)	0 (0.0)
	Caspofungin	11	0.12 to 2	0.25	0.25	10 (90.9)		0 (0.0)	1 (9.1)		
	Anidulafungin	7	≤0.015 to 0.5	ND	ND	6 (85.7)		1 (14.3)	0 (0.0)		
	Micafungin	7	0.06 to 0.5	ND	ND	6 (85.7)		1 (14.3)	0 (0.0)		

(Continued on following page)

TABLE 2 (Continued)

Species	Antifungal agent	No. of isolates tested	MIC (μg/ml)[a]			No. (%) of isolates in the indicated susceptibility category by CBP[b]				No. (%) of isolates by ECV[b]	
			Range	50%	90%	S	S-DD	I	R	Wild type	Non-wild type
C. lusitaniae	Amphotericin B	9	0.03 to 0.5	ND	ND					9 (100)	0 (0.0)
	Flucytosine	9	≤0.06 to 1	ND	ND					9 (100)	0 (0.0)
	Fluconazole	9	0.25 to 4	ND	ND					8 (88.9)	1 (11.1)
	Itraconazole	9	≤0.015 to 0.12	ND	ND					9 (100)	0 (0.0)
	Voriconazole	9	≤0.008 to 0.03	ND	ND					9 (100)	0 (0.0)
	Posaconazole	6	≤0.008 to 0.03	ND	ND					6 (100)	0 (0.0)
	Caspofungin	8	0.03 to 0.25	ND	ND					8 (100)	0 (0.0)
	Anidulafungin	3	0.03 to 0.12	ND	ND					3 (100)	0 (0.0)
	Micafungin	3	0.03 to 0.06	ND	ND					3 (100)	0 (0.0)
C. neoformans	Amphotericin B	7	≤0.12 to 0.5	ND	ND						
	Flucytosine	7	4 to 32	ND	ND						
	Fluconazole	7	4 to 64	ND	ND					5 (71.4)	2 (28.6)
	Itraconazole	7	0.03 to 0.25	ND	ND					7 (100)	0 (0.0)
	Voriconazole	7	0.06 to 0.25	ND	ND					7 (100)	0 (0.0)
	Posaconazole	4	0.03 to 0.5	ND	ND					4 (100)	0 (0.0)
	Caspofungin	6	≥8	ND	ND						
	Anidulafungin	4	≥8	ND	ND						
	Micafungin	4	≥8	ND	ND						
Other yeasts[d]	Amphotericin B	36	≤0.12 to 2	0.25	1						
	Flucytosine	36	≤0.06 to 16	0.06	4						
	Fluconazole	36	0.12 to 128	4	128						
	Itraconazole	36	≤0.015 to 2	0.12	0.5						
	Voriconazole	36	≤0.008 to 2	0.06	0.5						
	Posaconazole	25	0.015 to 4	0.25	1						
	Caspofungin	32	0.03 to ≥8	2	≥8						
	Anidulafungin	20	≤0.015 to ≥8	0.5	≥8						
	Micafungin	20	≤0.008 to ≥8	0.5	≥8						

[a] MICs are reported as the range, MIC_{50}, and MIC_{90}. The MIC_{50}s and MIC_{90}s were calculated only for those species with at least 10 isolates tested. ND, not determined.
[b] Clinical breakpoints (CBPs) for susceptible (S), susceptible dose dependent (S-DD), intermediate (I), and resistant (R) were those of the CLSI (28, 29). In the absence of CBPs for amphotericin B, flucytosine, itraconazole, and posaconazole and the five most common species of *Candida* (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*), as for the voriconazole and *C. glabrata* combination, for which no CBPs were assigned by the CLSI (26, 29), isolates were classified as having the WT and non-WT drug susceptibility phenotypes according to the epidemiological cutoff values (ECVs) recently proposed by CLSI (29). In lieu of CBPs, ECVs were also used for the amphotericin B, flucytosine, triazole, and echinocandin antifungal agents to identify isolates of *C. guilliermondii* with the non-WT phenotype (excluding echinocandins), *C. lusitaniae*, and other listed *Candida* species, such as *C. dubliniensis*, *C. kefyr*, and *C. pelliculosa* (see footnote *d* below) (29). Among the non-*Candida* yeasts (see footnote *d* below), ECVs were used only for *Cryptococcus neoformans*, as specified in the text.
[c] Includes two isolates that were identified as *C. orthopsilosis* since their isolation from the respective patients' blood cultures in 2010 and 2011, which was subsequent to the MALDI-TOF mass spectrometry implementation in the clinical microbiology laboratory. These isolates were classified as resistant to fluconazole according to the *C. parapsilosis* species-specific CBP mentioned in footnote *b* above, or as having the non-WT phenotype for susceptibility to fluconazole according to the established ECV (>2 μg/ml) (29). Two of the remaining four fluconazole-resistant isolates initially designated to be *C. parapsilosis* species complex were analyzed using MALDI-TOF mass spectrometry at the time of the present study and were identified as *C. parapsilosis sensu stricto*.
[d] Other *Candida* and non-*Candida* species included isolates of *Blastoschizomyces capitatus* ($n = 6$), *Rhodotorula mucilaginosa* ($n = 7$), *C. famata* ($n = 4$), *C. rugosa* ($n = 3$), *C. dubliniensis* ($n = 2$), *C. lipolytica* ($n = 2$), *C. pelliculosa* ($n = 2$), *Candida utilis* ($n = 2$), and 1 isolate each of *C. intermedia*, *C. kefyr*, *C. lambica*, *C. norvegensis*, *Rhodotorula dairenensis*, *Rhodotorula glutinis*, *Saccharomyces cerevisiae*, and *Trichosporon asahii* (see also Table S2 in the supplemental material).

icin B and flucytosine susceptibility; no isolates had amphotericin B MICs above the ECV, whereas 34 isolates across *C. albicans* (4/718 isolates, 0.6%), the *C. parapsilosis* complex (5/262 isolates, 1.9%), *C. tropicalis* (19/102 isolates, 18.6%), and *C. guilliermondii* (6/20 isolates, 30%) were found to have the non-WT phenotype for flucytosine susceptibility. The remaining 17 isolates belonged to those *Candida* species (e.g., *C. kefyr*, *C. pelliculosa*) for which amphotericin B or flucytosine ECVs were not defined (29). Tentative ECVs for the SYO method were recently proposed, and the median values obtained by the five approaches employed for flucytosine and *C. albicans*, *C. parapsilosis*, and *C. tropicalis* were almost identical to those obtained with the CLSI method (41). For these species, the MIC ranges of flucytosine obtained in the present study were similar to those obtained in two earlier surveys, both of which used the SYO method (20, 40), but a higher proportion of our *C. parapsilosis* complex or *C. tropicalis* isolates exhibited flucytosine MICs greater than the CLSI ECVs.

With regard to *C. albicans*, 2 (0.3%) isolates were resistant to fluconazole and 2 (0.3%) isolates were susceptible dose dependent to voriconazole, whereas 5 (0.7%) isolates and 4 (0.8%) isolates had the non-WT phenotype for itraconazole and posaconazole susceptibility, respectively. With regard to the *C. parapsilosis* complex, 6 (2.3%) isolates were resistant and 12 (4.6%) isolates were susceptible dose dependent to fluconazole and 2 (0.8%) isolates

were susceptible dose dependent to voriconazole; no isolates with itraconazole or posaconazole MICs greater than the ECVs were found. With regard to *C. tropicalis*, 2 (2.0%) isolates and 1 (1.0%) isolate were resistant and susceptible dose dependent to fluconazole, respectively, and 1 (1.0%) isolate was susceptible dose dependent to voriconazole, whereas 12 (16%) isolates had the non-WT phenotype for posaconazole susceptibility. With regard to *C. glabrata*, 3 (3.7%) isolates were resistant to fluconazole, 3 (3.7%) isolates had the non-WT phenotype for itraconazole or voriconazole susceptibility, and 3 (5.7%) had the non-WT phenotype for posaconazole susceptibility. All *C. krusei* isolates in this study were susceptible to voriconazole and had the WT phenotype for itraconazole and posaconazole susceptibility. The isolates of *C. neoformans* (the most represented among the non-*Candida* species studied) showed high MIC values only to fluconazole, with 2 (28.6%) of 7 isolates classified as having the non-WT phenotype for susceptibility to this antifungal agent.

Among the 11 remaining *Candida* species studied, 1 (11.1%) isolate of *C. lusitaniae* had the non-WT phenotype for fluconazole susceptibility, whereas 2 isolates of *C. pelliculosa* and 1 isolate of *C. kefyr* had fluconazole MICs that were below the ECVs established for this antifungal agent (see Table S2 in the supplemental material). In contrast, fluconazole MICs were consistently ≥2 μg/ml for *C. famata* (3 of 4 isolates), *C. rugosa* (2 of 3 isolates), *Candida lipolytica* (1 of 2 isolates), *Candida lambica* (1 isolate), and *Candida norvegensis* (1 isolate) (see Table S2 in the supplemental material). Otherwise, lower MICs of itraconazole and voriconazole were seen for *C. famata* (0.25 and ≤0.12 μg/ml, respectively), *C. rugosa* (0.06 and ≤0.06 μg/ml, respectively), *C. lipolytica* (0.25 and ≤0.12 μg/ml, respectively), *C. lambica* (0.12 and 0.03 μg/ml, respectively), and *C. norvegensis* (0.25 and 0.12 μg/ml, respectively); similarly, the MICs of posaconazole, when tested, were 0.12 μg/ml for *C. famata* (1 isolate), 0.25 μg/ml and 0.5 μg/ml for *C. lipolytica* (2 isolates), and 0.25 μg/ml for *C. norvegensis* (1 isolate) (see Table S2 in the supplemental material).

Among the *Candida* isolates tested (1,024 isolates for caspofungin and 718 isolates for both anidulafungin and micafungin across *C. albicans*, *C. parapsilosis* complex, *C. tropicalis*, *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. lusitaniae*, *C. dubliniensis*, *C. kefyr*, and *C. pelliculosa* isolates), susceptibility to echinocandins was very high. Despite this, the rates at which isolates were nonsusceptible (either intermediate or resistant) to echinocandins were 0.2% (1/619) for *C. albicans*, 5.3% (1/19) for *C. guilliermondii*, and 9.1% (1/11) for *C. krusei* (only to caspofungin), but no resistance was found among *C. glabrata* and *C. tropicalis* isolates. The *C. albicans* isolate was found to harbor a point mutation (S645F) in HS1 of *fks1*, whereas the *C. guilliermondii* isolate (except for a constitutive polymorphism) and the *C. krusei* isolate were wild type for the *fks* gene; of note, the *C. guilliermondii* isolate showed an intermediate phenotype for susceptibility to caspofungin and anidulafungin (Table 3). It was noticed that adoption of the revised CLSI CBPs for caspofungin may overstate the rates at which isolates are nonsusceptible (especially intermediate) to caspofungin among *C. glabrata* and *C. krusei* isolates (18), and the interlaboratory variability in caspofungin MICs for *C. albicans*, *C. glabrata*, *C. tropicalis*, and *C. krusei* may considerably limit the use of both the CLSI and EUCAST reference methods (42). Thus, while clinical microbiology laboratories should use micafungin or anidulafungin as a surrogate marker to predict caspofungin susceptibility (43, 44), the use of SYO assays was recently advised for hospitals that routinely perform echinocandin susceptibility testing of bloodstream isolates (18). This advice was provided to overcome the variability in caspofungin MICs that occurs when *Candida* species are tested by the reference methods. To support this concept, we observed low variability among the caspofungin MICs obtained for isolates of the most common *Candida* species, even through the testing performed with different SYO batches throughout the study period (see Fig. S1 in the supplemental material).

The percentages of resistance reported in our study are similar to those reported from two recent Spanish studies (38, 45), showing that resistance to echinocandins is not emerging like it is in other geographical areas, such as the United States (15, 35). In addition, it is notable that antifungal susceptibility testing in those studies was performed by using EUCAST and CLSI reference procedures, with comparable results being obtained between the two methods (45), and it is notable that our findings are also similar to those reported after analyzing yeast isolates collected from all over the world (SENTRY Program 2010-2011), using CLSI broth microdilution methods (30). In a study by Pfaller et al., decreased susceptibility to posaconazole was prominently (>5%) observed in 8.3% of the isolates of *C. albicans* (ECV, 0.06 μg/ml) and 7.1% of the isolates of *C. krusei* (ECV, 0.5 μg/ml) that were obtained from European laboratories (30). Interestingly, in that study (30) the *C. krusei* isolates for which posaconazole MICs were >0.5 μg/ml (non-WT phenotype) yet which had the WT phenotype for voriconazole susceptibility are reminiscent of *C. tropicalis* isolates for which posaconazole MICs were >0.12 μg/ml (non-WT phenotype) yet were classified as having the WT phenotype for voriconazole susceptibility in the present study. This provides further support for the concept that posaconazole ECVs for *C. krusei* and other common species of *Candida* may be set too low, perhaps because the ECVs were derived from MIC distributions which were obtained from a single laboratory (30). However, ECVs for MIC distributions originating from ≥6 laboratories for posaconazole remained substantially unchanged for eight species of *Candida*, including *C. albicans*, *C. tropicalis*, and *C. krusei* (46).

Overall (only *Candida* species), the rate of susceptibility was 97.5% (1,196/1,226 isolates) for fluconazole and 99.7% (1,032/1,035 isolates) for caspofungin. Among the fluconazole-resistant isolates, 16 isolates were *C. krusei* and the remaining 14 isolates were the *C. parapsilosis* complex (6 isolates, including 2 *Candida orthopsilosis* isolates), *C. glabrata* (3 isolates), *C. albicans* (2 isolates), *C. tropicalis* (2 isolates), and *C. lusitaniae* (1 isolate) (Table 3). Five isolates (3 *C. glabrata* and 2 *C. orthopsilosis* isolates) were resistant (non-WT phenotype) to fluconazole and voriconazole, and 3 isolates (all *C. glabrata*) were resistant (non-WT phenotype) to the other three azoles. Two *C. albicans* isolates were cross-resistant to fluconazole and itraconazole, and 1 *C. albicans* isolate and 2 *C. tropicalis* isolates exhibited a non-WT phenotype for posaconazole susceptibility. Overall (all isolates), the rate of resistance to fluconazole and echinocandin antifungals was 3.9%, as reflected by the number of BSI episodes caused by species with decreased susceptibility to fluconazole or by intrinsically resistant species, such as *C. neoformans*, *Rhodotorula* spp., or *Trichosporon asahii*. Even though these species are regarded to be rare pathogens, they merit particular attention because their challenging intrinsic susceptibility pattern often leads to delayed appropriate antifungal treatment (4).

Table 3 also shows the characteristics of patients with BSIs

TABLE 3 Characteristics of BSI episodes caused by *Candida* isolates shown to be fluconazole resistant or echinocandin nonsusceptible *in vitro*[h]

Date of BSI (day/mo/yr)	*Candida* species	Underlying condition(s)	Antifungal therapy	Duration (days)[a]	Resistance (non-WT phenotype) profile of the isolate[b]	MIC of the isolate (μg/ml)[c]							Source control (hour timing)[d]	Prior antifungal treatment	Outcome[e]
						FLC	ITC	VRC	POS	CAS	ANF	MCF			
14/11/2005	*C. lusitaniae*	Gastric lymphoma	FLC	7	FLC	4	0.12	0.03	ND				Yes (72)	Azoles (FLC)	Deceased
16/12/2005	*C. parapsilosis* complex[f]	Acute myeloid leukemia	AMB	16	FLC	8	0.12	0.12	ND				Yes (96)	Azoles (ITC)	Alive
20/6/2006	*C. parapsilosis* complex[f]	Small intestine syndrome	AMB	24	FLC	16	0.5	**0.25**	ND				Yes (72)	Azoles (FLC)	Alive
27/9/2006	*C. albicans*	Cholecystitis	FLC	10	FLC, ITC	16	1	**0.5**	ND				No	No	Deceased
6/6/2007	*C. parapsilosis* complex[f]	Hematopoietic stem cell transplantation	AMB	22	FLC	8	0.25	0.12	ND				Yes (48)	AMB and azoles (ITC, FLC)	Alive
29/8/2007	*C. parapsilosis* complex[f]	Anorexia, peripheral nervous system involvement	FLC	15	FLC	8	0.12	0.12	ND				Yes (48)	No	Alive
7/4/2008	*C. glabrata*	Staphylococcal prosthetic valve endocarditis	CAS	35	FLC, ITC, VRC, POS	256	>16	4	8				No	Azoles (FLC)	Alive
16/4/2008	*C. glabrata*	Chronic lymphoid leukemia, COPD	CAS	5	FLC, ITC, VRC, POS	128	16	4	8				No	Azoles (FLC)	Deceased
13/7/2009	*C. tropicalis*	Hydrocephalus	VRC	21	FLC, POS	16	0.25	**0.25**	0.25				Yes (48)	Azoles (FLC)	Alive
13/8/2009	*C. tropicalis*	Glioblastoma	VRC	5	FLC, POS	8	0.12	0.12	0.25				Yes (72)	No	Deceased
13/5/2010	*C. albicans*	Uncontrolled diabetes	ANF	19	FLC, ITC, POS	16	1	0.12	1				No	Azoles (FLC)	Alive
22/12/2010	*C. orthopsilosis*[g]	Pharyngeal cancer	CAS	31	FLC, VRC	8	0.12	0.25	0.06				Yes (48)	Azoles (FLC) and candins (CAS)	Alive
10/10/2011	*C. orthopsilosis*[g]	Renal transplantation	ANF	18	FLC, VRC	8	0.12	0.25	0.12				Yes (24)	Azoles (FLC)	Alive
8/1/2013	*C. glabrata*	Thyroiditis	CAS	4	FLC, ITC, VRC, POS	256	16	4	>8				No	Azoles (FLC)	Deceased
21/4/2008	*C. albicans*	Systemic lupus erythematosus	FLC	23	CAS					4	1	1	No	Candins (CAS)	Alive
6/10/2008	*C. guilliermondii*	Preterm newborn	AMB	17	CAS					**4**	**4**	2	Yes (48)	No	Alive
3/8/2010	*C. krusei*	Acute lymphoblastic leukemia	AMB	4	CAS					1	0.25	0.12	Yes (72)	Candins (CAS)	Deceased

[a] All patients who died within 7 days after infection were not treated appropriately with respect to the duration of antifungal therapy.

[b] Resistant isolates (with the non-WT phenotype) include isolates from normally susceptible *Candida* species that showed antifungal MICs above the CBPs or ECVs used in this study (see the text for details). All 16 *C. krusei* isolates studied (MIC range, 16 to 64 μg/ml) were considered intrinsically fluconazole resistant and also are not listed. Nonsusceptible includes either intermediate or resistant to echinocandins.

[c] The MICs of the following antifungal agents were determined: fluconazole, itraconazole; voriconazole, posaconazole, amphotericin B, caspofungin, anidulafungin, and micafungin. Boldface denotes intermediate susceptibility. The isolates of *C. albicans* and *C. guilliermondii* (both with caspofungin MICs of 4 μg/ml) were tested for susceptibility to anidulafungin and micafungin only later, because the two echinocandins were not available at the date of their isolation (the year 2008).

[d] Adequate source control was defined as removal of any preexisting central vein catheters or other fluid collections thought to be the source of *Candida* infection within 48 h of the onset of BSI, as determined by the positivity of the patient's blood culture(s) (47).

[e] Deaths were recorded at ≤8 days (for 5 patients) and 11 days (for 1 patient) after the first positive blood culture, whereas survival at 30 and 45 days was recorded for the remaining patients.

[f] Isolates from the *C. parapsilosis* species complex could be not differentiated as *C. parapsilosis sensu stricto*, *Candida metapsilosis*, or *C. orthopsilosis*, because MALDI-TOF mass spectrometry was not available in the clinical microbiology laboratory until the end of 2009.

[g] Two isolates were identified as *C. orthopsilosis* by MALDI-TOF mass spectrometry. These isolates were classified as WT for susceptibility to itraconazole (MIC, 0.12 μg/ml) using the ECV of ≤0.5 established for *C. parapsilosis sensu lato* (29).

[h] Abbreviations: FLC, fluconazole; ITC, itraconazole; VRC; voriconazole; POS, posaconazole; AMB, amphotericin B; CAS, caspofungin; ANF, anidulafungin; MCF, micafungin; COPD, chronic obstructive pulmonary disease; ND, not determined.

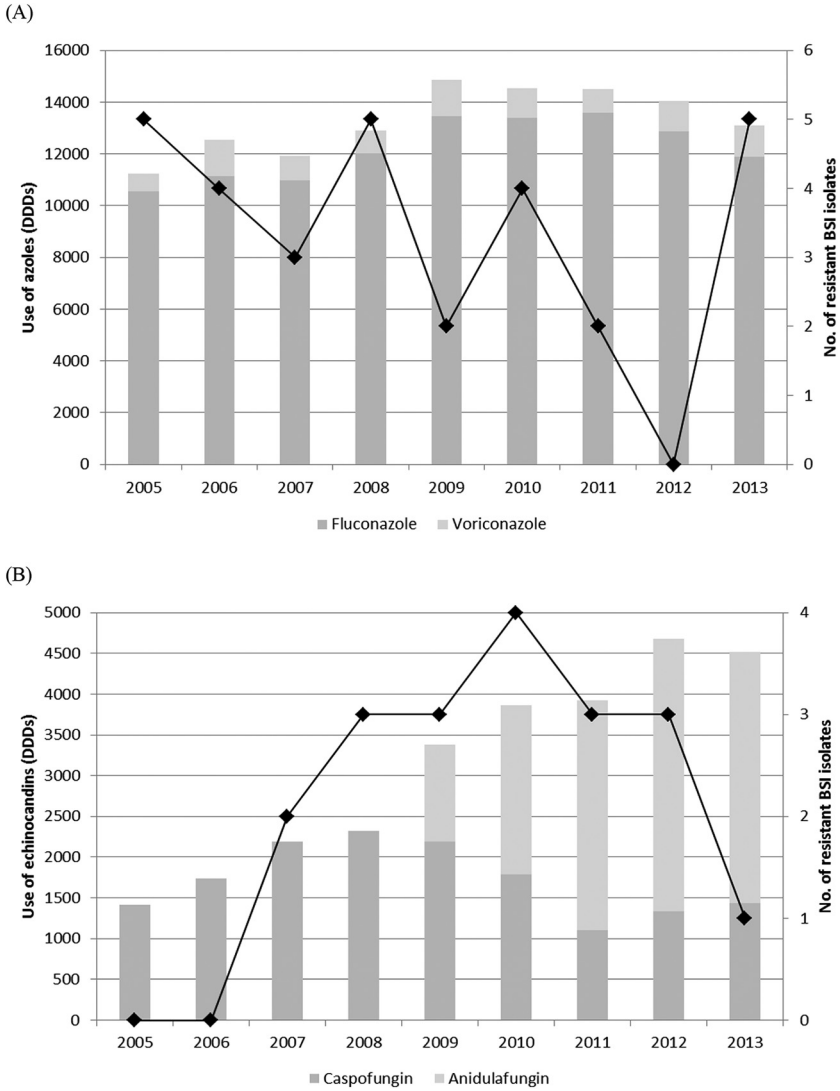

FIG 1 Trends of azole (A) and echinocandin (B) consumption (in DDDs) in the UCSC hospital over the study period (2005 to 2013). The overall distribution of BSI episodes caused by *Candida* and non-*Candida* isolates with intrinsic or acquired fluconazole (A) or echinocandin (B) resistance in the same years is denoted by a black line.

caused by *Candida* isolates found to be nonsusceptible to an azole(s) or echinocandin(s) *in vitro*. Among 14 patients infected with fluconazole-resistant *Candida* species, 13 (92.8%) were adults (age range, 22 to 91 years) and 8 (57.1%) were male. One of three patients with a BSI caused by an echinocandin-resistant *Candida* species was a newborn who was infected with *C. guilliermondii*. Excluding the last patient and 3 other patients (1 infected with *C. albicans*, 1 infected with the *C. parapsilosis* complex, and 1 infected with *C. tropicalis*), all the remaining patients had experienced prior exposure to azoles ($n = 9$, 64.2%) or echinocandins ($n = 2$, 14.2%) alone; 1 patient (infected with *C. orthopsilosis*) had previously been treated with either an azole or an echinocandin antifungal agent, and another patient (infected with an isolate of the *C. parapsilosis* complex) had previously been treated with either amphotericin B or azoles (both fluconazole and itraconazole). Six of 17 patients died, and in 5 of these patients the death occurred ≤8 days after initiation of antifungal therapy. Three of 14 patients infected with fluconazole-resistant isolates were treated with fluconazole, and 2 of them (i.e., 1 with a *C. lusitaniae* BSI and 1 with a *C. albicans* BSI) died after only 7 and 10 days of antifungal therapy, respectively; the third patient (with a *C. parapsilosis* complex BSI) survived after 15 days of antifungal therapy. The patients infected with echinocandin-nonsusceptible isolates were treated with fluconazole (1 patient) and amphotericin B (2 patients), but in one of them (i.e., the patient infected with *C. krusei*), the amphotericin B therapy was administered only for 4 days because the death occurred early. Furthermore, 6 of 17 patients had not received adequate control of the source infection, and half of these patients did not survive, according to previously published observations (47). The 17 BSI episodes described in Table 3 were distributed uniformly over the time period from 14 November 2005 to 8 January 2013, with 3 episodes (in the years 2005 and 2008) to 1 episodes (in the years 2006, 2007, 2009, and 2010) occurring per year (Fig. 1). This was despite the persistently high rate of fluconazole consumption during the study period (10,542 DDDs in 2005 to 11,889 DDDs in 2013); in contrast, the

hospital use of echinocandins had greatly increased during the same 9-year period, ranging from 1,414 DDDs (only caspofungin) in 2005 to 4,522 DDDs (both caspofungin and anidulafungin) in 2013. Of note, the echinocandin DDD ratio, which was 1.8 for caspofungin/anidulafungin in 2009, was noticed to reverse in favor of anidulafungin in 2010 and to reach values of 2.5 in 2011, which remained stable until 2013 (Fig. 1). No DDDs of micafungin were shown because this echinocandin was not included in the formulary of the hospital.

A limitation of the present study is that no comparisons with the CLSI broth microdilution methods were made, but previous studies have documented that antifungal MICs generated by the SYO are in good essential agreement with those obtained by the CLSI methodology, from which SYO is adapted (48, 49). However, the categorical agreement may be lower, especially for some fungal species-antifungal drug combinations (19). We applied the CLSI CBPs where applicable, yet we were aware that the SYO method should really be employed to screen fungal isolates showing high MICs of antifungal agents. In this context, ECVs for *Candida* species based on the SYO method have been set up, but though they are within 1 2-fold dilution of those determined by the CLSI reference method (41, 50), they need to be further validated for routine use.

In conclusion, the present study shows that the development of secondary antifungal resistance among common *Candida* species is not a growing threat in our hospital but that the emergence of *Candida* or non-*Candida* species with intrinsically reduced susceptibility or resistance needs to be continuously monitored. This emphasizes the necessity to perform locally relevant epidemiological studies as well as antifungal susceptibility studies, which in turn will reinforce the role of the clinical microbiology laboratory in assisting clinicians with the treatment of invasive fungal infections.

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