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ORIGINAL ARTICLE

Distribution and drug susceptibilities of *Candida* species causing candidemia from a medical center in central Taiwan

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Abstract Invasive fungal infections have increased significantly in the past few decades because of the increase in high-risk populations. To investigate the distribution and drug susceptibilities of such infections, we analyzed all 152 *Candida* isolates causing candidemia from 2004 to 2006 at the China Medical University Hospital, a medical center in central Taiwan. *Candida albicans* was the most common species, accounting for 52.6 % of the isolates, followed by *C. tropicalis* (19.7 %), *C. parapsilosis* (14.5 %), *C. glabrata* (8.6 %), *C. guilliermondii* (3.9 %), and *C. pelliculosa* (0.7 %). All isolates were susceptible to amphotericin B, anidulafungin, micafungin, and voriconazole according to minimum inhibitory concentrations (MICs) after a 24-h incubation; 0.7 %, 6.6 %, and 7.9 % of isolates were resistant to amphotericin B, fluconazole, and voriconazole, respectively, after 48-h incubation. Both *C. albicans* and *C. parapsilosis* had high degrees of agreement for azoles between 24- and 48-h incubation periods, whereas *C. glabrata* (38.5–46.2 %) and *C. tropicalis* (56.7–63.3 %) did not. The majority of the isolates with high azole MICs displayed a trailing growth phenotype. Hence, the MICs of different drugs after 24-h incubation may be considered for prognosis of candidemia.

Keywords Amphotericin B · Azoles · *Candida* species · Candidemia · Drug susceptibility · Echinocandins

Introduction

The epidemiology of invasive fungal infections has become important in the past few decades because of the increased number of immunocompromised patients, the augmentation of invasive medical devices, and the extensive use of broad-spectrum antibiotics [1–3]. *Candida* species are the most frequently isolated fungal pathogens,

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responsible for significant morbidity and mortality [4–9], and the leading causes of bloodstream infections globally [2, 8–14]. According to the results of hospital-based surveillance, European countries reported 0.5–0.7 cases per 10,000 patient-days in association with invasive candidiasis [15, 16], and in the United States, it was 1.5 cases per 10,000 patient-days. Globally, Brazil has the highest reported incidence of 3.7 cases per 10,000 patient-days [11, 17]. On average, it is about 1.5 cases per 10,000 patient-days in Taiwan [18–20].

Hundreds of species of *Candida* have been described, but only 30–40 species among these have been reported to cause diseases in humans [21, 22]. *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, *Candida parapsilosis*, and *Candida krusei* are the five most common species causing candidemia, in combination accounting for more than 95 % of the cases [2, 9, 11, 15].

Azole (fluconazole and voriconazole), echinocandin (anidulafungin, caspofungin, and micafungin), and polyene (amphotericin B) are the three classes of drugs most commonly prescribed for treating systemic fungal infections. The emergence of fungal pathogens resistant to these drugs is a growing concern [3, 23, 24]. Hence, we have conducted studies on 152 *Candida* isolates causing candidemia in central Taiwan, collected from 2004 to 2006, to determine the species distribution and their susceptibility profiles to five antifungal agents (amphotericin B, anidulafungin, fluconazole, micafungin, and voriconazole). We also compared the categorical agreement of minimum inhibitory concentrations (MICs) of amphotericin B, fluconazole, and voriconazole after 24- and 48-h incubation.

Materials and methods

Clinical isolates and patient age groups

All isolates causing candidemia were collected from October 2004 to December 2006 in central Taiwan at the China Medical University Hospital (CMUH), a medical center with approximately 2,000 beds. A total of 152 *Candida* isolates were collected and characterized in the present study. In addition to the sources of isolates, the patient age data were also recorded, and the patients were divided into three groups by age: <18 years, 19–65 years, and >65 years [25, 26].

Identification of isolates

All *Candida* isolates were first identified by the CMUH and reassessed in the laboratory at National Health Research Institutes (NHRI). The identification procedure at NHRI for the candidemia isolates was based on our previous reports [27–29]. All isolates were tested with the VITEK Yeast Biochemical Card (YBC) (bioMérieux, Marcy l'Etoile, France) for species identification. When the YBC identification probability was less than 90 % or when the identification of an organism was inconsistent between the hospital and the NHRI laboratories, sequences of the internal transcribed spacer (ITS) region and/or the D1/D2 region of ribosomal DNA were used for species identification. The ITS region was amplified with the primers ITS1, 5′-TCCGTAGGTGAACCTGCGG-3, and ITS4 5′-TCCTCCGCTTATTGATATGC-3′, and the D1/D2 region was amplified with the primers NL1 5′-GCATATCAAT AAGCGGAGGAAAAG-3′ and NL4 5′-GGTCCGTG TTTCAAGACGG-3′ [30, 31].

Antifungal susceptibility testing

The MICs of the five agents were determined by the same in vitro antifungal susceptibility testing established in our laboratory [32], according to the guidelines of M27-A3 recommended by the Clinical and Laboratory Standards Institute (CLSI) [33]. RPMI medium 1640 (31800-022; Gibco BRL, Gaithersburg, MD, USA) was used for growth and dilution of the yeasts. Strains from the American Type Culture Collection (ATCC), including *C. albicans* (ATCC 90028), *C. krusei* (ATCC 6258), and *C. parapsilosis* (ATCC 22019), were used as the standard controls. Growth of each isolate was measured by the Biotrak II plate spectrophotometric reader (Amersham Biosciences, Biochrom, Cambridge, England) after incubation at 35 °C for 24 h for all five drugs and 48 h for amphotericin B, fluconazole, and voriconazole.

Standard powders of amphotericin B kindly provided by Bristol Myers Squibb, anidulafungin, fluconazole, and voriconazole by Pfizer, and micafungin by Astellas Pharms were dissolved in dimethyl sulfoxide (DMSO). The final concentrations of anidulafungin, micafungin, and voriconazole were 0.0156–8 mg/l, amphotericin B, 0.0313–16 mg/l, and fluconazole, 0.125–64 mg/l.

The newly defined species-specific breakpoints after 24-h incubation for the five common *Candida* species *C. albicans*, *C. krusei*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* were applied in the present study [34]. For the species for which clinical breakpoints have not been established, we applied epidemiological cutoff values instead [34].

The MICs were defined as the concentration of drugs capable of reducing the turbidity of cells to greater than 50 % for anidulafungin, fluconazole, micafungin, and voriconazole and completely inhibiting cell growth for amphotericin B. The MICs of 50 % and 90 % of the total population were defined as MIC_{50} and MIC_{90}, respectively.

The epidemiological cutoff value of amphotericin B was 2 mg/l. For fluconazole, with *C. albicans*, *C. tropicalis*, and

C. parapsilosis after 24-h incubation, MICs ≤2 mg/l were considered to be susceptible, ≥8 mg/l resistant, and 4 mg/l susceptible-dose dependent (S-DD); for *C. glabrata*, MICs ≤32 mg/l were susceptible and ≥64 mg/l resistant after 24 h. The epidemiological cutoff values for *Candida guilliermondii* and *Candida pelliculosa* were 8 and 4 mg/l, respectively. When the incubations were extended to 48 h, then MICs ≤8 mg/l were susceptible, ≥64 mg/l resistant, and 16–32 mg/l S-DD for all species.

For voriconazole, with *C. albicans*, *C. tropicalis*, and *C. parapsilosis*, MICs ≤0.125 mg/l were susceptible, ≥1 mg/l resistant, and 0.25–0.5 mg/l intermediate after 24-h incubation. The epidemiological cutoff value for *C. guilliermondii* and *C. pelliculosa* was the same, 0.25 mg/l. MICs <1 mg/l were susceptible, 2 mg/l intermediate, and ≥4 mg/l resistant after 48 h.

Susceptibilities to echinocandins were suggested to be determined after 24-h incubation. For *C. albicans* and *C. tropicalis*, MICs ≤0.25 mg/l were susceptible, ≥1 mg/l resistant, and 0.5 mg/l intermediate. For *C. parapsilosis* and *C. guilliermondii*, the breakpoints were changed to <2 mg/l susceptible, 4 mg/l intermediate, and >8 mg/l resistant. For anidulafungin, with *C. glabrata*, MICs ≤0.125 mg/l were susceptible, ≥0.5 mg/l resistant, and 0.25 mg/l intermediate. For micafungin, MICs ≤0.06 mg/l were susceptible, ≥0.25 mg/l resistant, and 0.125 mg/l intermediate.

Data analysis

Categorical agreement (CA) was assigned to compare the susceptibility testing results with the MIC values at 24 h and at 48 h for amphotericin B, fluconazole, and voriconazole that fell within the same interpretive categories. Agreement was defined as discrepancies between each MIC pair compared within ±1 twofold dilution. We developed the saw-like calculation to describe the CA by using R statistics software (R Foundation for Statistical Computing, Vienna, Austria). The statistical significance of the differences in frequencies and proportions was determined by the chi-square test with Mantel–Haenszel correction.

Results

Species distribution

We recovered 15, 69, and 68 isolates in 2004, 2005, and 2006, respectively. A total of 9 isolates were speciated differently by the hospital and the NHRI laboratories: 3 *C. tropicalis* isolates were identified as *Candida intermedia* by CMUH, 2 *C. glabrata*, 1 *C. guilliermondii*, and 1 *C. tropicalis* as *C. albicans*, 1 *C. parapsilosis* as *C. sake*, and 1 *C. parapsilosis* as *C. tropicalis*. VITEK failed to identify 14 isolates, including 8 *C. parapsilosis*, 5 *C. guilliermondii*, and 1 *C. tropicalis*. All these 23 isolates were speciated according to their rDNA sequences.

Of the 152 isolates, *C. albicans* was the most common species, accounting for 52.6 %, followed by *C. tropicalis* (19.7 %), *C. parapsilosis* (14.5 %), *C. glabrata* (8.6 %), *C. guilliermondii* (3.9 %), and *C. pelliculosa* (0.7 %) (Table 1). Patient age ranged from 1 to 91 years (average age, 58 ± 24.7 years). Of the isolates, 11.2 %, 39.5 %, and 49.3 % were recovered from patients in the age groups of ≤18, 19–65, and ≥66 years, respectively (Table 2). Among different species, the majority of the *C. tropicalis* isolates (18/30, 60 %; $p = 0.04$) were recovered from patients in the age group 19–65 years. More *C. parapsilosis* were recovered from younger patients than from other age groups (10/17 vs. 12/135; $p < 0.001$). Among the 17 isolates collected from patients aged ≤18 years, 10 (58.8 %) were *C. parapsilosis*.

Susceptibility to amphotericin B

The range of amphotericin B MICs of the 152 isolates was from 0.03 to 1 mg/l and 0.125 to 4 mg/l after 24- and 48-h incubations, respectively (Table 1). The MIC_{50} values for amphotericin B were 0.125 and 0.5 mg/l after 24- and 48-h incubations, respectively. Only 1 isolate of *C. glabrata* had an 4 mg/l amphotericin B MIC after 48-h incubation.

Susceptibility to azole drugs

The range of fluconazole MICs of the 152 isolates was from 0.125 to 64 mg/l (Table 1). The values of MIC_{50} for fluconazole were 0.125 and 0.25 mg/l after 24- and 48-h incubations, respectively. One *C. parapsilosis* isolate (8 mg/l MIC) was resistant to fluconazole after 24-h incubation. Nevertheless, there were 10 isolates, including 7 *C. tropicalis*, and 1 each *C. albicans*, *C. glabrata*, and *C. parapsilosis*, with fluconazole MICs ≥64 mg/l after 48-h incubation. The range of voriconazole MICs of the 152 isolates was 0.03–0.5 mg/l after 24-h incubation and 0.03–16 mg/l after 48-h incubation (Table 1). The values of MIC_{50} for voriconazole remained the same, staying at 0.03 mg/l, after 24- and 48-h incubations. No isolate was resistant to voriconazole according to the MICs after 24-h incubation. After 48-h incubation, 10 *C. tropicalis* and 2 *C. albicans* had voriconazole MICs of 16 mg/l.

Susceptibility to echinocandins

MICs of echinocandins were determined after 24-h incubation only [35]. The values of MIC_{50} for echinocandin of

Table 1 Susceptibilities to various antifungal drugs of the 152 isolates causing candidemia

Type of drug	Drug	Incubation (h)	MIC (mg/l)				
			Range	MIC_{50}	MIC_{90}	%R	%CA
Total ($n = 152$)							
Polyene	AMB	24	0.03–1	0.125	1	0	72.4
		48	0.125–4	0.5	1	0.7	
Azole	FLU	24	0.125–32	0.125	2	1.3	88.8
		48	0.125–64	0.25	8	6.6	
	VOR	24	0.03–0.5	0.03	0.03	0	82.2
		48	0.03–16	0.03	0.5	7.9	
Echinocandin	ANI	24	0.016–2	0.016	0.5	0	94.7
	MICA	24	0.016–1	0.016	0.25	0	95.4
Candida albicans ($n = 80$)							
Polyene	AMB	24	0.03–1	0.125	0.5	0	76.3
		48	0.25–1	0.5	1	0	
Azole	FLU	24	0.125–1	0.125	0.25	1.2	90.0
		48	0.125–64	0.125	1	1.2	
	VOR	24	0.03	0.03	0.03	0	91.3
		48	0.03–16	0.03	0.06	2.5	
Echinocandin	ANI	24	0.016–0.03	0.016	0.016	0	95.0
	MICA	24	0.016	0.016	0.016	0	97.5
Candida tropicalis ($n = 30$)							
Polyene	AMB	24	0.125–0.5	0.125	0.5	0	56.7
		48	0.25–1	0.5	1	0	
Azole	FLU	24	0.125–0.25	0.125	0.25	0	63.3
		48	0.125–64	0.5	64	23.3	
	VOR	24	0.03	0.03	0.03	0	56.7
		48	0.03–16	0.03	16	33.3	
Echinocandin	ANI	24	0.016–0.06	0.03	0.03	0	93.3
	MICA	24	0.016–0.03	0.016	0.016	0	93.3
Candida parapsilosis ($n = 22$)							
Polyene	AMB	24	0.06–1	0.125	0.5	0	68.2
		48	0.125–2	0.5	1	4.5	
Azole	FLU	24	0.125–8	0.5	2	4.5	95.5
		48	0.25–64	0.5	2	4.5	
	VOR	24	0.03–0.125	0.03	0.03	0	95.5
		48	0.03–0.5	0.03	0.125	0	
Echinocandin	ANI	24	0.06–2	0.5	1	0	86.4
	MICA	24	0.016–1	25	1	0	72.7
Candida glabrata ($n = 13$)							
Polyene	AMB	24	0.125–1	1	1	0	92.3
		48	0.5–4	1	2	15.4	
Azole	FLU	24	1–32	2	4	0	46.2
		48	1–64	8	16	7.7	
	VOR	24	0.03–0.5	0.03	0.06	0	38.5
		48	0.03–2	0.25	0.5	0	
Echinocandin	ANI	24	0.03–0.06	0.06	0.06	0	100
	MICA	24	0.016–0.03	0.016	0.016	0	100
Others ($n = 7$)							
Polyene	AMB	24	0.06–0.25	0.125	0.25	0	ND

Table 1 continued

Type of drug	Drug	Incubation (h)	MIC (mg/l)				
			Range	MIC_{50}	MIC_{90}	%R	%CA
		48	0.25–0.5	0.25	0.5	0	ND
Azole	FLU	24	1–8	2	8	0	ND
		48	1–16	4	16	0	ND
	VOR	24	0.03–0.06	0.03	0.06	0	ND
		48	0.03–0.25	0.06	0.25	0	ND
Echinocandin	ANI	24	0.016–2	0.25	2	0	ND
	MICA	24	0.016–0.25	0.125	0.25	0	ND

h hours, *MIC* minimum inhibitory concentration, *n* number of isolates, *%R* percent resistant, *%CA* percent categorical agreement, *AMB* amphotericin B, *ANI* anidulafungin, *FLU* fluconazole, *MICA* micafungin, *VOR* voriconazole

Table 2 Distribution of *Candida* species according to the age of patients

Age (years)	*C. albicans*	*C. tropicalis*	*C. glabrata*	*C. parapsilosis*	Others	Total
≤18	5 (29.4)[a]	1 (5.9)	0	10 (58.8)*	1 (5.9)	17
19–65	30 (50)	18 (30)*	3 (5)	5 (8.3)	4 (6.7)	60
≥66	45 (60)	11 (14.7)	10 (13.3)	7 (9.3)	2 (2.7)	75
Total	80 (52.6)	30 (19.7)	13 (8.6)	22 (14.5)	6 (3.9)	152

[a] Number of isolates (percentage in each age group)

*p-value less than 0.05, considered significant

the 152 isolates ranged from 0.016 to 2 mg/l (Table 1). The MIC_{50s} of both anidulafungin and micafungin were the same at 0.016 mg/l. All isolates were susceptible to both anidulafungin and micafungin.

Categorical agreement between 24- and 48-h incubations

According to the guidelines, the interpretations of drug susceptibilities were not completely consistent after 24- and 48-h incubations. In the present study, we used categorical agreement to test the consistency between the MICs after 24- and 48-h incubations. Of the 152 isolates, consistency for azoles ranged from 82.2 % to 88.8 %, and for amphotericin B, it was 72.4 %. Even though the agreement was not perfect for amphotericin B, the majority of the 152 isolates were susceptible to amphotericin B after 24- and 48-h incubations. The agreement of azoles between the two incubation periods varied in different species. It showed good agreement for *C. albicans* (90–91.3 %), and *C. parapsilosis* (95.5 %). In contrast, agreement was poor for *C. glabrata* (38.5–46.2 %) and *C. tropicalis* (56.7–63.3 %).

Discussion

In the present study, 95 % of the 152 isolates belonged to one of the four major species: *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*. Although the prevalence of candidemia caused by non-*albicans Candida* species has increased, *C. albicans* is still the most common species causing candidemia, which is consistent with most reports [2, 8, 9, 36]. Among the phenomena associated with drug resistance, 'trailing' describes the reduced but persistent growth that some isolates exhibit at drug concentrations above the MIC in broth dilution tests [29, 37]. When the MIC of an isolate after 48-h incubation is approximately fourfold higher than that at the 24-h point, the isolate is defined to have trailing growth [29, 37]. In the present study, the trailing growth phenotype was more prevalent in *C. tropicalis* than in other species, which is consistent with our previous findings in a national surveillance, Taiwan Surveillance of Antimicrobial Resistance of Yeasts (TSARY) [29]. In the present study, no *C. albicans* was detected to be azole resistant after 24-h incubation. Hence, the isolates with high MICs after 48-h incubation may be caused by trailing growth. Furthermore, a "true" azole-resistant *C. albicans* isolated from blood was not detected in TSARYs, in 2006 and 2010 [29, 38].

The observation in the present study that a high prevalence of infections are caused by *C. parapsilosis* in younger patients is consistent with the previous finding that *C. parapsilosis* was the most common species causing invasive candidiasis in children [36].

The prevalence of non-*albicans Candida* species varies among different areas. In the present study, *C. tropicalis* was

the most common non-*albicans Candida* species causing candidemia, which is consistent with previous reports in Taiwan [4, 6, 9], as well as in other areas, such as Brazil (21.7 %) [39]. In contrast, *C. glabrata* is the most common non-*albicans Candida* species causing candidemia in Western countries. For example, in the study conducted by Pfaller et al. [40], among the 4,067 isolates causing candidemia, 26.7 % were *C. glabrata*, whereas only 8.7 % were *C. tropicalis*. In Taiwan, the low prevalence of candidemia was caused by *C. glabrata*. The majority of *C. glabrata* collected in TSARYs were from urine [28, 29, 40]. Thus, only 11.2 % (55/492) of cases of candidemia were caused by *C. glabrata* [9, 39]. In the present study, only 8.6 % of the 152 isolates were *C. glabrata*.

No *C. krusei* was collected in the present study. The prevalence of candidemia caused by *C. krusei* in Taiwan was 0.8 % (4/492) in the previous three TSARYs [9, 38], 0.5 % in a medical center in southern Taiwan [4], and none in northern Taiwan [6]. In the study of Pfaller et al. [40], among the 4,067 isolates causing candidemia, 3.4 % were *C. krusei*. Furthermore, the prevalence of candidemia caused by *C. krusei* was 3.5 % in another study conducted in Brazil from 2006 to 2010. Hence, the prevalence of candidemia caused by *C. krusei* in Taiwan is lower than that in other areas [8/1,615 (0.5 %) vs. 149/4,380 (3.4 %); $p < 0.001$]. Azole prophylaxis is one risk factor for *C. glabrata* and *C. krusei* infections [41]. The low prevalence of *C. glabrata* and *C. krusei* infections in Taiwan may be a result of infrequent fluconazole prophylaxis [42].

Epidemiological cutoff values and species-specific clinical breakpoints for fluconazole [43], voriconazole [44], and the echinocandins [35] for some *Candida* species have been established to minimize the trailing and to support a shorter time for reporting MICs. In conclusion, our results suggest that the MICs of different drugs after 24-h incubation may be adequate for predicting the prognosis of candidemia.

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Conflict of interest The authors declare that they have no conflict of interest.

References

1. Pappas PG, Kauffman CA, Andes D, Benjamin DK Jr, Calandra TF, Edwards JE Jr, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin Infect Dis. 2009;48:503–35.
2. Pfaller MA, Diekema DJ. Epidemiology of invasive mycoses in North America. Crit Rev Microbiol. 2010;36:1–53.
3. Yang YL, Lo HJ. Mechanisms of antifungal agent resistance. J Microbiol Immunol Infect. 2001;34:79–86.
4. Chen PL, Lo HJ, Wu CJ, Lee HC, Chang CM, Lee NY, et al. Species distribution and antifungal susceptibility of blood *Candida* isolates at a tertiary hospital in southern Taiwan, 1999–2006. Mycoses. 2011;54:e17–23.
5. Chen S, Slavin M, Nguyen Q, Marriott D, Playford EG, Ellis D, et al. Active surveillance for candidemia, Australia. Emerg Infect Dis. 2006;12:1508–16.
6. Cheng MF, Yu KW, Tang RB, Fan YH, Yang YL, Hsieh KS, et al. Distribution and antifungal susceptibility of *Candida* species causing candidemia from 1996 to 1999. Diagn Microbiol Infect Dis. 2004;48:33–7.
7. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007;20:133–63.
8. Warnock DW. Trends in the epidemiology of invasive fungal infections. Nippon Ishinkin Gakkai Zasshi. 2007;48:1–12.
9. Yang YL, Cheng MF, Wang CW, Wang AH, Cheng WT, Lo HJ, et al. The distribution of species and susceptibility of amphotericin B and fluconazole of yeast pathogens isolated from sterile sites in Taiwan. Med Mycol. 2010;48:328–34.
10. Chuang YC, Chen YC, Chang SC, Sun CC, Chang YY, Chen ML, et al. Secular trends of healthcare-associated infections at a teaching hospital in Taiwan, 1981–2007. J Hosp Infect. 2010;76:143–9.
11. Colombo AL, Nucci M, Park BJ, Nouer SA, Arthington-Skaggs B, da Matta DA, et al. Epidemiology of candidemia in Brazil: a nationwide sentinel surveillance of candidemia in eleven medical centers. J Clin Microbiol. 2006;44:2816–23.
12. Edwards EJJ. *Candida* species. In: Mandell GL, Bennett JE, Dolin R, editors. Principles and practice of infectious diseases. New York: Churchill Livingstone; 1995. p. 2289–306.
13. Ruan SY, Hsueh PR. Invasive candidiasis: an overview from Taiwan. J Formos Med Assoc. 2009;108:443–51.
14. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis. 2004;39:309–17.
15. Almirante B, Rodriguez D, Park BJ, Cuenca-Estrella M, Planes AM, Almela M, et al. Epidemiology and predictors of mortality in cases of *Candida* bloodstream infection: results from population-based surveillance, Barcelona, Spain, from 2002 to 2003. J Clin Microbiol. 2005;43:1829–35.
16. Marchetti O, Bille J, Fluckiger U, Eggimann P, Ruef C, Garbino J, et al. Epidemiology of candidemia in Swiss tertiary care hospitals: secular trends, 1991–2000. Clin Infect Dis. 2004;38:311–20.
17. Hajjeh RA, Sofair AN, Harrison LH, Lyon GM, Arthington-Skaggs BA, Mirza SA, et al. Incidence of bloodstream infections due to *Candida* species and in vitro susceptibilities of isolates collected from 1998 to 2000 in a population-based active surveillance program. J Clin Microbiol. 2004;42:1519–27.
18. Chen LY, Liao SY, Kuo SC, Chen SJ, Chen YY, Wang FD, et al. Changes in the incidence of candidaemia during 2000–2008 in a tertiary medical centre in northern Taiwan. J Hosp Infect. 2011;78:50–3.
19. Liu CY, Liao CH, Chen YC, Chang SC. Changing epidemiology of nosocomial bloodstream infections in 11 teaching hospitals in Taiwan between 1993 and 2006. J Microbiol Immunol Infect. 2010;43:416–29.
20. Wu CJ, Lee HC, Lee NY, Shih HI, Ko NY, Wang LR, et al. Predominance of gram-negative bacilli and increasing antimicrobial resistance in nosocomial bloodstream infections at a university hospital in southern Taiwan, 1996–2003. J Microbiol Immunol Infect. 2006;39:135–43.

21. Johnson EM. Rare and emerging *Candida* species. Curr Fungal Infect Rep. 2009;3:152–9.
22. Richardson MD. Opportunistic and pathogenic fungi. J Antimicrob Chemother. 1991;28(suppl A):1–11.
23. Sanglard D, Odds FC. Resistance of *Candida* species to antifungal agents: molecular mechanisms and clinical consequences. Lancet Infect Dis. 2002;2:73–85.
24. White TC, Marr KA, Bowden RA. Clinical, cellular, and molecular factors that contribute to antifungal drug resistance. Clin Microbiol Rev. 1998;11:382–402.
25. Yang YL, Cheng HH, Lo HJ. Distribution and antifungal susceptibility of *Candida* species isolated from different age populations in Taiwan. Med Mycol. 2006;44:237–42.
26. Yang YL, Hsieh LY, Wang AH, Lo HJ. Characterization of *Candida* species from different populations in Taiwan. Mycopathologia. 2011;172:131–9.
27. Lo HJ, Ho AH, Ho M. Factors accounting for mis-identification of *Candida* species. J Microbiol Immunol Infect. 2001;34:171–7.
28. Yang YL, Li SY, Cheng HH, Lo HJ. Susceptibilities to amphotericin B and fluconazole of *Candida* species in TSARY 2002. Diagn Microbiol Infect Dis. 2005;51:179–83.
29. Yang YL, Wang AH, Wang CW, Cheng WT, Li SY, Lo HJ, et al. Susceptibilities to amphotericin B and fluconazole of *Candida* species in TSARY 2006. Diagn Microbiol Infect Dis. 2008;61:175–80.
30. Leaw SN, Chang HC, Barton R, Bouchara JP, Chang TC. Identification of medically important *Candida* and non-*Candida* yeast species by an oligonucleotide array. J Clin Microbiol. 2007;45:2220–9.
31. Leaw SN, Chang HC, Sun HF, Barton R, Bouchara JP, Chang TC. Identification of medically important yeast species by sequence analysis of the internal transcribed spacer regions. J Clin Microbiol. 2006;44:693–9.
32. Yang YL, Ho YA, Cheng HH, Ho M, Lo HJ. Susceptibilities of *Candida* species to amphotericin B and fluconazole: the emergence of fluconazole resistance in *Candida tropicalis*. Infect Control Hosp Epidemiol. 2004;25:60–4.
33. Clinical Laboratory Standard Institute. Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard-third edition. CLSI document M27-A3. Wayne, PA: Clinical Laboratory Standard Institute; 2008.
34. Pfaller MA, Diekema DJ. Progress in antifungal susceptibility testing of *Candida* spp. by use of Clinical and Laboratory Standards Institute broth microdilution methods, 2010 to 2012. J Clin Microbiol. 2012;50:2846–56.
35. Pfaller MA, Diekema DJ, Andes D, Arendrup MC, Brown SD, Lockhart SR, 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 Update. 2011;14:164–76.
36. Peman J, Canton E, Linares-Sicilia MJ, Rosello EM, Borrell N, Ruiz-Perez-de-Pipaon MT, et al. Epidemiology and antifungal susceptibility of bloodstream fungal isolates in pediatric patients: a Spanish multicenter prospective survey. J Clin Microbiol. 2011;49:4158–63.
37. Arthington-Skaggs BA, Lee-Yang W, Ciblak MA, Frade JP, Brandt ME, Hajjeh RA, et al. Comparison of visual and spectrophotometric methods of broth microdilution MIC end point determination and evaluation of a sterol quantitation method for in vitro susceptibility testing of fluconazole and itraconazole against trailing and nontrailing *Candida* isolates. Antimicrob Agents Chemother. 2002;46:2477–81.
38. Yang YL, Chen HT, Lin CC, Chu WL, Lo HJ, TSARY Hospitals. Species distribution and drug susceptibilities of *Candida* isolates in TSARY 2010. Diagn Microbiol Infect Dis. 2013;76:182–6.
39. Moretti ML, Trabasso P, Lyra L, Fagnani R, Resende MR, de Oliveira Cardoso LG, et al. Is the incidence of candidemia caused by *Candida glabrata* increasing in Brazil? Five-year surveillance of *Candida* bloodstream infection in a university reference hospital in southeast Brazil. Med Mycol. 2013;51:225–30.
40. Pfaller M, Neofytos D, Diekema D, Azie N, Meier-Kriesche HU, Quan SP, et al. Epidemiology and outcomes of candidemia in 3648 patients: data from the Prospective Antifungal Therapy (PATH Alliance(R)) registry, 2004–2008. Diagn Microbiol Infect Dis. 2012;74:323–31.
41. Krcmery V, Barnes AJ. Non-*albicans Candida* spp. causing fungaemia: pathogenicity and antifungal resistance. J Hosp Infect. 2002;50:243–60.
42. Cheng MF, Yang YL, Yao TJ, Lin CY, Liu JS, Tang RB, et al. Risk factors for fatal candidemia caused by *Candida albicans* and non-*albicans Candida* species. BMC Infect Dis. 2005;5:22.
43. 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 Update. 2010;13:180–95.
44. Pfaller MA, Andes D, Arendrup MC, Diekema DJ, Espinel-Ingroff A, Alexander BD, 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;70:330–43.