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Distribution and antifungal susceptibility of *Candida* species causing candidemia from 1996 to 1999

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Abstract

Susceptibilities to amphotericin B and fluconazole of 383 *Candida* species isolated from blood were determined. *Candida albicans* was the most common species (55.6%), followed by *Candida parapsilosis* (17.5%), *Candida tropicalis* (16.5%), *Candida glabrata* (5.2%), *Candida guilliermondii* (2.3%), and others (2.9%). All but three isolates, *Candida ciferrii*, *C. tropicalis*, and *C. glabrata*, one each, were susceptible to amphotericin B. A total of 367 (95.8%) and 15 (4.2%) isolates were susceptible and susceptible-dose dependent to fluconazole, respectively. Only one isolate, a *C. glabrata*, was resistant to fluconazole. Few patients (13%) having prior fluconazole treatments may explain the low rate of resistance to fluconazole in this study.

Keywords: *Candida* species; Candidemia; Antifungal susceptibility; Resistance

1. Introduction

Candida species now rank as the fourth most common cause of nosocomial bloodstream infections in the United States (Jarvis, 1995). Non-albicans *Candida* species, including uncommon *Candida krusei*, *Candida guilliermondii*, and *Candida ciferrii*, have played an important role in candidemia in the past decade (de Gentile et al., 1991; Furman and Ahearn, 1983; Pfaller et al., 2003). The increase in the proportion of bloodstream infections by non-albicans *Candida* species is likely to associate with following causes: improvement in diagnostic procedures; increasing number of critically ill patients; surgical procedures; cytotoxic therapy with prolonged neutropenia; other immunosuppressive therapies; use of broad-spectrum antibiotics and indwelling invasive devices; and intensive care support (Yang and Lo, 2001; Verduyn Lunel et al., 1999).

Due to differences in antifungal practices and infection control strategies, there are some variations in the distribution of *Candida* species and their susceptibility to fluconazole among isolates from different institutions, localities, and countries (Sanglard and Odds, 2002; St. Germain et al., 2001). Totally, 6% of *Candida* species were fluconazole resistant in one of teaching hospitals in Taiwan and in India (Hsueh et al., 2002; Chakrabarti et al., 2002). Among 88 non-albicans *Candida* species causing candidemia, 17% were fluconazole resistant in the Slovak Republic (Kovacicova et al., 2000). In order to understand the spectrum of *Candida* species involved and the emergence of antifungal resistance, we retrospectively surveyed the clinical *Candida* species isolated from blood over a 4-year period (1996–1999) at Veterans General Hospital-Taipei (VGH-TPE), Taiwan.

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2. Materials and methods

2.1. Strains and media

Strains (one strain per species per patient) of *Candida* species isolated from blood were collected from April 1996 to December 1999 at VGH-TPE, a teaching hospital in Taiwan with 2800 beds. This hospital provides both primary and tertiary medical care, admitting an average of 69,000 patients each year. The isolates were first subcultured on Sabouraud dextrose agar (SDA, BBL, Becton Dickinson and Company, Cockeysville, MD) plates to check for purity. Pure isolates were labeled and stored at −70°C for further analysis. Brain heart infusion agar or broth (BHI, DIFCO, Becton Dickinson and Company, Sparks, MD), SDA plates, and RPMI 1640 (GibcoBRL, Inchinnan, Paisley, UK) were used routinely for inoculation.

Organisms were identified to species level by germ tube test, morphology evaluation on cornmeal-Tween 80 agar, carbohydrate assimilation tests with API -32C strips (bioMérieux Vitek, Inc., Hazelwood, Mo.), and Yeast Biochemical Card (YBC, bioMérieux Vitek, Inc., Hazelwood, Mo.) in the laboratory at VGH-TPE and/or at National Health Research Institutes (NHRI), Taipei, Taiwan.

Of 415 Candida isolates, 30 isolates were not recovered from −70°C and two failed to grow in the RPMI 1640 medium. A total of 383 *Candida* species were enrolled into this study.

2.2. Antifungal susceptibility testing

Susceptibility to antifungal drugs was determined in the laboratory at NHRI. Minimum inhibitory concentrations (MIC) to amphotericin B and fluconazole were determined by a broth microdilution method according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS), approved standard M27-A (National Committee for Clinical Laboratory Standards, 1997). *Candida albicans* ATCC 14053, *C. krusei* ATCC 6258, *Candida parapsilosis* ATCC 22019, and *Candida glabrata* ATCC 9003 were quality control strains in each batch of clinical isolates.

The MIC to amphotericin B was defined as minimum inhibitory concentration of amphotericin B reducing the turbidity to greater than 95%, after 48 h of incubation at 35°C. The MIC to fluconazole was defined at 50% reduction of turbidity. The interpretative breakpoints for both amphotericin B and fluconazole were determined according to the NCCLS guidelines. Isolates with resistance to amphotericin B were defined as MIC ≧2 μg/ml. Resistant, susceptible-dose dependent, and susceptible to fluconazole were defined as MICs ≧64 μg/ml, 16–32 μg/ml, and ≦8 μg/ml, respectively. The MIC of 50% of all isolates was defined as MIC_{50}.

2.3. Clinical data collection

Since there were isolates of *C. albicans, C. ciferrii C. glabrata, C. guilliermondii,* and *C. tropicalis* having fluconazole MICs ≧16 μg/ml, all 92 patients infected with *C. glabrata, C. guilliermondii,* or *C. tropicalis* and two patients infected with either *C. albicans* or *C. ciferrii* who showed fluconazole MICs ≧16 μg/ml were reviewed. The clinical data were collected retrospectively and inclusion criteria for patients were based on the presence of at least one positive blood culture with *Candida* species from April 1996 to December 1999 at VGH-TPE. The predisposing events within 30 days before developing candidemia were evaluated and the laboratory data within seven days that the first positive blood cultures were analyzed.

3. Results

3.1. Distribution of species

A total of 436 fungal pathogens causing fungemia were collected from April 1996 to December 1999 in the microbiology laboratory at VGH-TPE. *Candida* species were the most common pathogens, accounting for 415 isolates (95.3%), followed by 11 (2.5%) *Trichosporon* species, 8 (1.8%) *Cryptococcus neoformans*, 1 (0.2%) *Sporothrix schenckii*, and 1 (0.2%) Zygomycetes. On average, there were 1.3 fungal infections per 1000 discharges during the study period.

Of 383 *Candida* species tested for susceptibility to antifungal agents, *C. albicans* was the most common species (213, 55.6%), followed by *C. parapsilosis* (67, 17.5%), *C. tropicalis* (63, 16.5%), *C. glabrata* (20, 5.2%), *C. guilliermondii* (9, 2.3%), *C. peniculosa* (7, 1.8%), *C. famata* (3, 0.8%), and *C. ciferrii* (1, 0.3%) (Table 1).

3.2. Susceptibility to amphotericin B and fluconazole

The MICs of the *Candida* species to amphotericin B and fluconazole are listed in Table 1. The range of MICs of amphotericin B was 0.25 to 2 μg/ml, and the MIC_{50} was 0.5 μg/ml. Three isolates, one of each *C. ciferrii, C. glabrata, and C. tropicalis* had MICs of amphotericin B equal to 2 μg/ml.

The range of MICs to fluconazole was from 0.125 to 128 μg/ml, and the MIC_{50} was 0.5 μg/ml. A total of 15 (4.2%) isolates were susceptible-dose dependent. They included nine fatality cases (one *C. tropicalis*, two *C. guilliermondii*, and six *C. glabrata*) and six cured cases (one *C. ciferrii*, one *C. albicans*, one *C. glabrata*, and three *C. guilliermondii*). Only one isolate, a *C. glabrata*, was resistant to fluconazole with its MIC equal to 128 μg/ml (Table 2).

Table 1
In vitro susceptibilities of *Candida* species to amphotericin B and fluconazole

Species		Amphotericin B			Fluconazole		
	No. of isolates	MIC (μg/ml)		No. (%) of which MICs $\geq$ 2 μg/ml	MIC (μg/ml)		No. (%) of which MICs $\geq$ 16 μg/ml
		Range (mean)	MIC_{50}		Range (mean)	MIC_{50}	
C. albicans	213	0.25–1 (0.5)	0.5	0	0.125–32 (0.6)	0.25	1 (0.5)
C. parapsilosis	67	0.5–1 (0.7)	0.5	0	0.125–2 (1.1)	1	0
C. tropicalis	63	0.25–2 (0.7)	0.5	1 (1.6)	0.125–8 (1.6)	11	1 (1.6)
C. glabrata	20	0.5–2 (1.0)	1	1 (5)	2–128 (16.5)	8	8 (40)
C. guilliermondii	9	0.25–0.5 (0.4)	0.25	0	2–32 (12.2)	16	5 (55.5)
C. peniculosa	7	0.25–0.5 (0.4)	0.25	0	1–4 (3.3)	4	0
C. famata	3	0.25–1 (0.6)	0.5	0	0.255–8 (4.1)	4	0
C. ciferrii	1	2		1 (100)	32		1 (100)
Total	383	0.25–2 (0.6)	0.5	3 (0.8)	0.125–128 (2.1)	0.5	16 (4.2)

MIC, minimum inhibitory concentrations.

3.3. Clinical data

All 16 patients infected by *Candida* species having fluconazole MICs $\geq$16 μg/ml are listed in Table 2. *Candida glabrata* and *C. ciferrii* had higher amphotericin B MICs than other *Candida* species. Of these 16 patients, 13 had catheters, and only 3 patients were previously treated with antifungal drugs.

4. Discussion

It has been reported that beside *C. parapsilosis*, non-albicans *Candida* species including *C. krusei* (75%), *C. glabrata* (35%), *C. tropicalis* (10–25%), and *Candida lusitaniae* (10–25%) causing candidemia having had higher resistance rates to fluconazole than *C. albicans* (Krcmery and Barnes, 2002; Pfaller et al., 2003). Of 383 isolates collected from the VGH-TPE, only one was resistant to fluconazole. Of 92 reviewed patients infected with *C. glabrata*, *C. guilliermondii*, or *C. tropicalis*, only 13% of the patients (12/92) had prior fluconazole treatments. This fact may explain the low rate of resistance to fluconazole in this study. This is consistent with the idea that continuous exposure to azoles seems to have an essential role in developing resistance to fluconazole in *Candida* species (Kontoyiannis, 2002).

Although *C. albicans* remains the most common species causing candidemia, the prevalence of non-albicans *Candida* species has increased over the last decade (Kao et al., 1999; Pfaller et al., 1999; Sanglard and Odds, 2002). The prevalence of *C. glabrata* candidemia has been increasing due to its high MIC to fluconazole. It is the most common *non-albicans Candida* species in some other studies (Sanglard and Odds, 2002; Pfaller et al., 1999). However, *C. glabrata* caused only 5.2% of candidemia in this study, which is relatively low compared to recent studies (Sanglard and Odds, 2002). There was no *C. krusei* isolated in this

Table 2
Information of patients infected by *Candida* species having fluconazole MICs $\geq$ 16 μg/ml

patient	year	species	MIC (Flu)*	MIC (AmB)	Outcome	treatment	Immunosupression	Gender	Catheters	prior treatment
1	1997	cal	32	0.5	Cured	Flu	No	Female	Yes	No
2	1996	cci	32	2	Cured	no	No	Male	Yes	No
3	1997	cgl	16	1	Dead	Flu	No	Male	Yes	No
4	1997	cgl	128	1	Cured	Flu	No	Female	No	No
5	1997	cgl	16	2	Dead	no	Yes	Male	Yes	Itr
6	1998	cgl	16	1	Dead	AmB	No	Male	Yes	No
7	1998	cgl	16	1	Dead	AmB	No	Female	Yes	No
8	1998	cgl	16	1	Dead	Flu	No	Female	Yes	No
9	1998	cgl	16	1	Cured	no	No	Male	Yes	No
10	1999	cgl	16	1	Dead	Flu	No	Male	No	No
11	1997	cgu	32	0.25	Cured	AmB	No	Female	No	No
12	1997	cgu	16	0.5	Dead	Flu	No	Male	Yes	No
13	1997	cgu	16	0.5	Cured	AmB	No	Female	Yes	No
14	1999	cgu	16	0.5	Dead	Flu	No	Male	Yes	Flu and AMB
15	1999	cgu	16	0.25	Cured	no	No	Male	Yes	No
16	1996	ctr	16	0.5	Dead	Flu	Yes	Female	Yes	Flu

MIC, minimum inhibitory contributions; AmB, amphotericin B; Flu, fluconazole; Itr, itraconazole

study. This indicates that infection of *C. krusei* has remained a rare cause even though it is intrinsically resistant to fluconazole (Colombo et al., 1999; Kao et al., 1999; Sandven et al., 1998). Azole prophylaxis is one risk factor for *C. glabrata* and *C. krusei* infections (Krcmery and Barnes, 2002). Low prevalence of *C. glabrata* and *C. krusei* infections in this study may be a result of infrequent fluconazole prophylaxis during the study period.

Mardani et al. reported nine candidemia caused by *C. guilliermondii* from 1988 to 1998 in cancer patients. Five of those candidemia (55.6%) occurred as a breakthrough infection while patients were on antifungal prophylaxis with agents such as fluconazole, itraconazole, or amphotericin B (Mardani et al., 2000). Poor responses to antifungal therapy in vivo and emergence of resistance to amphotericin B during therapy had also been described by other studies (Tietz et al., 1999; Bulmer et al., 1999; Karlowsky et al., 1997). In this study, *C. guilliermondii* had the highest susceptible-dose dependent rate to fluconazole, which is consistent with a recent report concerning one hospital in Taiwan (Lee et al., 2002).

It has been suggested that *C. ciferrii* was an etiologic agent of superficial yeast infections in humans even though this species was thought to be a saprophyte previously (Furman and Ahearn, 1983; de Gentile et al., 1995). This species has been reported causing invasive fungal infections in humans in Australia and being a fluconazole-resistant fungus (Gunsilius et al., 2001). In this study, only one *C. ciferrii* was isolated and it was resistant to amphotericin B and susceptible-dose dependent to fluconazole.

In addition to the common non-albicans *Candida* species, including *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*, the increasing trend of unusual *Candida* species, such as *C. lusitaniae*, *C. guilliermondii* and *C. ciferrii* should be noted (Krcmery and Barnes, 2002). Previous study also showed that these rare *Candida* species appear to be more resistant to currently antifungal agents (Pfaller et al., 2003). Following works would be to determine the risk factors for non-albicans *Candida* species infections.

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