Susceptibilities of *Candida* Species to Amphotericin B and Fluconazole: The Emergence of Fluconazole Resistance in *Candida tropicalis* •

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SUSCEPTIBILITIES OF *CANDIDA* SPECIES TO AMPHOTERICIN B AND FLUCONAZOLE: THE EMERGENCE OF FLUCONAZOLE RESISTANCE IN *CANDIDA TROPICALIS*

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ABSTRACT

OBJECTIVE: To determine the susceptibilities of *Candida* species isolated from Taiwan to amphotericin B and fluconazole.

DESIGN: Prospective surveillance study.

METHODS: Each hospital was asked to submit up to 10 *C. albicans* and 40 non-*albicans Candida* species during the collection period, from April 15 to June 15, 1999. One isolate was accepted from each episode of infection. The broth microdilution method was used to determine susceptibilities to amphotericin B and fluconazole.

RESULTS: Only 3 of 632 isolates, one each of *C. famata*, *C. krusei*, and *C. tropicalis,* were resistant to amphotericin B. A total of 53 (8.4%) of 632 clinical yeast isolates, consisting of 4% *C. albicans*, 8% *C. glabrata*, 15% *C. tropicalis*, and 70% *C. krusei*, were resistant to fluconazole. In contrast, no *C. parapsilosis* isolate was resistant to fluconazole. Isolates from tertiary-care medical centers had higher rates of resistance to fluconazole than did those from regional and local hospitals (11.4% vs 6.6%). Isolates from different sources showed different levels of susceptibility to fluconazole. All of the isolates with the exception of *C. tropicalis* and *C. krusei* isolated from blood were susceptible to fluconazole. A pattern of co-resistance to both amphotericin B and fluconazole was observed.

CONCLUSIONS: Non-*albicans Candida* species had higher rates of resistance to fluconazole than did *C. albicans* (44 of 395 [11.2%] vs 9 of 237 [3.8%]; $P = .002$). The increasing rate of fluconazole resistance in *C. tropicalis* (15%) is important because *C. tropicalis* is one of the most commonly isolated non-*albicans Candida* species (*Infect Control Hosp Epidemiol* 2004;25:60-64).

In the past decade, nosocomial infections caused by yeast have increased significantly. In the United States, yeast infections rank as the fourth most common cause of nosocomial bloodstream infection.[1,2] The prevalence of nosocomial candidemia increased 27-fold from 1981 through 1993 at a hospital in Taiwan.[3,4] The dramatic increases in the prevalence of fungal infections are probably the result of alterations in immune status associated with the acquired immunodeficiency syndrome epidemic, cancer chemotherapy, organ and bone marrow transplantation,[5] frequent broad-spectrum antibacterial therapy, and invasive hospital procedures.

Currently available antifungal drugs can have troublesome side effects, are ineffective against some fungi, and lead to the development of resistance. Antifungal drug resistance has become an important issue for a variety of fungal infections. Oropharyngeal candidiasis due to drug-resistant fungi is a major problem for patients infected with human immunodeficiency virus.[6] One-third of patients with late-stage acquired immunodeficiency syndrome had drug-resistant strains of *Candida albicans* in their mouth in one report.[7] The rate of invasive fungal infection among liver transplant patients in another study was 11% and 40% of the patients with systemic fungal infection died. Seventy-two percent of these deaths were attributed to the fungal infection.[8]

Candida species have varying degrees of susceptibility to common antifungal agents. *C. lusitaniae* is relatively resistant to amphotericin B.[9] *C. krusei* and *C. glabrata* are less susceptible to fluconazole than are other *Candida* species.[10-12] Although *C. tropicalis* is less commonly isolated from clinical specimens than is *C. albicans*, it is one of the most common non-*albicans Candida* species[13,14] and it is always associated with diseases.[15,16] Among non-*albicans Candida* species, *C. tropicalis* is considerably clinically important because it develops fluconazole resistance rapid-

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TABLE 1
SUSCEPTIBILITIES TO AMPHOTERICIN B

MIC (μg/mL)	*C. albicans*	*C. tropicalis*	*C. glabrata*	*C. parapsilosis*	*C. krusei*	Others	Total
0.06	1	0	0	0	0	1	2
0.125	3	0	0	1	0	0	4
0.25	44	13	9	13	0	4	83
0.5	149	95	101	20	3	5	373
1	40	54	46	17	6	4	167
2	0	1	0	0	1	1	3
Total	237	163	156	51	10	15	632
MIC_{50}	0.5	0.5	0.5	0.5	1	0.5	0.5
MIC_{90}	1	1	1	1	2	1	1

MIC = minimum inhibitory concentration.

TABLE 2
SUSCEPTIBILITIES TO FLUCONAZOLE

MIC (μg/mL)	*C. albicans*	*C. tropicalis*	*C. glabrata*	*C. parapsilosis*	*C. krusei*	Others	Total
0.125	83	4	0	2	0	0	89
0.25	108	8	0	3	0	1	120
0.5	16	19	1	17	0	4	57
1	4	36	1	19	0	1	61
2	1	42	7	6	0	2	58
4	2	8	67	2	0	4	83
8	7	12	45	1	0	1	66
16	4	10	18	1	1	2	36
32	3	0	4	0	2	0	9
⩾ 64	9	24	13	0	7	0	53
Total	237	163	156	51	10	15	632
MIC_{50}	0.5	2	8	1	64	2	1
MIC_{90}	4	64	32	2	64	16	16

MIC = minimum inhibitory concentration.

ly[17] and the rate of resistance to fluconazole of clinical *C. tropicalis* isolates is increasing.[18-21]

We previously reported a national survey in Taiwan in which 22 hospitals contributed 660 clinical yeast isolates.[22] The current study showed that the levels of susceptibility to fluconazole of *Candida* species were different among different species and also among the same species from different sources. Our data suggested that, in addition to *C. krusei* and *C. glabrata*, *C. tropicalis* should be on the list of yeast for which fluconazole resistance is common.

METHODS

Organisms and Medium

Yeast isolates were collected from 22 hospitals participating in Taiwan Surveillance of Antimicrobial Resistance of Yeasts (TSARY). Six were tertiary-care medical centers, 14 were regional hospitals, and 2 were local hospitals. Each hospital was asked to submit up to 10 *C. albicans* and 40 non-*albicans Candida* species during the collection period, from April 15 to June 15, 1999. One isolate was accepted from each episode of infection. Isolates were stored frozen at -70°C in bead-containing Microbank cryovials (PRO-LAB Diagnostics, Austin, TX). At the end of the collection period, isolates were kept frozen and transported to the National Health Research Institutes laboratory within 24 hours. The isolates were first subcultured on Sabouraud dextrose agar (BBL Becton Dickinson, Cockeysville, MD) to check for purity and identification. Pure isolates were labeled and stored in vials containing 50% glycerol at -70°C for further analysis.

Antifungal Susceptibility Testing

The minimum inhibitory concentration (MIC) to amphotericin B or fluconazole of each yeast was determined by in vitro antifungal susceptibility testing according to guidelines published by the National Committee for Clinical Laboratory Standards.[23] The powder of RPMI medium 1640 was provided by Gibco BRL (Cat.# 31800-

TABLE 3
SUSCEPTIBILITIES OF *CANDIDA* SPECIES FROM DIFFERENT SOURCES TO FLUCONAZOLE

Source	*C. albicans*	*C. tropicalis*	*C. glabrata*	*C. parapsilosis*	*C. krusei*	Others	Subtotal
Blood							
S	100 (15)*	58.4 (7)	87.5 (7)	100 (13)	0	100 (2)	86.3 (44)
SDD	0	8.3 (1)	12.5 (1)	0	0	0	3.9 (2)
R	0	33.3 (4)	0	0	100 (1)	0	9.8 (5)
Sputum							
S	90.2 (74)	81.4 (35)	55 (11)	100 (3)	0	66.7 (2)	82.2 (125)
SDD	3.7 (3)	4.7 (2)	30 (6)	0	0	33.3 (1)	7.9 (12)
R	6.1 (5)	13.9 (6)	15 (3)	0	100 (1)	0	9.9 (15)
Urine							
S	94.9 (75)	75.3 (55)	80.2 (85)	85.7 (6)	0	100 (3)	82 (224)
SDD	3.8 (3)	6.9 (5)	12.3 (13)	14.3 (1)	40 (2)	0	8.8 (24)
R	1.3 (1)	17.8 (13)	7.5 (8)	0	60 (3)	0	9.2 (25)
Wound							
S	90 (18)	92.3 (12)	100 (4)	100 (11)	0	0	91.8 (45)
SDD	0	7.7 (1)	0	0	0	100 (1)	4.1 (2)
R	10 (2)	0	0	0	0	0	4.1 (2)
Others							
S	95.2 (39)	91 (20)	77.8 (14)	100 (17)	0	100 (6)	89.7 (96)
SDD	2.4 (1)	4.5 (1)	11.1 (2)	0	33.3 (1)	0	4.7 (5)
R	2.4 (1)	4.5 (1)	11.1 (2)	0	66.7 (2)	0	5.6 (6)
Subtotal							
S	93.2 (221)	79.2 (129)	77.6 (121)	98 (50)	0	86.7 (13)	84.5 (534)
SDD	3 (7)	6.1 (10)	14.1 (22)	2 (1)	30 (3)	13.3 (2)	7.1 (45)
R	3.8 (9)	14.7 (24)	8.3 (13)	0	70 (7)	0	8.4 (53)
Total	37.5 (237)	25.8 (163)	24.7 (156)	8.1 (51)	1.6 (10)	2.3 (15)	100 (632)

S = susceptible; SDD = susceptible-dose dependent; R = resistant.
*Percentage (number of isolates).

022; Becton Dickinson, Sparks, MD). Several strains from the American Type Culture Collection were used as standard controls. The final growth of each isolate was measured by a Spectra MAX Plus (Molecular Devices Corp., Sunnyvale, CA) after 48 hours of incubation at 35°C. The MIC of each isolate was measured at least twice. When the results of two susceptibility tests differed by more than onefold dilution, they were retested. MICs of some isolates were measured by Etest (AB Biodisk, Solna, Sweden) to confirm our microdilution results.

The interpretation of MICs was according to the guidelines of the National Committee for Clinical Laboratory Standards. The MICs to amphotericin B and fluconazole were defined as those that could reduce the turbidity of cells by greater than 95% and 50%, respectively. Isolates with a MIC of 2 μg/mL or greater were considered to be resistant to amphotericin B. Isolates with a MIC of 1 μg/mL or less were considered to be susceptible. Isolates with a MIC of 64 μg/mL or more were considered to be resistant to fluconazole. Isolates with a MIC of 8 μg/mL or less were considered to be susceptible. Isolates with a MIC between 16 and 32 μg/mL were susceptible-dose dependent. The MICs of 50% and 90% of the total population were defined as MIC_{50} and MIC_{90}, respectively.

Database and Analysis

In addition to identifying isolates, contributing hospitals also provided the following information about each isolate: location and type of hospital, genus and species as identified by each hospital, source (urine, sputum, blood, wound, and 48 other sources), and procedures for identification. An analysis was performed using Epi-Info software (version 6.04; Centers for Disease Control and Prevention, Atlanta, GA).[24] The significance of differences in proportions was determined by the chi-square test with Yate's correction.

RESULTS

Candida *Species From Different Sources*

A total of 632 isolates were analyzed for their susceptibilities to amphotericin B and fluconazole. *C. albicans* was the most common species (37.5% of all isolates). *C. tropicalis* (25.8%) and *C. glabrata* (24.7%) were the two most common non-*albicans Candida* species followed by *C. parapsilosis* (8.1%), *C. krusei* (1.6%), and others (2.3%). When classified according to the sources, 273 (43.2%) of the isolates were from urine, 152 (24.1%) were from sputum, 51 (8.1%) were from blood, 49 (7.8%) were from wounds, and 107 (16.8%) were from other sites.

TABLE 4
SUSCEPTIBILITIES OF *CANDIDA* SPECIES FROM DIFFERENT SOURCES TO FLUCONAZOLE

	C. albicans			*C. tropicalis*			*C. glabrata*			*C. parapsilosis*		
Source	**MICs (μg/mL)**	**MIC_{50}**	**MIC_{90}**	**MICs**	**MIC_{50}**	**MIC_{90}**	**MICs**	**MIC_{50}**	**MIC_{90}**	**MICs**	**MIC_{50}**	**MIC_{90}**
Blood	0.125–0.50	0.25	0.5	0.5–64	5	64	4.0–16	4	16	0.125–2	0.5	1
Sputum	0.125–512	0.25	8	0.25–64	2	64	1.0–64	8	64	0.5–2	1	2
Urine	0.125–64	0.25	2	0.125–64	2	64	0.5–128	4	24	0.5–16	1	8
Wound	0.125–64	0.25	1	0.25–16	2	2	4.0–8	4	8	0.5–4	1	2

MIC = minimum inhibitory concentration.

Susceptibilities to Amphotericin B and Fluconazole

The susceptibilities to amphotericin B are listed in Table 1. Only 3 of 632 isolates, one each of *C. famata*, *C. krusei*, and *C. tropicalis*, were resistant to amphotericin B. The MICs ranged from 0.06 to 2 μg/mL. The MIC_{50} of these isolates was 0.5 μg/mL and the MIC_{90} was 1 μg/mL. *C. krusei* were less susceptible to amphotericin B than were the other species, with MICs ranging from 0.5 to 2 μg/mL, a MIC_{50} of 1 μg/mL, and a MIC_{90} of 2 μg/mL.

The susceptibilities to fluconazole are listed in Table 2. A total of 53 (8.4%) and 45 (7.1%) isolates were resistant and susceptible-dose dependent. The MIC_{50} of these isolates was 1 μg/mL and the MIC_{90} was 16 μg/mL. *C. krusei* (70%), *C. tropicalis* (15%), and *C. glabrata* (8%) isolates had higher rates of resistance to fluconazole than did *C. albicans* (4%) and other *Candida* species (0%). No *C. krusei* isolate was susceptible to fluconazole and the MIC_{50} of this species was 64 μg/mL. Approximately 78% and 79% of *C. glabrata* and *C. tropicalis* isolates, respectively, were susceptible to fluconazole. No *C. parapsilosis* isolate was resistant to fluconazole. Non-*albicans Candida* species had higher rates of resistance to fluconazole than did *C. albicans* (44 of 395 [11.2%] vs 9 of 237 [3.8%]; $P = .002$). Isolates from hospitals affiliated with tertiary-care medical centers had a higher rate of resistance to fluconazole than did those from regional and local hospitals (25 of 219 [11.4%] vs 27 of 410 [6.6%]; $P = .05$).

Species and Sources Accounting for Susceptibilities to Fluconazole

Candida species isolated from different sources had different susceptibilities to fluconazole (Table 3). All isolates from blood, with the exception of 4 *C. tropicalis* isolates and 1 *C. krusei* isolate, were susceptible to fluconazole (Table 3). *Candida* species isolated from different sources had different MICs (MIC_{50} and MIC_{90}) to fluconazole (Table 4). The MIC_{50} of isolates from urine (4 μg/mL) was higher than that of isolates from other sources (0.5 to 1 μg/mL). The MIC_{90} of isolates from wounds (8 μg/mL) was lower than that of isolates from blood, sputum, and urine (16 to 32 μg/mL).

Co-resistance to Both Amphotericin B and Fluconazole

The trend of co-resistance to amphotericin B and fluconazole is outlined in Table 5. Fewer isolates with MICs to amphotericin B of 0.5 μg/mL or less were resistant to fluconazole compared with isolates with higher MICs (1 μg/mL or greater) to amphotericin B (30 of 462 vs 23 of 170; $P = .007$). Isolates with lower MICs to amphotericin B (0.5 μg/mL or less) had lower MIC_{50} and MIC_{90} to fluconazole than did isolates with higher MICs (1 μg/mL or greater) to amphotericin B (1 vs 2 μg/mL and 16 vs 64 μg/mL, respectively).

TABLE 5
SUSCEPTIBILITIES TO BOTH AMPHOTERICIN B AND FLUCONAZOLE

Amphotericin B	**Fluconazole**			
MIC (μg/mL)	**MIC_{50}**	**MIC_{90}**	**MIC ⩾ 64**	**MIC ⩽ 32**
⩽ 0.5	1	2	30 (6.5%)	432 (93.5%)
⩾ 1	16	64	23 (13.5%)	147 (86.5%)

MIC = minimum inhibitory concentration.

DISCUSSION

According to in vitro antifungal susceptibility testing, 0.5% and 8.4% of isolates were considered to be resistant to amphotericin B and fluconazole, respectively. The difference in rate of resistance between fluconazole and amphotericin B is a result of different mechanisms of antifungal activity,[6,25] frequency of use, different molecular mechanisms of drug resistance,[5,26] or all three. Two of three isolates resistant to amphotericin B (one *C. krusei* and one *C. tropicalis*) were also resistant to fluconazole. Although the rate of resistance to amphotericin B was low, there was a trend of co-resistance to amphotericin B and fluconazole.

Fungal infections caused by non-*albicans Candida* species have been increasing dramatically.[27-29] *C. krusei* and *C. glabrata* have been considered intrinsically more resistant to fluconazole.[10,11] In this study, no *C. krusei* isolate was susceptible to fluconazole, but *C. glabrata* was less resistant to fluconazole than in other studies (8% vs 13% or 45.4%).[19,30] Overall, non-*albicans Candida* species had higher rates of resistance to fluconazole than did *C. albicans*. Most of the *Candida* species isolates from blood

were susceptible to fluconazole. However, 4 (33.3%) of 12 *C. tropicalis* isolates from blood were resistant to fluconazole. The increasing rate of fluconazole resistance in *C. tropicalis* (15%) is important because *C. tropicalis* is one of the most commonly isolated non-*albicans Candida* species.[13,14] *C. tropicalis* develops drug resistance in the presence of fluconazole much more rapidly than does *C. albicans.*[17,31] These findings may explain why *C. tropicalis* (15%) had a higher rate of resistance to fluconazole than did *C. albicans* (4%) and *C. glabrata* (8%).

Isolates of the same species from different sources showed different susceptibilities to fluconazole. For example, *C. glabrata* isolates from sputum had a higher rate of resistance (15%) to fluconazole than did those from blood (0%) or urine (7.5%), whereas *C. tropicalis* isolates from blood had a higher rate of resistance (33.3%) to fluconazole than did those from sputum (13.9%) or urine (17.8%). The fact that isolates from tertiary-care medical centers had higher rates of resistance to fluconazole than did those from regional and local hospitals needs to be investigated further.

Different levels of susceptibility to fluconazole in the same species from different sources complicates diagnosis and treatment in the clinical setting. Hence, accurate identification to the species level and susceptibility testing are crucial for clinical management, especially for the emerging non-*albicans Candida* species that are resistant to fluconazole.

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