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Susceptibilities to amphotericin B and fluconazole of *Candida* species in TSARY 2002

TSARY Hospitals

Yun-Liang Yang[a], Shu-Ying Li[b], Hsiao-Hsu Cheng[c], Hsiu-Jung Lo[c,*]

[a]*Department of Biological Science and Technology, National Chiao Tung University, Hsinchu 300, Taiwan*

[b]*Laboratory for Mycopathogen, Chlamydia, and Mycoplasma, Division of Laboratory Research and Development, Center for Disease Control, Taipei 11561, Taiwan*

[c]*Division of Clinical Research, National Health Research Institutes, Miaoli County 350, Taiwan*

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Abstract

Susceptibilities to amphotericin B and fluconazole of 909 *Candida* species collected during the Taiwan Surveillance of Antimicrobial Resistance of Yeasts (TSARY) in 2002 were determined by the broth microdilution method. There were 395 (43.5%) *Candida albicans*, 244 (26.8%) *C. tropicalis*, 187 (20.6%) *C. glabrata*, 63 (6.9%) *C. parapsilosis*, 9 (1%) *C. krusei*, and 11 (1.2%) others. Among them, 23 (2.5%) isolates were resistant to amphotericin B. They consisted of 10 *C. glabrata*, 6 *C. krusei*, 3 *C. albicans*, 1 *C. tropicalis*, 1 *C. parapsilosis*, and 2 others. The resistance rate to amphotericin B has increased compared with that of TSARY 1999 (2.5% versus 0.5%). There were 7 *C. krusei*, 5 *C. albicans*, 3 *C. glabrata*, and 2 others isolates resistant to fluconazole. The resistance rate to fluconazole has decreased from 8.4% in 1999 to 1.9% in 2002. A pattern of coresistance to both amphotericin B and fluconazole was observed.

Keywords: *Candida*; Susceptibility; Resistance

1. Introduction

Nosocomial infections caused by yeasts have increased significantly in the past 2 decades. The prevalence of nosocomial candidemia increased 27-fold from 1981 through 1993 at a major hospital in Taiwan (Chen et al., 1997; Hung et al., 1996). In the United States, yeast infection also ranks as the fourth most common cause of nosocomial bloodstream infection (Beck-Sague and Jarvis, 1993; Pfaller et al., 1998). The dramatic increase in the prevalence of fungal infections is probably the result of alterations in immune status and invasive hospital procedures (White et al., 1998; Yang and Lo, 2001). Thus, infections caused by *Candida* species are becoming important causes of morbidity and mortality in immunocompromised patients. The major issues concerning currently available antifungal drugs include side effects and ineffectiveness against certain fungi. Because of broad prophylactic use and long-term treatment with antifungal drugs, drug resistance has become an important issue in various fungal infections, which have profound effects on human health (Marr et al., 2001; Pfaller et al., 2003; Yang et al., 2004b).

Candida species have various degrees of susceptibility to common antifungal drugs. For instance, *Candida lusitaniae* is relatively resistant to amphotericin B (Hadfield et al., 1987), whereas *C. krusei* and *C. glabrata* are less susceptible to fluconazole than other *Candida* species (Akova et al., 1991; Orozco et al., 1998; Yang et al., 2004b). This phenomenon emphasizes the importance of identification and surveillance of the *Candida* species in the clinical settings.

As part of the national survey Taiwan Surveillance of Antimicrobial Resistance of Yeasts (TSARY) in 1999, 22 hospitals have contributed 660 clinical yeast isolates to our study (Lo et al., 2001). Among the 632 tested isolates, 0.5% and 8.4% of isolates were resistant to amphotericin B and fluconazole, respectively. We have also shown that the levels of susceptibility to fluconazole of *Candida* species are different among different species (Yang et al., 2004a; Yang et al., 2004b). The aim of this study is to determine the susceptibilities to amphotericin B and fluconazole of isolates

* Corresponding author. Tel.: +886-37-246-166, ext. 35516; fax: +886-37-568-4457.

E-mail address: hjlo@nhri.org.tw (H.-J. Lo).

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collected in a follow-up survey in 2002, TSARY 2002, and to investigate the change of susceptibility to antifungal drugs of *Candida* species in Taiwan from 1999 to 2002.

2. Materials and methods

2.1. Organisms and media

Yeast isolates were collected from 24 hospitals that participated in TSARY 2002. Each hospital was asked to submit all yeast pathogens from blood and up to 10 *C. albicans* and 40 non-*albicans Candida* species isolates from non-blood sites from June to August in 2002. Only one isolate was accepted during each episode of infection. Isolates were stored frozen at −70°C in bead-containing Microbank cryovials (PRO-LAB Diagnostics, Austin, TX). The isolates were first subcultured on Sabouraud dextrose agar (BBL, Becton Dickinson Cockeysville, MD) to assess the purity and identification after they were sent to the laboratory at National Health Research Institutes (NHRI). Pure isolates were labeled and stored in vials containing 50% glycerol at −70°C awaiting further analysis.

2.2. Identification

The identifications of the isolates were reassured in the laboratory at the NHRI. The identification procedure for the yeast isolates was performed as previously described (Lo et al., 2001). Isolates identified as *C. albicans* by participating hospitals were first subjected to the germ tube test in brain heart infusion (BHI, BBL) medium containing 10% goat serum (GibcoBRL 16210-064, Grand Island, NY) at 37°C for 2 to 3-h (Larone, 1995). The VITEK Yeast Biochemical Card (bioMérieux, St. Louis, MO) was then used to identify the isolates appearing to be negative by the germ tube assay in the NHRI laboratory and the isolates were identified as non-*albicans Candida* species by participating hospitals. API32C (bioMérieux) was used to assess the NHRI result when the VITEK Yeast Biochemical Card showed less than 90% confidence.

2.3. Antifungal susceptibility testing

The minimum inhibitory concentration (MIC) to amphotericin B and fluconazole of each yeast was determined by in vitro antifungal susceptibility testing according to the guidelines of M27-A published in 1997 by the National Committee for Clinical Laboratory Standards (1997). The RPMI medium 1640 (31800-022) provided by GibcoBRL was used for the testing. Strains from American Type Culture Collection were used as the standard controls. The final growth of each isolate was measured by a Spectra MAX Plus (Molecular Devices Corp, Sunnyvale, CA) after 48-h incubation at 35°C. We also measured MICs of some isolates by Etest (AB Biodisk Solna, Sweden) to assess the results of the broth microdilution method.

The interpretation of MICs was according to the guidelines of the National Committee for Clinical Laboratory Standards (1997). The MICs to amphotericin B and fluconazole were defined as the MICs of drugs capable of reducing the turbidity of cells to greater than 95% and 50%, respectively. Isolates with MIC ≥ 2 μg/mL were considered

Table 1
The susceptibility to fluconazole of *Candida* species from different sources

Susceptibility	*C. albicans*	C. tropicalis	C. glabrata	C. parapsilosis	C. krusei	Others	All
Urine							
S	98 (93.3)	107 (95.5)	71 (52.2)	8 (100)	0	0	284 (78.2)
SDD	5 (4.8)	5 (4.5)	63 (46.3)	0	0	0	73 (20.1)
R	2 (1.9)	0	2 (1.5)	0	2 (100)	0	6 (1.7)
Sputum							
S	112 (93.3)	56 (96.6)	5 (35.7)	1 (100)	0	2 (50)	176 (87.6)
SDD	6 (5)	2 (3.4)	8 (57.2)	0	1 (25)	1 (25)	18 (8.9)
R	2 (1.7)	0	1 (7.1)	0	3 (75)	1 (25)	7 (3.5)
Blood							
S	55 (98.2)	30 (96.8)	6 (46.2)	24 (100)	0	0	115 (91.3)
SDD	0	1 (3.2)	7 (53.8)	0	0	0	8 (6.3)
R	1 (1.8)	0	0	0	1 (100)	1 (100)	3 (2.4)
Wound							
S	21 (95.5)	9 (100)	0	12 (100)	0	2 (100)	44 (95.7)
SDD	1 (4.5)	0	1 (100)	0	0	0	2 (4.3)
R	0	0	0	0	0	0	0
Others							
S	87 (94.6)	33 (97.1)	11 (47.8)	18 (100)	1 (50)	3 (75)	153 (88.4)
SDD	5 (5.4)	1 (2.9)	12 (52.2)	0	0	1 (25)	19 (11)
R	0	0	0	0	1 (50)	0	1 (0.6)
All							
S	373 (94.4)	235 (96.3)	93 (49.7)	63 (100)	1 (11.1)	7 (63.6)	772 (84.9)
SDD	17 (4.3)	9 (3.7)	91 (48.7)	0	1 (11.1)	2 (18.2)	120 (13.2)
R	5 (1.3)	0	3 (1.6)	0	7 (77.8)	2 (18.2)	17 (1.9)

S, susceptible; SDD, susceptible-dose-dependent; R, resistant. Data are given as number of isolates (%).

Table 2
The susceptibility to amphotericin B of *Candida* species

MIC (μg/mL)	*C. albicans*	C. tropicalis	C. glabrata	C. parapsilosis	C. krusei	Others	Total
0.06	3 (0.8)	1 (0.4)	0	0	0	0	4 (0.4)
0.125	1 (0.2)	0	0	0	0	0	1 (0.1)
0.25	6 (1.5)	1 (0.4)	1 (0.5)	6 (9.5)	0	1 (9.1)	15 (1.7)
0.5	218 (55.2)	126 (51.7)	33 (17.6)	27 (42.9)	1 (11.1)	4 (36.4)	409 (45)
1	164 (41.5)	115 (47.1)	143 (76.5)	29 (46)	2 (22.2)	4 (36.4)	457 (50.3)
2	3 (0.8)	1 (0.4)	10 (5.4)	1 (1.6)	6 (66.7)	2 (18.1)	23 (2.5)
Total	395	244	187	63	9	11	909
MIC_{50}	0.5	0.5	1	0.5	2	1	1
MIC_{90}	1	1	1	1	2	2	1

Data are given as number of isolates (%).

resistant to amphotericin B. Isolates with MIC ≤ 1 μg/mL were considered susceptible. Isolates with MIC ≥ 64 μg/mL were considered resistant to fluconazole, whereas isolates with MIC ≤ 8 μg/mL were considered to susceptible. Isolates of which the MICs fell in between (16–32 μg/mL) were fluconazole-susceptible-dose-dependent. MIC_{50} and MIC_{90} were defined as the MICs of 50% and 90% of the total population.

2.4. Database and analysis

The database for this study contained the characteristic information of each submitted isolate: hospital origin, location and type of the hospital, and identification and source of the isolate. The statistical significance of the differences in frequencies and proportions was determined by the χ^2 test with Yates' correction.

3. Results and discussion

3.1. Susceptibilities to fluconazole of Candida species from different sources

A total of 909 isolates listed in Table 1 were analyzed for their susceptibilities to amphotericin B and fluconazole. *C. albicans* was the most common species among the isolates (43.5%). *C. tropicalis* (26.8%) and *C. glabrata* (20.6%) were 2 most common non-*albicans Candida* species followed by *C. parapsilosis* (6.9%), *C. krusei* (1%), and others (1.2%). When classified according to the sources, there were 363 (39.9%) isolates from urine, 201 (22.1%) from sputum, 126 (13.9%) from blood, 48 (5.3%) from central venous line, 46 (5.1%) from wound, 33 (3.6%) from ascites, 14 (1.5%) from pus, and 78 (8.6%) from other sources.

A total of 772 (84.9%), 120 (13.2%), and 17 (1.9%) isolates were fluconazole-susceptible, fluconazole-susceptible-dose-dependent, and fluconazole-resistant, respectively. The MIC_{50} and MIC_{90} of these isolates were 1 and 16 μg/mL, respectively. The 17 fluconazole-resistant isolates consisted of 7 from sputum, 6 from urine, 3 from blood, and 1 from perineum. Fewer isolates (1.9%) from TSARY 2002 were resistant to fluconazole than that in TSARY 1999 (8.4%, $P < 0.05$) (Yang et al., 2004b). In contrast, higher percentage of isolates from TSARY 2002 (13.2%) were susceptible-dose dependent than that in TSARY 1999 (7.1%, $P < 0.05$). Consequently, there were similar portions of isolates susceptible to fluconazole in both surveys. The fluconazole resistance rate of isolates from blood in TSARY 2002 was 2.4%, which is higher than what has been reported from one major hospital in Taiwan (1.3%) (Chen et al., 1996). The MIC_{50} of *C. krusei* was 64 μg/mL. *C. krusei* (77.8%) had the highest resistance rate to fluconazole than any other species studied, which is consistent with previous report (Akova et al., 1991; Yang et al., 2004b). Although only 1.6% of *C. glabrata* were resistant to fluconazole, less than half of the isolates (49.7%) from this species were susceptible to fluconazole. The MIC_{50} and MIC_{90} of it were 16 and 32 μg/mL, respectively. In contrast, all of the *C. parapsilosis* isolates were susceptible to fluconazole, which is consistent with the previous report that *C. parapsilosis* is the most susceptible species to fluconazole (Yang et al., 2004b).

Table 3
The coresistance to amphotericin B and fluconazole

MIC of amphotericin B	MIC of fluconazole										Total
	0.125	0.25	0.5	1	2	4	8	16	32	64	
0.06		1	1	1		1					4
0.125	1										1
0.25		4	2	5	1	1		1		1	15
0.5	27	139	75	57	40	25	17	14	10	5	409
1	26	87	51	50	45	44	59	59	31	5	457
2		2	2	1	1	4	2	3	2	6	23
Total	54	233	131	114	87	75	78	77	43	17	909

3.2. Susceptibilities to amphotericin B of Candida species

The range of MICs to amphotericin B was from 0.06 to 2 μg/mL (Table 2). *C. krusei* was less susceptible to amphotericin B than any other species because the MIC_{50} of this species was 2 μg/mL. A total of 23 (2.5%) isolates were resistant to amphotericin B. Fungal infections caused by non-*albicans Candida* species have increased dramatically (Abi-Said et al., 1997; Slavin et al., 1995; Walsh et al., 2004), which was also reflected in the distribution of resistant isolates (Slavin et al., 1995; Walsh et al., 1998). Of the 23 amphotericin B-resistant isolates, 20 isolates were non-*albicans Candida* species. The distributions were 10 *C. glabrata*, 6 *C. krusei*, 1 *C. tropicalis*, 1 *C. parapsilosis*, and 2 others. Higher percentage of isolates (2.5%) from TSARY 2002 were resistant to amphotericin B than that in TSARY 1999 (0.5%, $P < 0.05$) (Yang et al., 2004b).

3.3. Coresistance to both amphotericin B and fluconazole

The phenomenon of coresistance has been reported for many pathogens. The trend of coresistance to amphotericin B and fluconazole is shown in Table 3. A total of 1.6% (12/772) of fluconazole-susceptible, 4.2% (5/120) of fluconazole-susceptible-dose-dependent, and 35.3% (6/17) of fluconazole-resistant isolates were resistant to amphotericin B. A total of 1.2% (11/886) and 26.1% (6/23) of isolates with MICs to amphotericin B of ≤1 and 2 μg/mL, respectively, were resistant to fluconazole.

A total of 11.1% of *C. krusei* and 49.7% of *C. glabrata* were susceptible to fluconazole, which is consistent with the previous report that both species were less susceptible to fluconazole than other *Candida* species (Akova et al., 1991; Orozco et al., 1998; Yang et al., 2004b). Thus, fluconazole is not a drug recommended to treat infections caused by these 2 species. Amphotericin B appears to be the drug of choice for the treatments. However, along with increased usage of amphotericin B, more *C. krusei* were resistant to it in TSARY 2002 (66.7%) than in TSARY 1999 (10%). This is also the case for *C. glabrata* (5.4% versus 0%). The coresistance to both amphotericin B and fluconazole of *C. krusei* and *C. glabrata* may become an issue for treatment of infections caused by them.

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