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Distribution and susceptibility to amphotericin B and fluconazole of *Candida* spp. isolated from Taiwan

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SUMMARY

Susceptibilities to amphotericin B and fluconazole of 628 clinical yeast strains collected from 22 hospitals in Taiwan were determined. A total of 53 isolates (8·4%) were resistant to fluconazole. Each hospital had different resistance rate to fluconazole ranging from 0% to 24%. None of the 186 isolates from eight of the 22 hospitals was resistant to fluconazole. In contrast, isolates from nine of the remaining 14 hospitals had greater than 10% resistance rate to fluconazole. Consistently, 88·9% (8/9) fluconazole-resistant *C. albicans* isolates were from hospitals having a high resistance rate to fluconazole. The prevalence of various *Candida* spp. in each hospital was different. A positive association was found between the prevalence of *C. tropicalis* and the resistance rate to fluconazole for individual hospitals. Although only three isolates (0·5%) were resistant to amphotericin B, a co-resistance to both amphotericin B and fluconazole was observed, which highlights the emerging problem of drug resistance.

INTRODUCTION

In the past two decades, nosocomial infections caused by fungi have been increasing significantly. On the healthy host, opportunistic pathogens including *Candida* spp. are commensal fungi commonly colonizing human mucosal surfaces. Fungal pathogens cause from relatively trivial conditions such as thrush in babies and vaginal infections in women to fatal, systemic infections in immunocompromised patients [1]. The dramatic increase in the prevalence of yeast infections is probably due to the AIDS epidemic, cancer chemotherapy, organ and bone-marrow transplantation, and invasive hospital procedures [2, 3]. Coinciding with the increased usage of antifungal agents, especially fluconazole, the incidence of drug resistance has increased [3]. Oropharyngeal candidiasis due to drug-resistant fungi is a major problem for patients infected with HIV [4].

Candida spp. have various degrees of susceptibility to common antifungal agents. *C. lusitaniae* is less susceptible to amphotericin B [5] and *C. krusei*, *C. glabrata*, and *C. tropicalis* are less susceptible to fluconazole than other *Candida* spp. [6–10]. The present study was carried out to determine the distribution and susceptibility profiles to amphotericin B and fluconazole of the 628 *Candida* spp. isolates collected from the 22 hospitals in the Taiwan Surveillance of Antimicrobial Resistance of Yeasts (TSARY) in 1999 [11].

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† The hospitals participating in the Taiwan Surveillance of Antimicrobial Resistance of Yeasts (TSARY) are listed in the Appendix.

METHODS

Organisms and medium

Yeast isolates were collected from 22 hospitals, consisting of six medical centres and 16 regional hospitals, in Taiwan. Each hospital was asked to submit up to 50 clinically significant yeast isolates including 10 *C. albicans* and 40 non-albicans *Candida* spp. during the collection period, from 15 April to 15 June in 1999 [11]. One isolate was accepted from each episode of infection. A total of 660 isolates were collected originally. However, there were 24 non-*Candida* spp. and eight *Candida* spp. failed to grow on RPMI medium 1640 (31800-022; Gibco, Paisley, Scotland, UK). The remaining 628 isolates were analysed for their susceptibility to amphotericin B and fluconazole.

After being collected, isolates were stored frozen at $-70\,^{\circ}C$ in bead-containing Microbank cryovials (Pro-Lab Diagnostics, Austin, TX, USA) in every hospital. At the end of the collection period, isolates were kept frozen and transported by an express delivery company to the laboratory at the National Health Research Institutes (NHRI) within 24 h. The isolates were first subcultured on Sabouraud dextrose agar (SDA, BBL Becton Dickinson & Co., Cockeysville, MD, USA) to check for purity and identification. Pure isolates were labelled and stored in vials containing glycerol at $-70\,^{\circ}C$ for further analysis. We have described the procedure for identification of *Candida* spp. previously [11]. In the NHRI laboratory, all isolates were first subjected to the germ tube test. Subsequently, Vitek yeast biochemical card (YBC, bioMérieux, St. Louis, MO, USA) and API-32C (bioMérieux) were used to identify isolates failing to form germ tubes in the test.

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 the National Committee of Clinical Laboratory Standards (NCCLS) as described previously [12]. The RPMI medium was used for dilution and growth of yeast culture. The final growth of each isolate was measured by a Spectra max Plus (Molecular Devices Corp., Sunnyvale, CA, US) after incubation at $35\,^{\circ}C$ for 48 h.

The interpretation of MICs was according to the guidelines of the NCCLS [12]. According to these guidelines, the MIC to amphotericin B was defined as the concentration that reduces the growth of cells down to 90% and the MIC to fluconazole was defined as the concentration that reduces the growth of cells down to 50%. Isolates with MIC $\geqslant 2\,\mu g/ml$ were considered to be resistant to amphotericin B, whereas isolates with MICs $\geqslant 64$, 16–32 and $\leqslant 8\,\mu g/ml$ were defined as resistant, susceptible-dose dependent, and susceptible to fluconazole respectively. The control strains for the study were *C. albicans* (ATCC 90028), *C. krusei* (ATCC 6258), and *C. parapsilosis* (ATCC 22019).

Data analysis

The information about each isolate in the database included location and type of hospital, genus and species as identified by each hospital and the NHRI laboratory, and source of the isolate (from urine, sputum, blood, wound, and others). The significance of differences in frequencies and proportions was determined by the χ^2 test with Yates' correction.

RESULTS

There were 11, 5, 3 and 3 hospitals participating in TSARY in 1999 located in the North, South, Middle, and East regions of Taiwan respectively. The distribution of tested *Candida* spp. is listed in Table 1. A total of 292, 165, 120, and 51 isolates were from hospitals in the North, South, Middle, and East regions respectively. Among them, 68·5, 64·7, 52·5, and 51% isolates from hospitals in the South, North, Middle, and East regions were non-albicans *Candida* spp. *C. tropicalis* was the most frequently isolated species among non-albicans *Candida* spp. in the North, South, and East regions, whereas *C. glabrata* was the one most frequently isolated in the Middle region. Interestingly, only 25·4% of isolates from the Middle region were *C. tropicalis*, which was significantly lower than other regions and also below the national average of 40·9%.

Although all hospitals were asked to submit 10 *C. albicans* isolates, the final number of isolates from each hospital varied. Three hospitals (nos. 5, 8 and 21) submitted less than 10 *C. albicans* isolates which may be due to a low prevalence of fungal infections in those hospitals. Some isolates from certain hospitals (nos. 1, 10, 13, 15 and 19) failed to grow on the RPMI medium. Nine hospitals had more than 10 *C. albicans* isolates for testing since their submission of non-albicans *Candida* isolates were *C. albicans*. The prevalence of non-albicans *Candida* spp. in different

Table 1. *The distribution of tested* Candida *spp.* (*n* = *628*)

Hosp. no.	Region (type)	Cal	Non-alb	*C. tropicalis*	*C. glabrata*	*C. parapsilosis*	*C. krusei*	Others
1	N (RH)	9	15	3 (20·0)*	10 (66·7)	2 (13·3)	0	0
2	N (MC)	10	32	7 (21·9)	14 (43·8)	7 (21·9)	1 (3·1)	3 (9·4)
3	N (RH)	11	8	3 (37·5)	1 (12·5)	2 (25·0)	1 (12·5)	1 (12·5)
4	N (RH)	11	17	9 (52·9)	4 (23·5)	3 (17·6)	1 (5·9)	0
5	N (RH)	8	6	2 (33·3)	2 (33·3)	1 (16·7)	0	1 (16·7)
6	N (RH)	10	10	5 (50·0)	5 (50·0)	0	0	0
7	N (MC)	10	18	1 (5·6)	8 (44·4)	8 (44·4)	1 (5·6)	0
8	N (RH)	5	2	0	0	2 (100·0)	0	0
9	N (MC)	10	39	20 (51·3)	9 (23·1)	6 (15·4)	2 (5·1)	2 (5·1)
10	N (RH)	9	13	6 (46·2)	5 (38·5)	1 (7·7)	0	1 (7·7)
11	N (RH)	10	29	15 (51·7)	10 (34·5)	2 (6·9)	1 (3·4)	1 (3·4)
12	M (RH)	14	30	4 (13·3)	21 (70·0)	4 (13·3)	0	1 (3·3)
13	M (MC)	10	17	7 (41·2)	6 (35·3)	3 (17·6)	0	1 (5·9)
14	M (RH)	33	16	5 (31·3)	10 (62·5)	1 (6·3)	0	0
15	S (RH)	8	23	11 (47·8)	7 (30·4)	3 (13·0)	2 (8·7)	0
16	S (RH)	13	14	3 (21·4)	9 (64·3)	0	0	2 (14·3)
17	S (MC)	12	37	18 (48·6)	13 (35·1)	3 (8·1)	0	3 (8·1)
18	S (RH)	10	9	8 (88·9)	1 (11·1)	0	0	0
19	S (MC)	9	30	19 (63·6)	10 (33·3)	0	1 (3·3)	0
20	E (RH)	13	14	8 (57·1)	4 (28·6)	2 (14·3)	0	0
21	E (RH)	2	1	1 (100·0)	0	0	0	0
22	E (RH)	10	11	5 (45·5)	6 (54·5)	0	0	0
Area								
North		103	189	71 (37·6)	68 (36)	34 (18)	7 (3·7)	9 (4·7)
Middle		57	63	16 (25·4)	37 (58·7)	8 (12·7)	0	2 (3·2)
South		52	113	59 (52·2)	40 (35·4)	6 (5·3)	3 (2·7)	5 (4·4)
East		25	26	14 (53·8)	10 (38·5)	2 (7·7)	0	0
Total		237	391	160 (40·9)	155 (39·6)	50 (12·8)	10 (2·6)	16 (4·1)

N, North; M, middle; S, south; E, east; RH, regional hospital; MC, medical centre; Cal, *C. albicans*; Non-alb, non-albicans.
* Number of isolates (percentage).

hospitals varied. The range was from 0% to 70% for *C. glabrata*, from 0% to 100% for *C. parapsilosis*, and from 0% to 100% for *C. tropicalis*.

Among the 628 isolates tested for susceptibility, 530 (84·4%), 45 (7·2%), and 53 (8·4%) isolates were susceptible, susceptible-dose dependent, and resistant to fluconazole respectively (Table 2). A total of 21·6, 12·1, 6·5 and 2·5% of the isolates from hospitals in the East, South, North, and Middle regions were resistant to fluconazole. The resistance rate to fluconazole of each hospital varied, ranging from 0% to 23·8%. Worthy of note is that none of the isolates from eight of the hospitals, out of the total of 186 isolates, was resistant to fluconazole. The localities of those hospitals were five in the North region, two in the Middle region, and one in the East region. In contrast, isolates from nine of the remaining 14 hospitals had resistance rates to fluconazole greater than 10%. Their locations were three in the South region, three in the North region, two in the East region, and one in the Middle region.

Hospitals without fluconazole-resistant isolates have contributed 94 non-albicans *Candida* spp. while those with resistant isolates have contributed 297 non-albicans *Candida* spp. (Table 1). Of the eight hospitals without fluconazole-resistant isolates, *C. glabrata* (50%) was the most frequently isolated species followed by *C. tropicalis* (23·4%), *C. parapsilosis* (20·2%), *C. krusei* (2·1%), and others (4·3%). Of the remaining 14 hospitals with fluconazole-resistant isolates, *C. tropicalis* (48·5%) was the most frequently isolated species followed by *C. glabrata* (36·4%), *C. parapsilosis* (10·4%), *C. krusei* (2·7%), and others (4%). The prevalence of *C. parapsilosis* and resistance rate to fluconazole had a negative association, whereas the prevalence of *C. tropicalis* and resistance rate to fluconazole had a positive association ($P < 0{\cdot}05$).

Table 2. *The distribution of susceptibility to fluconazole*

Hosp. no.	Region (type)	Susceptible	Susceptible-dose dependent	Resistant
1	N (RH)	19 (79·2)*	2 (8·3)	3 (12·5)
2	N (MC)	37 (88·1)	2 (4·8)	3 (7·1)
3	N (RH)	18 (94·7)	1 (5·3)	0
4	N (RH)	26 (92·9)	0	2 (7·1)
5	N (RH)	14 (100)	0	0
6	N (RH)	15 (75·0)	3 (15·0)	2 (10·0)
7	N (MC)	25 (89·3)	3 (10·7)	0
8	N (RH)	7 (100)	0	0
9	N (MC)	41 (83·7)	3 (6·1)	5 (10·2)
10	N (RH)	22 (100)	0	0
11	N (RH)	31 (79·5)	4 (10·3)	4 (10·3)
12	M (RH)	42 (95·5)	2 (4·5)	0
13	M (MC)	22 (81·5)	2 (7·4)	3 (11·1)
14	M (RH)	49 (100)	0	0
15	S (RH)	27 (87·1)	2 (6·5)	2 (6·5)
16	S (RH)	21(77·8)	3 (11·1)	3 (11·1)
17	S (MC)	33 (67·3)	6 (12·2)	10 (20·4)
18	S (RH)	16 (84·2)	2 (10·5)	1 (5·3)
19	S (MC)	30 (76·9)	5 (12·8)	4 (10·3)
20	E (RH)	20 (74·1)	1 (3·7)	6 (22·2)
21	E (RH)	3 (100)	0	0
22	E (RH)	12 (57·1)	4 (19·0)	5 (23·8)
Area				
North		255 (87·3)	18 (6·2)	19 (6·5)
Middle		113 (94·2)	4 (3·3)	3 (2·5)
South		127 (77)	18 (19·9)	20 (12·1)
East		35 (68·6)	5 (9·8)	11 (21·6)
Total		530 (84·4)	45 (7·2)	53 (8·4)

N, North; M, middle; S, south; E, east; RH, regional hospital; MC, medical centre.
* Number of isolates (percentage).

Of the 53 fluconazole-resistant isolates, 25, 15, 5, 2 and 6 isolates were from urine, sputum, blood, wound, and others respectively (Table 3). A total of 24 *C. tropicalis*, 13 *C. glabrata*, 9 *C. albicans*, and 7 *C. krusei* isolates were resistant to fluconazole. Of the nine fluconazole-resistant *C. albicans* isolates, only one was from a hospital (no. 18) having a resistance rate to fluconazole of less than 10% (Table 2) while three out of the seven fluconazole-resistant *C. krusei* isolates were from hospitals with resistance rates greater than 10%.

Of the 628 isolates, 88 (14·0%), 372 (59·2%), 165 (26·3%), and 3 (0·5%) isolates had a MIC for amphotericin B (MICs $\leqslant 0{\cdot}25$, 0·5, 1 and 2 μg/ml respectively) (Table 4). The resistant isolates were one each of *C. famata, C. krusei*, and *C. tropicalis*. There were 43·3% (23/53) of fluconazole-resistant isolates whose MICs to amphotericin B were $\geqslant 1$ μg/ml, only 24·9% (132/530) of the susceptible isolates had MICs for amphotericin B $\geqslant 1$ μg/ml ($P < 0{\cdot}05$).

DISCUSSION

C. krusei, *C. glabrata* and *C. tropicalis* were less susceptible to fluconazole than other *Candida* spp. [6, 7, 9]. Furthermore, these non-albicans *Candida* spp. are often associated with diseases rather than colonization as commensal-like *C. albicans* [13]. On the other hand, though we requested clinically significant isolates, fluconazole-resistant isolates from non-sterile sites such as sputum and urine could be argued as being commensal. Even so, it is still noteworthy that approximately 10% of the common *Candida* spp. were resistant to fluconazole [9]. Those colonization strains would cause diseases when opportunity arises [14–16]. Since we requested 10 *C. albicans* isolates during the collection period, the prevalence of *C. albicans* in the present study may be underestimated.

Isolates from the Middle (2·5%) and North (6·5%) regions have a lower ratio of resistance to fluconazole than isolates from the South (11·8%) and East (21·6%) regions [9, 10]. There are significant differences in the prevalence of *C. parapsilosis* and *C. tropicalis* between hospitals without and with fluconazole-resistant isolates. Hospitals having more *C. tropicalis* and less *C. parapsilosis* had a tendency to have more fluconazole-resistant isolates. Although *C. glabrata* has been recognized as less susceptible to fluconazole than other *Candida* spp. [17], there was no relationship between the prevalence of *C. glabrata* and the resistance rate to fluconazole. The resistance rate to fluconazole of *C. glabrata* isolates is lower in the present study than reported elsewhere (8% *vs.* from 18% to 50%) [18]. Due to the difference in antifungal practices and infection control strategies, there are some variations of distribution of species and susceptibility to fluconazole among different institutions, localities, and countries [19, 20]. In order to understand the spectrum of *Candida* spp. involved and the emergence of antifungal resistance, it is necessary to further the study to investigate factors involved in the resistance rate to fluconazole in individual hospitals.

According to the *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 resistance rates between fluconazole and amphotericin B may be the result of different mechanisms for antifungal activity

Table 3. *Sources of fluconazole-resistant* Candida *spp.* (*n* = *53*)

Hosp. no.	Resistance rate	Region (type)	Urine				Sputum				Blood		Wound	Others				Total
			Cal	Cgl	Ckr	Ctr	Cal	Cgl	Ckr	Ctr	Ckr	Ctr	Cal	Cal	Cgl	Ckr	Ctr	
1	12·5	N (RH)		1									1	1				3
2	7·1	N (MC)		1	1							1						3
4	7·1	N (RH)							1	1								2
6	10	N (RH)		1		1												2
9	10·2	N (MC)		2	2			1										5
11	10·3	N (RH)				2				1							1	4
13	11·1	M (MC)				2									1			3
15	6·5	S (RH)														2		2
16	11·1	S (RH)		1		1	1											3
17	20·4	S (MC)		2		2	1	2		2		1						10
18	5·3	S (RH)	1															1
19	10·3	S (MC)				1					1		1		1			4
20	22·2	E (RH)				4	1					1						6
22	23·8	E (RH)					2			2		1						5
Total	8·4		1	8	3	13	5	3	1	6	1	4	2	1	2	2	1	53

Cal, *C. albicans*; Ctr, *C. tropicalis*; Cgl, *C. glabrata*; Cpa, *C. parapsilosis*; Ckr, *C. krusei*.
N, North; M, middle; S, south; E, east; RH, regional hospital; MC, medical centre.

Table 4. *Susceptibility to both amphotericin B and fluconazole*

	Amphotericin B (μg/ml)						
Fluconazole	0·06	0·125	0·25	0·5	1	2	Total
Susceptible	2 (0·4)*	4 (0·8)	76 (14·3)	316 (59·6)	131 (24·7)	1 (0·2)	530
Susceptible-dose dependent	0	0	4 (8·9)	29 (64·4)	12 (26·7)	0	45
Resistant	0	0	2 (3·8)	28 (52·8)	21 (39·6)	2 (3·8)	53
Total	2 (0·3)	4 (0·6)	82 (13·1)	373 (59·4)	164 (26·1)	3 (0·5)	628

* Number of isolates (percentage).

(fungistatic *vs.* fungicidal) [4, 21], frequency of usage, and/or different molecular mechanisms of drug resistance [2, 22]. Although there were only three isolates with a MIC to amphotericin B at 2 μg/ml, there were 165 (26·3%) isolates with MICs to amphotericin B at 1 μg/ml. Since there is a trend of co-resistance between amphotericin B and fluconazole, it is advisable that public health authorities as well as clinicians should be alerted to the possibility of emerging amphotericin B resistance, especially for multidrug-resistant isolates.

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APPENDIX. Taiwan Surveillance of Antimicrobial Resistance of Yeasts (TSARY) hospitals

We express our appreciation to all 22 TSARY participating hospitals for providing the isolates and information related to these isolates. They were: Chang Gung Memorial Hospital at Linkou, Chang Gung Memorial Hospital at Keelung, Hsin-Chu Hospital, Lo-Tung Poh Ai Hospital, St. Mary Hospital, Taipei Municipal Yang-Ming Hospital, Taipei Municipal Zen Ai Hospital, Tao-Yuan General Hospital, Taiwan Adventist Hospital, Koo Foundation Sun Yat-sen Cancer Center, Tri Service General Hospital, Kuan-Tien General Hospital, Veterans General Hospital-Taichung, Zen Ai General Hospital, Chi Mei Hospital, Kaohsiung Medical College Chung-Ho Memorial Hospital, Kaohsiung Military Hospital, Tainan Municipal Hospital, Veterans General Hospital-Kaohsiung, Buddhist Tzu-Chi General Hospital,

Hua-Lien Hospital, and Mackay Memorial Hospital Taitung Branch.

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