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Fluconazole resistance rate of *Candida* species from different regions and hospital types in Taiwan

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From April 15 to June 15, 1999, 581 clinical *Candida* isolates from 19 hospitals in Taiwan were collected and susceptibilities to fluconazole of these isolates were determined by a broth microdilution method. A total of 42 (7.2%) isolates were resistant to fluconazole. Isolates from medical centers had a higher resistance rate to fluconazole than those from regional hospitals (10.7% vs 4.9%). *Candida* species isolated from different regions had different degrees of susceptibility to fluconazole. Approximately 2.5%, 6.5%, and 11.8% of *Candida* isolates from middle, north, and south regions, respectively, were resistant to fluconazole. The prevalence of the combination of *Candida glabrata*, *Candida krusei*, and *Candida tropicalis* infections were 44.5%, 49.8%, and 62.7% in middle, north, and south regions, respectively. There is an association between the rate of fluconazole resistance and the number of non-*albicans Candida* species collected from different regions and hospital types.

Key words: *Candida*, fluconazole, resistance

In the past decade, *Candida* infections increased significantly. The prevalence of nosocomial candidemia increased 27-fold from 1981 through 1993 at a teaching hospital in Taiwan [1,2]. In the United States, *Candida* is the 4th leading cause of nosocomial bloodstream infections [3,4]. The dramatic increase in the prevalence of fungal infections is probably due to alterations in immune status associated with the acquired immunodeficiency syndrome (AIDS) epidemic, cancer chemotherapy, organ and bone marrow transplantation, and invasive hospital procedures [5].

Even though *Candida albicans* is still the most common pathogen among *Candida* species, the spectrum of *Candida* infections has been increased to include more of other species [3,6]. The emergence of non-albicans *Candida* species infections may be due to an increasing population of immunosuppressed patients, an increased usage of antifungal agents, and an improved diagnosis of *Candida* species. Currently available antifungal drugs have several problems including side effects, ineffective against some fungi, and drug resistance [5]. Oropharyngeal candidiasis due to drug-resistant fungi is a major problem for patients infected with human immuno-deficiency virus (HIV) [7,8]. One-third of the late-stage AIDS patients have drug-resistant strains of *C. albicans* in their oral cavities [9]. Coinciding with the increased usage of antifungal agents, especially fluconazole, the number of drug-resistant cases has increased. *Candida* species have various degrees of susceptibility to common antifungal agents. *C. glabrata*, *C. krusei*, and *C. tropicalis* are less susceptible to fluconazole than other *Candida* species [10-12].

We have reported a Taiwan Surveillance of Antimicrobial Resistance of Yeasts (TSARY) in 1999, in which 22 hospitals contributed 660 clinical yeast isolates [13]. There were approximately 4%, 15%, 8%, and 70% of *C. albicans*, *C. tropicalis*, *C. glabrata*, and *C. krusei* isolates, respectively, that are resistant to fluconazole. In contrast, none of the *Candida parapsilosis* isolate was resistant to fluconazole (unpublished data). The present study was carried out to determine if isolates from different types of hospitals in different regions have different degrees of susceptibility to fluconazole and whether susceptibility is associated with different prevalence of non-*albicans Candida* species infections.

Materials and Methods

Organisms and medium

Yeast isolates were collected from 22 hospitals in Taiwan including 6 medical centers, 14 regional

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hospitals, and 2 local teaching hospitals. Each hospital was asked to submit 10 *C. albicans* and 40 yeast pathogens but not *C. albicans* during the collection period, from April 15 to June 15, 1999 [13]. One isolate was accepted from each episode of infection. Isolates were stored at –70°C in bead-containing Microbank cryovials (PRO-LAB Diagnostics, Austin, TX, US). 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 within 24 h. The isolates were first subcultured on Sabouraud Dextrose Agar (SDA, BBL Becton Dickinson and Company Cockeysville, MD, US) 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 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) [14]. The powder of RPMI medium 1640 (31800-022) was provided by Gibco BRL (Becton Dickinson Sparks, MD, US). The fluconazole (Pfizer Inc. Groton, CT, US) was serially 2-fold diluted and kept in 96-well Costar 3599 plate (Corning Inc. New York, US). The range of concentration of fluconazole was from 0.125 μg/mL to 64 μg/mL. The cells were inoculated to a final inoculum from 0.5 to 2.5 x 10^3 colony forming unit (CFU)/mL. The final growth of each isolate was measured by a Spectra MAX Plus (Molecular Devices Cop. Sunnyvale, CA, US) after incubation at 35°C for 48 h. The MIC was defined as the concentration that reduces the growth of cells down to 50% according to the guidelines of the NCCLS. Isolates with resistant, susceptible-dose dependent, and susceptible to fluconazole were defined as MICs ≥64 μg/mL, 16 to 32 μg/mL, and ≤8 μg/mL, respectively. *C. albicans* (ATCC 90028), *C. krusei* (ATCC 6258), and *C. parapsilosis* (ATCC 22019) were used as control strains. The MIC of each isolate was measured at least twice. Fluconazole susceptibility of some isolates was also determined by Etest (AB Biodisk Solna, Sweden) to assess the microdilution results.

Data analysis

The significance of differences in frequencies and proportions was determined by the chi-square test with Yates' correction or Fisher exact correction. The significance of difference in resistance rate was determined by comparing rates. The association between prevalence and resistance rate was computed by Pearson's correlation coefficient.

Results

During the study period, a total of 581 clinical isolates from 19 hospitals including 6 medical centers and 13 regional hospitals from north, middle, and south regions were characterized. The distribution of 581 *Candida* species from 19 hospitals is summarized in Table 1. *C. albicans* was the most common species provided by hospitals, with 36.5% (212/581) of the total isolates, followed by *C. tropicalis* (149/581, 25.7%), *C. glabrata* (146/581, 25.1%), *C. parapsilosis* (49/581, 8.4%), *C. krusei* (10/581, 1.7%), and others (15/581, 2.6%). The combination of *C. tropicalis* and *C. glabrata* accounted for 80% (295/369) of total non-*albicans Candida* species.

The distribution of *Candida* species at different locations and types of hospital is summarized in Table 2. Hospitals in the north and middle had less non-*albicans Candida* species, consisting of *C. glabrata*, *C. krusei*, and *C. tropicalis*, than hospitals in the south (49.8% and 44.5% vs 62.7%, $p<0.01$). Hospitals in the north contributed more *C. parapsilosis* than hospitals in south (11.9% vs 3.6%, $p<0.005$) especially those in the rank of medical centers (12.5% vs 3.4%, $p<0.05$).

The susceptibilities to fluconazole of 581 isolates from 19 hospitals located in 3 different regions in Taiwan were summarized in Table 3. A total of 42 (7.2%) and 40 (6.9%) isolates are resistant and susceptible dose dependent to fluconazole, respectively. Isolates from the north and middle had lower resistance rates than those from the south. A total of 2.5% (3/119), 6.5% (19/293), and 11.8% (20/169) isolates from the middle, north, and south, respectively, were resistant to fluconazole. Approximately 10.7% (25/234) and 4.9% (17/347) isolates from medical centers and regional hospitals were resistant to fluconazole, respectively ($p<0.01$). Isolates from medical centers in the middle and south had higher resistance rates to fluconazole than those from regional hospitals (11.5% vs 0% and 15.9% vs 7.4%).

There is significant difference in the prevalence of non-*albicans Candida* species including *C. glabrata*, *C. krusei*, and *C. tropicalis* between the north (49.8%) and south (62.7%) ($p<0.02$) as well as between the middle (44.5%) and south (62.7%) ($p<0.005$). Resistance rates of these non-*albicans Candida* species also differ significantly between the north (7.2%) and south (11.8%) ($p<0.05$) as well as between the middle (2.5%) and south (11.8%) ($p<0.005$). These results suggest that there is an association between the rate of

Table 1. Distribution of *Candida* species in 19 hospitals

Hospital no.	Region	No. of isolates						Total
		C. albicans	*C. tropicalis*	*C. glabrata*	*C. parapsilosis*	*C. krusei*	Others	
1	North	9	6	5	1	0	1	22
2	North	11	3	1	2	1	1	19
3	North	10	7	14	7	1	3	42
4	North	9	3	10	2	0	0	24
5	North	8	2	2	1	0	1	14
6	North	11	20	9	7	2	1	50
7	North	11	9	4	3	1	0	28
8	North	4	0	0	2	0	0	6
9	North	10	5	5	0	0	0	20
10	North	10	1	8	8	1	0	28
11	North	11	15	10	2	1	1	40
12	Middle	14	4	21	4	0	1	44
13	Middle	33	5	10	1	0	0	49
14	Middle	9	7	6	3	0	1	26
15	South	8	13	7	3	2	0	33
16	South	12	18	13	3	0	3	49
17	South	13	4	10	0	0	2	29
18	South	9	19	10	0	1	0	39
19	South	10	8	1	0	0	0	19
Total		212	149	146	49	10	15	581

resistance to fluconazole and the prevalence of non-*albicans Candida* species ($p<0.05$). This association is also found between medical centers and regional hospitals (60.6% vs 47.3% of *C. glabrata*, *C. krusei*, and *C. tropicalis* and 10.7% vs 4.9% resistance rate).

Discussion

According to the *in vitro* antifungal susceptibility testing, 42 of 581 (7.2%) isolates were considered to be resistant to fluconazole. Even though the prevalence of *C. albicans* infections is high, the resistance rate to fluconazole of this species is still low, suggesting that there is no correlation between the prevalence and resistance rate in *C. albicans*.

Among the clinical isolates from hospitals in the south, 3.6% were *C. parapsilosis* and 11.8% were resistant to fluconazole. In contrast, among the clinical isolates from hospitals in the north, 11.9% were *C. parapsilosis* and 6.5% were resistant to fluconazole. There is a negative correlation between susceptibility to fluconazole and the prevalence of *C. parapsilosis* infections. This observation is consistent with our data and others that *C. parapsilosis* is susceptible to fluconazole [15].

Table 2. Distribution of *Candida* species in different regions and types of hospitals (n = 581)

	No. of hospitals	No. of isolates (%)						
		C. albicans	*C. tropicalis*	*C. glabrata*	*C. parapsilosis*	*C. krusei*	Others	*C. tropicalis* *C. glabrata* *C. krusei*
North	11	104 (35.5)	71 (24.2)	68 (23.2)	35 (11.9)	7 (2.4)	8 (2.7)	146 (49.8)
Medical Center	3	31 (27.7)	32 (28.6)	28 (25.0)	14 (12.5)	3 (2.7)	4 (3.6)	63 (56.3)
Regional Hospital	8	73 (40.3)	39 (21.5)	40 (22.1)	21 (11.6)	4 (2.2)	4 (2.2)	83 (45.9)
Middle	3	56 (47.1)	16 (13.4)	37 (31.1)	8 (6.7)	0	2 (1.7)	53 (44.5)
Medical Center	1	9 (34.6)	7 (26.9)	6 (23.1)	3 (11.5)	0	1 (3.8)	13 (50.0)
Regional Hospital	2	47 (50.5)	9 (9.7)	31 (33.3)	5 (5.4)	0	1 (1.1)	40 (43.0)
South	5	52 (30.8)	62 (36.7)	41 (24.3)	6 (3.6)	3 (1.8)	5 (3.0)	106 (62.7)
Medical Center	2	21 (23.9)	37 (42.0)	23 (26.1)	3 (3.4)	1 (1.1)	3 (3.4)	61 (69.3)
Regional Hospital	3	31 (38.3)	25 (30.9)	18 (22.2)	3 (3.7)	2 (2.5)	2 (2.5)	45 (55.6)
Total	19	212 (36.5)	149 (25.6)	146 (25.1)	49 (8.4)	10 (1.7)	15 (2.6)	305 (52.5)
Medical Center	6	61 (27.0)	76 (23.6)	57 (25.2)	20 (8.8)	4 (1.8)	8 (3.5)	137 (60.6)
Regional Hospital	13	151 (42.5)	73 (20.6)	89 (25.1)	29 (8.2)	6 (1.7)	7 (2.0)	168 (47.3)

Table 3. Correlation between the susceptibility to fluconazole and the type and location of hospitals

Hospital location	No. of isolates (%)		
	Susceptible	Susceptible, dose-dependent	Resistant
North	258 (88.0)	16 (5.5)	19 (6.5)
Medical Center	105 (87.5)	7 (5.8)	8 (6.7)
Regional Hospital	153 (88.4)	9 (5.2)	11 (6.4)
Middle	112 (94.1)	4 (3.4)	3 (2.5)
Medical Center	23 (88.5)	0	3 (11.5)
Regional Hospital	91 (97.9)	2 (2.1)	0
South	130 (76.9)	19 (11.3)	20 (11.8)
Medical Center	63 (71.6)	11 (12.5)	14 (15.9)
Regional Hospital	67 (82.7)	8 (9.9)	6 (7.4)
Total	499 (85.9)	40 (6.9)	42 (7.2)
Medical Center	189 (80.8)	20 (8.6)	25 (10.7)
Regional Hospital	310 (89.3)	20 (5.8)	17 (4.9)

The non-*albicans Candida* species are often associated with diseases rather than colonization as commensal like *C. albicans* [16]. The frequency of candidiasis caused by non-*albicans Candida* species among different institutes ranged from as low as 0% to as high as 70% [17]. Different species had different susceptibilities to antifungal agents. The observation that there is an association between the rate of fluconazole resistance and the number of non-*albicans Candida* species is consistent with the report that *C. glabrata*, *C. krusei*, and *C. tropicalis* were considered having high resistance rates to fluconazole [10,11,18]. The finding that the prevalence of non-*albicans Candida* species infections varied among different hospitals in this study may be due to the differences in patient population, antimicrobial usage, and/or antifungal therapy. To unveil the actual causes, further investigation is required.

There are many mechanisms contributing to drug-resistant phenotypes in infecting organisms. To date, the identified mechanisms are as follows: (1) reduction of drug accumulation by preventing the import of drug into the cell and activating the efflux of drug from the cell; (2) alteration of drug target including mutating the target of drug, overexpressing the target, and bypassing the requirement of the drug-targeted enzyme by changing other enzymes in the same enzymatic pathway; and (3) inactivation of the drug including modifying and degrading of drugs [5,19-21]. The knowledge gained from identification of the molecular mechanisms contributing to fluconazole resistance of these clinical isolates may help to prevent fungal pathogens from developing drug resistance.

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