Characterization of *Candida* Species from Different Populations in Taiwan

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Abstract The opportunistic *Candida* species existing as part of commensal microbiota in humans are usually the etiological agents causing infections. We investigated whether isolates collected from different age groups, hospital units, and sources have distinct characteristics. A total of 913 isolates comprising 395 *Candida albicans*, 230 *Candida tropicalis,* 202 *Candida glabrata*, 62 *Candida parapsilosis*, 13 *Candida krusei*, and 11 of other six species were analyzed. Urine was the most common source (41.2%), followed by sputum (16.3%), blood (15.2%), and others (27.3%). *Candida albicans* and *C. parapsilosis* were more prevalent in the working group [from 19 to 65 years], whereas *C. tropicalis* and *C. glabrata* were more prevalent in the elder one ($\geq$ 66 years). We found that the age of patients and the source of isolates affect the distribution of species. On the other hand, the drug susceptibility of isolates was associated with fungal species and whether patients were hospitalized.

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Introduction

Due to the increased size of populations at risk, the prevalence of fungal infections has increased significantly. *Candida* species are the most frequently isolated fungal pathogens, causing morbidity and mortality in seriously immunocompromised hosts [1–4]. Overall, the five most frequently isolates of *Candida* species are *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, *Candida parapsilosis*, and *Candida krusei*.

The opportunistic *Candida* species existing as parts of commensal microbiota in humans are usually the etiological agents causing infections [5–8]. Thus, it is important to investigate whether isolates from various sources and various patients with different ages and in different hospital units have distinct characteristics. However, most available epidemiological data were related to candidemia [1–4, 9]. Thus, a national survey, Taiwan Surveillance of Antimicrobial Resistance of Yeasts (TSARY), was initiated in 1999 to analyze yeasts isolated from different sources [10]. In the present study, a continuous study of TSARY 2006 [11] was conducted to investigate whether isolates from sterile sites vs. non-sterile ones and hospitalized patients vs. outpatients have distinct characteristics. Furthermore,

the distribution and drug susceptibility of *Candida* species from different age groups, hospital units, and sources were also analyzed.

Materials and Methods

Organisms and Media

Yeast isolates were collected in the previous study from the 22 hospitals participating in TSARY 2006 [11]. Each hospital was asked to document total number of yeast isolates isolated from July to September in 2006. For further analysis, all yeast pathogens from sterile sites and up to ten *C. albicans* and 40 non-albicans *Candida* species isolates from non-sterile sites were obtained from each hospital. In principle, only one isolate was accepted from each specimen. Nevertheless, when there were multiple species isolated from the same specimen, one isolate from each species was analyzed. After their arrival at the laboratory at National Health Research Institutes (NHRI), these isolates were first sub-cultured on Sabouraud dextrose agar (SDA, BBL, Becton–Dickinson Cockeysville, MD, USA) to assess the purity and identification. Pure isolates were labeled and stored in vials containing 50% glycerol at −70°C awaiting further analysis. One hospital did not provide sufficient information. Thus, 913 patients from 21 participating hospitals were analyzed.

Identification

The identifications of the isolates were reassessed in the laboratory at the NHRI. The identification procedure for the yeast isolates was described in our previous report [11]. All isolates were subjected to analyses with ID 32C (bioMérieux, Marcy l'Etoile, France). The VITEK Yeast Biochemical Card (YBC, bioMérieux, Marcy l'Etoile, France) was then used for the identification of the isolates when the result of ID 32C showed less than 90% probability and of those with discrepancy from the information provided by the hospitals. The sequences of internal transcribed spacer regions (ITS) and the D1/D2 region of ribosomal DNA [12] were subjected to further assessment when both ID 32C and YBC failed to reach a probability greater than 90%.

Antifungal Susceptibility Testing

The minimum inhibitory concentrations (MICs) of antifungal agents were determined according to the procedures in our previous studies [13, 14], which followed the guidelines of Clinical and Laboratory Standards Institute [15]. The RPMI medium 1640 (Gibco BRL 31800-022) was used for the testing. Strains from American Type Culture Collection, including *C. albicans* (ATCC 90028), *C. krusei* (ATCC 6258), and *C. parapsilosis* (ATCC 22019), were used as the standard controls. The final growth of each isolate was measured by Biotrak II plate spectrophotometric reader (Amershan Biosciences, Biochrom Ltd., Cambridge England) after incubation at 35°C for 24 and 48 hours (h).

The MICs for amphotericin B and azoles were defined as the lowest concentrations capable of preventing any discernible growth and of reducing the turbidity of cells more than 50%, respectively. For amphotericin B, MICs of ≥ 2 μg/ml were considered resistant and ≤ 1 μg/ml were considered susceptible. Isolates with fluconazole MICs ≥ 64 μg/ml were considered to be resistant, whereas those ≤ 8 μg/ml were susceptible, and those in the range of 16–32 μg/ml were susceptible dose dependent. Isolates with voriconazole MICs ≥ 4 μg/ml were considered to be resistant, whereas those ≤ 1 μg/ml were susceptible, and those with 2 μg/ml was susceptible dose dependent.

Among the phenomena associated with drug resistance, "trailing" describes the reduced but persistent growth that some isolates exhibit at drug concentrations above the MIC in broth dilution tests with azole antifungal agents, such as fluconazole [16]. When the MIC of an isolate after 48-h incubation is approximately fourfold higher than that at the 24-h point, the isolate is defined to have trailing growth [11, 17].

Database and Analysis

The database for this study contained the characteristic information of each submitted isolate. The patients were divided into three groups according to our previous criterion [18], including younger group with age ≤ 18 years (y), working one with age from 19 to 65 years, and elder one with age ≥ 66 years. The statistic significance of the differences in frequencies

and proportions was determined by the chi-square test with Mantel–Haenszel's correction or Fisher exact correction. Logistic regression was applied to assess the independent effect of factors, including age, hospital unit, source, and species.

Results

The Distribution of *Candida* Species

A total of 3,767 yeast isolates were isolated at the 22 hospitals participating in TSARY from July to September in 2006. Among the 3,501 isolates identified as *Candida* species by the hospitals, *C. albicans* (72.3%) was the most common species, followed by *C. tropicalis* (12.3%), *C. glabrata* (11.7%), *C. parapsilosis* (2.7%), *C. krusei* (0.7%), and others (0.3%). The medical information of 913 isolates was provided by 21 of the hospitals and analyzed in the present study. The average number of isolates contributed per hospital was 43 (ranging from 11 to 80). There were 395 (43.3%) *C. albicans*, 230 (25.2%) *C. tropicalis*, 202 (22.1%) *C. glabrata*, 62 (6.8%) *C. parapsilosis*, 13 (1.4%) *C. krusei*, 4 (0.4%) *Candida lusitaniae*, 3 (0.3%) *Candida metapsilosis*, and one isolate each of *Cryptococcus curvatus*, *Candida guilliermondii*, *Candida lipolytica*, and *Candida rugosa* (Table 1).

Since each hospital was asked to submit up to ten *C. albicans* isolates from non-sterile sites during collection period, the proportion of *C. albicans* was significantly underestimated (43.3% in the 913 isolates vs. 72.3% in the 3,501 isolates). Consequently, the source distribution of this species was not calculated.

As expected, majority of the patients (83.8%, $P = 0.008$) were hospitalized when the isolates were recovered. There were 286 and 479 patients in ICU and non-ICU, respectively. A total of 113 and 405 non-albicans *Candida* species were recovered from sterile and non-sterile sites, respectively. A higher proportion of *C. tropicalis* was from sputum ($P = 0.0007$), whereas the majority of *C. glabrata* in the present study were isolated from urine (67.8%, $P = 0.00002$). Consequently, significantly less *C. glabrata* were isolated from sputum ($P = 0.02$) and pus ($P = 0.01$). In addition, only 19 (13.7%) candidemia were caused by *C. glabrata*.

The Distribution of Patient Ages

More than half of the isolates were from the elder group, accounting for 58.7%, followed by the working one (38.2%) and the younger one (3.1%) (Table 2). With the exception of *C. krusei*, the distributions of other species between the working group and the elder one were significantly different. *Candida albicans* ($P = 0.05$) and *C. parapsilosis* ($P = 0.000002$) were more prevalent in the working group, whereas *C. tropicalis* ($P = 0.04$) and *C. glabrata* ($P = 0.02$) were more prevalent in the elder one.

The distribution of isolates from urine and blood were different among different age groups. There were 14.3, 20.3, and 11.9% isolates recovered from the blood of patients with age ≤ 18, 19–65, and ≥ 66, respectively. There were more isolates from blood in the working group than those in others ($P = 0.0008$). A total of 42.9, 26.6, and 50.6% of isolates from patients with age ≤ 18, 19–65, and ≥ 66, respectively, were from urine. Furthermore, nine of the ten isolates ($P = 0.05$) from ear and all nine isolates from genital tract ($P = 0.02$) were recovered from the working group. A total of 89.3, 74.5, and 89.6% of patients with age ≤ 18, 19–65, and ≥ 66 were hospitalized, respectively. As expected, the working group had a lower rate of hospitalization than others ($P < 0.000001$).

The Distribution of Sources

Of all sources, urine was the most common one, accounting for 41.2% of total isolates, followed by sputum (16.3%), blood (15.2%), wound (4.7%), pus (4.3%), ascites (4.2%), tip (3.2%), catheter (1.6%), ear (1.1%), genital tract (1%), and others (6.4%). There were 267 (29.2%) isolates recovered from 17 sterile sites, and blood was the most common source (52.1%) followed by pus (14.6%), ascites (14.2%), catheter (5.6%), and others (13.5%) (Table 3). Among the 20 non-sterile sites contributing 646 isolates, urine was the most common source (58.2%) followed by sputum (23.1%), wound (6.7%), and others (12%) (Table 3).

The distribution of sources were different between outpatients and hospitalized patients. There were more isolates from sterile sites in hospitalized patients than those in outpatients ($P = 0.05$), especially for blood ($P = 0.002$). Nevertheless, there were more isolates from sputum ($P = 0.0006$) in hospitalized

Table 1 Characteristics of 913 isolate by species

	C. albicans	C. tropicalis	C. glabrata	C. parapsilosis	C. krusei	Others	Total
Total	395	230	202	62	13	11	913
Source							
Sterile							
Blood	80	29 (12.6)[a]	19 (9.4)	9 (14.5)	1 (7.7)	1 (9.1)	139 (15.2)
Pus	20	8 (3.5)	2 (1)[b]	6 (9.7)[b]	2 (15.4)[b]	1 (9.1)	39 (4.3)
Ascites	24	7 (3)	4 (2)	2 (3.2)	1 (7.7)	0	38 (4.2)
Catheter	7	5 (2.2)	1 (0.5)	2 (3.2)	0	0	15 (1.6)
Bile	7	0	1 (0.5)	0	0	0	8 (0.9)
Others	16	4 (1.7)	4 (2)	4 (6.5)	0	0	28 (3.1)
Subtotal	154	53 (23)	31 (15.3)	23 (37.1)[b]	4 (30.8)	2 (18.2)	267 (29.2)
Non-sterile							
Urine	118	99 (43)	137 (67.8)[b]	13 (21)[b]	5 (38.5)	4 (36.4)	376 (41.2)
Sputum	63	63 (27.4)[b]	19 (9.4)[b]	2 (3.2)[b]	2 (15.4)	0	149 (16.3)
Wound	21	6 (2.6)	6 (3)	5 (8.1)	1 (7.7)	4 (36.4)	43 (4.7)
Tip	15	8 (3.5)	3 (1.5)	3 (4.8)	0	0	29 (3.2)
Ear	2	0	0	8 (12.9)[b]	0	0	10 (1.1)
Genital tract	6	0	3 (1.5)	0	0	0	9 (1)
Others	16	1 (0.4)	3 (1.5)	8 (12.9)	1 (7.7)	1 (9.1)	30 (3.3)
Subtotal	241	177 (77)	171 (84.7)	39 (62.9)	9 (69.2)	9 (81.8)	646 (70.8)
MICs (µg/ml)							
Amp 48 h $\geq$ 2	1 (0.3)	11 (4.8)	1 (0.5)	0	2 (15.4)	1 (9)	16 (1.8)
Flu 24 h $\geq$ 64	10 (2.5)[b]	40 (17.4)[b]	5 (2.5)[b]	0[b]	5 (38.5)[b]	0	60 (6.6)
Flu 48 h $\geq$ 64	10 (2.5)[b]	123 (53.5)[b]	7 (3.5)[b]	0[b]	13 (100)[b]	0	153 (16.8)
Vor 24 h $\geq$ 4 ($N = 911$)[c]	9 (2.3)[b]	39 (17)[b]	4 (2)[b]	0	0	0	52 (5.7)
Vor 48 h $\geq$ 4	14 (3.5)[b]	124 (53.9)[b]	6 (3)[b]	0[b]	0	0	144 (15.8)
Unit							
ICU	120	85	60	14	6	1	286
Flu 24 h $\geq$ 64	4 (1.4)	14 (4.9)	2 (0.7)	0	2 (0.7)	0	22 (7.7)
Vor 24 h $\geq$ 4	2 (1.7)	16 (18.8)	2 (3.3)	0	0	0	20 (7)
Non-ICU	217	123	102	23	5	9	479
Flu 24 h $\geq$ 64	4 (0.8)	23 (4.8)	2 (0.4)	0	3 (0.6)	0	32 (6.7)
Vor 24 h $\geq$ 4	5 (2.3)	21 (17.1)	2 (3.3)	0	0	0	28 (5.8)
Outpatient	58	22	40	25	2	1	148
Flu 24 h $\geq$ 64	2 (1.4)	3 (2)	1 (0.7)	0	0	0	6 (4.1)
Vor 24 h $\geq$ 4 ($N = 146$)[c]	2 (3.4)	2 (9.1)	0	0	0	0	4 (2.7)

Amp amphotericin B, *Flu* fluconazole, *ICU* intensive care unit, *MIC* minimum inhibitory concentration, *Vor* voriconazole

[a] Number of isolate (percentage in the same species group)

[b] $P \leq 0.05$, comparison of percentage for each characteristic/species group vs. percentage in total population

[c] MICs of one of each *C. glabrata* and *C. parapsilosis from outpatients* grew too slowly to be determined

patients than those in outpatients. Furthermore, all 27 isolates from tip were from hospitalized patients and there were more isolates from ear or genital tract in outpatients than those in hospitalized patients ($P < 0.000001$). It is not surprising that more non-sterile site isolates were recovered from outpatients since they were relatively less ill and few to have blood drawn for culture.

	≤ 18 years	19–65 years	≥ 66 years	Total
Table 2 Characteristics of 913 isolates by age group of patients				
Total	28	349	536	913
Species				
C. albicans	15 (53.6)[a]	164 (47)	216 (40.3)	395 (43.3)
C. tropicalis	4 (14.3)	76 (21.8)	150 (28)	230 (25.2)
C. glabrata	3 (10.7)	63 (18.1)	136 (25.4)	202 (22.1)
C. parapsilosis	3 (10.7)	41 (11.7)[b]	18 (3.4)[b]	62 (6.8)
C. krusei	1 (3.6)	3 (0.9)	9 (1.7)	13 (1.4)
Others	2 (7.1)	2 (0.6)	7 (1.3)	11 (1.2)
Source				
Sterile				
Blood	4 (14.3)	71 (20.3)[b]	64 (11.9)	139 (15.2)
Pus	2 (7.1)	22 (6.3)	15 (2.8)	39 (4.3)
Ascites	1 (3.6)	20 (5.7)	17 (3.2)	38 (4.2)
Catheter	1 (3.6)	4 (1.1)	10 (1.9)	15 (1.6)
Bile	0	3 (0.9)	5 (0.9)	8 (0.9)
Others	4 (14.3)	14 (4)	10 (1.9)	28 (3.1)
Subtotal	12 (42.9)	134 (38.4)[b]	121 (22.6)[b]	267 (29.2)
Non-sterile				
Urine	12 (42.9)	93 (26.6)[b]	271 (50.6)[b]	376 (41.2)
Sputum	3 (10.7)	42 (12)	104 (19.4)	149 (16.3)
Wound	0	23 (6.6)	20 (3.7)	43 (4.7)
Tip	1 (3.6)	13 (3.7)	15 (2.8)	29 (3.2)
Ear	0	9 (2.6)	1 (0.2)[b]	10 (1.1)
Genital tract	0	9 (2.6)	0[b]	9 (1)
Others	0	26 (7.4)	4 (0.7)	30 (3.3)
Subtotal	16 (57.1)	215 (61.6)[b]	415 (77.4)[b]	646 (70.8)
Unit				
ICU	12 (42.9)	65 (18.6)[b]	209 (39)[b]	286 (31.3)
Non-ICU	13 (46.4)	195 (55.9)	271 (50.6)	479 (52.5)
Outpatient	3 (10.7)	89 (25.5)[b]	56 (10.4)[b]	148 (16.2)
MICs (µg/ml) at 24 h				
Flu ≥ 64	1 (3.6)	25 (7.2)	34 (6.3)	60 (6.6)
Vor ≥ 4 (N = 911)[c]	1 (3.6)	20 (5.7)	31 (5.8)	52 (5.7)

Amp amphotericin B, *Flu* fluconazole, *ICU* intensive care unit, *MIC* minimum inhibitory concentration, *Vor* voriconazole

[a] Number of isolate (percentage in the same age group)

[b] $P \le 0.05$, comparison of percentage for each characteristic/age group vs. percentage in total population

[c] MICs of one of each 19–65 years and ≥66 years grew too slowly to be determined

Antifungal Susceptibilities of Yeasts

After 48-h incubation, the overall ratio of resistant isolates to amphotericin B was 1.8%. The 16 amphotericin B-resistant isolates were consisted of 11 *C. tropicalis*, 2 *C. krusei*, and one isolate each of *C. albicans*, *C. glabrata*, and *C. curvatus* (Table 1): a total of 2, 6, and 8 isolates from patients with age ≤ 18, 19–65, and ≥ 66, respectively (Table 2).

Of the 153 isolates, comprising 123 *C. tropicalis*, 13 *C. krusei*, 10 *C. albicans*, and 7 *C. glabrata*, having fluconazole MICs ≥ 64 µg/ml after 48-h incubation, 98 (64%) showed trailing phenotype. They included 87 *C. tropicalis*, 7 *C. albicans*, and 4 *C. glabrata*. The issue of trailing is problematic for interpreting the results of fluconazole MICs at 48 h. Therefore, in the present study, we analyzed whether there were any characteristics associated with isolates having fluconazole MICs ≥ 64 µg/ml after 24-h incubation, considered as resistant. The percentage of fluconazole-resistant isolates was not different among different age populations (Table 2). The proportion

Table 3 Characteristics of 913 isolates by sources

	Unit			Total	MICs at 24 h (µg/ml)	
	ICU	Non-ICU	Outpatient		Flu ≥ 64	Vor ≥ 4 (N = 911)[c]
Total	286	479	148	913	60 (6.6)	52 (5.7)
Sterile						
Blood	45 (32.4)[a]	84 (60.4)	10 (7.2)[b]	139	8 (5.8)	7 (5)
Pus	6 (15.4)	22 (56.4))	11 (28.2)	39	0	0
Ascites	16 (42.1)	18 (47.4)	4 (10.5)	38	1 (2.6)	0
Catheter	7 (46.7)	6 (40)	2 (13.3)	15	3 (20)	2 (13.3)
Bile	1 (12.5)	6 (75)	1 (12.5)	8	0	0
Others	7 (25)	16 (57.1)	5 (17.9)	28	2 (7.2)	1 (3.6)
Subtotal	82 (30.7)	152 (56.9)	33 (12.4)	267	14 (5.2)	10 (3.8)
Non-sterile						
Urine	130 (34.6)	184 (48.9)	62 (16.5)	376	26 (6.9)	20 (5.3)[c]
Sputum	57 (38.3)	82 (55)	10 (6.7)[b]	149	15 (10.1)	16 (10.7)
Wound	6 (14)	31 (72)	6 (14)	43	2 (4.7)	2 (4.7)
Tip	10 (34.5)	19 (65.5)	0[b]	29	0	2 (6.9)
Ear	0	1 (10)	9 (90)[b]	10	0	0
Genital tract	0	0[b]	9 (100)[b]	9	1 (11.1)	1 (11.1)
Others	1 (3.3)	10 (33.3)	19 (63.4)	30	2 (6.7)	1 (3.3)
Subtotal	204 (31.6)	327 (50.6)	115 (17.8)	646	46 (7.1)	42 (6.5)

Amp amphotericin B, *Flu* fluconazole, *ICU* intensive care unit, *MIC* minimum inhibitory concentration, *Vor* voriconazole

[a] Number of isolate (percentage in the same source group)

[b] $P \leq 0.05$, comparison of percentage for each characteristic/source group vs. percentage in total population

[c] MICs of one of each isolate from pus and urine grew too slowly to be determined

of the fluconazole-resistant isolates from hospitalized patients was higher than that from outpatients (54/765 vs. 6/148, $P = 0.05$) (Table 1). As expected, different species had different percentage of fluconazole-resistant isolates. The species of *C. krusei* (38.5%) had the highest rate to be fluconazole resistant, followed by *C. tropicalis* (17.4%) and *C. glabrata* (2.5%). All *C. parapsilosis* were susceptible to fluconazole. Based on multivariate analysis, *C. tropicalis* was the only independent risk factor associated with fluconazole resistance (odds ratio, 7.85; 95% confidence interval, 13.8–16.2).

Trailing phenotype was also observed for voriconazole susceptibility. One *C. glabrata* isolate had voriconazole MICs ≥ 4 µg/ml after 48-h incubation, whereas the MIC after 24-h incubation was not detected due to slow growth. Of the remaining 143 isolates comprising 124 *C. tropicalis*, 14 *C. albicans*, and 5 *C. glabrata*, having voriconazole MICs ≥ 4 µg/ml after 48-h incubation, 101 (70.6%) showed trailing phenotype. They included 89 *C. tropicalis*, 10 *C. albicans*,

and 2 *C. glabrata*. Again, we analyzed whether there were any characteristics associated with isolates having voriconazole MICs ≥ 4 µg/ml after 24-h incubation, considered as resistant. The percentage of voriconazole-resistant isolates was not different among different age populations (Table 2). Unlike susceptibility to fluconazole, the proportion of the voriconazole-resistant isolates from hospitalized patients was not significantly different from that from outpatients (48/765 vs. 4/146, $P = 0.1$) (Table 1). Furthermore, different species had different percentage of voriconazole-resistant isolates. The species of *C. tropicalis* (17%) had the highest rate to be voriconazole resistant, followed by *C. albicans* (2.3%) and *C. glabrata* (2%).

Discussion

In the past, nearly 80% of candidemia could be attributed to *C. albicans* [19]. Although *C. albicans* is still the most common single species causing

candidemia in our study (57.6%) as well as other studies [1, 3, 9, 20], prevalence of non-*C. albicans Candida* has indeed increased. Consistent with previous observation that *C. glabrata* is the most common cause of candiduria [13, 14, 21], the majority of this species in the present study were isolated from urine. Even though this species is the second most common cause of candidemia in the USA and the Netherlands [3, 22], only 19 (13.7%) candidemia were caused by *C. glabrata* in the present study. Even though isolates sent to NHRI had clinical significance, we still cannot rule out the possibility that isolates from non-sterile sites are colonizers. *Candida* species colonizing in human are usually the causes of infections when patients become immunocompromised. Thus, it is important to investigate isolates from non-sterile sites as well as those from sterile ones for prevention and control strategies.

In our previous report, 58 *C. parapsilosis* isolates were collected in 2002 and blood was the most common source, accounting for 36.2%, followed by wound (20.7%) [18]. In the present study, urine became the most common source, accounting for 21% of the 62 recovered isolates, followed by blood (14,5%), ear (12.9%), pus (9.7%), and wound (8.1%). One explanation for the different source distribution of *C. parapsilosis* from other species is that it can be part of the normal flora on the human skin [23] and has a wide distribution in environments [24, 25]. Nevertheless, factors contributed to the different source distribution of this species between these two studies need further investigation. Among the 515 isolates from either sputum or urine, 375 (72.8%) were from the elder group. We found that the majority of *C. glabrata* (114/136, 83.8%) and *C. tropicalis* (122/150, 81.3%) from the elder group were isolated from either sputum or urine. This observation may explain why both *C. glabrata* and *C. tropicalis* are more prevalent in elderly patients in the present study. Whether the elderly patients are more susceptible to *C. glabrata* and *C. tropicalis* infections needs further investigation.

Overexpression of fluconazole transporters and the modification of the target enzymes are the two major mechanisms contributing to fluconazole resistance [26, 27]. It has been shown that multiple molecular mechanisms contribute to a stepwise development of fluconazole resistance in *C. albicans* [28]. Even though isolates have trailing phenotype and are not resistant to fluconazole yet, we still cannot ignore those isolates since they are prone to be resistant. The observation that only 2.5% of *C. glabrata* having fluconazole MICs ≥ 64 μg/ml after 24-h incubation is different from the results in European countries and the United States. One explanation is that in Taiwan, physicians do not administer fungal prophylaxis as frequently as those in other regions [29–31]. *Candida glabrata* is much more ready to display variation in phenotypes contributing to drug resistance partially due to its haploid nature [32]. Hence, we do not suggest physicians to administer fluconazole therapy for infections caused by *C. glabrata,* even though very few isolates of this species recovered in the present study having fluconazole MICs ≥ 64 μg/ml. Furthermore, a correlation of susceptibility to fluconazole and voriconazole was observed in the present study as described in our previous study [33]. Interestingly, no *C. krusei* was resistant to voriconazole, despite that it is intrinsically resistant to fluconazole.

In conclusion, we have analyzed the species, culture source (sterile, non-sterile), patient location (ICU, non-ICU, outpatient), patient age group, and amphotericin B/fluconazole/voriconazole susceptibility for *Candida* isolates. We found that the age of patients is associated with species distribution. So is the source of isolates. Furthermore, species and whether the patients were hospitalized are associated with fluconazole susceptibility of the isolates.

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