J Microbiol Immunol Infect
2005;38:200-210

Epidemiologic analysis and antifungal susceptibility of *Candida* blood isolates in southern Taiwan

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Received: July 23, 2004 Revised: September 6, 2004 Accepted: September 30, 2004

Candidemia is a clinically important disease which has increased in incidence worldwide in recent decades. In order to identify the risk factors for mortality in candidemic patients and to elucidate the role of antifungal susceptibility testing, a retrospective cohort study was performed of 56 episodes of candidemia in 1998 at a medical center in southern Taiwan. The minimal inhibitory concentration (MIC) of these isolates was determined by E-test. Malignancy and alimentary diseases (42.9%) were the most common underlying conditions of these patients. There was no difference of *Candida* spp. distribution among patients treated in medical or surgical departments, except that all 5 isolates of *C. intermedia* were found in patients treated in medical departments (p=0.02) and 50% of candidemic infants had *C. parapsilosis* isolates (p=0.046). Among all *Candida* isolates, 3 (5.4%) were fluconazole non-susceptible. *C. tropicalis* had a significantly higher rate of amphotericin B resistance than the other species (p=0.007). Thirty four patients died and 70.6% of these deaths were attributable to candidemia. Thrombocytopenia, septic shock at the date of candidemia onset, C-reactive protein >100 mg/L, blood urea nitrogen >20 mg/dL, length of stay <60 days, and Acute Physiology and Chronic Health Evaluation II score ≥10 points were significantly associated with the death attributable to candidemia. Thrombocytopenia was the only independent predictor for mortality in the multivariate analysis. When the breakpoint of fluconazole was set at 2 μg/mL, as opposed to 8 μg/mL as in the National Committee for Clinical Laboratory Standards (NCCLS) criteria, the clinical outcome of death was significantly correlated to the MICs of the blood isolates. The correlation between MIC of fluconazole determined by E-test data, which is more easily obtainable than with NCCLS methods, and outcome requires larger scale investigation.

Key words: Antifungal agents, candidiasis, fungemia, mortality

Candida bloodstream infection (candidemia), a significant cause of morbidity and mortality in critically ill patients and immunocompromised hosts, contributes to increased length of hospital stay and medical costs [1-3]. In recent decades, the incidence of candidemia in the United States has increased from 0.1 to 0.5 per 1000 discharges [4] and *Candida* spp. have become the fourth leading cause (8-15%) of bloodstream infection [2,3]. In Taiwan, *Candida* spp. are the third and the sixth most common pathogen of nosocomial infections in medical centers and regional hospitals, respectively [5]. The incidence of nosocomial candidemia in National Taiwan University Hospital (NTUH), a tertiary care hospital in northern Taiwan, increased 36-fold from 1981 to 2000 [6-8]. The incidence of nosocomial candidemia in Kaohsiung Medical University Hospital (KMUH), a 1200-bed tertiary care hospital in southern Taiwan, rose from 0.13 to 0.77 per 1000 discharges from 1992 to 1999 [9,10]. The global trend of increasing incidence might be attributable to numerous factors, including the growing population of patients receiving immunosuppressive agents or chemotherapy, increased numbers of patients with cancer, acquired immunodeficiency disease syndrome (AIDS) or diabetes, increased use of broad-spectrum antibiotics, increased numbers of invasive procedures and increased use of intensive life support systems [2,4,11-15]. The importance of these factors may depend on the hospital's size, available facilities, and the severity of disease in the patient population.

The emergence of antifungal resistance and the increasing percentage of intrinsically resistant *Candida* spp. have raised concern about the limitations of susceptibility testing, including delay in obtaining results, costs, availability and lack of well-established

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correlation between minimal inhibitory concentration (MIC) and clinical response for agents for treating various candidal infections [16]. The resistance rate to fluconazole was 6% among the *Candida* blood isolates in NTUH during 1998-2001 [6]. In 1999, the Taiwan Surveillance of Antimicrobial Resistance of Yeasts reported resistance rates to fluconazole of 10.7% and 4.9% of isolates from medical centers and regional hospitals, respectively [17]. However, epidemiologic data on candidemia, antifungal susceptibility and clinical outcome are limited from hospitals in southern Taiwan. This retrospective cohort study investigated the species distribution and antifungal susceptibility of blood *Candida* isolates and the risk factors for mortality in patients with candidemia.

Materials and Methods

Study population

All patients who had *Candida* spp. isolated from blood between January 1, 1998 and December 31, 1998 were included in the study. Chart review was performed to collect demographic information including medical history, invasive procedures, medications, laboratory data and outcome.

Definitions

All blood isolates fit the United States Centers for Disease Control and Prevention definition of nosocomial bloodstream infection [18]. Invasive procedures or medications performed up to 2 weeks prior to the onset of candidemia were recorded. The types and numbers of antibiotics used after admission were recorded. Vital signs and laboratory data on the date of candidemia onset, including white blood cell count, platelets, C-reactive protein, blood urea nitrogen and creatinine, were also collected. Candidemia was considered as the cause of death if the patients died within 7 days of fungemia without other major causes of mortality, or remained in a septic condition until death [7]. Lack of appropriate antifungal therapy was defined as no treatment with antifungal agent or use of an antifungal agent to which the *Candida* isolate was not susceptible. Concomitant bacteremia was defined as isolation of bacteria from blood within 24 hours of the initial positive fungal blood culture [7].

Species identification and antifungal susceptibility testing

Candida spp. were isolated using the BacT/Alert automatic blood culture system (Organon Teknika, Durham NC, USA), and identified to the species level by colony characteristics, microscopic features, carbohydrate assimilation-fermentation studies and germ tube test, and rechecked by ID 32 C system (bioMerieux Inc., Hazelwood, MO, USA). Antifungal susceptibility testing was performed and MIC of amphotericin B and fluconazole were determined by the E-test method (AB Biodisk, Solna, Sweden), according to the manufacturer's instructions. RPMI 1640 agar buffered to pH 7.0 with MOPS (morpholine-propanesulfonic acid) was prepared for use in the E-test. 0.5 McFarland standard inocula were applied to RPMI agar with a cotton swab. The MIC endpoints were read after 48 h of inoculation at 35°C [19-21]. *C. albicans* (ATCC 90028), *C. krusei* (ATCC 6258), and *C. parapsilosis* (ATCC 22019) were used as control strains. Results of susceptiblity were considered eligible only when the MICs of the control strains were all within the standard range [22]. Interpretative criteria of susceptibility of *Candida* spp. to fluconazole were in accordance with the National Committee for Clinical Laboratory Standards (NCCLS) guidelines [22]. Resistance, susceptible-dose dependence, and susceptibility to fluconazole in isolates were defined as MIC ≥64 μg/mL, 16-32 μg/mL, and ≤8 μg/mL, respectively. Amphotericin B-resistant *Candida* isolates were defined as those with an amphotericin B MIC >1 μg/mL [12,19,23-31].

Statistical analysis

All data were analyzed by SPSS version 11.0 (SPSS Inc., Chicago, IL, USA). Categorical variables were compared by univariate analysis with chi-squared test or Fisher's exact test when 20% of the expected count was less than 5 in the statistics. Continuous variables were analyzed with Student's *t* test or one-way analysis of variance. A *p* value of <0.05 was considered statistically significant (2-tailed). Multivariate analyses were conducted by a logistic regression model for the variables which were significant in the univariate analysis.

Results

A total of 56 patients (32 male and 24 female patients) with a diagnosis of nosocomial candidemia during the study period were included. The median age of these patients was 59.5 years (range, 16 days to 99 years). The proportion of infants less than 1 year old and of elderly patients was 10.7% and 30.4%, respectively (Table 1). The percentage of candidemic patients treated in intensive care units (ICUs), surgical wards and

Table 1. Demographic features and outcome of 56 patients with candidemia

Age (years)	49.8 ± 25.7 (1-99)
Pediatric (<18 years)	10 (17.9%)
Elderly (>65 years)	17 (30.4%)
Gender (male/female)	32/24 (57.1%/42.9%)
APACHE II score	14.11 ± 6.06 (2-29)
0-4/5-9/10-14/15-19/20-24/25-29	2/8/15/14/3/4
Proportion with APACHE II score ≥20	7/46[a] (15.2%)
Length of stay (days)	73.63 ± 68.61 (15-359)
Hospital days before candidemia (days)	41.07 ± 43.95 (4-216)
Ward	27.23 ± 38.00 (4-177)
ICU	13.84 ± 25.92 (3-161)
Mortality	
Total deaths	34 (60.7%)
Proportion of deaths due to candidemia	24/34 (70.6%)

Abbreviations: APACHE = Acute Physiology and Chronic Health Evaluation; ICU = intensive care unit

[a]APACHE II scores determined only in adult patients.

medical/pediatric wards was 39.3%, 30.4% and 30.4%, respectively (Table 2). Most of the patients with malignancy (19/24, 79.2%) were admitted to the medical and surgical wards instead of ICU (Table 3).

Predisposing factors for candidemia

The most common underlying diseases of the candidemic patients were alimentary diseases (42.9%) and malignant diseases (42.9%). One-fourth of the patients with malignancy had hematologic malignancy. Diabetes mellitus was presented in 25% of patients and auto-immune disease in 5.4%. Thirty six patients had more than 1 underlying disease at admission. Blood transfusion, central venous catheterization, nasogastric tube insertion, antacid use and Foley catheterization were the 5 most common predisposing factors for nosocomial candidemia (Table 4). Eighty two percent of candidemic patients received more than 2 types of antibiotics. Antifungal agents were used prior to the onset of candidemia in 10 episodes, including nystatin oral suspension for oral candidiasis in 6 episodes, oral fluconazole for candiduria in 2, amphotericin B combined with nystatin suspension in 1, and several courses of parenteral fluconazole followed by amphotericin B in 1 uremic patient. This uremic patient was receiving continuous ambulatory peritoneal dialysis at the time multiple episodes of *Candida* infection occurred, with isolates of different species in urine, dialysate and PD wound, and the candidemia developed despite treatment with amphotericin B.

Clinical manifestations

The most common clinical manifestation was fever (44/56, 78.6%), followed by gastrointestinal bleeding

Table 2. Distribution of infection with different *Candida* spp. by treatment setting and in premature infants

Candida spp.	Total	Medical[a]/surgical	Ward/ICU[a]	Infants[b] (number with prematurity)
C. albicans	17	6/11	12/5	0
C. tropicalis	17	11/6[c]	8/9	1
C. glabrata	2	0/2	1/1	0
C. parapsilosis	9	3/6	7/2	3[e] (2)
C. intermedia	5	5/0[d]	3/2	0
C. sake	4	1/3	1/3	1 (1)
C. humicola	1	0/1	1/0	0
C. guilliermondii	1	1/0	1/0	1

Abbreviation: ICU = intensive care unit

[a]Including pediatric ward and pediatric ICU.

[b]Including infants less than 1 year old.

[c]Including 1 patient with burns over 50% of total body surface area.

[d]p=0.02 (significant difference in proportion of *C. intermedia* among *Candida* spp. between medical patients and surgical patients).

[e]p=0.046 (significant difference in proportion of *C. parapsilosis* between infants and non-infants).

Table 3. Distribution of infection with different *Candida* spp. in patients with malignancy

Candida spp.	Total	Patients without malignancy	Solid tumor	Hematologic malignancy
C. albicans	17	11	6	0
C. tropicalis	17	10	4	3 (3)[a]
C. glabrata	2	0	2	0
C. parapsilosis	9	5	4	0
C. intermedia	5	3	1	1 (1)[a]
C. sake	4	2	1	1 (1)[a]
C. humicola	1	1	0	0
C. guilliermondii	1	0	1 (1)[a]	0

[a]Number with neutropenia. Neutropenia was defined as absolute neutrophil count less than 500/mm^3.

(60.7%), acute renal failure (46.4%), diarrhea (25%) and acute respiratory distress syndrome (16.1%). Twelve patients had a body temperature of less than 38°C at the time blood was collected for culture. Fifty five percent of candidemic patients had leukocytosis (white blood cell count over 10,000/mm^3) and 45% patients had thrombocytopenia (platelet count below 150,000/mm^3). Thirteen patients (23.2%) had septic shock at the time when culture evidence of candidemia was obtained. Central venous catheters were removed in 35 of 46 patients at a mean of 4 days later. Ten *Candida* spp. were isolated from 35 catheter tips and all of them had the same species as in the patient's blood sample. Twenty four patients (42.9%) had either oral candidiasis or candiduria prior to developing candidemia in this study. Nine *Candida* isolates were detected from non-blood specimens and all of these were species different from those isolated in subsequent blood culture results.

Table 4. Association of *Candida* spp. with predisposing factors

	Total	*C. albicans*	Non-albicans *Candida* spp.	*p*
Invasive procedures				
Central venous catheter	46	16	30	0.25
Nasogastric tube	40	12	28	1.00
Endotracheal tube	28	8	20	1.00
Foley catheter	31	10	21	0.96
Non-abdominal major operation	20	5	15	0.73
Abdominal surgery	19	6	13	1.00
Arterial line	13	4	9	1.00
Chemotherapy	10	0	10	0.02
Venous port	5	0	5	0.31
Pulmonary artery catheter	5	1	4	1.00
Double-lumen catheter for hemodialysis	5	0	5	0.31
Radiotherapy	2	2	0	0.09
Antibiotic treatment				
Use more than 2 types of antibiotics	46	16	30	0.25
Third-generation cephalosporin	29	10	19	0.69
Aminoglycoside	47	15	32	0.71
β-Lactamase inhibitor-penicillin[a]	45	13	22	0.26
Glycopeptide	9	2	7	0.71
Carbapenem	6	1	5	0.66
Antifungal agent use prior to candidemia	10	1	9	0.25
Other treatments				
Blood transfusion	52	17	35	0.30
Antacid	39	13	26	0.68
H_2-blocker	31	8	23	0.59
Parenteral nutrition	25	8	17	1.00
Steroid	12	3	9	0.74

[a]Including ampicillin-sulbactam, amoxicillin-clavulanate, ticarcillin-clavulanate, piperacillin-tazobactam.

Correlation of *Candida* spp. isolated with underlying disease and predisposing factors

C. albicans and C. tropicalis were the most common isolated species (both 30.4%). There was no significant difference in species distribution between patients without and with either solid tumor or hematologic malignancy, as well as between those treated in wards versus ICUs. All 5 isolates of *C. intermedia* were found among patients treated in medical as opposed to surgical departments (p=0.02). *C. parapsilosis* was more prevalent in infants less than 1 year old (p=0.046) [Table 2, Table 3].

The patients who had received chemotherapy were more likely to develop non-albicans candidemia than *C. albicans* fungemia (p=0.02). The major C*andida* spp. of candidemia in uremic patients with an indwelling hemodialysis catheter was *C. tropicalis* (4 patients with *C. tropicalis* vs 1 with *C. intermedia*, p=0.03). *C. albicans* candidemia developed in 2 patients, who had received radiotherapy (p=0.09) [Table 4]. Three patients developed concomitant bacteremia, including 2 infected with *Pseudomonas aeruginosa* and 1 with *Escherichia coli*. There was no significant relationship between types of antibiotics used and the species distribution of candidemia.

Antifungal susceptibility

C. tropicalis had a significantly higher resistance rate to amphotericin B than other species, including 6 isolates of *C. tropicalis* and each 1 of *C. sake* and *C. intermedia* (p=0.007). Only 1 *C. albicans* and 1 *C. sake* isolate were resistant to fluconazole (MIC >256 μg/mL) and 1 isolate of *C. glabrata* exhibited dose-dependent susceptibility (MIC = 48 μg/mL) [Table 5]. No isolate was resistant to both amphotericin B and fluconazole. Reduced susceptibility to amphotericin B or fluconazole was not significantly related to prior use of these 2 antifungal agents. No antifungal agent was used in the 3 patients with fluconazole-non-susceptible *Candida* isolates obtained before the onset of candidemia. However, 2 patients with amphotericin B-resistant isolates had received empiric treatment with amphotericin B and fluconazole. Although development of resistance of *Candida* isolates to amphotericin B and fluconazole was not correlated with ICU stay (4/22 vs 4/30, p=0.69 and 3/22 vs 0/34, p=0.06, respectively), patients with amphotericin B and fluconazole non-susceptible isolates tended to have longer ICU stay (27.25 ± 55.15 days vs 11.6 ± 17.26 days, p=0.45 and 22.67 ± 7.64 days vs 13.34 ± 25.53 days, p=0.55, respectively).

Predictors of mortality in univariate and multivariate analysis

The overall case fatality rate was 60.7% (34/56) and mortality directly attributable to candidemia (proportionate mortality) was 70.6% (24/34). Two patients died within 72 h after diagnosis of candidemia and neither of them received antifungal therapy. Among those patients whose cause of death was indirectly related to candidemia, 5 were due to septic shock caused by other pathogens, 2 died of hepatic failure and 3 died of cardiogenic shock, intracranial hemorrhage and acute respiratory distress syndrome due to *Klebsiella pneumoniae*, respectively. There was no significant difference in mortality between patients infected with different *Candida* spp..

Patients whose cause of death was classified as candidemia had a higher MIC of fluconazole than patients who survived or died of causes unrelated to candidemia (p=0.02). All 3 patients infected with

Table 5. Susceptibility and minimal inhibitory concentration (MIC) of different *Candida* spp. to antifungal agents

Candida spp.	MIC (μg/mL) of amphotericin B			Susceptibility to amphotericin B		MIC (μg/mL) of fluconazole			Susceptibility to fluconazole[a]		
	MIC_{50}	MIC_{90}	Range	S	R	MIC_{50}	MIC_{90}	Range	S	S-DD	R
C. albicans	0.25	0.55	0.047-0.75	17	0	2	104.8	1->256	16	0	1
C. tropicalis	1	1.6	0.002-2	11	6	2	4	1-4	17	0	0
C. glabrata	0.31	0.5	0.125-0.5	2	0	2.45	48	1-48	1	1	0
C. parapsilosis	0.38	0.75	0.094-0.75	9	0	1.5	4	0.38-4	9	0	0
C. intermedia	1	1.5	0.5-1.5	4	1	1.5	3	1-3	5	0	0
C. sake	0.75	2	0.25-2	3	1	1.75	>256	0.5->256	3	0	1
C. humicola	-	-	0.38	1	0	-	-	4	1	0	0
C. guilliermondii	-	-	0.19	1	0	-	-	4	1	0	0

Abbreviations: MIC_{50} = MIC for 50% of isolates; MIC_{90} = MIC for 90% of isolates; S = susceptible; S-DD = dose-dependent susceptibility; R = resistant

Table 6. Univariate analysis of microbiologic factors associated with mortality attributable to candidemia

Characteristic	Survivors or mortality unrelated to candidemia (n = 32)	Mortality attributable to candidemia (n = 24)	Odds ratio	95% CI	*p*
Candida spp.					
C. albicans	10 (31.3%)	7 (29.2%)	0.906	0.285-2.875	1.00
C. tropicalis	7 (21.9%)	10 (41.7%)	2.551	0.794-8.192	0.15
C. parapsilosis	7 (21.9%)	2 (8.33%)	0.32	0.03-1.99	0.27
Other *Candida* spp.	8 (25.0%)	5 (20.8%)	0.79	0.17-3.29	0.96
Susceptible to fluconazole	32 (100.0%)	21 (87.5%)	0.00	0.00-1.75	0.07
MIC for fluconazole (μg/mL)	1.50 ± 1.76	3.63 ± 5.39			0.02[b]
Susceptible to amphotericin B	26 (81.3%)	21 (87.5%)	0.62	0.09-3.36	0.72
MIC for amphotericin B (μg/mL)	0.38 ± 2.81	0.32 ± 4.26			0.61
Antifungal agent use prior to candidemia	6 (18.8%)	4 (16.7%)	0.867	0.215-3.490	1.00
Antifungal agents use	25 (78.1%)	20 (83.3%)	1.400	0.359-5.465	0.74
Fluconazole	20 (62.5%)	19 (79.2%)	2.280	0.675-7.705	0.24
MIC for fluconazole in treated patients (μg/mL)	1.54 ± 1.80	3.27 ± 4.30			0.04[b]
Amphotericin B	8 (25.0%)	3 (12.5%)	0.429	0.100-1.828	0.32
MIC for amphotericin B in treated patients (μg/mL)	0.48 ± 4.40	0.41 ± 4.03			0.88
Lack of appropriate antifungal therapy[a]	10 (31.3%)	7 (29.2%)	1.10	0.30-4.09	0.89

Abbreviations: CI = confidence interval; MIC = minimal inhibitory concentration

[a]Patients who did not use any antifungal agents and those treated with fluconazole or amphotericin B who had a blood isolate resistant to the specific antifungal agent.

[b]$p<0.05$.

fluconazole non-susceptible isolates died of candidemia (p=0.07). Among the patients who received fluconazole therapy, the MICs for fluconazole in patients who died of candidemia were significantly higher than the MICs of patients with non-candidemia-related mortality (p=0.04). The MICs of amphotericin B did not differ significantly between survivors and non-survivors. There was no correlation between mortality and patients with isolates resistant to antifungal agents or lack of antifungal treatment (p=0.89) [Table 6].

Other significant prognostic factors of mortality in the univariate analysis included the total length of hospital stay, Acute Physiology and Chronic Health Evaluation (APACHE) II score, platelet count, C-reactive protein level, blood urea nitrogen level, and septic shock on the date of diagnosis of candidemia (Table 7).

Of all the variables significantly associated with mortality in the univariate analysis, thrombocytopenia was the only predictive factor of mortality in the multivariate analysis (p=0.013, odds ratio 23.103, 95% confidence interval 1.939-275.235) [Table 8].

Discussion

In this study, reduced susceptibility to fluconazole had a significant impact on mortality due to candidemia in the univariate analysis. The MIC of fluconazole ≥2 μg/mL determined by E-test was associated with a 5-fold higher risk of candidemia-attributable death (p=0.012, odds ratio 5.00, 95% confidence interval 1.36-19.26). The breakpoints of MICs from NCCLS M27-A were based on studies of adult patients with oropharyngeal and esophageal candidiasis (for fluconazole and itraconazole) and patients with invasive candidiasis (mostly neutropenic patients with candidemia; for fluconazole only) [22,30,32]. However, good correlation between the clinical response and MICs was generally found only for oropharyngeal candidiasis in AIDS patients. The clinical correlates of MICs in systemic and deep-seated *Candida* infections are still controversial [23,29-37]. Several studies showed that the clinical outcome of candidemia was highly correlated to the results of antifungal susceptibility testing following the NCCLS criteria for the MIC of fluconazole [30,35,37].

The MIC of fluconazole ≥1 μg/mL determined by flow cytometry, not 8 μg/mL as in the NCCLS criteria, was reported to be associated with mortality due to candidemia in 1 study [33]. In our study, when the breakpoint of fluconazole susceptibility was set at 2 μg/mL, mortality was significantly correlated to the MICs of the blood isolates. Among the patients who received fluconazole treatment, candidemia-attributable

Table 7. Univariate analysis of factors associated with mortality attributable to candidemia

Characteristic	Survivors or mortality unrelated to candidemia (n = 32)	Mortality attributable to candidemia (n = 24)	Odds ratio	95% CI	*p*
Age (years)	45.40 ± 4.49	55.67 ± 5.19			0.14
Gender (male/female)	18/14	14/10	1.089	0.373-3.177	1.00
Treatment setting					
Medical/surgical	14/18	13/11	0.619	0.209-1.832	0.55
ICU/ward	11/21	13/11	1.519	0.524-4.404	0.62
APACHE II score	12.44 ± 5.53	16.10 ± 6.18			0.04[a]
Total LOS (days)	89. 91 ± 77.47	51.92 ± 48.03			0.03[a]
Total LOS before candidemia (days)	43.69 ± 42.94	37.58 ± 45.96			0.61
ICU stay before candidemia (days)	13.72 ± 30.87	14.00 ± 17.96			0.97
Ward stay before candidemia (days)	29.97 ± 35.33	23.58 ± 41.78			0.54
Underlying diseases					
Hematologic	12 (37.5%)	12 (50.0%)	1.667	0.570-4.876	0.51
Solid tumor/hematologic malignancy	11/1	8/4	0.182	0.017-1.951	0.32
Neutropenia	2 (6.3%)	4 (16.7%)	3.000	0.501-17.954	0.39
Invasive procedures					
CVC	26 (81.3%)	20 (83.3%)	1.154	0.287-4.646	1.00
CVC removal	20/26 (76.9%)	15/20 (75.0%)	0.900	0.230-3.516	1.00
Days after candidemia onset	5.13 ± 7.13	2.77 ± 3.54			0.29
Endotracheal tube with ventilator	14 (43.8%)	14 (58.3%)	1.800	0.617-5.251	0.42
Foley catheter	16 (50.0%)	15 (62.5%)	1.667	0.567-4.900	0.51
Arterial line	7 (21.9%)	6 (25.0%)	1.190	0.342-4.145	1.00
Pulmonary artery catheter	3 (9.4%)	2 (8.3%)	0.879	0.135-5.719	1.00
Double lumen catheter for dialysis	2 (6.3%)	3 (12.5%)	2.143	0.329-13.960	0.64
Venous port	3 (9.4%)	2 (8.3%)	0.879	0.135-5.719	1.00
Antibiotic treatment					
Days of antibiotics use before candidemia	33.88 ± 30.10	31.00 ± 35.53			0.75
Number of antibiotics used before candidemia	5.75 ± 0.60	5.54 ± 0.69			0.82
Other treatments					
Parenteral nutrition	14 (43.8%)	11 (45.8%)	1.088	0.375-3.153	1.00
Steroid	6 (18.8%)	6 (25.0%)	1.444	0.401-5.202	0.81
Manifestations					
ARDS	3 (9.4%)	6 (25.0%)	3.222	0.715-14.521	0.15
Acute renal failure	13 (40.6%)	13 (54.2%)	1.727	0.593-5.030	0.46
Septic shock	2 (6.3%)	11 (45.8%)	12.692	2.459-65.509	0.002[a]
Laboratory data					
Albumin level before candidemia (g/dL)	3.45 ± 0.62	3.13 ± 0.48			0.06
White blood cell counts (/mm^3)	13,021 ± 8042	11,258 ± 6738			0.39
Platelet counts (/mm^3)	270,355 ± 184,174	134,333 ± 110,356			0.001[a]
C-reactive protein (mg/L)	89.2 ± 63.4	138.9 ± 99.0			0.05[a]
Blood urea nitrogen (mg/dL)	37.2 ± 34.9	60.7 ± 45.1			0.04[a]
Creatinine (mg/dL)	2.06 ± 1.94	2.70 ± 2.10			0.26

Abbreviations: CI = confidence interval; ICU = intensive care unit; APACHE = Acute Physiology and Chronic Health Evaluation; LOS = length of stay; CVC = central venous catheter; ARDS = acute respiratory distress syndrome
[a]$p<0.05$.

mortality was also correlated with reduced fluconazole susceptibility. The geometric means of MICs for fluconazole in patients with candidemia-attributable mortality and in patients who survived or whose mortality was unrelated to candidemia were both lower than 8 μg/mL, the breakpoint of the NCCLS criteria. This suggests that the adoption of the breakpoint of 8 μg/mL would not significantly influence mortality.

Table 8. Multivariate logistic regression analysis of risk factors associated with mortality in candidemic patients

Variable	Odds ratio	95% CI	*p*
Length of stay <60 days	1.360	0.210-8.806	0.747
Septic shock	4.928	0.466-52.155	0.185
Platelet <150,000/μL	23.103	1.939-275.235	0.013
C-reactive protein >100 mg/L	3.424	0.651-17.996	0.146
Blood urea nitrogen >20 mg/dL	1.339	0.177-10.105	0.777
MIC of fluconazole ≥2 μg/mL	7.717	0.699-85.229	0.095

Abbreviations: CI = confidence interval; MIC = minimal inhibitory concentration

Minimal lethal concentrations of amphotericin B >1 μg/mL were reported to be predictive of mortality in candidemic patients receiving amphotericin B, especially those infected with non-*C. albicans* spp. [28], and NCCLS M27-A2 pointed out that an amphotericin B MIC >1 μg/mL indicated the likely resistance of the isolates [22]. Our results did not confirm this observation, possibly because of the small number of patients who received amphotericin B therapy. This study did not find a correlation of mortality rate and patients with *Candida* spp. non-susceptible to antifungal agents according to the NCCLS criteria (i.e., microbiologic failure did not correlate to clinical failure). However, susceptibility testing may be helpful for patients with persistent or breakthrough fungemia to determine whether clinical failure is resulting from microbiologic resistance or problems with drug delivery [29].

In contrast to previous findings that bacterial bloodstream infection isolates in ICUs were less susceptible to antimicrobial agents than non-ICU isolates [36], we found that neither prior empiric antifungal therapy nor ICU stay were associated with increased MICs of antifungal agents. This is in agreement with the previous findings that *Candida* isolates from non-ICU patients were equally or even less susceptible to fluconazole than isolates from an ICU setting [6,36]. Previous study from Taiwan found that increasing use of fluconazole did not correlate with the stable susceptibility of *Candida* blood isolates to this agent [6,38]. These results suggest that *Candida* spp. had different thresholds for or mechanisms of resistance than bacteria exposed to antimicrobial agents.

In addition to reduced susceptibility of fluconazole, we also found that thrombocytopenia, C-reactive protein >100 mg/L, septic shock, blood urea nitrogen >20 mg/dL, length of stay less than 60 days and higher APACHE II score were predictors of mortality in the univariate analysis. Among them, thrombocytopenia was an independent risk factor for mortality in the multivariate analysis. Several independent risk factors for mortality have been reported in the other studies, including sustained candidemia, neutropenia, steroid therapy, lack of antifungal therapy, central venous catheter not changed, elderly, acute renal failure and the severity of illness [13-15]. Three studies from Taiwan also showed underlying conditions (aged, malignancy), disease severity (high APACHE II score, septic shock and azotemia at the onset of candidemia) and delayed antifungal therapy were the most important factors for determining clinical outcome [7,9,39].

This study found a higher proportion of non-albicans *Candida* spp. (69.6%) than most of the previous studies of candidemia from Taiwan (33-70.6%) [6,38-41]. *C. tropicalis* was the major strain in non-albicans *Candida* (30.4%) in this study as in previous studies [6,38-41]. Several studies have reported that azole prophylaxis is a risk factor for *C. glabrata* and *C. krusei* infection [40,42] and candidemia with these 2 strains had a higher rate of resistance and mortality [43]. However, no *C. krusei* isolates and only 2 *C. glabrata* isolates in our study might have influenced the resistance rate, *Candida* spp. distribution and candidemia-attributable mortality. Although several studies noted a relationship of the bloodstream infection by *C. tropicalis*, *C. krusei*, *C. parapsilosis* and hematologic malignancy and between *C. albicans*, *C. glabrata* and solid tumor [44-46], this was not found in our study. We found a different epidemiologic result in that patients receiving chemotherapy were more likely to have non-albicans *Candida* infection (p=0.02). This study also found that *C. tropicalis* was the main pathogen associated with an indwelling hemodialysis catheter, unlike previous studies which found that *C. parapsilosis* was the most common *Candida* spp. in nosocomial CVC-related bloodstream infections (p=0.03) [43,44].

The MICs for amphotericin B or fluconazole and *Candida* spp. distribution in this study were similar to the results of previous reports from Taiwan [6,38-41]. *C. tropicalis* and *C. krusei* had a higher resistance rate

to amphotericin B than other species in these studies. Eight (14.3%) of 56 *Candida* blood isolates were resistant to amphotericin B with the breakpoint of 1 μg/mL by the criteria of NCCLS M27-A2. If the breakpoint was set at 2 μg/mL, as in some studies, the resistant rate to amphotericin B was 3.6%, which is similar to the results of those studies [41,42]. Yang et al [17] found significant differences in the distribution of *Candida* spp. at different locations and types of hospitals in Taiwan. Compared to hospitals in northern Taiwan, more *C. tropicalis* and less *C. parapsilosis* were isolated from hospitals in southern Taiwan. The resistance rate to fluconazole was higher in hospitals in southern Taiwan. Our study also found a higher proportion of *C. tropicalis* isolates, but the finding that only 3 *Candida* isolates (5.4%) were not susceptible to fluconazole showed that the predominant species and resistance pattern may vary between hospitals.

In conclusion, underlying condition and disease severity are the major factors associated with mortality attributable to candidemia. Reduced susceptibility to fluconazole (MIC ≥2 μg/mL) significantly predicts candidemia mortality. This study also supports previous findings that candidemia due to non-albicans *Candida* spp. is more prominent in hospitals in southern Taiwan. Identification of these predictors may alert clinicians to increased risk of candidemia-related mortality in critically ill patients. The correlation of the MIC of fluconazole with mortality in this study when the breakpoint was set at 2 μg/mL suggests the need for further investigation of the clinical correlation of MIC determined with different methodologies to antifungal resistance in candidemia.

Acknowledgments

We would like to thank Ms. Shu-Ping Wu for her help in collecting data and Ms. Hwei-Hsiang Lee for her assistance in *Candida* spp. identification.

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