Invasive *Candida* infections in children: the clinical characteristics and species distribution and antifungal susceptibility of *Candida* spp.

Nurşen Belet[1], Ergin Çiftçi[2], Derya Aysev[3], Haluk Güriz[3], Zümrüt Uysal[4], Nurdan Taçyıldız[5] , Begüm Atasay[6], Figen Doğu[7], Tanıl Kendirli[8], Zarife Kuloğlu[9], Erdal İnce[2], Ülker Doğru[2]

[1]Department of Pediatric Infectious Diseases, Ondokuz Mayıs University Faculty of Medicine, Samsun, and Departments of [2]Pediatric Infectious Diseases, [3]Pediatrics, [4]Pediatric Hematology, [5]Pediatric Oncology, [6]Neonatology, [7]Pediatric Immunology and Allergy, [8]Pediatric Intensive Care Unit, and [9]Pediatric Gastroenterology, Ankara University Faculty of Medicine, Ankara, Turkey

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The aims of the study were to examine the distribution of Candida spp. isolated from sterile body sites, the antifungal susceptibility of the isolates to amphotericin B, fluconazole, voriconazole, and caspofungin, and factors affecting mortality with invasive Candida infections in children. Thirty-five children with invasive candidiasis between January 2004 and January 2008 were evaluated retrospectively. The antifungal susceptibility of isolated Candida species was studied by Etest. Of the invasive Candida infections, 65.7% were due to *C. albicans*. The second most common isolated species was *C. parapsilosis* (11.4%). The rates of resistance to fluconazole, amphotericin B and voriconazole were 8.5%, 2.8% and 5.7%, respectively. Caspofungin was the most effective antifungal agent. 22.8% of the patients died in the first 30 days. In univariate analyses, increased mortality was associated with stay in the intensive care unit, the presence of central venous catheter (CVC), failure to remove CVC, and mechanical ventilation.

Key words: invasive candidiasis, children, risk factors, antifungal susceptibility.

The incidence of fungal infections due to Candida spp. has been increasing in recent years. Most of the invasive Candida infections are caused by *Candida albicans*, although non-albicans species have been reported with increasing frequency in recent years. Studies have shown that the epidemiology of invasive Candida infections may differ between geographical locations and even between institutions within the same location, highlighting the importance of studying the local epidemiology. Another serious problem is posed by the increased resistance to antifungal drugs commonly used in clinical practice[1-6].

Surveillance studies are necessary to follow the epidemiologic changes and changes in antifungal sensitivity among Candida species. There is limited data about Candida species causing invasive infections in children and their antifungal drug sensitivities in our country. The aims of the study were thus to examine the distribution of Candida spp. isolated from sterile body sites, the antifungal susceptibility of the isolates to amphotericin B, fluconazole, voriconazole, and caspofungin, and the factors affecting mortality with invasive Candida infections in children.

Material and Methods

Patient Population and Definitions

A cross-sectional study followed by a built-in case control study was conducted between January 2004 and January 2008 at a university hospital. All hospitalized children with culture-proven invasive Candida spp. infection were

included, and data on invasive Candida infections were retrospectively collected. Invasive Candida spp. infections were defined as isolation of Candida spp. from sterile body areas. In patients with more than one episode, only the first episode was included. Patients with polymicrobial Candida infections were excluded. Each episode of invasive Candida spp. infection was classified as nosocomial or community-acquired. Infection was defined as community onset if the organism was isolated within 48 hours (h) from the initial hospitalization. Infection was defined as nosocomial onset if the organism was isolated ≥48 h from initial hospitalization. If a patient was transferred from another hospital, the duration of inpatient stay was calculated from the date of the first hospital admission.

Data of patients were collected from inpatient medical records by using a standardized questionnaire. This form was completed for each patient, including demographic characteristics, risk factors for fungal infection [underlying disease; receipt of broad-spectrum antibiotics, such as antipseudomonal penicillins, third- or fourth-generation cephalosporins, carbapenems, β-lactamase-resistant penicillins, quinolones, and glycopeptides; presence of central venous catheter (CVC); presence of urinary catheter; total parenteral nutrition (TPN); mechanical ventilation; surgical intervention; prolonged hospitalization; use of immunosuppressant medication (chemotherapy, posttransplant immunosuppressants and systemic steroids); neutropenia; bone marrow transplantation; solid organ transplantation; concurrent bacterial bloodstream infection; dialysis; previous hospitalization; and location in the intensive care unit (ICU) at the time of infection], length of hospital stay before invasive Candida spp. infection, details of antifungal therapy, removal or not of CVC, duration of hospital stay, and outcome.

Neutropenia was defined as polymorphonuclear neutrophils ≤500/mm^3. Neutropenia and use of immunosuppressant drugs were analyzed if present for at least two weeks before invasive fungal infection. Prematurity was defined as gestational age of ≤37 weeks. Concomitant bacteremia was defined as the isolation of bacterial species from blood within 24 h of the initial positive fungal culture. Prolonged hospitalization was defined as more than 30 days. Presence of CVC and urinary catheter was considered if present for at least seven days of invasive candidiasis. History of hospitalization was analyzed if present for three months before invasive fungal infection. The other potential risk factors were evaluated by analyzing the clinical history of the patients in the four weeks before invasive candidiasis was diagnosed. Urinary tract infection was defined as growth of a single organism at 10^5 cfu ml^{-1} in urine collected during midstream voiding after appropriate cleaning or ≥10^4 cfu ml^{-1} in urine collected by catheterization. Candidemia was defined as a blood culture from either a peripheral vessel or a CVC from which any Candida species was isolated. Catheter-associated candidemia was defined as the isolation of Candida spp. from any culture of blood from a patient with a CVC or as the growth of Candida spp. from a catheter-tip culture[7]. There was no consistent policy about the removal of CVCs in this study. Initial therapy was considered inadequate when more than 72 h elapsed between the time a culture was obtained and initiation of treatment with an antifungal and/or when the infecting organism was resistant to the antifungal agent used.

The main outcome measured was the mortality at 30 days after the initiation of infection. According to this outcome, survivor and exitus patient groups were compared.

Identification of Organism and Susceptibility Testing

Blood, cerebrospinal fluid (CSF), peritoneal fluid, and pericardial fluid cultures were processed by the BACTEC 9240 system (Becton-Dickinson, Cockeysville, MD, USA) and were then cultured with Sabouraud's dextrose agarose culture medium. The other samples were inoculated into blood agar, chocolate agar and eosin-methylene blue agar plate with subsequent passages to Sabouraud dextrose agar. The isolates were cultured from various clinical specimens (24 blood, 15 CVC, 3 urine, 3 abscess, 2 peritoneal fluid, 2 drain, 1 pericardial fluid, 1 CSF, 1 penile exudates). Only one isolate from each patient was included. All isolates were stored -20°C in glycerol stocks 20% until use. Yeast identification was based on

germ tube formation, microscopic morphology on corn agar and API 20CAUX bioMérieux.

The susceptibility of Candida isolates to four antifungal agents (amphotericin B, fluconazole, voriconazole, and caspofungin) was tested by use of the Etest (AB Biodisk, Solna, Sweden) in accordance with the manufacturer's instructions. The minimum inhibitory concentrations (MICs) were read visually after 24 h of incubation at 35°C. For the azoles and caspofungin, an 80% inhibition in growth was used as the MIC cut-off (microcolonies were ignored), and for amphotericin B, the MIC endpoint was defined as the lowest concentration with complete (100%) growth inhibition. MIC interpretive criteria for fluconazole, voriconazole and caspofungin were those published by the Clinical and Laboratory Standards Institute (CLSI) and were as follows: fluconazole susceptible (MIC ≤8 mg/L); susceptible-dose dependent (MIC 16-32 mg/L); resistant (MIC ≥64 mg/L); voriconazole susceptible (MIC ≤1 mg/L); susceptible-dose dependent (MIC 2 mg/L); resistant (MIC ≥4 mg/L); caspofungin susceptible (MIC ≤2 mg/L); and resistant (MIC >2 mg/L) (M27-A3)[8]. Interpretative breakpoint criteria for amphotericin B have not been determined. For this study, we determined isolates that were inhibited by ≥1 μg/ml to be resistant as described by Pfaller et al.[9] Isolates of *C. krusei* were accepted as fluconazole-

Table I. The Demographic and Clinical Characteristics of the Patients

Patients	
Age [median (25-75%)]	245 days (16-1825 days)
Sex (Female/Male)	9/26
Nosocomial infection (n, %)	33, 94.2
Time to infection (median, 6-270 days)[a]	16 (11-35.5)
Hospital ward	n, %
Neonatal intensive care	9 (25.7)
General pediatrics	8 (22.8)
Intensive care unit (ICU)	7 (20)
Pediatric surgery ICU	4 (11.4)
Pediatric surgery	3 (8.5)
Hematology-Oncology	4 (11.4)
Underlying disease[b]	n, %
Neonates	9 (25.7)
Prematurity	9
Non-neonates	26 (74.2)
Surgery	13
Malignancy	5
Solid tumor	2
Leukemia	3
Congenital heart disease	3
Renal disease	3
Chronic renal failure	1
Congenital hydronephrosis	2
Nervous system disease	3
SSPE	2
SMA	1
Transplantation	3
Liver transplantation	2
Bone marrow transplantation	1
Immunodeficiency	1
Severe combined immune deficiency	1
Other[c]	7

SSPE: Subacute sclerosing panencephalitis. SMA: Spinal muscular atrophy.
[a]The time from admission to the date of the first positive culture for nosocomial acquired infection only.
[b]In some patients, there was more than one diagnosis.
[c]Other diseases (Crohn disease, metabolic disorders, Down syndrome, chronic lung disease, multiple anomaly, neonatal hepatitis, Wilson disease).

Table II. Risk Factors for Invasive Candida spp. Infection in 35 Patients

Risk factors	No. (%) of patients
Underlying disease	35 (100)
Antimicrobial therapy	33 (94.2)
Location in the ICU at the time of infection	20 (57.1)
Central venous catheter	20 (57.1)
Hospitalization before infection	18 (51.4)
Total parenteral nutrition	15 (42.8)
Mechanical ventilation	14 (40)
History of surgery	12 (34.2)
Prolonged hospitalization	11 (31.4)
Immunosuppressant therapy	10 (28.5)
Urinary catheter	9 (25.7)
Concomitant bacteremia	8 (22.8)
Dialysis	4 (11.4)
Neutropenia	3 (8.5)
Transplantation	3 (8.5)

resistant regardless of their fluconazole MICs. C. albicans ATCC 90028 was included in each run of susceptibility tests for quality control.

The descriptive data of the study group were given as frequency and median (25-75%). Chi-square, Fisher's exact test and Cox regression analysis were used for evaluation of the data. Results with $p<0.05$ were considered statistically significant.

Results

Patients' Characteristics

During the four-year period, there were 37 patients with invasive candidiasis. A second episode of invasive fungal infection was found in 2 patients. The first episode of these patients was included. Two patients were excluded for polymicrobial candidemia. The number of evaluable patients was 35, with a median age of 245 days (range: 5 days to 13 years) (Table I). The risk factors for invasive candidiasis of the patients are shown in Table II.

Species Distribution and Antifungal Susceptibility of the Isolates

C. albicans was the most common agent of invasive candidiasis, accounting for 23 cases (65.7%) (Table III). *C. albicans* was isolated in all the newborns and 80% of the patients with malignancy (4 of 5). The susceptibility of the isolates to the antifungal agents for which CLSI breakpoints are available (fluconazole, voriconazole, and caspofungin) is shown in Table IV.

Therapy

A total of 7 patients (20%) received antifungal therapy as prophylactic or empirical use and for pulmonary aspergillosis at the time of invasive candidiasis detection: 5 fluconazole, 1 amphotericin B and 1 itraconazole. All of the therapies except amphotericin B were changed after invasive candidiasis was determined. Different types of antifungal regimens were used (Table V). They included monotherapy (a single antifungal drug), sequential monotherapy (more than 1 antifungal drug but at different times) and combination therapy (concomitant administration of more than 1 antifungal drug). The median length of antifungal therapy was 20.5 days (range: 1-95 days).

Initial therapy was evaluated as inadequate in 4 patients (11.7%). In 3 of them, antifungal therapy was started 3 days after culture positivity. A patient who had high amphotericin B MIC values was treated with amphotericin B. CVCs were withdrawn in 15 of 19 patients with catheter-related candidemia (78.9%) between days 1-26. Four patients in whom the catheter was not withdrawn died.

Outcome

Eight of the patients died within the 30 days following a positive culture, resulting in an overall mortality rate of 22.8%. The median length of hospital stay after onset of invasive fungal infection was 26 days (range: 1-185 days). The demographic and clinical characteristics of the patients, risk factors for fungal infections, Candida species, fluconazole

susceptibility of the isolates, and the nature of the antifungal therapy were compared between surviving patients and those who died. The following variables reached significance in the univariate analysis: location in the ICU at the time of infection, the presence of CVC, CVC not replaced, and mechanical ventilation ($p<0.05$) (Table VI). However, none of them was significant in the multivariate analysis. In addition, the patients with invasive candidiasis caused by *C. albicans* and non-albicans Candida spp. were compared using univariate analysis. Of these patients, demographic and clinical characteristics, therapy, susceptibility of the isolate to fluconazole, and outcome were compared, and no statistically significant differences were determined between these variables ($p>0.05$, data not shown).

Discussion

We found that *C. albicans* was the most common yeast species responsible for invasive candidiasis in children. *C. parapsilosis* was the second most common Candida species identified in children. Caspofungin was the most active agent against all Candida species.

The risk factors associated with candidiasis are presence of underlying disease, prematurity, surgery (especially gastrointestinal surgery), malignancy, immunodeficiency, transplantation, neutropenia, bacterial infections, broad-spectrum antibiotics, colonization with Candida spp., corticosteroids and chemotherapeutic agents, CVCs, endotracheal intubation, hyperalimentation, dialysis, and stay in the ICU[1,3,10-15]. Risk factors for invasive candidiasis of patients in this study were similar to those reported in previous studies, and there was an underlying disease in all patients. The most common underlying diseases were surgical diseases and prematurity. Most of our patients received broad-spectrum antibiotic treatment and had CVCs. Stay in the ICU during invasive Candida spp. infection, hospitalization before invasive candidiasis, TPN, and mechanical ventilation were the common risk factors.

C. albicans is the most common Candida species in pediatric patients and is reported to be responsible for 30-76% of invasive Candida spp. infections. *C. parapsilosis* is reported to be the most common non-albicans Candida spp.[1,9,16,17]. In this study, the most common species causing invasive candidiasis was *C. albicans,* and it was responsible for 65.7% of all isolates. Similar to previous studies, *C. parapsilosis* was the second most commonly isolated agent (11.4%). Many physicians expect that infections due to non-albicans Candida spp. will occur in patients given prior antifungal therapy. Invasive candidiasis developed in 20% of patients during antifungal therapy in our study. There was no statistical difference for frequency of *C. albicans* and non-albicans Candida spp. between patients who received or did not receive antifungal therapy ($p<0.05$, data not given in results).

The Etest is a practical agar-based diffusion method that is reliable for susceptibility testing of Candida spp. Since it is especially able to differentiate species that are susceptible or resistant to amphotericin B, it is preferred to the microdilution reference method[18-23]. To our knowledge, there has been no previous study evaluating *in vitro* susceptibility of yeasts in children in our country.

Invasive fungal infections due to amphotericin B-resistant Candida species are increasing[24-26] and range between 0-3%[13,24-30]. In the literature, the highest resistance to amphotericin B was reported in *C. krusei* (66.7%), and *C. krusei* species resistant to amphotericin B, especially among malignancy patients, were found[24,28,30]. 94-97% of Candida species are inhibited in ≤1.0 μg/mL concentration of amphotericin B.

Table III. Candida Species Isolated from Sterile Body Areas

Candida spp.	No. of isolates (%)
C. albicans	23 (65.7)
Non-albicans Candida spp.	12 (34.2)
C. parapsilosis	4 (11.4)
C. tropicalis	3 (8.5)
C. krusei	2 (5.7)
C. glabrata	2 (5.7)
C. pelliculosa	1 (2.8)

Table IV. The In Vitro Susceptibility of Isolates to Fluconazole, Voriconazole and Caspofungin

Species	No. of isolates	No. Susceptibility							
		Fluconazole			Voriconazole			Caspofungin	
		S	SDD	R	S	SDD	R	S	NS
C. albicans	23	20 (86.9%)	2 (8.6%)	1 (4.3%)	21 (91.3%)		2 (8.6%)	23 (100%)	
C. parapsilosis	4	4 (100%)			4 (100%)			4 (100%)	
C. tropicalis	3	3 (100%)			3 (100%)			3 (100%)	
C. krusei	2			2 (100%)	2 (100%)			2 (100%)	
C. glabrata	2	1 (50%)	1 (50%)		2 (100%)			2 (100%)	
C. pelliculosa	1	1 (100%)			1 (100%)			1 (100%)	
[a]Total (%)	35	29 (82.8%)	3 (8.5%)	3 (8.5%)	33 (94.2%)		2 (5.7%)	35 (100%)	

S: Susceptible. SDD: Susceptible dose-dependent. R: Resistant. NS: Non-susceptible.

In this study, amphotericin B in ≤1 μg/ml concentration inhibited 97.1% of isolates. There was one *C. krusei* isolate with MIC value >1 μg/ml, and the amphotericin B resistance rate was 2.8%. Amphotericin B resistance was detected in *C. krusei* species isolated in a child receiving chemotherapy for Ewing sarcoma, similar to the literature. This patient was treated with liposomal amphotericin B and discharged. The present data fail to demonstrate a correlation between higher MICs for amphotericin B and outcome for patients treated with amphotericin B and emphasize the importance of the host condition in predicting the clinical outcome[23,31].

It was reported in four children's hospital in the United States that fluconazole susceptibility in *C. albicans* isolates was 92%, dose-dependent susceptibility was 6% and fluconazole susceptibility in non-albicans Candida isolates, especially in *C. glabrata* species, was low[17]. Fluconazole susceptibility, dose-dependent susceptibility and resistance to Candida spp. were found as 82.8%, 8.5% and 8.5%, respectively, in our study, and these values were within the limits reported in the literature. Fluconazole resistance among *C. albicans* isolates (4.3%) was lower than among non-albicans Candida isolates (16.6%); however, the difference was not statistically significant ($p>0.05$). Fluconazole resistance among non-albicans Candida species was detected in *C. krusei*. *C. krusei* is a species intrinsically resistant to fluconazole and must be regarded as resistant independently from MIC values, as suggested by Pfaller et al.[32]. When we used CLSI breaking points, one of the *C. krusei* species was susceptible and the other was dose-dependent susceptible. Similar *C. krusei* species were reported in the literature, and the frequency of *C. krusei* with a MIC value of 64 μ/mL was reported as 34-100%[30,33,34].

Expanded spectrum triazoles are more effective in species with decreased susceptibility to fluconazole, like *C. krusei*. Zaoutis et al.[17] reported that voriconazole susceptibilities among 176 Candida spp. isolated from sterile body regions in children for *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei* were 92%, 80%, 71%, 100%, and 100%,

Table V. Antifungal Therapy

Antifungal therapy	No. (%) of patients
Monotherapy	23 (65.7)
Amphotericin B	14
Fluconazole	9
Sequential therapy	7 (20)
Fluconazole/amphotericin B	6
Amphotericin B/voriconazole	1
Combination therapy	4 (11.4)
Amphotericin B + fluconazole	2
Amphotericin B + fluconazole + irrigation of the bladder with amphotericin B	1
Fluconazole + irrigation of the bladder with amphotericin B	1
None	1 (2.8)

Table VI. Univariate Analysis of Factors Associated with Mortality in Patients with Invasive Candida spp. Infection

	Survived (n=27)	Died (n=8)	Total	p value
Boys	20	6	26	>0.05
Age [days, median (25-75%)]	304 (34.5-1642)	168 (19.5-3376.2)		>0.05
Time to infection [days, median (25-75%)][a]	17 (11.5-37.25)	16 (8.75-31.5)		>0.05
Hospital ward				
ICU[b]	12	8	20	>0.05
Non-ICU	15	-	15	<0.05
Clinical characteristics				
Prematurity	7	2	9	>0.05
Malignancy	4	1	5	>0.05
Transplantation	1	2	3	>0.05
Neutropenia	1	2	3	>0.05
Immunosuppressive therapy	7	3	10	>0.05
Total parenteral nutrition	11	4	15	>0.05
Previous receipt of antifungal therapy	4	3	7	>0.05
History of surgery	11	1	12	>0.05
Central venous catheter (CVC)	13	7	20	<0.05
Broad-spectrum antibiotics	25	8	33	>0.05
Urinary catheter	5	4	9	>0.05
Mechanical ventilation	6	8	14	<0.05
Prolonged hospitalization	9	2	11	>0.05
Concomitant bacteremia	7	1	8	>0.05
Previous hospitalization	14	4	18	>0.05
Dialysis	2	2	4	>0.05
Length of stay [days, median (25-75%)]	55 (37-181)	18.5 (14.25-58)		<0.05
Candida spp. isolated				
C. albicans	17	6	23	>0.05
Non-albicans Candida spp	10	2	12	>0.05
Therapy				
Inadequate initial therapy	2	2	4	>0.05
Removal of CVC	13	3	16	>0.05
No removal of CVC	0	4	4	<0.05
Amphotericin B	9	5	14	>0.05
Fluconazole	8	1	9	>0.05
Sequential therapy	6	1	7	>0.05
Combination therapy	4	-	4	>0.05
No drug	-	1	1	>0.05
Fluconazole susceptibility				
Fluconazole resistance	3	0	3	>0.05
S-DD fluconazole resistance	2	1	3	>0.05
Fluconazole susceptibility	22	7	29	>0.05

[a]The time from admission to the date of the first positive culture for nosocomial acquired infection only.
[b]Newborn, Pediatric Surgery, and Pediatric Intensive Care Units.
S-DD: Susceptible dose-dependent, ICU: intensive care unit.

respectively. High voriconazole MIC values in *C. albicans*, *C. glabrata* and *C. tropicalis* isolates were defined in some studies. High MIC values for voriconazole among *C. albicans* and other species were detected usually in patients who received salvage treatment and were unresponsive to one or more than one antifungal therapy[35]. Voriconazole susceptibility and resistance to Candida isolates were 94.3% and 5.7%, respectively, in our study. Voriconazole susceptibility was 91.3% and 100% in *C. albicans* species and non-albicans Candida spp., respectively. Voriconazole resistance was detected in two *C. albicans* isolates. None of these patients received prior

antifungal therapy, one of them was a newborn, and the other, who had Crohn disease, was receiving immunosuppressive treatment.

Echinocandin antifungal agents are effective against not only azole-susceptible Candida isolates but also on azole-resistant Candida species[10,36,37]. High caspofungin MIC values are detected among *C. parapsilosis* and *C. guilliermondii* spp. in the literature[17,38-40]. Susceptibility of caspofungin to Candida isolates was reported as 100% in studies performed among children[17,41]. In our study, caspofungin was 100% susceptible to all Candida isolates, and it was also effective against amphotericin B and azole-resistant species. Similar to the literature, *C. parapsilosis* isolates had higher caspofungin MIC values (1.5 and 2 µg/ml) than other species.

The mortality rates in children and infants were reported as 19-26% and 43-54%, respectively[16]. Mortality related to invasive candidiasis in our study was 22.8%, and this was within the limits reported in the literature. Risk factors associated with mortality are malnutrition, presence of underlying disease, immunosuppression, stay in the ICU, CVCs, endotracheal intubation, prolonged antibiotic treatment, parenteral nutrition, prolonged candidemia, and type of Candida species[13,42,43]. *C. albicans* and *C. tropicalis* are the most aggressive isolates. According to univariate analysis, mortality-associated risk factors in our study were stay in the ICU, presence of CVC, failure to remove the CVC, and mechanical ventilation during invasive Candida spp. infection. However, none of the risk factors was found significant in the multivariate analysis. Similar to previous studies, *C. albicans* mortality (26%) was higher than the non-albicans Candida spp. mortality (16.6%); however, the difference was not statistically significant. The low number of cases in our study may prevent the appearance of a statistical difference.

It is reported that there are differences in risk factors, clinical features and outcomes between Candida species. Çelebi et al.[15] reported that urinary catheters and young age were important risk factors in *C. albicans* candidemia. In a study composed of children and adults, it was shown that patients with *C. albicans* infection had a higher mortality and received immunosuppressive treatment more commonly. Among the non-albicans Candida species, patients with *C. parapsilosis* infection had lower fatality and complication rates, whereas patients with *C. glabrata* infection had the highest fatality rate[6]. We did not find any difference between the invasive infections due to *C. albicans* versus non-albicans Candida species with respect to demographic and clinical features, treatment, fluconazole susceptibility, and mortality.

There are some limitations to our study. The first is that clinical isolates were collected from only one university hospital and thus do not represent the general antifungal resistance pattern in our country. The second is that our sample size might have been insufficient to detect a small difference, if present.

In summary, characteristics of invasive candidiasis may vary between centers, and knowledge of the local risk factors, species distribution and antifungal resistance patterns of Candida isolates is imperative for the establishment of efficient therapeutic and preventive strategies. In our study, the most common isolated species in invasive Candida spp. infections was *C. albicans,* and the most effective antifungal drug according to *in vitro* antifungal susceptibility tests was caspofungin.

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