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Original Article

Evaluation of *Candida species* and antifungal susceptibilities among children with invasive candidiasis

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Abstract

Aim: Non-albicans *Candida species* and resistant microorganisms have been more commonly isolated in invasive candidiasis in recent years. The aim of this study was to evaluate the distrubution of *Candida* spp and antifungal resistance in our clinic.

Material and Methods: Fifty-four Candida isolates and antifungal susceptibility results obtained from patients diagnosed as having invasive candidiasis between December 2012 and June 2016 were included. Clinical and laboratory data were retrospectively analyzed. E-test method was used in order to determine antifungal susceptibilities of *Candida spp* for amphotericin B, fluconazole, voriconazole, ketoconazole, itraconazole, anidulafungin, caspofungin, and flucytosine.

Results: The clinical diagnoses of the patients were candidemia (n=27, 50%), catheter-related blood stream infection (n=1, 1.8%), urinary tract infection (n=13, 24%), surgical site infection (n=4, 7.4%), intraabdominal infection (n=3, 5.5%), empyema (n=2, 3.7%), and pneumonia (n=4, 7.4%). The most common isolated agent was *C. albicans* (n=27, 50%) and the others were *C. parapsilosis* (n=13, 24%), *C. tropicalis* (n=6, 11.1%), *C. glabrata* (n=3, 5.6%), *C. lusitaniae* (n=2, 3.7%), and unspecified *Candida spp.* (n=3, 5.6%). Fluconazole resistance was 7.4% among all isolates. Resistance against itraconazole, ketoconazole, anidulafungin, voriconazole and caspofungin were 33.3%, 12.5%, 11.1%, 5%, and 2.5%, respectively. Isolates presented intermediate resistance against itraconazole (41.7%), voriconazole (5.6%), and amphotericin B (3.7%) to varying extents. All of the isolates were susceptible to flucytosine.

Conclusions: In our clinic, *C. albicans* and non-albicans *Candida species* were equally distributed and antifungal susceptibilities against major antifungal agents such as fluconazole, amphotericin B, and caspofungin were found considerably high.

Keywords: Antifungal susceptibility, child, invasive candidiasis

Introduction

Invasive candida infections (ICI) are one of the important causes of morbidity and mortality in immunosuppressed individuals and in hospitalized patients (1). Newborns, patients in intensive care units (ICU) and hematology-oncology wards, and patients who have undergone bone marrow transplantation are especially at risk in terms of invasive candidiasis. *Candida species* have recently been found to be the fourth leading cause of bloodstream infections (BSI) following coagulase negative staphylococcus (CNS), *Staphylococcus aureus*, and enterococci (2, 3). They are the second leading agent following CNS in central catheter-related BSI (4). Mortality rates may reach up to 40-50%, especially in newborns and infants, despite prophylactic antifungal use and appropriate treatment methods (5).

Currently, the most commonly isolated agent in cases of invasive candidiasis is *Candida albicans*, but the

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frequency of non-albicans species is gradually increasing. In studies conducted in our country, non-albicans candida (NAC) species in ICI have been reported with rates reaching up to 73% (6). Non-albicans candida species pose a high risk for newborns and patients with advanced age who have weakened immune systems. With the wide use of prophylactic fluconazole (FCZ) in patients in the risk group, an increase in FCZ-resistant species, especially *C. glabrata* and *C. krusei*, has been observed. The increase in the prevalence of these resistant species decreases treatment success in cases of invasive candidiasis.

It is important for physicians to know the distribution of *Candida species* and antifungal susceptibilities in their clinics in terms of empirical antifungal selection and treatment planning. Althought there are data reporting antifungal susceptibilities in adult cases of invasive candidiasis in the literature, the number of studies conducted with children in this area is limited. The species distribution and antifungal susceptibilities of the isolates obtained from patients with invasive candidiasis who were hospitalized in different wards in our pediatric clinic were evaluated in this study.

Material and Methods

Fifty-four patients aged 0-18 years who were followed up in our clinic between December 2012 and June 2016 with a diagnosis of invasive candidiasis, who were found to have positive growth of Candida in blood samples obtained from central venous catheter and peripheral vein and in urine, surgical area incision site, abscess fluid (intraabdominal abscess and empiyema) and tracheal aspirate cultures, and in whom species distribution and antifungal susceptibilities were studied, were included in this study. The patients' files and computer records were examined and the clinical and laboratory results were recorded. Ethics committee approval was obtained from the Local Ethics committee of Istanbul University, Istanbul Medical Faculty, for this study (2016: 421).

Definitions

The diagnosis was made according to the surveillance diagnostic criteria specified by the Centers for Diseases Control and Prevention (CDC) (7). According to these criteria, growth of at least one *Candida species* in blood cultures and the presence of signs of infection including fever, hypothermia, leukocytosis, increased acute-phase reactants, tachycardia, and hypotension in association was considered *candidemia*. Isolation of the same *Candida species* in blood cultures obtained simultaneously from peripheral blood and the central venous catheter (CVC) and growth in the catheter blood at least two hours earlier compared with the venous blood in the presence of CVC without any other infection focus was considered *catheter-related bloodstream infection*.

When *Candida species* were isolated in urine culture in the presence of at least one of the signs and symptoms related with urinary tract infection (temperature >38°C, dysuria, pollakiuria, and suprapubic tenderness), a diagnosis of symptomatic urinary tract infection (UTI) was made.

The presence of at least two of the signs and symptoms including a temperature above 38°C that could not be explained otherwise, nausea, vomiting, abdominal pain, and isolation of Candida species in surgical drain fluid culture was considered *intraabdominal infection*. Purulent fluid at the site of superficial or deep incision that developed within 30 days following surgery and isolation of *Candida species* in a culture of this fluid was considered *surgical site infection*.

In patients who were found to have new or progressing infiltration on lung X-ray, isolation of *Candida* in tracheal aspirate culture obtained in the presence of two or more of the criteria specified (temperature >38°C or hypothermia, leukocytosis or leukopenia, purulent secretion) was considered *pneumonia*. *Pleural space infection (empyema)* was defined as isolation of *Candida species* in pleural fluid in the presence of a temperature above 38°C that could not be explained otherwise, and symptoms and signs of pneumonia.

Mycologic examination

Slides were prepared with 10% potassium hydroxide and 0.1% calcofluor white from all samples excluding urine and swab samples, and examined under fluorescence microscopy in terms of presence of fungal hyphe and spores. Urine and swab samples were cultivated in Sabouraud Dextrose Agar (SDA) (Becton-Dickinson, Paris, France) with gentamycin (Sigma, St. Louis, MO, USA) (0.1 g/L) and chloramphenicol (Sigma, St.Louis, MO, USA) (0.05 g/L), and incubated at 37°C and 27°C for 10 days. The other systemic samples were cultivated in SDA, which included the same antibiotics and "brain heart infusion agar" (Becton-Dickinson, Paris, France) and incubated at 37°C and 27°C for 30 days. Samples suspicted of containing superficial mycosis such as skin samples were cultivated in SDA that included actidione (0.1 g/L) in addition to the same antibiotics, and incubated at 37°C and 27°C for 21-30 days.

Microscopic morphology was examined in agar-agar with cornflour and 1% Tween using the API ID 32 C yeast identification kit (bioMérieux, Marcy I'Etoile, France).

Antifungal susceptibility test

An Etest was used in accordance with the manufacturer's instructions to specify the amphotericin B (AMB), fluconazole, voriconazole, ketoconazole, itraconazole, anidulafungin, caspofungin, and flucytosine (FLU) susceptibilities of the species. For the objective of quality control, standard species including *C. albicans* ATCC 90028, *C. parapsilosis* ATCC 22019, and *C. krusei* ATCC 6258 were used.

A suspension of the *Candida species* was prepared in 0.85% sodium chloride according to a 0.5 McFarland turbidity standard and inoculated using RPMI-1640 (Sigma, St.Louis, MO, ABD) medium with a swab. After the agar plate surfaces were dried, Etest bands (bioMérieux, Marcy I'Etoile, France) containing AMB (0.002-32 µg/mL), anidulafungin (0.002-32 µg/mL), caspofungin (0.002-32 µg/mL), FCZ (0.016-256 µg/mL), itraconazole (0.002-32 µg/mL), ketoconazole (0.002-32 µg/mL), voriconazole (0.002-32 µg/mL) and FLU (0.002-32 µg/mL) were prepared. The plates were incubated at 37°C for 24 hours. If insufficient growth was present, the plates were incubated for 48 hours. After incubation, the concentration that enabled 100% inhibition for AMB, 80% inhibation for azoles and echinocandins, and 90% inhibition for FLU was specified as the minimum inhibitory concentration (MIC) (8).

Susceptibility was studied for FCZ and AMB in all isolates obtained, for caspofungin in 74%, for voriconazole in 74% (n=40), for itraconazole in 22.2% (n=12), for ketokonazole in 14.8% (n=8), for FLU in 14.8% (n=8), and for anidulafungin in 16.6% (n=9).

Results assessment

The MIC values specified were compared with the MIC values specified in the M27-A3 and M27-S4 guidelines prepared by the Clinical Laboratory Standards Institute (CLSI) and the species were specified as "susceptible," "susceptible-dose dependent," "moderately susceptible," and "resistant" to the antifungal substance studied (Table 1).

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS Inc; Chicago, IL, USA) 21 package program. Normality was tested using the Shapiro-Wilk and Kolmogorov- Smirnov tests.

Table 1. ***Candida species and antifungal susceptibility minimal inhibitory concentration values***

	S	SDD	MS	R
		Fluconazole		
C. albicans	≤2	4	-	≥8
C. parapsilosis	≤2	4	-	≥8
C. tropicalis	≤2	4	-	≥8
C. glabrata	-	≤32	-	≥64
C. krusei[a]	-	-	-	-
		Voriconazole		
C. albicans	≤0.12	0.25-0.50	-	≥1
C. parapsilosis	≤0.12	0.25-0.50	-	≥1
C. tropicalis	≤0.12	0.25-0.50	-	≥1
C. glabrata[b]	-	-	-	-
C. krusei	≤0.5	1	-	≥2
		Itraconazole		
Candida spp.	≤0.12	0.25-0.5	-	≥1
		Amphotericin B		
Candida spp.	≤1	-	-	>1
		Flucytosine		
Candida spp.	≤4	-	8-16	≥32
		Caspofungin-Anidulafungin		
C. glabrata	≤0.125	-	0.25	≥0.5
C. albicans				
C. tropicalis	≤0.25	-	0.5	≥1
C. krusei				
C. parapsilosis	≤2	-	4	≥8
C. guilliermondii	≤2	-	4	≥8

[a]*C. krusei* with innate resistance to fluconazole, *bC. glabrata*: WT ≤0.5 µg/mL; non-WT ≥1 µg/mL; MIC: minimum inhibitory concentration; MS: moderately susceptible ; R: resistant; S: susceptible; SDD: susceptible (dose-dependent)

Data are expressed as median, minimum-maximum, frequency, and precentage.

Ethics committee approval was obained for this study from the Ethics Committee of Istanbul University, Faculty of Medicine (2016: 421). Informed consent was not obtained because the study was conducted retrospectively.

Results

A total of 54 patients were diagnosed as having candidemia (n=27, 50%), catheter-related bloodstream infection (n=1, 1.8%), UTI (n=13, 24%), surgical site infection (n=4, 7.5%), intra-abdominal infection (n=3, 5.5%), empyema (n=2, 3.7%), and pneumonia (n=4, 7.5%) were

Table 2. Distribution of *Candida* species by units

Candida species	PICU (n=21)	NICU (n=13)	Other (n=20)
C. albicans (n=27)	10 (47.6)	7 (53.8)	10 (50)
C. parapsilosis (n=13)	7 (33.3)	1 (7.7)	5 (25)
C. tropicalis (n=6)	2 (9.5)	3 (23.1)	1 (5)
C. glabrata (n=3)	2 (9.5)	0	1 (5)
C. lusitaniae (n=2)	0	0	2 (10)
Unidentified (n=3)	0	2 (15.4)	1 (5)

PICU: Pediatric Intensive Care Unit; NICU: Neonatal Intensive Care Unit

Table 3. Distribution of *Candida* species by infection site

Candida species	Blood (n=27)	Urine (n=13)	Catheter (n=1)	Surgical area (n=4)	TAC (n=4)	Abscess (n=5)
C. albicans (n=27)	10 (37)	7 (53.8)	1 (100)	2 (50)	4 (100)	3 (60)
C. parapsilosis (n=13)	10 (37)	2 (15.4)	0	0	0	1 (20)
C. tropicalis (n=6)	2 (7.4)	3 (23.1)	0	1 (25)	0	0
C. glabrata (n=3)	2 (7.4)	0	0	1 (25)	0	0
C. lusitaniae (n=2)	2 (7.4)	0	0	0	0	0
Unidentified (n=3)	1 (3.7)	1 (7.7)	0	0	0	1 (20)

TAC: tracheal aspirate culture

Table 4. Antifungal susceptibility in all *Candida* isolates obtained

Antifungal agent (n)	S (n, %)	SDD (n, %)	RD (n, %)
Fluconazole (n=54)	47 (87)	3 (5.6)	4 (7.4)
Amphotericin B (n=54)	52 (96.3)	2 (3.7)	-
Caspofungin (n=40)	39 (97.5)	-	1 (2.5)
Voriconazole (n=40)	38 (95)	-	2 (5)
Itraconazole (n=12)	3 (25)	5 (41.7)	4 (33.3)
Ketoconazole (n=8)	7 (87.5)	-	1 (12.5)
Flucytosine (n=8)	8 (100)	-	-
Anidulafungin (n=9)	8 (88.9)	-	1 (11.1)

R: resistant; SDD: susceptible (dose dependent); S: susceptible

included in the study. The median age of the subjects was found as 19.5 months (range, 1-176 months), and 24 (44.4%) of the subjects were female. The median age was found as 14 months (range, 1-135 months) in the patients with *C. albicans* and 33 months (range, 1-176 months) in those with NAC.

The most commonly isolated agent was *C. albicans* in 50% of the subjects (n=27). Among the non-albicans candida species, the most commonly isolated was *C. parapsilosis* (n=13, 24%), followed by *C. tropicalis* (n=6, 11.1%), *C. glabrata* (n=3, 5.6%), *C. lusitaniae* (n=2, 3.7%), and unidentified *Candida* species (UCS) (n=3, 5.6%).

Twenty-one (38.9%) patients were hospitalized in the Pediatric Intensive Care Unit (PICU), 13 (24.1%) were hospitalized in the Neonatal Intensive Care Unit (NICU), and 20 (37%) were hospitalized in other pediatric wards. When the units were examined by the distribution of species, the most commonly found agent was again *C. albicans* in the PICU, NICU, and other wards. *C. parapsilosis* was the most commonly isolated *NAC* species in the PICU (n=7, 33.3%) and other wards (n=5, 25%). *C. tropicalis* was the most commonly isolated *NAC* species in the NICU (n=3, 23.1%) (Table 2).

Location of infection

Candida species were found peripheral blood samples of 27 patients, urine in 13, tracheal aspirate samples in four, abscess fluid in five, surgical incision sites in four, and in the blood sample obtained from the CVC in one patient. In all samples, the most commonly isolated agent was *C. albicans* (Table 3). *C. parapsilosis* was the most commonly isolated NAC in blood (n=10, 37%) and *C. tropicalis* was the most commonly isolated *NAC in urine* (n=3, 23.1%). All (n=4) agents grown in tracheal aspirate fluids were identified as *C. albicans*.

Facilitating factors

The most common facilitating factor in terms of invasive candidiasis was use of broad-spectrum antibiotics, which was found in 45 (83.3%) patients. The other facilitating factors included hospitalization in the ICU (n=37, 68.5%), presence of CVC (n=35, 64.8%), history of previous surgery (n=20, 37%), total parenteral nutrition (n=17, 31.4%), prophylactic FCZ treatment (n=6, 11.1%), and neutropenia (n=3, 5.5%).

Antifungal susceptibility

When all *Candida species* were examined, FCZ resistance was found with a rate of 7.4%. Susceptibility was not tested in all isolates. However, itraconazole, ketoconazole, anidulafungin, voriconazole, and caspofungin resistance was found at rates of 33.3%, 12.5%, 11.1%, 5%, and 2.5%, respectively. Dose- dependent susceptibility was found in 41.7% of isolates for itraconazole, and in 5.6% for voriconazole. Moderate susceptibility to AMB was found in 3.7%. All samples examined were susceptible to FLU (Table 4). The MIC values and susceptibility rates of *Candida species* to the selected antifungal drugs are shown in Table 5 in detail.

C. albicans

Fluconazole and AMB susceptibility was tested in all obtained *C. albicans* isolates (n=27). Fluconazole resistance was found with a rate of 7.4%, whereas all isolates were susceptible to AMB. Resistance was present in two

Table 5. Assessment of antifungal susceptibility of *Candida* species

Candida species (n, %)	Antifungal agent (number)	MIC value Median, range	R (n, %)	MS/SDD (n, %)	S (n, %)
C. albicans (n=27)	Fluconazole (27)	0.25 (0.02-64)	2 (7.4)	-	25 (92.6)
	Amphotericin B (27)	0.125 (0.02-0.64)	-	-	27 (100)
	Caspofungin (19)	0.047 (0.02-0.5)	-	-	19 (100)
	Voriconazole (22)	0.032 (0.04-32)	2 (9.1)-		20 (90.9)
	Itraconazole (6)	0.25 (0.02-32)	1 (16.7)	- / 3 (50)	2 (33.3)
	Ketoconazole (5)	0.012 (0.06-32)	1 (20)	-	4 (80)
	Flucytosine (5)	0.064 (0.047-0.190)	-	-	5 (100)
	Anidulafungin (4)	0.002 (0.002-0.018)		-	4 (100)
C. parapsilosis (n=13)	Fluconazole (13)	0.25 (0.032-2)	-	-	13 (100)
	Amphotericin B (13)	0.38 (0.004-1.5)	-	-	13 (100)
	Caspofungin (10)	0.025 (0.006-2)	1 (10)	-	9 (90)
	Voriconazole (8)	0.07 (0.032-0.94)	-	-	8 (100)
	Itraconazole (1)	0.75 (0.75-0.75)	-	- / 1 (100)	-
	Ketoconazole (1)	0.047 (0.047-0.047)	-	-	1 (100)
	Flucytosine (1)	0.023 (0.023-0.023)	-	-	1 (100)
	Anidulafungin (2)	0.005 (0.002-0.008)	-	-	2 (100)
C. tropicalis (n=6)	Fluconazole (6)	1.5 (0.38-2)	-	-	6 (100)
	Amphotericin B (6)	0.75 (0.094-1)	-	-	6 (100)
	Caspofungin (5)	0.032 (0.016-0.25)	-	-	5 (100)
	Voriconazole (5)	0.094 (0.012-0.125)	-	-	5 (100)
	Itraconazole (2)	0.236 (0.092-0.38)	-	- / 1 (50)	1 (50)
	Ketoconazole (1)	0.032 (0.032-0.032)	-	-	1 (100)
	Flucytosine (1)	0.038 (0.038-0.038)	-	-	1 (100)
	Anidulafungin (2)	0.004 (0.002-0.006)	-	-	2 (100)
C. glabrata (n=3)	Fluconazole (3)	1 (1-3)	-	- / 1 (33.7)	2 (66.7)
	Amphotericin B (3)	0.5 (0.25-0.5)	-	-	3 (100)
	Caspofungin (3)	0.125 (0.094-0.19)	-	-	3 (100)
	Voriconazole (-)	-	-	-	-
	Itraconazole (-)	-	-	-	-
	Ketoconazole (-)	-	-	-	-
	Flucitosin (-)	-	-	-	-
	Anidulafungin (-)	-	-	-	-
C. lusitaniae (n=2)	Fluconazole (2)	4 (0.016-8)	1 (50)	-	1 (50)
	Amphotericin B (2)	-	-	2 (100)	-
	Caspofungin (2)	0.078 (0.032-0.125)	-	-	2 (100)
	Voriconazole (2)	0.078 (0.032-0.125)	-	-	2 (100)
	Itraconazole (1)	2 (2-2)	1 (100)	-	-
	Ketoconazole (1)	0.064 (0.064-0.064)	-	-	1 (100)
	Flucitosin (1)	0.125 (0.125-0.125)	-	-	1 (100)
	Anidulafungin (1)	4 (4-4)	1 (100)	-	-
Unidentified (n=3)	Fluconazole (3)	4 (4-32)	1 (33.7)	2 (66.7)	-
	Amphotericin B (3)	0.25 (0.032-0.25)	-	-	3 (100)
	Caspofungin (1)	0.25 (0.25-0.25)	-	-	1 (100)
	Voriconazole (3)	0.125 (0.125-0.25)	-	-	3 (100)
	Itraconazole (2)	4 (4-4)	2 (100)	-	-
	Ketoconazole (-)	-	-	-	-
	Flucytosine (-)	-	-	-	-
	Anidulafungin (-)	-	-	-	-

R: resistant; SDD: susceptible (dose dependent); S: susceptible; MIC: minimum inhibitory concentration; MS: moderately susceptible

(9.1%) of 22 *C. albicans* isolates for which voriconazole susceptibility was studied. Two (3.3%) of six isolates for which itraconazole susceptibility was studied were susceptible, and three (50%) were susceptible in a dose-dependent manner; one (16.7%) was found resistant. On the other hand, resistance was found in one (20%) of five isolates for which ketoconazole susceptibility was studied. Caspofungin (n=19), FLU (n=5), and anidulafungin (n=4) resistance was not found among the isolates tested.

Non-albicans *Candida*

Fluconazole and AMB susceptibility was studied in all *Candida parapsilosis* isolates (n=13) and no resistance was found. No resistance was found in any isolates for which voriconazole (n=8), ketoconazole (n=1), FLU (n=1), and anidulafungin (n=2) susceptibilities were studied, whereas resistance was found in one (10%) of 10 isolates for which caspofungin susceptibility was studied. A single *C. parapsilosis* species was found susceptible to itraconazole in a dose-dependent manner (100%).

C. tropicalis isolates (n=6) for which antifungal resistance was studied were susceptible to FCZ, AMB (n=6), caspofungin (n=5), voriconazole (n=5), ketaconazole (n=1), FLU (n=1), and anidulafungin (n=2). One of the *C. tropicalis* isolates (n=2) was found susceptibile to itraconazole (50%), the other was susceptible in a dose-dependent manner.

Fluconazole, AMB, and caspofungin susceptibilities were studied for all *C. glabrata* isolates obtained (n=3). Only one isolate (33.3%) was found susceptible to FCZ in a dose-dependent manner.

All *C. Lusitaniae* isolates (n=2) were found as moderately susceptible to AMB. Fluconazole resistance was found in one (50%) of the isolates for which susceptibility was studied, itraconazole resistance was found in one (100%), and anidulafungin resistance was found in one (100%).

All unidentified *Candida species* were susceptible to AMB and voriconazole. One isolate (33.3%) was resistant to FCZ and two (66.6%) were susceptible in a dose-dependent manner. Caspofungin susceptibility was studied in one isolate and no resistance was found.

Discussion

Resistant species are currently found as causative agents with a gradually increasing frequency, and there has been an increase in invasive *Candida* infections, both of which are important problems. With the widespread use of prophylactic and empirical antifungal therapy, the rate of especially treatment-resistant NAC species is also increasing. However, it is also known that *Candida species* show variance depending on the geographic and climatic conditions. Although the most commonly isolated NAC species in adult patients in Canada, North Europe, and the United States of America is *C. glabrata*, *C. parapsilosis* is more common in Asia, South Europe, and South America (9). It has been reported that *C. Parapsilosis* is observed more commonly in children (10). Although variances have been observed in different studies conducted in our country, the frequency of *C. albicans* is approximately 39-65% in *Candida* infections (11, 12). The second most commonly isolated agent has been reported as *C. parapsilosis* in many studies (11-13). However, these studies include data related with adult patients. In our study, the distribution ratio for *C. albicans* and NAC was found to be half and half, and the most common type of NAC found was *C. parapsilosis* with a rate of 24.1%. *C. parapsilosis*, which has been reported to be transmitted by hyperalimentation fluids and intravascular devices, constitutes a significant problem, especially in patients in ICUs (14). Among our stubjects, the frequency of *C. parapsilosis* in the PICU was also found as high (33.3%). In our NICU, the most commonly observed NAC species was *C. tropicalis* (23,1%). However, statistical assessment revealed no difference between the units in terms of *Candida species*. This is probably related with the low number of isolates.

When *Candida* infections were examined in terms of infection site, candidemia and UTI were found to be the leading infections in different studies. Acar et al. (14) reported the rates of candidemia and UTI as 42.9% and 37.1%, respectively, in patients hospitalized in the ICU. In studies conducted by Çekin et al. (15) and Hazırolan et al. (16), UTI was found to be the leading infection before candidemia. In our study, candidemia (50%) and UTI (24%) were found to be the most common infections in accordance with the literature data. The frequency of UTIs was lower compared with previously reported frequencies. This may be related with the fact that there was a high risk of UTI in association with candidemia because our subjects were in the childhood age group. In the study conducted by Hazırolan et al. (16), *C. glabrata* was found with a high rate (78.9%) in urine. In our study, the most commonly isolated agent in all samples was *C. Albicans. C. parapsilosis* was the most common (37%) NAC species in blood, and *C. tropicalis* was the most common (23.1%) NCA species in urine.

Triazoles are the most commonly used antifungal drugs because they have high bioavaliability when used orally, and they generally have a favorable safety profile. Widespread use of triazoles in prophylactic and empirical treatment has led to an increase in *Candida species* with resistance to azoles. It was reported that previous FCZ treatment was an independent risk factor for candidemias, which are resistant to FCZ (17). Although differences are present in various studies, the rate of FCZ resistance ranges between 0.7% and 8.6% (18, 19). However, İris-efe et al. (13) reported a high rate of resistance to FCZ at 19%. In our study, the FCZ resistance rate was found as 7.4%, including two *C. albicans*, one *C. lusitaniae*, and one UCS. In the literature, it has been reported that reduced susceptibility to azoles is present in cases of FCZ resistance. Barchiesi et al. (20) reported higher MIC values for itraconazole in *C. albicans* species with FCZ resistance. In our study, the highest resistance was found against itraconazole among the isolates for which antifungal susceptibility was studied. A third (33.3%) of the isolates were found resistant to itraconazole and 41.7% were susceptible in dose- dependent manner. Similarly, Gültekin et al. (6) from our country and Tan et al. (21) from Singapore showed a high itraconazole resistance in their studies. Although there may be differences between studies, voriconazole resistance generally seems to be low. Çalışkan et al. (22) showed that all isolates in cases of candidemia were susceptible to voriconazole. Öztürk et al. (23) found voriconazole resistance only in *C. albicans* strains with a rate of 8%. Similarly, the rate of variconazole resistance was 5% in the prsent study, and the isolates were *C. albicans*.

AMB, which acts by binding to ergosterol in the cellular membrane and increasing cell permeability, is an antifungal agent with a considerably broad spectrum. The fact that it is fungicidal, depending on concentration, and has a post-antifungal effect increases its action (24). AMB resistance is generally rare in treatment of invasive candidiasis. Mechanisms including reduction in the ergosterol rate in fungal cells, variance in sterol or reduction in binding tendency are blamed for resistance. *Candida lusitaniae* and *C. guilliermondii* have innate resistance to AMB, whereas *C. glabrata* and *C. krusei* generally show susceptibility despite high MIC values. Although there are international publications and our country reporting that all *Candida species* are susceptible to AMB, some studies reported a high rate of resistance (16, 22). In the study conducted by Hazırolan et al. (16) in which the susceptibility of *C. albicans* to AMB was found as 92.8%, the resistance rate of *C. kefyr* was reported as 18.2%. In the present study, AMB susceptibility was 96.3% among all *Candida species* and two *C. lusitaniae* isolates (3.7%) were moderately susceptible. In light of the present information, it is observed that AMB treatment maintains its importance, especially in cases of invasive candidiasis with in-vitro or in-vivo FCZ resistance.

Drugs in the echinocandin group are not affected by resistance mechanisms against azoles, including changes in sterol structure and exclusion pump formation, because they do not enter the fungal cell (24). Their fungicidal effect on *Candida species* increases treatment success. There are studies reporting that all *Candida species* in our country are susceptible to caspofungin. On the other hand, the caspofungin resistance rate for *C. krusei* was documented as 12.5% in a multi-center study by Pfaller et al. (25). Similarly, some studies reported that the MIC values of echinocandins were higher for *C. parapsilosis* strains (26, 27). In our study, the caspofungin susceptibility rate was 97.5% and resistance was found in only one (2.5%) *C. parapsilosis* isolate. Generally, the rate of caspofungin susceptibility to predict anidulafungin and micafungin susceptibility is considered to be above 98% (28). Therefore, it has been stated that other echinocandins may be preferred in *Candida* infections that are known to be susceptible to caspofungin. However, anidulafungin resistance was found in a *C. lusitaniae* isolate that was susceptible to caspofungin in the present study. This shows that resistance to antifungal agents in the same group may develop with different mechanisms.

Flucytosine, which acts as a pyrimidine analogue, is frequently used in combination treatments in locations where AMB has low permeability including cerebrospinal fluid, cardiac valves, and fundus oculi. In publications reported from our country, FLU resistance was reported as low (0.4-3%) (22, 29). This is likely related with the fact that the drug is not commercially available in our country. Similarly, all *Candida* isolates for which resistance was tested in our study were susceptible to FLU.

In conclusion, *Candida* infections constitute a serious problem because of many factors including mortality, morbidity, and high cost throughout the country. The gradual increase in resistant strains decreases treatment efficiency and increases costs. It will be directive for physicians to know the distribution of *Candida species* and antifungal susceptibilities in their clinics, especially in terms of prophylactic and empirical antifungal selection. This is especially important in pediatric medicine. Multi-center studies are needed to obtain more elucidative data across our country.

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