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ORIGINAL ARTICLE

Candidemia Cases Caused by *Candida parapsilosis* Complex Species: Epidemiology and Antifungal Susceptibility of Strains

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SUMMARY

Background: ***Candida parapsilosis*** **is a common non-*albicans Candida* species isolated from blood cultures. The increase in fluconazole-resistant *C. parapsilosis* complex isolates is worrying, especially in strains with Y132F changes in the *ERG11* gene since this ultimately leads to outbreaks. This study aimed to investigate the distribution and antifungal susceptibility of *C. parapsilosis* complex species isolated from bloodstream, clinical characteristics of patients, prevalence of risk factors, and to determine *ERG11* gene region mutations in strains that were not susceptible to fluconazole.**
Methods: **Between 2014 and 2018, 96 patients with *C. parapsilosis* candidemia were evaluated. Thermo Scientific Sensititer™ YeastOne™ YO10 was used for antifungal susceptibility testing. The *ERG11* gene region sequence analysis was performed for fluconazole non-susceptible isolates.**
Results: **All the strains were defined as *C. parapsilosis* sensu stricto. The rate of fluconazole resistance was 6.3%, and that of susceptibility to fluconazole at an increased dose was 2.1%. Two isolates showed Y132F or G458S *ERG11* changes associated with azole resistance, with the most common change being identified as R398I, which was shown not to encode azole resistance. No resistance to echinocandins and amphotericin B was observed. The use of broad-spectrum antibiotics (83.3%) was the most common risk factor.**
Conclusions: **This study highlights the importance of susceptibility testing when making a decision to use fluconazole in the treatment of *C. parapsilosis* candidemia. The presence of resistance associated with *ERG11* Y132F changes indicated that azole resistance should be closely monitored. Increasing awareness of fluconazole-resistant *C. parapsilosis* candidemia will help identify strategies to overcome these infections.**
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KEYWORDS

Candida parapsilosis complex, candidemia, antifungal susceptibility, Sensititre Yeast One, fluconazole resistance, *ERG11* mutation, Y132F alteration, epidemiology

INTRODUCTION

Invasive candidiasis is an infection associated with high morbidity and mortality rates, and therefore early diagnosis and appropriate treatment are essential, especially in high-risk hosts, such as immunocompromised pa-

tients [1]. The most common clinical form of invasive candidiasis is candidemia [2]. Although studies agree that *Candida albicans* continues to be the main cause of hospital-acquired yeast infections, *Candida parapsilosis* is reported to be the second or third most frequently isolated species after *C. albicans* in candidemia cases [3, 4].

C. parapsilosis is the most common *Candida* species in intensive care units and pediatric patients, especially the infant group [5,6]. In 2005, Tavanti et al. named the species complex consisting of three subspecies: *C. parapsilosis* sensu stricto, *Candida orthopsilosis*, and *Candida metapsilosis* [7]. Although molecular methods are frequently preferred for the identification of the members of the species complex, in recent years, matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) has become one of the most commonly used techniques due to its cost-effectiveness and fast and accurate results [8].

The Clinical and Laboratory Standards Institute states that in cases where it is not possible to identify *C. orthopsilosis* and *C. metapsilosis* due to their low prevalence, the clinical breakpoints of *C. parapsilosis* can be used; however, if such an identification can be made, epidemiological cutoff values (rather than clinical breakpoints established for *C. parapsilosis*) are recommended to be used in *C. orthopsilosis* and *C. metapsilosis* [9].

This study aimed to perform the subtyping of candidemia agent *C. parapsilosis* complex strains with the MALDI-TOF MS method at Akdeniz University Hospital over a five-year period, investigate the antifungal susceptibility of these strains, examine *ERG11* gene region mutations in those that were found to be resistant or susceptible-increased exposure to azole agents, and determine the clinical characteristics of patients with candidemia.

MATERIALS AND METHODS

The authors confirm that the ethical policies of the Journal, as noted on the Journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received from the Clinical Research Ethics Committee of Akdeniz University with the decision number 2019/34.

Strain selection and definitions

The study included *C. parapsilosis* complex strains (n = 96) first isolated from the blood cultures of the patients followed up in inpatient wards and intensive care units of Akdeniz University Hospital from 1 January 2014 through 31 December 2018. The detection of *Candida* sp. in at least one blood culture sample at 48 hours after hospitalization was accepted as hospital-acquired candidemia in accordance with the Centers for Disease Control and Prevention criteria. Catheter-associated candidemia was considered if the same organism grew from at least 1 percutaneous blood sample culture and from the catheter tip. Patients who had negative blood cultures and regressed clinical symptoms and signs after receiving 14-day antifungal therapy were considered cured [10]. Early mortality was defined as death on the third to seventh day after diagnosis, and late mortality as death from eight to 30 days [11].

Microbiological methods

Strains stored at -80°C in tryptic soy broth with 15% glycerol were passaged into the Saboraud dextrose agar (SDA; BD, Germany) and Columbia blood agar (BD, Germany) media and incubated at 35 - 37°C for 24 - 48 hours. The isolates were identified in the Bruker Maldi Biotyper (Bruker Daltonics, Germany) system using the ethanol-formic acid extraction method according to the manufacturer's recommendations.

The *in vitro* susceptibility of each strain to anidulafungin, micafungin, caspofungin, 5-fluorocytosine, posaconazole, voriconazole, itraconazole, fluconazole, and amphotericin B was analyzed using the Thermo Scientific Sensititre™ YeastOne™ YO10 (SYO) (TREK Diagnostics, Thermofisher, United Kingdom) colorimetric microdilution test method following the manufacturer's recommendations. The determined minimum inhibitory concentration (MIC) values were interpreted according to the clinical breakpoints available in the *European Committee on Antimicrobial Susceptibility Testing* (EUCAST) v. 10.0 [12]. For 5-fluorocytosine, which has no clinical breakpoint, the epidemiological cutoff values (ECVs) were used to differentiate between wild and non-wild types [13]. *C. parapsilosis* ATCC 22019 was used as the quality control strain.

ERG11 gene region analysis

C. parapsilosis strains that were found to be resistant or susceptible-increased exposure to the azole group agents were passaged into SDA, and new passages were prepared. With these new passages, 200 µL of suspension prepared at 2 McFarland turbidity in sterile 0.9% saline was taken, and nucleic acid isolation was performed using the commercial column-based RTP pathogen kit (Stratec, Germany). The amount of DNA obtained was measured using the HS dsDNA protocol with a Qubit analyzer (Thermo Fisher, USA), and DNA samples were prepared with a final concentration of 2 ng/µL in TE buffer. DNA was fragmented by taking 50 µL of the prepared samples, sonicating it for 15 seconds, and waiting for 15 seconds (44 cycles). Fragmented DNA was measured again with the Qubit analyzer following the HS dsDNA protocol. The sample was prepared at a final concentration of 1 ng/µL in TE buffer was used to construct the library.

The Nextera XT DNA Library Preparation Kit (Illumina, USA) was used to construct the library. The tagmentation and barcoding processes of the DNA fragments were performed. A library pool was created with an initial concentration of 2 nM and a final concentration of 1.2 - 1.3 pM, together with other samples to be

entered into the device. This pool was then loaded into the MiniSeq device (Illumina, USA), and reading was performed. The alignment of the paired reading data files obtained from the device was undertaken with the Burrows-Wheeler Alignment software package using the *C. parapsilosis ERG11* gene sequence (LT596076) as a reference and the maximal exact matches (MEM) method. SAMtools software package was used to sequence and map the aligned sequences and determine variants. DeepM Aligner software running under Linux was used to run all procedures.

Examination and analysis of clinical data

The clinical data of the patients included in the study were retrospectively reviewed. The patients' demographic characteristics, underlying diseases, risk factors, duration of and reason for hospitalization, time from hospitalization to candidemia, candidemia treatment, agents used and their duration, and clinical outcomes were examined.

Data analysis was performed using SPSS software package (version 22). Pearson's chi-squared test was conducted to analyze relationships between categorical variables. Statistically, p-values of < 0.05 were considered significant.

RESULTS

Frequency of *Candida parapsilosis* complex candidemia

A total of 527 candidemia episodes were detected in our hospital between 2014 and 2018. During this five-year period, 40% of the candidemia cases were caused by *C. albicans* (n = 211), while non-*albicans Candida* species were found in 60%. *C. parapsilosis* complex (n = 99, 18.8%) was the most common causative agent of non-*albicans Candida* cases, followed by *C. tropicalis* (n = 72, 13.7%), *C. glabrata* (n = 63, 12%), *C. krusei* (n = 32, 6.1%), *C. kefyr* (n = 17, 3.2%), *C. lusitaniae* (n = 7, 1.3%), *C. dubliniensis* (n = 5, 0.9%), and other *Candida* species (n = 21, 4.1%). Three of the 99 strains defined as *C. parapsilosis* complex were excluded from the study because they did not grow in passages.

All 96 strains included in the study were defined as *C. parapsilosis* sensu stricto with the Bruker MALDI Biotyper, and no *C. orthopsilosis* and *C. metapsilosis* subtype was isolated.

Antifungal susceptibility test results

Table 1 presents the MIC value ranges and MIC_{50} and MIC_{90} values of the isolates for all antifungal agents, and Table 2 shows the distribution of MIC values of the *C. parapsilosis* sensu stricto strains according to various antifungals.

According to the EUCAST (v 10.0) breakpoints, all the *C. parapsilosis* sensu stricto strains were susceptible to micafungin and anidulafungin. EUCAST has proposed that isolates which are susceptible to anidulafungin as well as micafungin should be considered susceptible to caspofungin, because interpretive breakpoints have not been established for caspofungin. As a result, in this study, all isolates were susceptible to caspofungin. The amphotericin B MIC values of all the strains were $\leq$ 1 mg/L, and no resistance was found to this antifungal agent. Since there is no clinical breakpoint for 5-fluorocytosine in EUCAST, the ECV of $\leq$ 0.5 μg/mL was used in the evaluation, and 11 (11.5%) strains were determined as non-wild type [13].

According to EUCAST breakpoints, 88 (91.6%) strains were susceptible to fluconazole at the standard dose, two (2.1%) were susceptible at an increased exposure, and six (6.3%) were resistant to this agent. Three of the six fluconazole-resistant strains were found to be cross-resistant to voriconazole, posaconazole, and itraconazole. The remaining three fluconazole-resistant strains were susceptible to voriconazole at an increased exposure, and posaconazole and itraconazole at their standard doses. Table 3 presents the data on cross-resistance to voriconazole, posaconazole, and itraconazole among the strains that were found to be fluconazole-resistant or susceptible to this agent at an increased exposure, as well as *ERG11* mutations detected in these strains. Four (66.6%) of the six strains with fluconazole resistance were isolated from different intensive care units.

The *ERG11* gene region analysis of the eight strains showing susceptible at an increased dose or resistance to azole antifungal agents revealed that Y299N in isolate 2, A262D + F305L + H401Q + Y471F in isolate 7, Y132F + R274G + V282L + R398I in isolate 50, R398I + G458S in isolate 83, G89C + R398I in isolate 98, and R398I changes in isolates 46, 48, and 96 (Table 3).

Epidemiology and clinical results

Of the 96 patients included in the study, 61 (63.5%) were male and 35 (36.5%) were female. The mean age of all the patients was 40.75 ± 28.73 years, and the age range was 1 day - 97 years. Twelve (12.5%) patients were aged < 1 year, seven (7.3%) were 1 - 5 years, 10 (10.4%) were 5 - 18 years, 45 (46.9%) were 18 - 65 years, and 22 (22.9%) were > 65 years old.

Thirty-nine (40.6%) of the patients were followed up in surgical units, and at the time of the detection of candidemia, 48 (50%) of the patients were in different intensive care units. Eighty-seven (90.6%) of the cases were hospital-acquired, while the remaining cases were associated with healthcare. In patients with candidemia, the mean onset of infection was 38.14 ± 44.06 days (0 - 245 days) after hospitalization. The total number of hospitalization days of the patients was 73.64 ± 68.62 (7 - 437 days). It was determined that candidemia was catheter-related in 23 (24%) patients. The clinical features of the 96 patients with *C. parapsilosis* complex candidemia are shown in Table 4.

When the reasons for the hospitalization of the patients were examined, the most common was infection for any reason (24%), followed by surgical intervention (16.7%), cardiological causes (9.4%), trauma (8.4%),

Table 1. MIC value ranges and MIC_{50} and MIC_{90} values for the nine antifungal agents of *Candida parapsilosis* sensu stricto strains.

	Antifungal agents	MIC range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)
C. parapsilosis **(n = 96)**	**Anidulafungin**	**0.25 - 4**	**1**	**2**
	Micafungin	**0.25 - 2**	**0.5**	**1**
	Caspofungin	**0.06 - 1**	**0.25**	**0.5**
	5-fluorocytosine	**≤ 0.06 - 2**	**0.25**	**1**
	Posaconazole	**≤ 0.008 - 0.25**	**0.015**	**0.03**
	Voriconazole	**≤ 0.008 - 8**	**0.015**	**0.06**
	Itraconazole	**≤ 0.015 - 0.5**	**0.03**	**0.06**
	Fluconazole	**≤ 0.12 - > 256**	**1**	**2**
	Amphotericin B	**≤ 0.12 - 1**	**0.5**	**0.5**

MIC - minimum inhibitory concentrations.
mg/L - milligram/liter.

and oncological-hematological diseases (7.3%).

The patients with candidemia were found to have at least one of the risk factors investigated. The most common risk factor was the use of one or more broad-spectrum antibiotics (83.3%). The most frequently used antibiotics were meropenem, trimethoprim-sulfamethoxazole, vancomycin, colistin, and teicoplanin in that order. Of the patients included in the study, 76 (79.2%) received antifungal treatment for candidemia and 34 (44.7%) were cured. Antifungal therapy was initiated within a mean of 0.96 ± 1.65 (0 - 8) days after the exact diagnosis. The most preferred antifungal agent (47.9%) was fluconazole, followed by amphotericin B and echinocandins. For the patients receiving treatment, the mean duration of treatment was 15.89 ± 14.88 (1 - 68) days.

The mortality data were not available for six patients; therefore, mortality calculations were evaluated over the data of the remaining 90 patients. Accordingly, the rates of 30-day, early, and late mortality were determined as 36.7% (33/90), 20% (18/90), and 16.7% (15/90), respectively. While the rate of 30-day mortality was 29.6% (21/71) among the patients who received treatment, it was 63.1% (12/19) in those who did not receive treatment, and it was found that not receiving antifungal treatment statistically significantly increased mortality ($p = 0.007$). The 30-day mortality of patients infected with fluconazole resistant *C. parapsilosis* strains was 50% (3/6).

DISCUSSION

C. parapsilosis is among the five species that cause more than 90% of candidemia episodes. Although *C. albicans* continues to be the most common *Candida* species causing invasive infections in most clinical settings, there has been a noteworthy increase in infections associated with non-*albicans Candida* species, such as *C. parapsilosis* and *C. glabrata* in recent years [14]. Arikan-Akdağli et al., evaluating antifungal susceptibility in a multicenter study for the first time in Turkey, reported that following *C. albicans, C. parapsilosis* complex (n = 575) was the most common isolated cause of 1991 candidemia cases (1997 - 2017) [15]. In some studies, the frequency of *C. parapsilosis* is even observed to exceed that of *C. albicans* [16]. The increasing rate of *C. parapsilosis* infections is worrying because this strain can colonize in healthcare workers and lead to outbreaks [14].

Systemic antifungals that are currently in clinical use for the treatment of candidemia belong to only three main classes: triazoles, polyenes, and echinocandins. In the presence of a limited number of therapeutic targets, resistance to any class of drugs can significantly limit treatment options, while multi-drug resistance can even render fungal infections incurable.

In our study, we determined that 6.3% of the strains were resistant to fluconazole, and 2.1% were susceptible at an increased exposure. Although fluconazole resistance is not generally considered to be common among *C. parapsilosis* isolates, several studies have shown that fluconazole resistance in C. *parapsilosis* may occur due to selective pressure following fluconazole treatment or prophylaxis, followed by outbreaks with clonal spread [17-20]. It was even reported that azole-resistant *C. parapsilosis* isolates persisted for more than two years in an adult intensive care unit [21].

In a global surveillance study conducted from 2016 to 2017, it was reported that fluconazole resistance among *C. parapsilosis* isolates was the highest (15.0%) in those isolated from Europe, while this species was not observed in Asia-Pacific or Latin America [22]. In a multicenter study investigating in vitro resistance rates

Table 2. Minimum inhibitory concentrations (MIC) (mg/L) distribution of *Candida parapsilosis* sensu stricto strains.

Candida parapsilosis (n = 96)	MIC values (mg/L)																
	≤ 0.008	≤ 0.015	0.03	≤ 0.06	≤ 0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	> 256
Anidulafungin	-	0	0	0	0	3	28	37	27	1	0	-	-	-	-	-	-
Micafungin	0	0	0	0	0	7	43	44	2	0	0	-	-	-	-	-	-
Caspofungin	0	0	0	1	12	57	20	6	0	0	0	-	-	-	-	-	-
5-fluorocytosine	-	-	-	30	13	21	21	9	2	0	0	0	0	0	-	-	-
Posaconazole	3	59	26	5	2	1	0	0	0	0	0	-	-	-	-	-	-
Voriconazole	17	31	36	5	1	3	1	0	0	0	2	-	-	-	-	-	-
Itraconazole	-	8	53	30	2	2	1	0	0	0	0	0	-	-	-	-	-
Fluconazole	-	-	-	-	1	8	29	35	15	2	0	1	1	2	0	0	2
Amphotericin B	-	-	-	-	15	32	48	1	0	0	0	-	-	-	-	-	-

-: indicates the absence of wells examined for the antifungal agent at the specified MIC values, mg/L: milligram/liter.

Table 3. MIC (mg/L) values, mutations detected in the *ERG11* gene region, and isolation times and clinical units for the strains found to be susceptible or resistant to standard or increased doses of azole antifungal agents.

Strain number	Fluconazole		Voriconazole		Posaconazole		Itraconazole		*ERG11* mutations	Isolation time and unit
	MIC (mg/L)	EU	MIC (mg/L)	EU	MIC (mg/L)	EU	MIC (mg/L)	EU		
2	32	R	0.25	I	0.015	S	0.03	S	Y299N	October 2018 - newborn ICU
7	64	R	0.25	I	0.03	S	0.06	S	A262D + F305L + H401Q + Y471F	August 2018 - oncology clinic
46	4	I	0.03	S	0.015	S	0.03	S	R398I	October 2015 - pediatric clinic
48	16	R	0.25	I	0.03	S	0.06	S	R398I	October 2015 - anesthesia ICU
50	> 256	R	8	R	0.12	R	0.25	R	Y132F + R274G + V282L + R398I	September 2015 - pediatric clinic
83	> 256	R	8	R	0.25	R	0.	R	R398I + G458S	February 2014 - internal medicine clinic
96	64	R	0.5	R	0.12	R	0.25	R	R398I	December 2018 - chest diseases ICU
98	4	I	0.12	S	0.06	S	0.06	S	G89C + R398I	April 2016 - pediatric clinic

MIC - minimum inhibitory concentrations, mg/L - milligram/liter, EU - EUCAST (v10.0), S - susceptible at the standard dose, I - susceptible at an increased exposure, R - resistant, ICU - intensive care unit.

in candidemia isolates in Turkey, it was determined that the rate of resistance to fluconazole among *C. parapsilosis* complex isolates greatly varied from one center to another (0 - 47.1%), and the mean prevalence was reported as 7.7% [15].

We detected amino acid changes at position 132 of the *ERG11* gene in one of the fluconazole-resistant isolates (16.6%). In a previous study, the Y132F mutation was reported in 31 - 57% of the fluconazole-resistant *C. parapsilosis* isolates collected from different regions of the world, but the Y132F mutation was not detected in fluconazole-susceptible isolates, confirming that this mutation is the dominant fluconazole resistance mechanism for *C. parapsilosis* across the world [20]. In a two-year study of Castanheira et al., 80.4% of the strains that were not susceptible to fluconazole were Y132F isolates, and this change was detected in at least seven countries for both study years [22]. In another study covering a 21-year period, Demirci-Duarte et al. showed that in strains that were not susceptible to fluconazole, the most common mutation was Y132F (80.4%), followed by G458S (10.9%), and D421N (4.3%) [23]. In a clonal candidemia epidemic caused by fluconazole-resistant *C. parapsilosis* isolates, 90% of

Table 4. Clinical characteristics of patients with *Candida parapsilosis* complex candidemia.

Characteristic		n	%
Age, Mean ± SD 40.75 ± 28.73		96	100
Gender	Male	61	63.5
	Female	35	36.5
Underlying Disease			
Malignancy		29	30.2
Primary GIS malignancy		10	10.4
Diabetes mellitus		9	9.4
Chronic renal failure		4	4.2
Transplantation		3	3.1
Heart failure		3	3.1
COPD		2	2.1
Risk Factors			
Neutropenia		16	16.7
Total parenteral nutrition		16	16.7
Abdominal surgery		11	11.5
GIS bleeding		4	4.2
Antibiotic treatment *		80	83.3
Immunosuppressive drug treatment *		39	40.6
Antifungal treatment *		31	32.3
Chemotherapy *		10	10.4
Corticosteroid use *		37	38.5
Central venous catheterization		62	64.6
Urinary catheterization		63	65.6
Mortality (30 - day)		33	36.7

SD - standard deviation, GIS - gastrointestinal system, COPD - chronic obstructive pulmonary disease.
*** - Treatment applied before candidemia.**

the resistant isolates were determined to be clones containing known mutations in the *ERG11* gene, such as Y132F and Y132F + K143R [24].

The G458S mutation we detected in another isolate resistant to azoles was reported in fluconazole-resistant *C. parapsilosis* and *C. orthopsilosis* isolates in different studies, and it was suggested that this mutation might be associated with azole resistance [23]. In our study, the R398I mutation was present in four of the fluconazole-resistant isolates and two of those that were susceptible to fluconazole at an increased exposure. Similarly, in a study by Choi et al., R398I mutations were detected in resistant isolates, but this mutation was also reported in susceptible isolates [20]. Thomaz et al., evaluating azole-susceptible and non-susceptible isolates in Brazil, identified the R398I mutation in only one susceptible isolate [21]. The detection of this mutation in susceptible and resistant isolates suggests that it is not associated with azole resistance alone but may be a compensatory mutation [25].

The remaining polymorphisms we detected in *ERG11* have not been previously reported to be associated with azole resistance. We consider that apart from *ERG11* gene mutations, different molecular mechanisms that may cause azole resistance may play a role in our isolates with resistance.

In the current study, the fluconazole-resistant isolates were determined to have cross-resistance to all investigated triazoles and three also had cross-resistance to voriconazole. Cross-resistance was not found among the isolates susceptible to fluconazole at an increased exposure. Due to the common mechanisms of action of azole antifungal agents, there is a strong positive correlation between the MIC values of fluconazole and those of itraconazole, voriconazole and posaconazole among *Candida* species other than *C. krusei*, which indicates significant cross-resistance [26]. Therefore, fluconazole resistance can be used to predict the resistance of *Candida* species to other broad-spectrum triazoles [27]. *Candida parapsilosis* has naturally occurring polymor-

phisms in the FKS1 gene, leading to increased echinocandin MICs, the clinical significance of which is not established [28]. Although the emergence of echinocandin-resistant *C. parapsilosis* clinical isolates has been reported, acquired resistance to echinocandins has been rarely observed among *Candida parapsilosis* [29].

The identification of risk factors associated with candidemia can help reduce mortality, define measures, and initiate antifungal therapy early [30]. Similar to other studies [1,31], the most common risk factor in our study was the use of broad-spectrum antibiotics, followed by a history of invasive procedures, such as urinary catheterization and central venous catheter (CVC). However, unlike previous studies [11,31], we found the rate of total parenteral nutrition (TPN) use to be low. The difference may be due to the retrospective assessment of medical records.

Mortality rates in invasive candidiasis vary according to different patient groups. While low early mortality is associated with factors such as appropriate antifungal therapy and early removal of central venous catheter, late mortality is generally associated with host-related factors [6,32]. In a study evaluating mortality rates, the rate of 30-day mortality in *C. parapsilosis* complex candidemia was found to be lower when compared to other *Candida* species [31]. In another study, the early mortality rate was also found to be lower compared to *C. albicans* candidemia [11]. In the current study, it was determined that the rate of 30-day mortality (36.7%) was higher than reported by some studies in the literature [11] while lower compared to others [33]. In our study the mortality rate in fluconazole resistant *C. parapsilosis* candidemias (50%) was found to be higher than *C. albicans* candidemias in other studies [11,31]. There are a number of reasons for the differences between the studies that evaluated the mortality, including, but not limited to delayed initiation of empiric treatment or inadequate dosing of antifungal therapy, septic shock attributed to *Candida* infection, failure to remove a central venous catheter, increased severity of illness, and use of immunosuppressive therapy [32,34,35].

Our study has certain limitations, the primary being the single-center and retrospective design. In addition, molecular methods could not be used to identify strains, and identification was performed with the Bruker Maldi Biotyper (Bruker Daltonics, Germany), which received US Food & Drug Administration approval in 2018 for the differentiation of *C. parapsilosis* complex species [36]. Another limitation is that we did not investigate the clonal relationship between fluconazole-resistant strains, and we only screened *ERG11* mutations among the molecular mechanisms of fluconazole resistance.

In conclusion, we consider that it is necessary to at least determine the antifungal susceptibility of the invasive infectious agent *C. parapsilosis* isolates to fluconazole. Antifungal susceptibility testing should be performed for other triazoles in isolates that are found to be resistant to fluconazole given the possibility of cross-resistance. The rate of resistance to fluconazole, which is frequently used in empirical treatment in our hospital, was determined to be 6.3% in our study, which shows that the empirical antifungal regimen should be adjusted accordingly. Fluconazole should not be used in empirical treatment, especially in critically ill patients. We detected the Y132F mutation in only one strain, but since Y132F isolates, similar to the behavior of *C. auris*, can survive in the hospital environment, the rapid detection of these isolates is important for taking special precautions to prevent their spread in the hospital.

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Declaration of Interest:
The authors have no conflicts of interest to declare that are relevant to the content of this article**.**

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