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Investigation of Azole Susceptibility Profile and Virulence Factors of *Candida* Strains Isolated From Blood Culture

Kan Kültüründen İzole Edilen *Candida* Suşlarının Azol Duyarlılık Profili ve Virülans Faktörlerinin Araştırılması

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ABSTRACT

Introduction: *In our study, the azole group antifungal susceptibility status, proteinase, phospholipase, and coagulase enzyme activities, biofilm formation ability, and hemolytic activities of Candida species isolated from blood culture were investigated.*

Materials and Methods: *A total of 156 Candida species isolated from blood cultures were included in our study. Antifungal susceptibilities to fluconazole, voriconazole and posaconazole were determined by E-test method (Himedia, India). The modified tube adherence method was used to determine the biofilm production characteristics of the strains. Phospholipase activity was determined using egg yolk agar medium and proteinase activity was determined using 1% bovine serum albumin medium. Hemolytic activity was determined using Sabouraud Dextrose agar medium with 7% sheep blood. Human plasma was used for coagulase activity.*

Results: *Among C. albicans strains, 2% of them were found to be fluconazole-resistant, and 3% of them were voriconazole intermediate. While 7% of C. albicans strains, 2% of C. parapsilosis strains, and 5% of C. tropicalis strains were found as non-wild types for posaconazole, all C. glabrata strains were found as wild types for posaconazole. The highest biofilm formation rate was observed in C. tropicalis strains, the highest protease activity in C. albicans and C. parapsilosis strains, and the highest phospholipase activity in C. albicans strains.*

Conclusion: *As a result, it was found that hydrolytic enzymes such as proteinase and phospholipase were synthesized not only in C. albicans strains but also in non-albicans Candida strains. However, no significant relationship was found between the virulence factors examined in our study and azole resistance.*

Key Words: *Azole resistance; Blood culture; Candida; Virulence factors*

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ÖZ

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Giriş: *Çalışmamızda kan kültüründen izole edilen Candida türlerinin azol grubu antifungal duyarlılık durumu, proteinaz, fosfolipaz ve koagülaz enzim aktiviteleri, biyofilm oluşturma yeteneği ve hemolitik aktiviteleri araştırıldı.*

Materyal ve Metod: *Kan kültürlerinden izole edilen toplam 156 Candida türü çalışmamıza dahil edildi. Flukonazol, vorikonazol ve posakonazol için antifungal duyarlılıklar e-test yöntemiyle (Himedia, Hindistan) belirlendi. Suşların biyofilm üretim özelliklerini belirlemek için modifiye tüp yapışma yöntemi kullanıldı. Fosfolipaz aktivitesi, yumurta sarısı agar ortamı kullanılarak belirlendi ve proteinaz aktivitesi, %1 sığır serum albümin ortamı kullanılarak belirlendi. Hemolitik aktivite, %7 koyun kanı içeren Sabouraud Dekstroz agar ortamı kullanılarak belirlendi. Koagülaz aktivitesi için insan plazması kullanıldı.*

Bulgular: *C. albicans suşlarının %2'sinin flukonazole dirençli ve %3'ünün vorikonazol orta duyarlı olduğu bulundu. Posakonazol için C. albicans suşlarının %7'si, C. parapsilosis suşlarının %2'si, C. tropicalis suşlarının %5'i yabani olmayan tip olarak bulunurken, tüm C. glabrata suşları posakonazol için yabani tip olarak bulunmuştur. En yüksek biyofilm oluşum hızı C. tropicalis suşlarında, en yüksek proteaz aktivitesi C. albicans ve C. parapsilosis suşlarında ve en yüksek fosfolipaz aktivitesi C. albicans suşlarında gözlendi.*

Sonuç: *Sonuç olarak; Proteinaz ve fosfolipaz gibi hidrolitik enzimlerin sadece C.albicans suşlarında değil, albicans dışı Candida suşlarında da sentezlendiği bulundu. Ancak çalışmamızda incelenen virülans faktörleri ile azol direnci arasında anlamlı bir ilişki bulunamamıştır.*

Anahtar Kelimeler: *Azol direnci; Kan kültürü; Candida; Virülans faktörleri*

INTRODUCTION

As a result of treatments used for malignant diseases and Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS), intensive care unit interventions, and advances in organ transplantation, there has been an increase in opportunistic infections and most importantly fungal infections. *Candida* species are the most common pathogens among fungal infections. They are isolated from 8-10% of nosocomial bloodstream infections[1]. *Candida albicans* is the most frequently isolated species in invasive infections and is the causative pathogen in 37-50% of bloodstream infections. However, in recent years, the frequency of *C. albicans* species causing candidemia has been gradually decreasing and the frequency of non-albicans *Candida* species has increased. Although the frequency of *C. albicans* has decreased over the years, it is still the most frequently isolated species[1].

Prevention and treatment of candidemia usually require long-term drug use. For this purpose, azole group antifungals are preferred first. Due to the prolonged and frequent utilization of antifungals, the emergence of resistant *Candida* strains has become a common occurrence[2]. Consequently, the regular implementation of antifungal susceptibility tests is of utmost significance for guiding treatment decisions and monitoring resistance levels.

Adherence, slime factor, biofilm formation, proteinase and phospholipase secretion, yeast-hyphae transformation, cell surface composition, and phenotypic transformation are the main virulence factors of *Candida* species[3]. Extracellular hydrolytic enzymes of *Candida* species facilitate adhesion and penetration into tissues and therefore invasion of the host. In addition, it is known that *Candida* species evade immune response with biofilm production and they are more resistant to antimicrobial agents which causes ineffective treatment[4].

In our study, the azole group antifungal susceptibility status, proteinase, phospholipase, and coagulase enzyme activities, biofilm formation ability, and hemolytic activities of *Candida* species

isolated from blood cultures were investigated. In addition, we aimed to investigate whether there is a relationship between resistance to azole antifungal agents and virulence factors.

MATERIALS and METHODS

A total of 156 *Candida* species isolated from blood cultures and sent to the central laboratory of our hospital between November 2019 and January 2022 were included in our study. Candida identification was conducted using the YST diagnostic panel (Diagnostics, Slovenia) along with their characteristic appearance observed in cornmeal tween-80 medium (Himedia, India). Antifungal susceptibilities for fluconazole, voriconazole, and posaconazole were determined by the E-test method (Himedia, India) and interpreted using CLSIM27A S4 clinical breakpoints and CLSIM59 epidemiological cut-off values[5,6].

The modified tube adherence method was used to determine the biofilm production characteristics of the strains. For this, *Candida* species grown on Saboraud dextrose agar (SDA) were inoculated into 10 ml polystyrene falcon tubes containing a loopful of Sabouraud Dextrose broth (SDB) with 8% glucose and incubated at 35° C for 48 hours. At the end of the period, the liquid in the tube was drained and the tubes were washed twice with distilled water. Then, 1% saffron dye solution was put into the tubes and left for 30 minutes. Then the dye solution was drained, and the tubes were placed upside down on blotting paper and left to dry for 24 hours. Biofilm positivity was determined by evaluating the presence of a colored layer on the inner wall of the tubes after 24 hours. The biofilm activity was categorized as weak positive (1+), moderately positive (2+), or strongly positive (3+), based on the thickness of the formed layer. *Candida krusei* ATCC 6258 was used as positive control strain.

For phospholipase activity, egg yolk agar medium was prepared as described by Ardıç et al[7]. 10 µL of *Candida* suspension adjusted to 0.5 McFarland was taken and inoculated on egg yolk agar medium. It was incubated at 37° C for four days. At the end of the period, the plates were evaluated for phospholipase activity. For evaluation, the diameter of the annular precipitation zone around the yeast colony was measured. Each isolate was tested twice. *Candida albicans* ATCC 90028 was used as positive control strain. To measure phospholipase activity, the diameter of the colony was proportional to the total diameter of the colony plus the precipitation zone, and the Pz value was calculated. Pz values between 0.9-1 were categorized as 1+, strains between 0.8-0.89 were categorized as 2+, between 0.7-0.79 were categorized as 3+, and strains with <0.69 were categorized as 4+ for phospholipase activity.

For the investigation of proteinase activity, a medium containing 1% bovine serum albumin was used and prepared as described by Ardıç et al[7]. *Candida* colonies grown in SDA were inoculated into Yeast Pepton Dextrose broth (Conda, Spain) and incubated at 30° C for four hours. McFarland was set to 0.5 at the end of the duration. 10 µL was taken and inoculated on paper discs placed on a solid medium containing bovine serum albumin. It was incubated at 30° C for six days. At the end of the period, the melting zones around the disc were measured. Those with 1-2 mm melting zones were categorized as weakly positive 1+ and those with 3-5 mm melting zone were categorized as strongly positive 2+ in terms of proteinase activity. Those without melting zones were considered negative. Each isolate was tested twice. *Candida parapsilosis* ATCC 22019 strain was used as positive control strain.

To evaluate the hemolytic activity of *Candida* isolates, they were cultivated in SDA and incubated for 18 hours at 37° C. A suspension of 1 x 10^8 cells/mL was prepared in sterile saline. 10 µL of this suspension was taken and dripped into the SDA containing 3% glucose and 7% sheep blood. Plates were incubated for 48 hours at 37° C in the presence of 5% CO_2. The clear zone around the inoculum area was attributed to hemolytic activity. Hemolytic activity was determined by the ratio of the sum of the colony diameter and the clear zone to the colony diameter and was expressed in Hz. Hz values of 1.0 were categorized as; 0.9-1 as 1+; 0.89-0.8 as 2+; 0.79-0.7 as 3+ and <0.69 as 4+.

To evaluate coagulase activity, a loopful of Candida colonies grown in Sabouraud Dextrose

Agar was transferred to tubes containing 500 µL of human plasma. Suspensions were prepared from these colonies for further analysis. They were incubated at 37° C. At the end of 2, 4, 6, and 24 hours, they were checked for clot formation. *S. aureus* ATCC 25923 was used as positive control and *S. epidermidis* ATCC 14990 strain was used as negative control.

Data analysis was performed using GraphPad Prism (GraphPad Software, USA). To compare the resistance rates for each strain, a One-Way Analysis of Variance (ANOVA) test was employed. For comparing categorical variables, Fisher's exact test was utilized. The compatibility of the quantitative data with the normal distribution was evaluated with the Shapiro-Wilk test. The normally distributed data were expressed as "mean ± standard deviation", while the data that did not conform to a normal distribution were expressed as "median (25^{th} percentile-75^{th} percentile]". Ethics committee approval was obtained for this study with the decision number 22-KAEK-035 dated 17.02.2022 from the clinical research ethics committee of the medical faculty of Tokat Gaziosmanpaşa University.

RESULTS

A total of 156 *Candida* species isolated from blood cultures and sent to the central laboratory of our hospital between November 2019 and January 2022 were included in our study. Of the strains included, 38% were *C. albicans*, 28% were *C. parapsilosis*, 13% were *C. tropicalis*, 11% were *C. glabrata*, 4% were *C. lusitaniae*, 3% were *C. guilliermondii*, 2% were *C. krusei* and 1% were *C. kefyr*. Of the isolated strains, 83 (73%) were from patients hospitalized in the intensive care unit, 24 (21%) were from internal medicine patients and 7 (6%) were from surgical patients. The median age of the patients included in the study was 72 (60-80).

Of the *C. albicans* strains, 2% were fluconazole resistant, 3% voriconazole intermediate, and 7% posaconazole non-wild type. One *C. albicans* strain resistant to fluconazole showed cross-resistance to posaconazole and voriconazole. Fourteen percent of *C.parapsilosis* strains were fluconazole resistant, 2% voriconazole resistant and 2% posaconazole non-wild type. According to these results, it was concluded that voriconazole and posaconazole were more sensitive to *C. parapsilosis* strains than fluconazole. In *C. parapsilosis* strains, cross-resistance was found with voriconazole in five fluconazole-resistant strains and six fluconazole intermediate susceptible strains, while cross-resistance was detected with voriconazole and posaconazole in one fluconazole intermediate susceptible strain. Seventeen percent of *C. glabrata* strains were fluconazole-resistant, 56% of them were voriconazole non-wild type, and posaconazole was found as wild type for all strains. According to these results, it was concluded that posaconazole was more sensitive than fluconazole and voriconazole in *C. glabrata* strains. Cross-resistance to voriconazole was detected in all fluconazole resistant *C. glabrata* strains and in seven fluconazole intermediate susceptible *C. glabrata* strains. No cross-resistance was found to posaconazole. While all *C. tropicalis* strains were susceptible to fluconazole, 10% of the strains were found to be intermediate-susceptible to voriconazole, and 5% to posaconazole non-wild type. *C. tropicalis* strains were found to be more sensitive to fluconazole. No cross-resistance was observed in any of the *C. tropicalis* strains. MIC range, MIC50, MIC90, Mean, Geometric mean, and Modal MIC values and antifungal resistance rates of *Candida* species are shown in Table 1.

Considering the virulence factors of *Candida* strains, it was observed that *C. tropicalis* strains formed the highest rate of biofilm formation; *C. parapsilosis* strains were found to have the lowest rate of biofilm formation. While the highest proteinase activity was observed in *C. albicans* and *C. parapsilosis*, it was not found in any of the *C. glabrata* and *C. krusei* strains. When we look at the phospholipase activity, it was seen only in *C. albicans* and *C. glabrata* strains. While hemolytic activity was positive in all *C. guilliermondii* and *C. kefyr* strains and 96% of *C. albicans* strains, it was not observed in any of the *C. krusei* strains. While the highest coagulase activity was observed in *C. glabrata* and *C. parapsilosis* strains; it was not found in any of the *C. lusitanae, C. guilliermondii, C. krusei, C. kefyr* strains (Table 2).

Table 1. MIC range, MIC_{50}, MIC_{90}, arithmetic mean, geometric mean, modal MIC values and antifungal resistance rates of Candida species

	MIC Range (µg/mL)	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	Mean ± Standard error of mean (SEM)	Geometric mean	Modal MIC	WT (%)	Non-WT (%)	Sensitive (%)	Intermediate (%)	Susceptible Dose Dependent (SDD)	Resistant (%)
C. albicans (n= 60)												
Fluconazole	0.064-12	0.19	0.5	0.46 ± 0.19	0.23	0.5			59 (98%)			1 (2%)
Voriconazole	0.012-0.75	0.016	0.125	0.05 ± 0.013	0.02	0.012			58 (97%)	2 (3%)		
Posaconazole	0.023-0.38	0.047	0.064	0.05 ± 0.008	0.04	0.064	56 (93%)	4 (7%)				
C. parapsilosis (n= 43)												
Fluconazole	0.094->256	0.75	12	20.03 ± 65.44	9.98	0.5			26 (60%)		11 (26%)	6 (14%)
Voriconazole	0.006-2	0.125	0.75	0.22 ± 0.36	0.09	0.125			29 (68%)	13 (30%)		1 (2%)
Posaconazole	0.002-0.94	0.047	0.125	0.07 ± 0.02	0.04	0.047	42 (96%)	1 (2%)				
C. glabrata (n= 18)												
Fluconazole	6->256	8	16	49.56 ± 22.40	14.08	8					15 (83%)	3 (17%)
Voriconazole	0.38-3	0.5	1.5	0.99 ± 0.16	0.82	0.5	8 (44%)	10 (56%)				
Posaconazole	0.25-2	1	1.5	1.0 ± 0.13	0.83	1	18 (100%)					
C. tropicalis (n= 20)												
Fluconazole	0.25-1	0.5	1	0.54 ± 0.07	0.45	1			20 (100%)			
Voriconazole	0.008-0.19	0.094	0.125	0.08 ± 0.011	0.06	0.125			18 (90%)	2 (10%)		
Posaconazole	0.016-0.19	0.047	0.125	0.06 ± 0.009	0.05	0.047	19 (95%)	1 (5%)				
C. lusitaniae (n=6)												
Fluconazole	0.5-4	0.5	1	1.16 ± 0.57	0.79	0.5	5 (83%)	1 (17%)				
Voriconazole	0.032-0.125	0.032	0.125	0.06 ± 0.01	0.05	0.032						
C. guilliermondii (n= 4)												
Fluconazole	1-4	1	1	2 ± 1.00	1.58	1	4 (100%)					
Voriconazole	0.064-0.125	0.125	0.125	0.12 ± 0	0.12	0.125						
C. krusei (n= 3)												
Voriconazole	0.047-0.064	0.047	0.064	0.05 ± 0.006	0.05	0.047			3 (100%)			
C. kefyr (n= 2)												
Fluconazole	1	1	1	1 ± 0	1	1						
Voriconazole	0.12	0.12	0.12	0.12 ± 0	0.12	0.12						

WT: Wild type, Non-WT: Non-wild type; MIC: Minimum inhibitory concentration.

Table 2. Biofilm, proteinase, phospholipase, hemolytic activity, and coagulase positivity rates of Candida species

		C. albicans (n= 60)	*C. parapsilosis* (n= 43)	*C. glabrata* (n= 18)	*C. tropicalis* (n= 20)	*C. lusitaniae* (n= 6)	*C. guilliermondi* (n= 4)	*C. kefyr* (n= 2)	*C. krusei* (n= 3)
Biofilm	3+	2	-	3	6	-	2	-	1
	2+	7	-	2	4	-	-	-	1
	1+	16	5	4	5	2	-	1	-
	Total (%)	25 (42%)	5 (12%)	9 (50%)	15 (75%)	2 (33%)	2 (50%)	1 (50%)	2 (66%)
Proteinase	2+	23	15	-	3	-	2	-	-
	1+	33	25	-	6	4	2	1	-
	Total (%)	56 (93%)	40 (93%)	0 (0%)	9 (45%)	4 (67%)	4 (100%)	1 (50%)	0 (0%)
Phospholipase	4+	5	-	-	-	-	-	-	-
	3+	18	-	-	-	-	-	-	-
	2+	16	-	-	-	-	-	-	-
	1+	12	-	1	-	-	-	-	-
	Total (%)	51 (85%)	0 (0%)	1 (6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Hemolytic activity	4+	8	3	-	-	-	-	-	-
	3+	4	-	-	-	-	-	-	-
	2+	10	2	14	2	1	4	1	-
	1+	36	4	3	14	4	-	1	-
	Total (%)	58 (97%)	9 (21%)	17 (94%)	16 (80%)	5 (83%)	4 (100%)	2 (100%)	0 (0%)
Coagulase	+	5	14	6	5	-	-	-	-
	Total (%)	5 (8%)	14 (33%)	6 (33%)	5 (25%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

As a result of the statistical analysis, no significant difference was found between *C. albicans* (42%) and non-albicans *Candida* (35%) in terms of biofilm formation (p= 0.603). However, it was found that *C. albicans* strains (42%) formed a significantly higher rate of biofilm formation than *C. parapsilosis* strains (12%) (p< 0.001). Also, *C. tropicalis* strains (75%) formed a significantly higher rate of biofilm formation than *C. albicans* (42%) and *C. parapsilosis* strains (12%) (respectively p= 0.009; p< 0.001). It was determined that *C. glabrata* (50%) strains also formed a significantly higher rate of biofilm formation than *C. parapsilosis* strains (12%) (p= 0.001).

It was found that *C. albicans* strains (93%) showed significantly higher proteinase activity than *C. glabrata* (0%) and *C. tropicalis* (45%) strains (respectively p< 0.001; p< 0.001). It was also found that *C. parapsilosis* strains (93%) showed statistically higher proteinase activity than *C. glabrata* (0%) and *C. tropicalis* (45%) strains (respectively p< 0.001; p< 0.001). It was also found that *C. tropicalis* (45%) and *C. lusitaniae* strains (67%) had significantly higher proteinase activity than *C. glabrata* (0%) strains (respectively p= 0.001; p= 0.001). It was determined that *C. albicans* strains (85%) showed significantly higher phospholipase activity than *C. parapsilosis* (0%), *C. glabrata* (6%) and *C. tropicalis* (0%) strains (respectively p< 0.001; p< 0.001; p< 0.001). It was also found that *C. parapsilosis* strains (21%) showed significantly lower hemolysis positivity rates than *C. albicans* (97%), *C. glabrata* (94%), *C. tropicalis* (80%) and *C. lusitaniae* (83%) strains (respectively p< 0.001; p< 0.001; p< 0.001; p= 0.005). In the comparison of coagulase activities; It was found that *C. glabrata* (33%) and *C. parapsilosis* (33%) strains showed significantly higher coagulase positivity rates than *C. albicans* strains (8%) (respectively p= 0.007; p= 0.001).

When comparing azole resistance and virulence factors, our analysis revealed no significant relationship between azole resistance and virulence factor across any of the Candida strains.

DISCUSSION

Candida albicans is the most isolated species from invasive infections and is the causative pathogen in 37-50% of bloodstream infections. However, in recent years, the frequency of *C. albicans* species causing candidemia has been gradually decreasing and the frequency of Non-albicans *Candida* species has increased. Although the frequency of *C. albicans* has decreased over the years, it is still the most frequently isolated species[1]. In our study, the most isolated strain was *C. albicans* with a rate of 38%. In this study, non-albicans *Candida* species causing candidemia were *C. parapsilosis* (28%), *C. tropicalis* (13%), and *C. glabrata* (11%) in order of frequency. In a multicenter study by Cortes et al. in Colombia, *C. albicans* was found to be the most common cause of candidemia. However, the incidence of *C. albicans* has decreased over the years. *C. albicans* was seen with the highest rate (66.7%) between 2008-2009. It was found to be 40.8% in 2010-2011, and 41.2% in 2010-2013. In the same study, following *C. albicans*, the most commonly detected candidemia agents were C. *tropicalis* (23.4-10.6%), *C. parapsilosis* (26.5-13.7%), and *C. glabrata* (9.5-2.6%), respectively[8].

In this study, *C. albicans* strains were found to be resistant to fluconazole 2%, intermediate to voriconazole 3%, and posaconazole 7% non-wild type. In various studies conducted in our country, fluconazole resistance was found at rates ranging from 0-16.6%; voriconazole resistance was found to be between 0-14%[9-12]. Cicek-Kolak et al., in their study, reported that all *C. albicans* strains were classified as wild type for posaconazole. In contrast, Zhang et al., in their study examining 385 Candida strains, found a higher proportion (15.4%) of *C. albicans* strains exhibiting resistance to posaconazole compared to our study[11,13].

In our study, *C. glabrata* strains were found to be resistant to fluconazole by 17%, and resistant to voriconazole by 56%, while all strains were found to be susceptible to posaconazole. While fluconazole resistance has been reported at rates varying between 2.6-48% in various studies

conducted in our country and abroad[14-17]; posaconazole resistance was found similar to our study in various studies[18,19]. Cicek-Kolak et al. reported a 38.5% higher posaconazole resistance in *C. glabrata* strains than in our study[11].

C. parapsilosis strains were 14% resistant to fluconazole, 26% fluconazole dose-dependent susceptible, 2% voriconazole resistant, 30% voriconazole intermediate, and posaconazole 2% non-wild type. In other studies, fluconazole resistance was between 37.8% and 3.2%; voriconazole resistance was found at rates ranging from 4.35% to 28.2%[9-11,16,20,21]. Posaconazole resistance in *C. parapsilosis* strains was determined by Cicek-Kolak et al. 4.3%, Zhang et al. While 1.2% reported lower values than our study, Marti-Corrisoza et al. reported similar results to our findings[11,13,22].

In this study, all of the *C. tropicalis* strains were found to be susceptible to fluconazole, consistent with many previous studies[11,17,20,21]. Ten percent of the strains were found to be intermediate-susceptible to voriconazole and 5% to non-wild type for posaconazole. While voriconazole resistance was reported at 0-14.3% in *C. tropicalis* strains in various previous studies[9,10,21,23], posaconazole resistance reported by Cicek-Kolak et al. was 70.6%, and Mete et al. was 54%; which were higher than our study[11,23].

Proteinase and phospholipase are essential hydrolytic enzymes that play a role in the pathogenesis of *C. albicans*. However, various studies have shown that these enzymes are also present in non-albicans Candida strains[24-26]. Although non-albicans Candida strains also showed proteinase and phospholipase activity in our study, *C. albicans* strains had a significantly higher phospholipase activity rate than *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* strains. We also found that *C. albicans* strains showed significantly higher proteinase activity than *C. glabrata* and *C. tropicalis* strains.

In this study, 93% of *C. albicans* strains showed proteinase activity and 85% of them showed phospholipase activity. Atalay et al. reported these rates as 82% and 88.2% while Yenisehirli et al. reported 81% and 76%, respectively, which were lower than our study's results. Tay et al. reported 93.7% proteinase and 73.3% phospholipase activity in *C. albicans* strains[4,26,27]. In non-albicans Candida species, proteinase and phospholipase activity was found to be 93% and 0% in *C. parapsilosis* strains 45% and 0% in *C. tropicalis* strains, and 0-6% in *C. glabrata* strains, respectively. Atalay et al. reported a lower proteinase activity in *C. parapsilosis* and *C. tropicalis* strains (44% and 0%, respectively) than our study, and higher proteinase activity in *C. glabrata* strains than our study, with a rate of 28.6%. In addition, they reported higher phospholipase activity in *C. parapsilosis* and *C. glabrata* strains (11.1%-35.7%, respectively) than in our study[4]. Oksuz et al. reported 11.1% proteinase activity in *C. glabrata*, 33.3% in *C. parapsilosis*, and 75% in *C. tropicalis*[24]. Deorukhkar et al. reported much higher rates than our results with 15.2% phospholipase and 6.1% proteinase activity rates in *C. tropicalis* strains isolated from blood culture[28].

When analyzing the rates of biofilm formation, it was observed that *C. albicans* exhibited a rate of 42%, *C. parapsilosis* had a rate of 12%, *C. glabrata* showed a rate of 50%, and *C. tropicalis* demonstrated the highest rate at 75%. No significant difference was found between albicans and non-albicans Candida in terms of biofilm formation. In addition, no significant relationship was found between biofilm production and antifungal resistance. While Atalay reported 5.9% biofilm positivity in *C. albicans* strains and 77.7% in *C. parapsilosis* strains, they did not detect biofilm positivity in any of the *C. glabrata* and *C. tropicalis* strains[4]. In addition, in a study examining Candida isolated from blood culture, 95% of *C. glabrata* strains, 66.7% of C. parapsiolosis strains, 100% of *C. tropicalis* strains, and 40.3% of *C. albicans* strains produced biofilm, which was a much higher rate than our findings. It was reported that non-albicans Candida species produced a significantly higher rate of biofilm than *C. albicans* species[29]. In another study conducted in our country, it was reported that non-albicans Candida species formed a significantly higher rate

of biofilm formation than *C. albicans* species, unlike our study[30].

In our study, the lowest hemolysis positivity rate was observed in *C. parapsilosis* strains at 21%. Similarly, while Favero et al. detected hemolytic activity in all *C. albicans* and *C. tropicalis* strains, no hemolytic activity was detected in any of the *C. parapsilosis* strains[31]. Rossoni et al. detected hemolytic activity in all of the *C. albicans*, *C. tropicalis*, and *C. glabrata* strains, while they detected hemolytic activity in 40% of the *C. parapsilosis* strains[32]. Similarly, Yenisehirli et al. also found hemolytic activity in all of the examined *C. albicans* strains in their study[27].

CONCLUSION

As a result, the majority of candidemia cases were attributed to *C. albicans*, followed by *C. parapsilosis* strains, with a notable susceptibility to posaconazole. Furthermore, our study revealed that hydrolytic enzymes, such as proteinase and phospholipase, were synthesized not only in *C. albicans* strains but also in non-albicans Candida strains. Among the strains studied, *C. tropicalis* exhibited the highest rate of biofilm production at 75%. However, no significant association was found between the examined virulence factors and azole resistance in our study.

ETHICS COMMITTEE APPROVAL

This study was approved by the Tokat Gaziosmanpaşa University Clinical Research Ethics Committee (Decision no: 83116987-172, Date: 10.03.2022).

CONFLICT of INTEREST

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

AUTHORSHIP CONTRIBUTIONS

Concept and Design: AA, GY

Analysis/Interpretation: AA, GY

Data Collection or Processing: AA

Writing: AA, GY

Review and Correction: AA, GY

Final Approval: GY, AA

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