

Distribution and Antifungal Susceptibility of Candida Species Isolated from Blood Cultures

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What is already known on this topic?

- Candidemia is defined as the isolation of Candida species from blood and is an infection with high mortality and morbidity.
- Immediate initiation of appropriate antifungal treatment is very important in terms of prevention of antifungal resistance development, morbidity, and mortality.
- It is important for each center to determine its own incidence of candidemia, the change in incidence over the years, species distribution, and antifungal susceptibility pattern in candidemias in order to guide prophylactic and empirical treatment.

What this study adds on this topic?

- Our study, "Distribution and Antifungal Susceptibility of Candida Species Isolated from Blood Cultures," will contribute to the selection of empirical antifungal therapy and the development of infection control strategies by contributing to local epidemiological data.

Abstract

Objective: Candidemia is defined as the isolation of Candida species from blood and is an infection with high mortality and morbidity. Although *Candida albicans* is the most common causative agent of candidemia in blood cultures, the detection rate of non-albicans species in blood has increased in recent years. In this study, the aim was to determine the distribution and antifungal susceptibility patterns of Candida species isolated from blood cultures.

Methods: Candida species isolated from blood culture specimens received by the microbiology laboratory of the hospital between 2021 and 2023 were retrospectively analyzed. Species identification and antifungal susceptibility tests of Candida isolates were performed on the VITEK 2 automated system.

Results: Candidemia was detected in 123 different patients over a 3-year period and 132 Candida species isolated from these patients were included in the study. Of the 132 isolates, 88% belonged to patients hospitalized in the intensive care unit and 12% belonged to patients hospitalized in the other clinics. At the species level, *Candida albicans* was isolated 39.4%, *Candida parapsilosis* 30.3%, *Candida glabrata* 12.1%, *Candida tropicalis* 11.3%. The highest susceptibility rate was 98.4% to micafungin, while susceptibility rates for other antifungals were 97.6%, 97.2%, 94.4%, and 88.3% to caspofungin, voriconazole, amphotericin B, and fluconazole, respectively. Susceptibility rates were found to decrease in all other antifungals except amphotericin B in 2023 compared to 2021. This decrease in fluconazole was found to be statistically significant.

Conclusion: Candidemia is a clinical condition with high mortality and requires rapid diagnosis. In this study, it was found that *C. albicans* was most frequently isolated as the causative agent of candidemia, however, non-albicans Candida species were also important pathogens of candidemia and antifungal susceptibility rates decreased over the years. Therefore, it is important for each center to determine its own candidemia agent distribution and antifungal susceptibility pattern.

Keywords: Antifungal, Candida, susceptibility

Introduction

Candida species are important opportunistic pathogens causing infections found as normal flora elements in the gastrointestinal system, skin, mucosa, and vagina.¹ Candidemia is defined as the isolation of Candida species from the blood and is an infection with high mortality and morbidity.^{2,3} Candida species are the third most common cause of bloodstream infections.³ The most important risk factors leading to the development of candidemia include broad-spectrum antibiotic use, comorbidities, high-dose corticosteroid use, hemodialysis, parenteral nutrition, malignancy, neutropenia, prolonged hospitalization, mechanical ventilation, invasive interventions (such as urethral catheter, nasogastric catheter, central venous catheter), blood transfusion, surgical operations, and organ transplantation.^{2,4,5}

Although *Candida albicans* is the most common causative agent of candidemia detected in blood cultures, the detection rate of non-albicans species such as *Candida parapsilosis*, *Candida glabrata*, *Candida tropicalis*, and *Candida krusei* in blood is increasing.² Immediate initiation of treatment with appropriate antifungals, removal of the central venous catheter, if any, and control of the underlying disease are very important.⁵ According to Infectious Diseases Society of America

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(IDSA) guidelines, echinocandins are recommended as the initial therapy for most patients with candidemia. Fluconazole is the most widely used, low-cost antifungal from the triazole group and is considered an alternative to echinocandins in selected, non-critical patients with no prior azole exposure and a low risk of resistance. *Candida krusei* is naturally resistant to fluconazole; *C. glabrata* has low sensitivity to fluconazole. Voriconazole is a broad-spectrum antifungal belonging to the triazole group derived from fluconazole. It is effective against all *Candida* species, including *C. krusei* and *C. glabrata*, where the effect of fluconazole is limited. Due to the widespread prophylactic and empirical use of the azole group (especially fluconazole) and echinocandin group antifungal agents, acquired resistance to these drugs is increasing in *Candida* species. Resistance to the azole group and echinocandins is more common in non-albicans *Candida* species compared to *C. albicans*.^{1,4} Amphotericin B is another polyene-derived antifungal agent, and increasing resistance rates to amphotericin B have been reported in recent years.⁶

Immediate initiation of appropriate antifungal treatment is very important in terms of prevention of antifungal resistance development, morbidity, and mortality. Therefore, accurate identification of *Candida* at the species level and application of antifungal susceptibility tests are of great importance in determining appropriate antifungal treatment.^{2,3} The diversity of candidemia causative species and antifungal susceptibilities varies according to countries, centers, and time. It is important for each center to determine its own incidence of candidemia, the change in incidence over the years, species distribution, and antifungal susceptibility pattern in candidemias in order to guide prophylactic and empirical treatment. The aim of this study was to retrospectively determine the distribution and antifungal susceptibility patterns of *Candida* species isolated from blood cultures, to contribute to local epidemiological data, support empirical antifungal treatment decisions, and aid in the development of infection control strategies.

Methods

In this study, *Candida* species isolated from blood culture specimens received by the microbiology laboratory of the hospital between January 2021 and December 2023 were retrospectively analyzed. This hospital is an institution with 605 beds and 200 intensive care units (ICUs), which started to serve in 2021. While there were few hospitalizations in the hospital in 2021, the ICU started to provide tertiary health care services intensively in 2022 and beyond.

Blood culture samples were incubated in Bact/Alert® 3D (bioMérieux, Marcy l'Etoile, France) automated blood culture system for 5 days. Gram staining was performed on the blood culture specimens in which growth was detected, and the specimens in which yeast was detected were first reported to the relevant clinic for empirical antifungal initiation and inoculated simultaneously on compartmentalized agar (Thermo Fisher, 5% sheep blood agar and MacConkey agar) and *Candida* chrome agar (Thermo Fisher) and incubated at 36°C for 24-48 hours. At the end of incubation, the isolates identified as *Candida* according to Gram staining and colony morphology in the medium were studied in the VITEK 2 Compact automated system (bioMérieux, France). YST (identification card) and AST-YS08 (antifungal susceptibility card) were used for the identification of *Candida* species and antifungal susceptibility study (bioMérieux, France). Susceptibility results against caspofungin, micafungin, fluconazole, voriconazole, and amphotericin B were evaluated according to European Committee on Antimicrobial Susceptibility Testing (EUCAST)⁷ standards. Only Minimal inhibitory concentration (MIC) values were given for

antifungals without susceptible/resistant equivalents in EUCAST. These isolates and naturally resistant isolates were not included in the susceptibility rate calculation. Antifungals with a susceptibility result in category I (intermediate or susceptible, increased exposure) were calculated as S (susceptible).

In this study, the clinics where *Candida* isolates were isolated were evaluated, but the clinical characteristics of the patients were not included. The first isolate identified from each patient was evaluated, and isolates detected in repeated cultures of the same patient were not included in the study.

Statistical Analysis

Data were analyzed using SPSS 22.0 (SPSS Inc.; Chicago, IL, USA). Categorical variables were expressed as percentages. Chi-square test was used for comparisons between groups and post-hoc analyses were performed. A *P*-value < .05 was considered statistically significant.

Ethics committee approval of this study was obtained from Tekirdağ Dr İsmail Fehmi Cümalioglu City Hospital Clinical Research Ethics Committee (Date: December 1, 2023, and No: 2023/66). All series of steps were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Since the study used retrospective laboratory data, informed consent is not required.

Results

Within a 3-year period, candidemia was detected in 123 different patients and 132 *Candida* species isolated from these patients were included in the study. Two different types of *Candida* growth were detected in 9 patients and were included in the study as they were considered to be the causative agent. When the demographic data of the patients were analyzed, 8 of them were children, 115 of them were adults, 69 were male and 54 were female, and the age range of the patients was between 0 and 95 years.

When the distribution of 132 isolates was analyzed according to the clinics, 88% (*n* = 116) belonged to patients hospitalized in the ICU, 12% (*n* = 16) belonged to patients hospitalized in the clinics, and most of them were from the 3rd Step General ICU (Table 1). Analysis of the distribution of isolates at the species level showed that *C. albicans* was the most common isolate, followed by *C. parapsilosis* (Table 2).

16 isolates were detected in 2021, 72 isolates in 2022, and 44 isolates in 2023. Since the hospital began operating in 2021, both the number of blood culture requests and the number of isolates obtained from blood cultures in 2021 were lower than in other years. When the antifungal susceptibility rates of the isolates were analyzed, the highest susceptibility rate was found against micafungin with 98.4%. For the other antifungals, susceptibility rates were 97.6%, 97.2%, 94.4%, and 88.3% for caspofungin, voriconazole, amphotericin B, and fluconazole, respectively.

In the statistical analysis of susceptibility rates over the years, the lowest susceptibility rate for amphotericin B was detected in 2021, increased in 2022 and 2023, and no significant difference was observed between the years (*P* = .054). Susceptibility rates for voriconazole decreased over the years, but no statistically significant difference was detected (*P* = .062). Similarly, susceptibility rates for caspofungin were found to decrease over the years; although this decrease was considered significant in the overall evaluation (*P* = .028), no significant difference was detected between years in the post-hoc analysis. Fluconazole susceptibility remained at similar rates in 2021 and 2022, but decreased to 80.0% in 2023. This decrease was found to be statistically significant in both the overall assessment (*P* = .006) and the post-hoc analysis (*P* = .013). The

Table 1. Distribution of Isolated Candida Species According to Clinics (n/%)

Clinics	Frequency of Detection	
	n	%
3rd Step General ICU	69	52.3
Surgical ICU	35	26.5
Pediatric ICU	7	5.3
Medical Oncology Service	7	5.3
Internal Medicine Service	3	2.2
Neonatal ICU	2	1.7
Other services	6	4.5
Other ICUs	3	2.2
Total	132	100

Other services: Neurology, Infectious Diseases, Orthopedics, Palliative, Gastroenterological Surgery, Cardiology Service.
Other ICUs (intensive care units): Neurology ICU, 2nd Level General ICU, Coronary ICU.
ICU, intensive care unit.

susceptibility rate detected as 100% in 2021 and 2022 decreased to 94.9% in 2023 for micafungin, although this decrease was statistically significant in the general evaluation ($P = .028$), no significant difference was found in the post-hoc analysis ($P = .070$) (Table 3).

When the antifungal susceptibility rates of the isolates were analyzed according to the agent, it was observed that *C. tropicalis* had the highest susceptibility rate and *C. parapsilosis* had the lowest susceptibility rate (Table 4).

Discussion

The incidence of Candida infections has increased with the increase in predisposing factors such as invasive interventions including vascular and urinary catheterization,

Table 2. Species Distribution of Candida Isolates (n/%)

Microorganisms	Frequency of Detection	
	n	%
<i>Candida albicans</i>	52	39.4
<i>Candida parapsilosis</i>	40	30.3
<i>Candida glabrata</i>	16	12.1
<i>Candida tropicalis</i>	15	11.3
<i>Candida kefyr</i>	3	2.3
<i>Candida lusitanae</i>	2	1.5
<i>Candida krusei</i>	2	1.5
<i>Candida dubliniensis</i>	1	0.8
Total	132	100

Table 3. Antifungal Susceptibility Rates of Isolated Candida Species According to Years (%)

	2021 (n = 16)	2022 (n = 72)	2023 (n = 44)	Total (n = 132)	P
Amphotericin B	87.5	95.7	95.0	94.4	.054
Caspofungin	100	98.6	94.9	97.6	.028*
Fluconazole	91.7	93.2	80.0	88.3	.006
Micafungin	100	100	94.9	98.4	.006*
Voriconazole	100	98.3	94.7	97.2	.062

P-values in Post-Hoc analysis:

Caspofungin: 2021-2022: 1.00, 2022-2023: 0.214, 2021-2023: 0.070.

Fluconazole: 2021-2022: 1.00, 2022-2023: 0.013, 2021-2023: 0.024.

Micafungin: 2021-2022: 1.00, 2022-2023: 0.070, 2021-2023: 0.070.

*Although there was a significant difference in the general evaluation, no significant difference was found in the detailed Post-Hoc analysis according to years.

immunosuppressive treatment, ICU hospitalization, broad-spectrum antibiotic use, mechanical ventilation, and abdominal surgery.^{6,8} Candidemia is the most common clinical condition among invasive Candida infections.⁹ In this study, it was observed that 88% of the isolates included in the study were patients followed up in the ICU; approximately 50% were isolated from the tertiary general ICU. In the 3rd general ICU of the hospital, intubated patients with poor general condition who receive broad-spectrum antibiotic therapy are generally monitored. In similar studies, it has been reported that Candida isolates are frequently isolated from ICU patients,^{1,2} and these findings are consistent with the literature.

The most common causative agent of fungal infections is Candida species, frequently *C. albicans*. In recent years, there has been an increase in non-albicans Candida species. Especially the frequent use of azole group antifungal drugs has led to an increase in the frequency of azole resistant isolates.⁸⁻¹² Studies on the distribution of Candida species report *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei* as the most common species.^{13,14} In a multicenter study conducted in Türkiye, the most frequently isolated Candida species from blood cultures were reported as *C. albicans*, *C. parapsilosis*, *C. glabrata*, and *C. tropicalis*.¹⁵ Oner et al¹⁶ determined the species distribution as *C. albicans*, *C. parapsilosis*, *C. tropicalis* and *C. glabrata* in various clinical samples. In this study, the most frequently isolated species were *C. albicans*, *C. parapsilosis*, *C. glabrata*, and *C. tropicalis*, respectively, which constituted 93.1% of the isolates.

Table 4. Antifungal Susceptibility Profiles of Candida Species (%)

	<i>C. albicans</i> (n = 52)	<i>C. parapsilosis</i> (n = 40)	<i>C. glabrata</i> (n = 16)	<i>C. tropicalis</i> (n = 15)
Amphotericin B	94.2	92.5	100	100
Caspofungin	100	95.0	93.8	100
Fluconazole	94.2	80.0	–	100
Micafungin	100	95.0	100	100
Voriconazole	98.1	95.0	–	100

Due to the high mortality rate of invasive *Candida* infections, it is important to start empirical treatment as early as possible. In case of *Candida* growth in blood culture, fluconazole is frequently started as the first choice in stable patients who do not have a history of previous antifungal treatment and do not have organ dysfunction, and the continuation of treatment is reviewed according to antifungal susceptibility in culture.^{8,17} Beder et al¹ found fluconazole susceptibility in *Candida* species isolated from blood culture as 87% in *C. albicans*, 93.5% in *C. parapsilosis*, and 93.8% in *C. tropicalis*. Aydoğan et al² found fluconazole susceptibility of 93.7% in candidemia agents during the pandemic period. In this study, fluconazole was the antifungal with the lowest susceptibility rate, and it was found that the sensitivity rate decreased significantly over the years. When fluconazole susceptibility was evaluated both at the species level and by year, it was observed that susceptibility reached the 80% limit, which is important for empirical treatment. The fact that the use of fluconazole causes an increase in azole resistance and the risk of infection with isolates that are naturally resistant to fluconazole suggests that a next-line drug may be needed instead of fluconazole in the empirical treatment of invasive *Candida* infections over the years.

Voriconazole is another triazole group antifungal with a broad spectrum of activity. It is effective against other *Candida* species, especially *C. krusei* and *C. glabrata* species where the effect of fluconazole is limited. However, it is not preferred as the first choice in empirical treatment due to its high cost compared to fluconazole, high drug interactions, and side effects.^{1,17} Alci et al¹⁸ evaluated *Candida* species isolated from different clinical samples and found voriconazole susceptibility to be 94.9% in *C. albicans* and 91.9% in *C. parapsilosis*. In this study, the susceptibility to voriconazole was found to be 97.2% in total, which is compatible with the literature. However, when the course of voriconazole sensitivity was examined over the years, it was found that it decreased, although not statistically significant, and the *P*-value that was determined was close to the significant limit. Therefore, it is believed that fluconazole and its derivatives should be used more cautiously in the hospital to prevent azole resistance.

The other antifungal group preferred in the treatment of candidemia is echinocandins. The fact that caspofungin, anidulafungin, and micafungin in this group are more effective than other treatments and have fewer drug interactions increases their importance in the first-line treatment of candidemia infection. In the European Society of Clinical Microbiology and Infectious Diseases and IDSA guidelines for the treatment of candidemia, it is emphasized that azole group drugs have less efficacy and echinocandins should be preferred with priority. Recent studies report that echinocandins are the most effective treatment for candidemia.^{19–22} Er et al²³ found 1.2% anidulafungin resistance in *C. parapsilosis* isolates isolated from blood culture and 100% susceptibility in *C. albicans*, *C. tropicalis*, and *C. glabrata*. Batçık et al²⁴ reported caspofungin and micafungin susceptibility rates above 99% in *Candida* species isolated from various clinical samples except for *C. glabrata* and *C. krusei*. Sanlı et al¹⁰ determined the susceptibility rates of caspofungin and micafungin in *Candida* species isolated from blood cultures as 100% and 99.4% in *C. albicans*, 97.6% and 95.1% in *C. tropicalis*, 97.6% and 96%. In this study, susceptibility rates to caspofungin and micafungin were 97.6% and 98.4%, respectively, and although not statistically significant, susceptibility rates have decreased over the years. When susceptibility rates at the species level were observed, *C. albicans* and *C. tropicalis* were 100% susceptible, while resistance to echinocandins was detected in *C. glabrata* and *C. parapsilosis* isolates, and the rates that were detected are consistent with the literature.

More than half of the isolates included in this study were isolated in 2022. The increased resistance rates in 2023 could be due to the increased antifungal use in 2022. Amphotericin B is a polyene group antifungal agent used in the treatment of candidemia and effective against all *Candida* species except *C. lusitanae*. In studies, amphotericin B susceptibility rates have been reported between 85.4% and 100%.^{1,2,9,10,18,23} When the susceptibility rates observed in the present study were compared with the literature, rates were lower only in 2021 (compatible with the literature in other years), and it may be due to the low number of isolates in 2021. In addition, the broth microdilution method is accepted as the reference method in antifungal susceptibility tests by EUCAST. However, the VITEK 2 automated system is used in routine antifungal susceptibility studies in the laboratory due to its good reproducibility and more than 90% agreement with the broth microdilution method.²⁵ The isolates in which amphotericin B resistance was detected in this system could not be confirmed by the broth microdilution method and this is the limiting aspect of this study. Therefore, the amphotericin B susceptibility rate in this study may have been slightly lower than expected.

This study included *Candida* species isolated from both pediatric and adult patients. The lower number of pediatric patients compared to adult patients is a limiting factor for the study, as it may create heterogeneity. Additionally, the retrospective design of the study and the lack of access to clinical information on patients from whom *Candida* species were isolated are other limitations.

Candidemia is a clinical condition with high mortality and requires rapid diagnosis. Rapid reporting of blood culture Gram stain by the microbiology laboratory to the clinic is important in this respect. In this study, it was found that *C. albicans* was most frequently isolated as the causative agent of candidemia; however, non-*albicans* *Candida* species were also important pathogens of candidemia and antifungal susceptibility rates have decreased over the years. Therefore, each center should determine its own incidence of candidemia, species distribution, and antifungal susceptibility pattern in candidemias, and this should form the basis of the clinician's choice of empirical and maintenance treatment. In addition, the authors believe that antifungals should be used with caution to prevent the development of both resistance and infection with resistant isolates.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author.

Ethics Committee Approval: Ethical committee approval was received from the Tekirdağ Dr. İsmail Fehmi Cümaloğlu City Hospital Clinical Research Ethics Committee (Date: 01.12.2023; Approval No: 2023-66).

Informed Consent: Since the study used retrospective laboratory data, informed consent is not required.

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