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Antifungal Susceptibilities of Candida Species Isolated to Clinical Samples

Klinik Örneklerden İzole Edilen Candida Türlerinin Antifungal Duyarlılıkları

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ABSTRACT

Background: Candidiasis is a skin, mucosal, and organ infection caused by Candida fungi, with Candida albicans being the most common. These infections pose significant morbidity and mortality risks, necessitating rapid identification and characterization methods for diagnosis and personalized antifungal therapy based on Candida's pathogenic properties.

Materials and Methods: Candida species isolated from diverse clinical samples from patients were included in this study. A total of 86 isolates were analyzed at the genus and species levels using mass spectrometry, while susceptibility profiles to amphotericin B (AmB), anidulafungin (AND), fluconazole (FLC), and voriconazole were regulated using the gradient test method.

Results: Candida spp. were detected in 1254 clinical samples obtained from 736 patients. Antifungal susceptibility was tested on 86 isolated samples, including C. albicans, Candida glabrata, Candida tropicalis, Candida parapsilosis, Candida kefyr, Candida guilliermondii, and Candida krusei. The corresponding rates of resistance to AmB and FLC in C. albicans isolates were 2.2% and 13.3%. No resistance to AND was determined.

Conclusion: Candida species C. albicans is the most regularly isolated, but infections from other Candida species have increased. No resistance to AND was observed, but species-specific resistance to other antifungal agents was identified, emphasizing the need for continuous monitoring.

Keywords: Candida spp, antifungal susceptibility, fluconazole, voriconazole, anidulafungin

ÖZ

Amaç: Kandidiyaz, Candida mantarlarının neden olduğu bir tür cilt, mukoza ve organ enfeksiyonudur; en yaygın olanı Candida albicans'tır. Bu enfeksiyonlar önemli morbidite ve mortalite riskleri oluşturmakta, tanı için hızlı tanımlama ve karakterizasyon yöntemleri ve Candida'nın patojenik özelliklerine dayalı olarak kişiselleştirilmiş antifungal tedaviyi gerektirmektedir.

Gereç ve Yöntemler: Hastalardan alınan çeşitli klinik örneklerden izole edilen Candida türleri bu çalışmaya dahil edildi. Kütle spektrometresi kullanılarak cins ve tür düzeyinde toplam 86 izolat tanımlanırken, gradyan test yöntemi kullanılarak amfoterisin B (AmB), anidulafungin (AND), flukonazol (FLC) ve vorikonazole duyarlılık profilleri belirlendi.

Bulgular: Candida spp. 736 hastadan alınan 1254 klinik örnekte üreme tespit edildi. C. albicans, Candida glabrata, Candida tropicalis, Candida parapsilosis, Candida kefyr, Candida guilliermondii ve Candida krusei'nin de aralarında bulunduğu 86 izolat üzerinde antifungal duyarlılık testi yapıldı. C. albicans izolatlarında AmB ve FLC'ye direnç oranları sırasıyla %2,2 ve %13,3'tür. AND'ye karşı direnç tespit edilmedi.

Sonuç: Candida türü C. albicans en sık izole edilen türdür ancak diğer Candida türlerinden kaynaklanan enfeksiyonlar da artış göstermiştir. AND'ye karşı herhangi bir direnç gözlenmedi ancak diğer antifungal ajanlara karşı türe özgü direnç belirlendi ve bu da sürekli izleme ihtiyacını vurguladı.

Anahtar Kelimeler: Candida türleri, antifungal duyarlılık, flukonazol, vorikonazol, anidulafungin

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Introduction

Candidiasis is a prevalent infection caused by Candida fungi, and it affects the skin, mucosal membranes, and internal organs (1). These infections can occur at any age and are frequently linked to particular risk factors. Candida albicans is the predominant pathogen causing candidiasis globally, followed by Candida parapsilosis, Candida tropicalis, and Candida glabrata (1-3). Invasive candidiasis is a highly prevalent condition and is associated with high morbidity and mortality. Hence, it is imperative to develop effective methods for immediately detecting and classifying the condition to ensure precise diagnosis and appropriate antifungal therapy (4,5).

Several studies have examined the clinical significance and therapeutic consequences of different Candida species, highlighting the need to understand their separation, identification, and resistance to antifungal drugs (1,3). Candida species, particularly C. albicans, are frequently found in clinical samples. However, there has been a rise in infections caused by non-albicans species, such as C. parapsilosis, C. tropicalis, and C. glabrata (2,3,6,7). These non-albicans species possess unique characteristics that contribute to their ability to cause disease and respond to antifungal treatment. This highlights the importance of accurately identifying species and developing customized therapeutic strategies (1,2,8-10).

Multiple methodologies, including phenotypic, genotypic, and proteomic techniques, have been devised to precisely determine Candida species (11). Conventional phenotypic approaches have drawbacks in terms of precision and speed, but molecular techniques including polymerase chain reaction, DNA sequencing, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) have significantly transformed the area of Candida species identification (11-13). MALDI-TOF MS is a method that involves comparing databases of reference spectra to identify the protein fingerprints of different microorganisms. MALDI-TOF MS exhibits a sensitivity and specificity of up to 100%, enabling rapid and accurate detection and identification of Candida species (11,14,15). The utilization of modern techniques has improved our understanding of the dispersion and frequency of distinct Candida species in diverse clinical environments (16).

The management of candidiasis is increasingly influenced by the development of antifungal resistance. Candida species exhibit different levels of resistance to commonly prescribed antifungal drugs, such as azoles [e.g., itraconazole, voriconazole, fluconazole (FLC)], echinocandin [e.g., anidulafungin (AND), caspofungin], and polyenes [e.g., amphotericin B (AmB)] (9,13,17-19). Comprehending the resistance patterns exhibited by various Candida species is crucial for selecting the most suitable antifungal treatment and avoiding treatment ineffectiveness (1,6,10). Resistance mechanisms, including changes in drug targets, increased production of efflux pumps, and genetic mutations, have been discovered and are associated with decreased susceptibility to antifungal drugs (17,20,21). Candida species play a key role in human illness because of their capacity to create drug-resistant biofilms, which are highly resistant to antifungal treatments (22-24). Treatment options are complicated by Candida isolates that are resistant to many drugs, requiring the improvement of new antifungal techniques. The global prevalence of intrinsic resistance in Candida species, except for C. albicans, is increasing (18,25). Non-albicans have been rising regularly in the last decade. The major cause of therapy failure is the presence of Candida species that exhibit limited sensitivity, collected resistance, or inherent resistance to existing antifungal drugs (16). Overall, candidiasis poses a substantial therapeutic obstacle because of its varied symptoms and the development of resistance to antifungal treatments (19,26,27). To address these challenges, this study aimed to enhance our understanding of the isolation, identification, and antifungal resistance profiles of Candida species.

Materials and Methods

In this study, yeast isolates classified as members of Candida genus, which were obtained from various clinical samples submitted to the Central Laboratory Bacteriology Unit of Dicle University Hospital between 22.12.2019 and 22.10.2020, were included. Only the first isolate of duplicate yeast fungi isolated from the same patient was included in the study, and the isolated samples were stored at -80 °C until the time of analysis in a nutrient medium containing 16% glycerol.

This study was approved by the Dicle University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (approval number: 157, date: 20.06.2019) and informed consent was obtained from the participants.

Sample Cultivation and Selection Criteria of Subcultures

Cerebrospinal fluid, pleural and joint fluid, and peripheral and catheter blood samples were collected from adult and pediatric patients in Bactec Plus aerobic/F or Becton Dickinson Bactec Peds Plus/F tubes and incubated in the BACTEC FX system (Becton Dickinson, USA). Subcultures were performed on solid culture media including 5% Sheep Blood Agar (RTA, Türkiye), Eosin Methylene Blue Agar (RTA, Türkiye), or Sabouraud Dextrose Agar (SDA) (Oxford, UK) from bottles displaying growth. The study used quantitative methods for cultivating tracheal aspirates, bronchoalveolar

lavage, urine, and nephrostomy samples, with dilution for the other samples. Colonization was determined by the presence of yeast in respiratory samples, whereas pathogenic growth was detected by Gram staining of wound culture samples. Vaginal samples were stained using the Gram method and were assessed using the Nugent scoring system. For catheter-related bloodstream infections, simultaneous cultures were performed from the catheter tip, blood, and peripheral vein blood. If growth was observed in both cultures, the isolate was considered the causative agent. Colonies suspected of being infectious agents were identified via Gram staining and subcultures on SDA culture media.

Gram Staining

Candida species are identified as gram-positive budding yeast cells or pseudo-hyphae with regular constrictions when stained using the Gram method. Clinical samples or colony suspensions are fixed, stained with crystal violet, iodine solution, or fuchsin, and examined under a light microscope. The yeasts are then stained with a dark or light purple. The process involves spreading, drying, and fixing the samples before examination.

Identification of Fungal Species using MALDI-TOF MS

After 24-48 hours of incubation, the identification of the growing fungal species on subculture plates was performed using mass spectrometry. MALDI-TOF MS was used for the identification of yeast. Samples were prepared in accordance with the MALDI Biotyper standard protocol. Initially, a clean wooden stick was used to transfer the sample collected from the colony onto the circular area of the target plate. The MALDI-TOF MS target plate is a recyclable stainless steel plate with 96 circular areas for testing different colonies, each of which can be used. The sample cells were treated with 70% formic acid on the target plate for application as yeast and then dried. Subsequently, 1-2 µL of matrix solution was applied to the spot and dried. The plate was then placed in the ionization chamber of the mass spectrometer.

The MALDI-TOF MS system, using positive linear mode and 337 nm nitrogen laser ionization, generated data spectra in the mass range of 2-20 kDa to identify Candida species. Using Bruker Biotyper 3.1 software (Bruker Daltonics, Bremen, Germany), these data spectra were identified.

Antifungal Susceptibility Testing Using Gradient Tests

The antifungal susceptibility of the identified yeast species was determined using the gradient test (e-test). The agar-based gradient test is a quantitative method used to identify the minimum inhibitory concentration (MIC) of antifungal agents that inhibit the growth of Candida species, expressed in µg/mL (Table 1).

The e-tests consisted of a narrow and non-porous plastic strip. On one side of the strip (A), there is a MIC reading scale in µg/mL to indicate the identity of the antifungal agent, along with a two- or three-letter code. Four antifungal agents included in our study (FLC, voriconazole, AmB, AND) and their e-test codes are presented in Table 1. The other side of the strip (B) was affixed with a dried and stabilized antifungal agent, which was previously defined with an increasing gradient ranging from the maximum concentration to the minimum concentration (Figure 1).

Four antifungal agents were used in this study, and the MICs were determined for identified Candida spp. Prior to the study, each isolate was subcultured on an SDA plate. Purified isolates were suspended in physiological saline, homogenized using a vortex mixer, and adjusted to a McFarland of 0.5 using a nephelometer.

The prepared suspensions were poured onto RPMI 1640 agar plates (RPMI 1640 Agar w/MOPS and 2% Glucose) with a diameter of 90 mm, ensuring even distribution across the entire surface of the agar. The agar plates were dried, and the gradient test strips (FLC, voriconazole, AmB, AND) were maintained at room temperature until equilibrium was reached. The antifungal gradient was then placed on agar plates at increasing concentrations in a consistent pattern. The plates were then incubated at 37 °C after the process.

Statistical Analysis

The test was conducted following manufacturer's instructions, with areas of inhibition around the gradient test strips assessed after incubation. The MIC of the isolate for a specific antifungal agent was determined by comparing the strip with areas where growth was 80% inhibited. If the culture appeared mixed or faint, the test was repeated. The MICs were analyzed according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards. Culture and quality control for the test of antifungal resistance were performed using the C. albicans American Type Culture Collection 10231 standard strain. Table 1 presents the MIC values of the antifungal drugs used in the study according to EUCAST standards (28).

The study recorded patient information, clinical specimens, yeast species, and antifungal susceptibility results. The isolates were compared with four tested antifungal agents based on specimen type, patient settings, and fungal species. The data were evaluated to determine the effectiveness of the antifungal agents. One-Way analysis of variance was applied to data that is showing a normal distribution among the groups, whereas the Kruskal-Wallis is analysis of variance test was applied to data not showing a normal distribution. Additionally, descriptive statistical calculations were employed in the data analysis.

Table 1. The antifungal drugs used in this study and their characteristics. The EUCAST MIC breakpoint values for susceptibility (S) and resistance (R) of the specified Candida species are expressed

Candida spp.	Antifungal agents and EUCAST MIC breakpoints (mg/L)				
		AmB	AND	FLC	VRC
Candida albicans	S≤	1	0.03	2	0.06
	I			4	0.125-0.25
	R>	1	0.03	4	0.25
Candida glabrata	S≤	1	0.06	0.001	-
	I			≤16	-
	R>	1	0.06	16	-
Candida kefyr	S≤	-	-	-	-
	I	-	-	-	-
	R>	-	-	-	-
Candida krusei	S≤	1	0.06	-	-
	I			-	-
	R>	1	0.06	-	-
Candida parapsilosis	S≤	1	4	2	0.125
	I			4	0.25
	R>	1	4	4	0.25
Candida tropicalis	S≤	1	0.06	2	0.125
	I			4	0.25
	R>	1	0.06	4	0.25
Candida gulliermondii	S≤	1	0.03	2	0.06
	I				
	R>	1	4	4	0.25

AmB: Amphotericin B, EUCAST: European Committee on Antimicrobial Susceptibility Testing, AND: Anidulafungin, FLC: Flukonazol, VRC: Vorikonazol, MIC: Minimal inhibitory concentration

Results

Between December 2019 and November 2020, a total of 1254 Candida spp. isolates were obtained from various clinical samples from 736 patients (477 females, 269 males) from the clinics and outpatient departments of Dicle University Hospital. The clinic-specific distribution of samples collected from patients with positive Candida spp. cultures is presented in Table 2.

A total of 1254 samples were found to exhibit Candida spp. growth, with 756 (60.29%) obtained from patients in intensive care units, 269 (21.45%) from patients in regular hospital wards, and 229 (18.26%) from outpatient clinic patients. The superiority of Candida spp. isolates was determined from urine, vaginal, and blood samples.

In total, 270 clinical samples exhibiting Candida spp. growth were isolated from blood cultures. The number of blood culture samples was 13,213, with 2,094 cultures suspected of being causative agents. Among these suspected cultures, 12.9% were identified as Candida species. C. albicans was the most regularly isolated Candida species from blood cultures, accounting for 37.41%, followed by C. parapsilosis at 32.22%. Other Candida species isolated from blood samples included C. tropicalis (14.07%), C. glabrata (8.15%), Candida kefyr (5.18%), Candida lusitaniae (1.48%), Candida krusei (1.11%), and Candida guilliermondii (0.37%).

The number of urine samples was 11,391, with a total of 3,037 (26.7%) urine cultures yielding positive results, regardless of pathogenicity or colonization. Among the 669 isolates obtained, C. albicans was found in 324 samples (48.43%), making it the most identified Candida species in urine samples. C. tropicalis was the second most frequently isolated species, found in 192 samples (28.7%). C. parapsilosis was detected in 48 samples (7.17%), C. kefyr in 38 samples (5.68%), C. glabrata in 32 samples (3.78%), Cyberlindhera fabiani in 10 samples (1.5%), C. krusei in 8 samples (1.2%), C. lusitaniae in 4 samples (0.6%), and C. dubliniensis in 4 samples (0.6%). The yeast species in 9 samples could not be classified.

Out of 352 vaginal swab cultures performed, Candida species were isolated in 227 (64.5%) samples. C. albicans and C. glabrata accounted for more than 90% of the Candida species isolated from vaginal cultures. C. albicans was isolated in over half of the samples (144 samples, 63.44%), followed by C. glabrata (68 samples, 29.96%). Rarely isolated species in vaginal samples included C. krusei (4 samples, 1.76%), C. tropicalis (3 samples, 1.32%), C. kefyr (3 samples, 1.32%), and C. parapsilosis (1 sample, 0.44%), while 4 samples could not be classified.

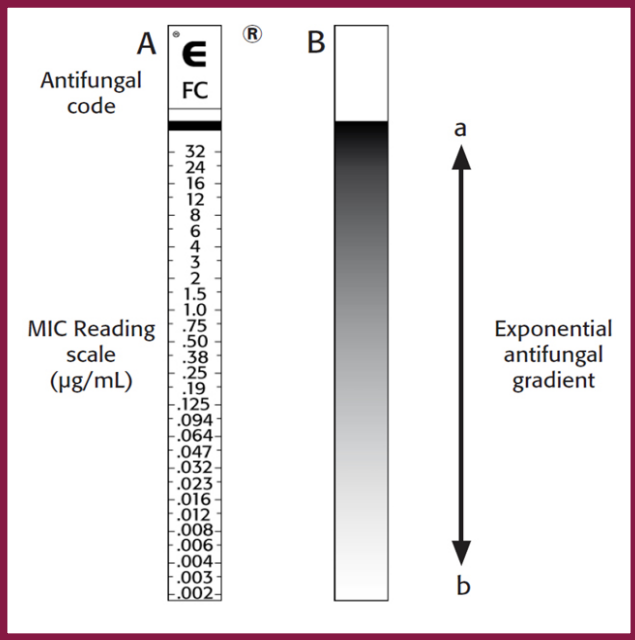

Figure 1. E-test strip for the antifungal agent gradient test used in this study. A) Front side of the strip containing the MIC reading scale. B) Backside of the strip containing an exponential antifungal gradient. Where a represents the maximum and b represents the minimum concentration of the antifungal agent MIC: Minimum inhibitory concentration

Antifungal Susceptibility Test

Out of the 86 Candida spp., the distribution of clinical samples in which isolated Candida species were tested for antifungal susceptibility is shown in Table 3.

From female patients, a total of 59 clinical samples were isolated, whereas a total of 27 samples were obtained from male patients with a mean age of 44.82. Significant differences were observed in terms of patient age and gender distribution among Candida species ($p<0.05$).

The Candida spp. isolates included in this study were obtained from different units, including clinical, intensive care, and outpatient departments. It was statistically significant that C. glabrata was more frequently isolated from outpatients, and C. tropicalis and C. parapsilosis were more frequently isolated from intensive care and clinical patients ($p<0.05$). Among the 45 C. albicans isolates in our study, 7 were derived from clinical samples, 13 from intensive care samples, and 25 were from outpatient sources. Of the C. glabrata isolates, 5 were from clinical samples, 2

Table 2. Distribution of samples obtained from patients with positive Candida spp. culture according to clinics. The number of isolates was determined according to the clinical sample type

Clinical sample type	Number of isolates	%
Urine	669	59.77
Blood	270	18.57
Vagina	227	15.61
Respiratory tract	35	2.41
Catheter	19	1.31
Wound	11	0.76
Abscess	8	0.55
Ear	5	0.34
Peritoneum	4	0.28
Pleura	3	0.21
Cerebrospinal fluid	3	0.24
Total	1254	100

Table 3. Clinical sample distribution for candida antifungal susceptibility testing. Distribution of clinical samples for antifungal susceptibility testing of isolated Candida species

Agent	Vagina	Blood	Urine	Abscess	Wound	Cerebrospinal fluid	Total
Candida albicans	25	14	3	1	1	1	45
Candida glabrata	15	3	1	1	-	-	20
Candida tropicalis	-	7	1	-	1	-	9
Candida parapsilosis	-	6	-	-	-	-	6
Candida kefyr	-	1	3	-	-	-	4
Candida guilliermondi	-	1	-	-	-	-	1
Candida krusei	-	1	-	-	-	-	1
Total	40	33	8	2	2	1	86

Table 4. Candida spp. MIC values and resistance profiles for antifungal agents. MIC values and resistance profiles of Candida spp. isolates based on gradient test results for antifungal agents

Candida spp. (n)	Antifungal drugs	MIC range (mg/L)	Susceptible (S) n (%)	Moderately susceptible (I) n (%)	Resistant (R) n (%)
Candida albicans	AmB	<0.002-1.5	44 (%97.8)	-	1 (2.2%)
	AND	<0.002-0.125	45 (100%)	-	-
	FLC	<0.016-> 56	39 (86.7%)	-	6 (13.3%)
	VRC	<0.002-0.25	39 (86.7%)	6 (13.3%)	-
Candida glabrata (20)	AmB	0.002-1	20 (100%)	-	-
	AND	<0.002-0.064	20 (100%)	-	-
	FLC	1-32	-	12 (60%)	8 (40%)
	VRC	0.5-8	-	-	-
Candida tropicalis (9)	AmB	0.25-3	8 (88.9%)	-	1 (11.1%)
	AND	<0.002	9 (100%)	-	-
	FLC	<0.016-8	8 (88.9%)	-	1 (11.1%)
	VRC	0.032-0.38	8 (88.9%)	-	1 (11.1%)
Candida parapsilosis (6)	AmB	<0.002-1	6 (100%)	-	-
	AND	<0.002-0.5	6 (100%)	-	-
	FLC	0.016-256	5 (83.3%)	-	1 (16.7%)
	VRC	<0.002-0.75	5 (83.3%)	1 (16.7%)	-
Candida kefyr (4)	AmB	0.12-1.5	-	-	-
	AND	<0.002-1	-	-	-
	FLC	0.125-1	4 (100%)	-	-
	VRC	0.032-0.125	-	-	-
Candida guilliermondi (1)	AmB	1	-	-	-
	AND	0.5	-	-	-
	FLC	2	1 (100%)	-	-
	VRC	0.125	-	-	-
Candida krusei (1)	AmB	0.5	1 (100%)	-	-
	AND	0.064	1 (100%)	-	-
	FLC	>256	-	-	1 (100%)
	VRC	0.094	-	-	-

AmB: Amphotericin B, AND: Anidulafungin, FLC: Flukonazol, VRC: Vorikonazol, MIC: Minimal inhibitory concentration

from intensive care samples, and 13 from outpatients. Four C. tropicalis isolates were obtained, 4 from clinical and 5 from intensive care patients. One C. parapsilosis isolate was derived from clinical samples, while 5 were from intensive care sources. Four isolates were examined for C. kefyr, including 2 clinical and 2 intensive care samples. One isolate was clinically confirmed as C. guilliermondii, while one isolate of C. krusei was obtained from the intensive care unit.

EUCAST reported susceptibility breakpoints for seven antifungals against C. albicans isolates, including AmB, AND, FLC, vorikonazol (VRC), itraconazole, posaconazole, and micafungin. The MICs of the isolates and the distribution of resistance profiles among Candida spp. based on gradient test results are shown in Table 4.

In this study, a total of 45 isolates were obtained, including one isolate from a burn intensive care unit patient. Among the 6 isolates showing resistance to AmB from the C. albicans species, it was determined that 4 were obtained from patients in the intensive care units and 2 were obtained from outpatient clinic patients. The test of antifungal susceptibility was shown that 8 out of 8 C. glabrata isolates tested had MIC values above 16 for FLC. Among these isolates, 1 was obtained from an intensive care unit patient and 4 were obtained from outpatient clinic patients, with 3 isolates identified as clinical isolates. One

C. tropicalis isolate showing resistance to AmP was isolated from an 81-year-old male patient in the internal medicine intensive care unit. This isolate also showed resistance to FLC and VRC agents. It was observed that the resistant C. krusei isolates identified in the antifungal susceptibility testing also showed resistance to VRC and were obtained from patients infected with C. parapsilosis in intensive care units. Intensive care units are noteworthy for being where both fungal infections and resistant isolates are detected.

Discussion

Candidiasis refers to various infections caused by C. fungi that affect the skin, mucous membranes, and deep-seated organs. Commonly caused by C. albicans, these invasive infections pose significant morbidity and mortality risks (6,19). The symptoms of candidiasis include oral lesions, redness, burning, bleeding, cracking, loss of taste, and spread into the esophagus in patients with weakened immune systems (28,29). Treatment for candidiasis depends on the type of infection. Antifungal medications such as FLC (oral antifungals), clotrimazole (antifungal lozenges), and nystatin (antifungal mouth wash) are used to treat fungal infections (28). Candida species can develop resistance to antifungal medications through efflux pump and biofilm formation, which protect cells against azoles. Candida biofilms, which are resistant to azoles, can also protect cells against antifungal medications by altering ergosterol (17,18,22).

Analysis of the clinical samples revealed the presence of Candida species in a substantial number of cases, with 1254 clinical samples yielding positive growth for Candida spp. Among the Candida isolates, C. albicans was the most frequently identified species, accounting for 46.8% of the isolates. However, infections caused by other Candida species demonstrated an increasing trend. Studies conducted worldwide have demonstrated variations in Candida species occurrence across regions. While C. albicans is frequently isolated as a pathogen in Northern and Central Europe as well as the United States, non-albicans Candida species are predominant in Asia, Southern Europe, and South America. The highest rate of C. glabrata isolates has been reported in Northern and Central Europe, whereas C. parapsilosis is most commonly found in Slovakia, Southern Europe, South America, and Asia. C. tropicalis, on the other hand, exhibits dominance in Eastern Asia and Argentina. In contrast, the prevalence of C. krusei is relatively low across all geographic regions. The underlying medical conditions of patients can also have an impact on the frequency of Candida species, in addition to regional variance, the administration of antifungal agents, and local factors related to the hospital environment (30).

When comparing the conducted studies, it was observed that the decreasing order of Candida species in the American population generally consisted of C. albicans, C. glabrata, C. parapsilosis, and C. tropicalis. In our study, the most commonly encountered Candida species was C. albicans (46.8%). On the other hand, when our study was compared with other studies, C. glabrata was not identified as the most frequently encountered non-albicans Candida species. The frequency of C. tropicalis and C. parapsilosis was higher than that of C. glabrata. The discrepancy in the ranking between C. glabrata, C. tropicalis, and C. parapsilosis in our study can be attributed to population differences. The epidemiology of candidemia varies according to region. As observed in the study conducted by Horn et al. (31) in 2009, our study also demonstrated a higher isolation rate of non-albicans Candida species from blood cultures (62.59%) (32,33).

As observed, different populations influence the frequency of Candida species occurrence. In a study conducted in Singapore, it was found that the most commonly encountered Candida species differed from our study, as C. albicans was not predominant (33). The frequency of non-C. albicans Candida species, particularly C. tropicalis and C. parapsilosis, appeared to be consistent with our findings.

In accordance with nearly all studies, C. albicans, C. tropicalis, C. parapsilosis, and C. glabrata, which exhibit variable rankings, were the most commonly encountered Candida species in our investigation, occupying the top four positions (34). Consistent with the prevalent Candida species observed in our study population, C. albicans emerged as the predominant species. Some studies conducted in Türkiye have demonstrated the occasional inclusion of C. krusei among the four most frequently isolated Candida species (35). However, contrary to these findings, C. krusei (1.29%) was the least regularly isolated species in our study.

In our study, consistent with previous studies, C. parapsilosis was found to be the third most frequently isolated species. C. parapsilosis has been reported as the third most common Candida species obtained from blood cultures in North America. However, in our study, C. parapsilosis was the second most common species isolated from blood cultures, despite ranking third overall. Growth of C. parapsilosis was observed in isolates obtained from blood cultures of intensive care unit patients, accounting for 90% of cases, and was suggested to be associated with catheter usage (33). Antifungal drug resistance can be categorized microbiologically or clinically, with microbiological resistance referring to a fungal pathogen's *in vitro* susceptibility to an antifungal agent (30). According to the Infectious Diseases Society of America guidelines,

FLC is recommended for less severe infections, whereas echinocandin are the first-line treatment for systemic candidiasis in patients with moderate-to-severe infections and those who have previously been exposed to azoles. The European Society of Clinical Microbiology and Infectious Diseases in Europe recommends echinocandin for all cases (30). Different varieties of Candida exhibit different levels of susceptibility to widely used antifungal medications. For instance, C. krusei is naturally resistant to FLC with a global resistance rate of 78.3%, whereas C. glabrata exhibits dose-dependent low susceptibility with a global resistance rate of 15.7% compared to other Candida species (30).

Fundamental resistance to FLC is known to be extraordinary in C. albicans (1.4%), C. parapsilosis (3.6%), and C. tropicalis (4.1%). In our study, the FLC resistance rates of C. albicans, C. parapsilosis, and C. tropicalis isolates were 13.3%, 16.7%, and 11.1%, respectively. This resistance profile may be attributed to the frequent use of FLC as a prophylactic treatment, particularly in hospitalized patients. Echinocandins have strong antifungal properties against most Candida species, except for C. parapsilosis, which has been reported to have higher MICs (30). None of the tested isolates in our study showed resistance to echinocandin. This finding is consistent with the existing literature. As observed in other studies (35), we did not observe resistance to echinocandin in our study. The most common resistance was found against FLC, both in C. albicans and non-albicans Candida species. The isolates from clinical samples showed an increasing prevalence of non-albicans Candida species, including C. parapsilosis, C. tropicalis, and C. glabrata. The observed increase is attributed to invasive procedures, prolonged stays, and long-term use of antibiotics. Prophylactic antifungal therapy may also contribute to antifungal resistance. Monitoring antifungal resistance profiles will help guide empirical fungal infections treatment, highlighting the need for more effective treatment strategies.

Conclusion

Candida species C. albicans is the most frequently isolated, but infections from other Candida species have increased. No resistance to AND was observed, but species-specific resistance to other antifungal agents was identified, emphasizing the need for continuous monitoring.

Ethics

Ethics Committee Approval: This study was approved by the Dicle University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (approval number: 157, date: 20.06.2019)

Informed Consent: Informed consent was obtained from all participants.

Authorship Contributions

Surgical and Medical Practices: N.K., S.A., N.Ö., Concept: N.K., S.A., N.Ö., Design: N.K., S.A., N.Ö., Data Collection or Processing: N.K., N.Ö., Analysis or Interpretation: N.K., S.A., Literature Search: N.K., N.Ö., Writing: N.K., S.A., N.Ö.

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REFERENCES

1. Pappas PG, Lionakis MS, Arendrup MC, Ostrosky-Zeichner L, Kullberg BJ. Invasive candidiasis. Nat Rev Dis Primers. 2018;4:18026. [Crossref]
2. Gómez-Gaviria M, Ramírez-Sotelo U, Mora-Montes HM. Non-albicans Candida species: immune response, evasion mechanisms, and new plant-derived alternative therapies. J Fungi (Basel). 2023;9:11. [Crossref]
3. Kadosh D, Mundodi V. A re-evaluation of the relationship between Morphology and Pathogenicity in Candida species. J Fungi (Basel). 2020;6:13. [Crossref]
4. Quindós G, Marcos-Arias C, San-Millán R, Mateo E, Eraso E. The continuous changes in the aetiology and epidemiology of invasive candidiasis: from familiar Candida albicans to multiresistant Candida auris. Int Microbiol. 2018;21:107-119. [Crossref]
5. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007;20:133-163. [Crossref]
6. Gonzalez-Lara MF, Ostrosky-Zeichner L. Invasive Candidiasis. Semin Respir Crit Care Med. 2020;41:3-12. [Crossref]
7. Singh DK, Tóth R, Gácser A. Mechanisms of pathogenic Candida species to evade the host complement attack. Front Cell Infect Microbiol. 2020;10:94. [Crossref]
8. Arendrup MC, Patterson TF. Multidrug-resistant Candida: epidemiology, molecular mechanisms, and treatment. J Infect Dis. 2017;216:S445-S451. [Crossref]
9. Fioriti S, Brescini L, Pallotta F, Canovari B, Morroni G, Barchiesi F. Antifungal combinations against Candida species: from bench to bedside. J Fungi (Basel). 2022;8:1077. [Crossref]
10. Kumar S, Kumar A, Roudbary M, Mohammadi R, Černáková L, Rodrigues CF. Overview on the infections related to rare Candida species. Pathogens. 2022;11:963. [Crossref]
11. Musinguzi B, J. Sande O, Mboowa G, Baguma A, Itabangi H, Achan B. Laboratory diagnosis of Candidiasis. In: Candida and Candidiasis. IntechOpen; 2023. [Crossref]
12. Clancy CJ, Nguyen MH. Diagnosing invasive Candidiasis. J Clin Microbiol. 2018;56:e01909-e01917. [Crossref]
13. Fang W, Wu J, Cheng M, Zhu X, Du M, Chen C, et al. Diagnosis of invasive fungal infections: challenges and recent developments. J Biomed Sci. 2023;30:42. [Crossref]
14. Paul S, Singh P, Shamanth AS, Rudramurthy SM, Chakrabarti A, Ghosh AK. Rapid detection of fluconazole resistance in Candida tropicalis by MALDI-TOF MS. Med Mycol. 2018;56:234-241. [Crossref]

15. Mehramouz B, Khodadadi E, Khodadadi E, Taghizadeh S, Ghanbarov K, Köse Ş, et al. The use of MALDI-TOF mass spectrometry technology in molecular analysis of microbial pathogenesis. Vol. 13, Biointerface Research in Applied Chemistry. AMG Transcend Association; 2023. [Crossref]
16. Aslani N, Kokabi R, Moradi F, Abbasi K, Vaseghi N, Afsarian MH. Characterization of Candida species isolated from vulvovaginal candidiasis by MALDI-TOF with *in vitro* antifungal susceptibility profiles. Curr Med Mycol. 2021;7:6-11. [Crossref]
17. Cowen LE, Sanglard D, Howard SJ, Rogers PD, Perlin DS. Mechanisms of antifungal drug resistance. Cold Spring Harb Perspect Med. 2014;5:a019752. [Crossref]
18. Kaur J, Nobile CJ. Antifungal drug-resistance mechanisms in Candida biofilms. Curr Opin Microbiol. 2023;71:102237. [Crossref]
19. Whaley SG, Berkow EL, Rybak JM, Nishimoto AT, Barker KS, Rogers PD. Azole antifungal resistance in Candida albicans and emerging non-albicans Candida species. Front Microbiol. 2017;7:2173. [Crossref]
20. Krishnasamy L, Krishnakumar S, Kumaramanickavel G, Saikumar C. Molecular mechanisms of antifungal drug resistance in Candida species. Vol. 12, Journal of Clinical and Diagnostic Research. 2018. [Crossref]
21. Lee Y, Puumala E, Robbins N, Cowen LE. Antifungal drug resistance: molecular mechanisms in Candida albicans and beyond. Chem Rev. 2021;121:3390-3411. [Crossref]
22. Silva S, Rodrigues CF, Araújo D, Rodrigues ME, Henriques M. Candida species biofilms' antifungal resistance. J Fungi (Basel). 2017;3:8. [Crossref]
23. Rodrigues CF, Rodrigues ME, Silva S, Henriques M. Candida glabrata biofilms: how far have we come? J Fungi (Basel). 2017;3:11. [Crossref]
24. Mohammadi F, Hemmat N, Bajalan Z, Javadi A. Analysis of biofilm-related genes and antifungal susceptibility pattern of vaginal Candida albicans and non-Candida albicans species. Biomed Res Int. 2021;2021:5598907. [Crossref]
25. Sobel JD. Role of antifungal susceptibility tests in the treatment of vulvovaginal Candidiasis. Vol. 25, Current Infectious Disease Reports. Springer. 2023;25:29-32. [Crossref]
26. Pristov KE, Ghannoum MA. Resistance of Candida to azoles and echinocandins worldwide. Clin Microbiol Infect. 2019;25:792-798. [Crossref]
27. Arendrup MC, Friberg N, Mares M, Kahlmeter G, Meletiadis J, Guinea J, et al. How to interpret MICs of antifungal compounds according to the revised clinical breakpoints v. 10.0 European committee on antimicrobial susceptibility testing (EUCAST). Clin Microbiol Infect. 2020;26:1464-1472. [Crossref]
28. Taylor M, Brizuela M, Raja A. Oral Candidiasis. StatPearls. 2023 Mar 19. [Crossref]
29. Hu L, He C, Zhao C, Chen X, Hua H, Yan Z. Characterization of oral candidiasis and the Candida species profile in patients with oral mucosal diseases. Microb Pathog. 2019;134:103575. [Crossref]
30. Sanguinetti M, Posteraro B, Lass-Flörl C. Antifungal drug resistance among Candida species: mechanisms and clinical impact. Mycoses. 2015;58 Suppl 2:2-13. [Crossref]
31. Horn DL, Neofytos D, Anaissie EJ, Fishman JA, Steinbach WJ, Olyaei AJ, et al. Epidemiology and outcomes of candidemia in 2019 patients: data from the prospective antifungal therapy alliance registry. Clin Infect Dis. 2009;48:1695-1703. [Crossref]
32. Bhattacharya S, Sae-Tia S, Fries BC. Candidiasis and mechanisms of antifungal resistance. Antibiotics (Basel). 2020;9:312. [Crossref]
33. Pfaller M, Neofytos D, Diekema D, Azie N, Meier-Kriesche HU, Quan SP, et al. Epidemiology and outcomes of candidemia in 3648 patients: data from the Prospective Antifungal Therapy (PATH Alliance®) registry, 2004-2008. Diagn Microbiol Infect Dis. 2012;74:323-331. [Crossref]
34. Teo JQM, Candra SR, Lee SJY, Chia SYH, Leck H, Tan AL, et al. Candidemia in a major regional tertiary referral hospital-epidemiology, practice patterns and outcomes. Antimicrob Resist Infect Control. 2017;6:27. [Crossref]
35. Badiee P, Alborzi A. Susceptibility of clinical Candida species isolates to antifungal agents by E-test, Southern Iran: A five year study. Iran J Microbiol. 2011;3:183-188. [Crossref]