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Öz

Giriş: Klinik açıdan önemli mayaların antifungal duyarlılık paternlerini ve minimum inhibitör konsantrasyonlarını (MIC) değerlendirmek.

Gereç ve Yöntem: Bu laboratuvar tabanlı gözlemsel çalışma, prospektif olarak toplanan maya izolatlarını ve anonimleştirilmiş laboratuvar verilerinin retrospektif analizini içermekte olup, Mart-Aralık 2024 tarihleri arasında üçüncü basamak bir kanser hastanesinde gerçekleştirilmiştir. Toplam 100 adet tekrarlanmayan maya izolatu dahil edilmiştir. Maya izolatları başlangıçta klinik mikrobiyoloji laboratuvarında MALDI-TOF MS kullanılarak tanımlanmıştır. Tüm izolatlar, mantar rDNA'sının ITS bölgesini hedefleyen PCR-RFLP kullanılarak yeniden tanımlanmıştır. PCR-RFLP'nin doğruluğu, nadir ve yaygın olmayan türler de dahil olmak üzere seçilen izolatların moleküler dizilemesi ile doğrulanmıştır. Antifungal duyarlılık testi, Sensititre YeastOne Antifungal Duyarlılık Testi sistemi kullanılarak gerçekleştirildi. MIC değerleri, ilgili CLSI türe özgü klinik kırılma noktalarına göre yorumlandı. MIC₅₀ ve MIC₉₀ hesaplandı.

Bulgular: *Candida albicans* (%37) ve *Candida tropicalis* (%32) en sık izole edilen türlerdi. Kan, en yaygın örnek kaynağıydı (%43), bunu idrar (%37) izledi. Ekinokandinler, tüm türlerde en düşük MIC değerlerini göstererek tutarlı *in vitro* aktivite sergiledi. Flukonazol, en geniş MIC aralığını (0,12-256 µg/mL) ve özellikle *Candida glabrata* ve *Candida krusei* üzerinde en yüksek MIC₉₀ değerlerini gösterdi. Azol ajanları güçlü pozitif MIC korelasyonları gösterirken, amfoterisin B diğer antifungal sınıflarla zayıf korelasyon gösterdi.

Sonuç: *Candida albicans* dışı türler, maya izolatlarının önemli bir bölümünü oluşturmuş ve değişken azol duyarlılığı göstermiştir. Bu bulgular, mevcut tedavi kılavuzlarıyla uyumludur; ancak klinik yönetim, klinik bağlama göre bireyselleştirilmelidir. Antifungal tedaviyi en verimli hale getirmek için, sürekli yerel izleme ve rutin duyarlılık testleri hâlâ büyük önem taşımaktadır.

Anahtar Kelimeler: *Candida*, ekinokandin, azol direnci, antifungal duyarlılık, Sensititre YeastOne

Introduction

Invasive fungal infections are a major cause of morbidity and mortality among hospitalized patients, particularly those with malignancies, immunosuppression, or prolonged hospital stays^[1,2]. Patients undergoing chemotherapy or intensive care are at increased risk of developing opportunistic fungal infections. Among these pathogens, *Candida* species are the leading cause of invasive fungal infections worldwide.

Over the past two decades, the epidemiology of candidiasis has changed substantially, with an increasing proportion of infections being caused by non-*albicans* *Candida* species. These species frequently exhibit reduced susceptibility to commonly used antifungal agents, particularly azoles, which may complicate the selection of appropriate antifungal therapy^[3,4].

Several surveillance studies have demonstrated geographic variation in the distribution of *Candida* species. However, local data from cancer centers in Pakistan remain limited, particularly with respect to minimum inhibitory concentration (MIC) distributions and cross-class MIC correlations. In several Asian countries, *Candida tropicalis* has emerged as one of the predominant causes of candidemia^[5,6].

Accurate identification of *Candida* species is essential because antifungal susceptibility profiles can vary substantially among species. Conventional phenotypic identification methods may not reliably distinguish closely related species, potentially affecting the selection of appropriate antifungal therapy. Molecular techniques, such as polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), provide rapid and reliable identification of medically important yeast species^[7,8].

Antifungal susceptibility testing is critical for guiding appropriate therapy. The reference broth microdilution method recommended by the Clinical and Laboratory Standards Institute (CLSI) is widely used for determining antifungal susceptibility; however, commercial systems such as the Sensititre YeastOne Antifungal Susceptibility Test offer a more convenient alternative and have demonstrated good correlation with reference methods^[9,10]. Therefore, we aimed to characterize the species distribution, antifungal MIC patterns, and cross-drug MIC correlations among clinical yeast isolates obtained from a tertiary care cancer hospital.

Materials and Methods

Study Design and Sample Size

This laboratory-based observational study involved the prospective collection of yeast isolates and retrospective analysis of anonymized laboratory data. It was conducted from March to December 2024 at a tertiary care cancer hospital. A total of 100 non-duplicate clinical yeast isolates were included during the study period. The sample size was determined based on the feasibility of collection within the defined study period and is acknowledged as a limitation of the study. Ethical approval was obtained from Shaukat Khanum Memorial Trust Review Board (protocol number: IRB-23-46, date: 11.12.2023). Patient consent was waived by the Institutional Review Board because the patient data were analyzed retrospectively and all identifiers were removed before analysis.

Identification of Yeasts

Yeast isolates obtained from clinical specimens, including blood, urine, pus, tissue, and body fluids, were included in the study. All isolates were cultured on Sabouraud dextrose agar

and incubated at 35-37 °C for 24-48 hours before identification and antifungal susceptibility testing.

Initial species identification was performed using matrix-assisted laser desorption ionization–time-of-flight mass spectrometry in the clinical microbiology laboratory. All isolates were subsequently reidentified using PCR-RFLP targeting the internal transcribed spacer (ITS) region of fungal ribosomal DNA, a widely accepted universal DNA barcode marker for fungi^[7,8]. PCR amplification was performed using the universal primers ITS1 and ITS4. The amplified products were digested with the restriction enzyme MspI to generate species-specific fragment patterns, which were analyzed by agarose gel electrophoresis and compared with reference profiles for species identification.

Rare and uncommon yeast species, such as *Kodamaea ohmeri* and *Dirkmeia churashimaensis*, were confirmed by Sanger sequencing.

Reference strains of *Candida albicans* (ATCC 10231), *Candida parapsilosis* (ATCC 90018), *Candida glabrata* (ATCC 15126), *Candida tropicalis* (ATCC 1369), and *Candida auris* (CDC B11903) were used as quality-control strains.

Antifungal Susceptibility Testing

Antifungal susceptibility testing of clinically significant yeasts was performed using the Sensititre™ YeastOne™ system (Thermo Fisher Scientific), which provides standardized, reproducible, and quantitative MIC results for multiple antifungal agents^[9]. The antifungal panel included fluconazole, voriconazole, itraconazole, posaconazole, 5-flucytosine, amphotericin B, and the echinocandins caspofungin, micafungin, and anidulafungin.

A broad range of *Candida* species, including *Candida albicans*, *Candida famata*, *Candida glabrata*, *Candida guilliermondii*, *Candida krusei*, *Candida orthopsilosis*, *Candida parapsilosis*, *Candida pelliculosa*, and *Candida tropicalis*, as well as non-*Candida* yeasts such as *Papiliotrema laurentii*, *Cryptococcus neoformans*, and *Dirkmeia churashimaensis*, were tested.

A yeast suspension equivalent to 0.5 McFarland was prepared from a pure 24-hour culture. Subsequently, 20 µL of the suspension was transferred into 11 mL of YeastOne broth. Using the Sensititre™ AIM system, 100 µL of the inoculated broth was dispensed into each well of the Sensititre plate. The plates were sealed and incubated at 35 °C in ambient air and were read after 24 hours using the Sensititre™ Vizion system.

Individual MIC values were recorded for each antifungal agent. MIC results obtained using the Sensititre™ YeastOne™ system were interpreted using species-specific CLSI clinical breakpoints outlined in CLSI M60, Performance Standards for *Antifungal Susceptibility Testing of Yeasts*, with categorical interpretations

performed only when established breakpoints were available^[10,11].

CLSI clinical breakpoints have been established primarily for selected *Candida* species and specific antifungal agents. For non-*Candida* yeasts and organism–drug combinations for which validated breakpoints were unavailable, MIC values were reported descriptively without categorical interpretation. Where applicable, epidemiological cutoff values were considered to distinguish wild-type from non-wild-type isolates.

Statistical Analysis

MIC values were log₂-transformed before analysis. MIC₅₀, MIC₉₀, and geometric mean MIC values were calculated for each antifungal agent.

Isolates with missing MIC values for a given antifungal agent were excluded from the calculations for that specific drug. All analyses were performed at the overall isolate level and were not stratified by clinical setting (e.g., intensive care unit, inpatient, or outpatient).

Pearson correlation coefficients were calculated to assess relationships among log₂-transformed MIC values for azole and echinocandin agents. Amphotericin B was excluded from the correlation analysis because cross-class MIC correlations with azoles have limited clinical interpretability and no validated CLSI clinical breakpoint is available for amphotericin B in *Candida* species.

Results

Species Distribution and Specimen Sources

A total of 100 yeast isolates were included in the study. Blood (43%) and urine (37%) were the predominant specimen sources, followed by pus, tissue, tips, and other specimens.

The species distribution was as follows: *Candida albicans* (n=37, 37%), *Candida tropicalis* (n=32, 32%), *Candida krusei* (n=6, 6%), *Candida famata* (n=5, 5%), *Candida parapsilosis* (n=5, 5%), *Candida glabrata* (n=4, 4%), and *Candida pelliculosa* (n=3, 3%). Less frequently isolated species included *Cryptococcus neoformans* (n=2), *Candida orthopsilosis* (n=1), *Candida guilliermondii* (n=1), *Papiliotrema laurentii* (formerly *Cryptococcus laurentii*) (n=1), *Kodamaea ohmeri* (n=1), and *Dirkmeia churashimaensis* (n=1). One mixed yeast infection was also identified. No clinical *Candida auris* isolates were identified during the study period.

Overall Antifungal Susceptibility Profile

The overall *in vitro* antifungal activity against all isolates is summarized in Table 1 and Figure 1.

The distribution of MIC values (Figure 1) demonstrated substantial variability for fluconazole, whereas the echinocandins exhibited tightly clustered low MIC values, indicating consistent *in vitro* activity.

Amphotericin B showed relatively narrow variability, suggesting consistent antifungal activity.

Fluconazole MICs ranged from 0.12 to 256 µg/mL, with an MIC₅₀ of 1 µg/mL, an MIC₉₀ of 4 µg/mL, and a geometric mean MIC of 1.56 µg/mL. Voriconazole, itraconazole, and posaconazole demonstrated low MIC₅₀ values (≤0.06 µg/mL) with narrow

Table 1. Overall geometric mean minimum inhibitory concentrations (MICs) of the antifungal agents tested against yeast isolates.

Drug	n	MIC ₅₀	MIC ₉₀	Geometric mean (rounded)
Fluconazole	89	1	4	1.5
Voriconazole	90	0.06	0.5	0.07
Caspofungin	90	0.03	0.12	0.04
Micafungin	90	0.03	0.06	0.04
Anidulafungin	90	0.03	0.12	0.03
Amphotericin B	90	0.25	0.5	0.3
Itraconazole	90	0.06	0.25	0.07
Posaconazole	90	0.06	0.25	0.05

n varies by antifungal agent because of missing values. MIC values were generated using the Sensititre™ YeastONE™ system; MIC₅₀ and MIC₉₀ values are reported using the nearest twofold dilution step available on the YeastONE tray.

MIC distributions. All three echinocandins—caspofungin, micafungin, and anidulafungin—showed excellent *in vitro* activity, with MIC₅₀ values of 0.03 µg/mL and low geometric mean MICs. Amphotericin B retained consistent activity, with an MIC₅₀ of 0.25 µg/mL.

MIC analysis included 89–90 isolates per antifungal agent, depending on the availability of valid MIC results. Isolates with missing MIC values were excluded only from the analysis of the respective drug.

CLSI categorical interpretations were applied only to *Candida* species for which established clinical breakpoints were available. For non-*Candida* yeasts, including *Cryptococcus* and other rare species, MIC values are presented descriptively because validated clinical breakpoints are unavailable.

Based on applicable CLSI species-specific clinical breakpoints, most *Candida albicans* isolates were categorized as susceptible to echinocandins and azoles for which applicable CLSI clinical breakpoints were available. Reduced fluconazole susceptibility was observed primarily among non-*albicans Candida* species. For *Candida glabrata*, fluconazole results were categorized as susceptible-dose dependent or resistant according to CLSI criteria.

Candida albicans: Isolates generally demonstrated low MICs for azoles and echinocandins. Fluconazole MICs ranged from 0.12 to 8.0 µg/mL, with an MIC₅₀ of 1 µg/mL and an MIC₉₀ of 4 µg/mL. All echinocandins demonstrated potent activity, with

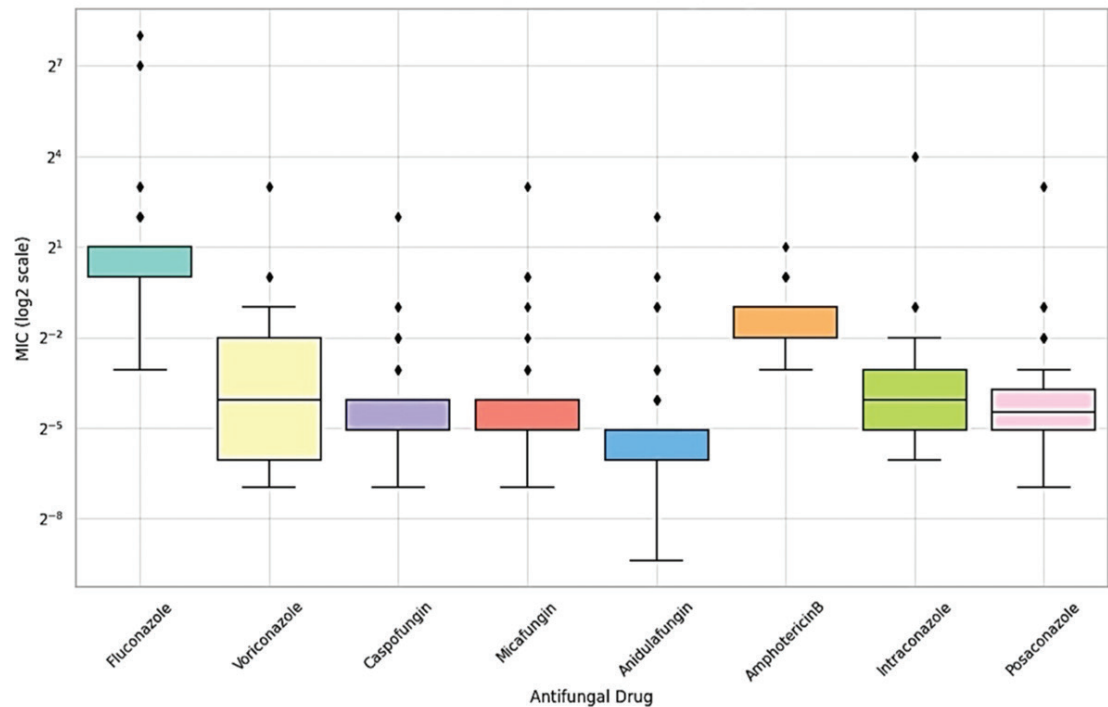


Figure 1. Boxplot showing the overall distribution of minimum inhibitory concentration (MIC) values (log₂ scale) for antifungal agents.

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MIC₅₀ values of ≤ 0.03 $\mu\text{g/mL}$ (Table 2). Amphotericin B also demonstrated consistent activity.

Candida tropicalis: Isolates demonstrated preserved susceptibility to most antifungal agents. Fluconazole MICs ranged from 0.5 to 8.0 $\mu\text{g/mL}$, with MIC₅₀ and MIC₉₀ values of 1 $\mu\text{g/mL}$ and 4 $\mu\text{g/mL}$, respectively.

Candida krusei: Isolates demonstrated intrinsically elevated fluconazole MICs (1–128 $\mu\text{g/mL}$), with an MIC₅₀ of 64 $\mu\text{g/mL}$ and an MIC₉₀ of 128 $\mu\text{g/mL}$. Voriconazole and the echinocandins demonstrated lower MIC values.

Candida glabrata: Isolates exhibited intrinsically reduced susceptibility to fluconazole, with MICs ranging from 4 to 256 $\mu\text{g/mL}$ and a high geometric mean MIC of 11.31 $\mu\text{g/mL}$.

Cryptococcus neoformans: Isolates showed a narrow fluconazole MIC range (0.5–1.0 $\mu\text{g/mL}$).

Other non-*Candida* species and rare yeasts demonstrated variable MIC distributions across the antifungal agents tested. For these yeasts, MIC values are presented descriptively only and should not be interpreted as indicating clinical susceptibility.

Most *Candida albicans* and *Candida tropicalis* isolates remained susceptible to fluconazole and the echinocandins, whereas *Candida glabrata* predominantly exhibited susceptible-dose dependent or resistant fluconazole phenotypes. *Candida krusei* was intrinsically resistant to fluconazole.

Percentages were calculated only for isolates with valid MIC results for which categorical interpretation was possible. Therefore, the totals may not correspond to the total number of isolates within a species because isolates with missing MIC values or without applicable CLSI breakpoints were excluded from the categorical analysis.

Comparative Analysis

Figure 2 presents a bubble plot showing the geometric mean MICs of the antifungal agents across yeast species.

Species-specific comparisons showed higher fluconazole MIC values for *Candida glabrata* and *Candida krusei*, whereas the echinocandins maintained low MIC values across most species. This pattern highlights the variability in azole susceptibility and the consistently low MICs observed with echinocandins.

Antifungal MIC Correlation Analysis

Figure 3 illustrates the pairwise Pearson correlation coefficients among antifungal MIC values. Strong correlations were observed only between certain azole pairs (fluconazole–itraconazole $r = 0.78$, fluconazole–posaconazole $r = 0.78$, itraconazole–posaconazole $r = 0.86$). Other azole pairs showed weaker correlations (e.g., voriconazole–itraconazole $r = 0.20$, voriconazole–posaconazole $r = 0.20$). Echinocandin correlations ranged from weak to moderate ($r = -0.02$ to 0.65). Amphotericin B was excluded from the correlation

Table 2. Species-specific geometric mean MICs ($\mu\text{g/mL}$) of antifungal agents against clinically relevant yeast species.

Species (n)	Amphotericin B	Anidulafungin	Caspofungin	Fluconazole	Itraconazole	Micafungin	Posaconazole	Voriconazole
<i>Candida albicans</i> (n = 37)	0.291	0.025	0.033	1.084	0.057	0.036	0.038	0.045
<i>Candida famata</i> (n = 5)	0.214	0.03	0.03	2.297	0.07	0.04	0.08	0.14
<i>Candida glabrata</i> (n = 4)	0.595	0.072	0.061	11.31	0.49	0.102	0.291	0.589
<i>Candida krusei</i> (n = 6)	0.376	0.034	0.046	13.45	0.16	0.046	0.092	0.162
<i>Candida orthopsilosis</i> (n = 1)	0.5	0.12	0.03	4	0.12	0.03	0.25	1
<i>Candida parapsilosis</i> (n = 5)	0.25	0.015	0.015	2	0.12	0.03	0.06	0.25
<i>Candida pelliculosa</i> (n = 3)	0.5	0.015	0.021	0.707	0.042	0.03	0.042	0.021
<i>Candida tropicalis</i> (n = 32)	0.284	0.029	0.04	1.368	0.072	0.042	0.051	0.058
<i>Candida guilliermondii</i> (n = 1)	NA	NA	NA	NA	NA	NA	NA	NA
<i>Cryptococcus neoformans</i> (n = 2)	0.25	0.042	0.015	0.707	0.042	0.03	0.03	0.03
<i>Papiliotrema laurentii</i> (n = 1)	NA	NA	NA	NA	NA	NA	NA	NA
<i>Dirkmeia churashimaensis</i> (n = 1)	0.25	0.015	0.03	4	0.25	0.03	0.5	0.12
<i>Kodamaea ohmeri</i> (n = 1)	0.25	0.03	0.03	1	0.03	0.06	0.008	0.03
Mixed yeast infection (n = 1)	NA	NA	NA	NA	NA	NA	NA	NA

NA, Not applicable geometric mean MIC could not be calculated because susceptibility results were unavailable for the isolate(s).

analysis because cross-class MIC correlations with azoles were considered to have limited clinical interpretability in the absence of validated clinical breakpoints and treatment-outcome data.

Discussion

The antifungal susceptibility patterns observed in this study support the continued role of echinocandins in the management of invasive candidiasis. Consistently low MIC values across most *Candida* species indicate preserved *in vitro* activity of this antifungal class, consistent with existing evidence demonstrating their potent activity and the globally low prevalence of echinocandin resistance, which is largely attributable to the low frequency of *FKS* gene mutations^[12,13].

The relatively higher MICs observed in the *Candida parapsilosis* complex are consistent with its intrinsically reduced susceptibility, which is associated with naturally occurring polymorphisms in the β -(1,3)-D-glucan synthase enzyme rather than acquired resistance mechanisms^[12,13].

The categorical susceptibility findings (Table 3) further support the preserved activity of echinocandins against the major *Candida* species. However, the greater variability in azole susceptibility, particularly among *Candida glabrata* and *Candida krusei*, underscores the importance of species-level identification and susceptibility-guided therapy.

Echinocandin resistance and antifungal susceptibility trends among *Candida* species have been widely studied in recent years, highlighting the emergence of resistance mechanisms

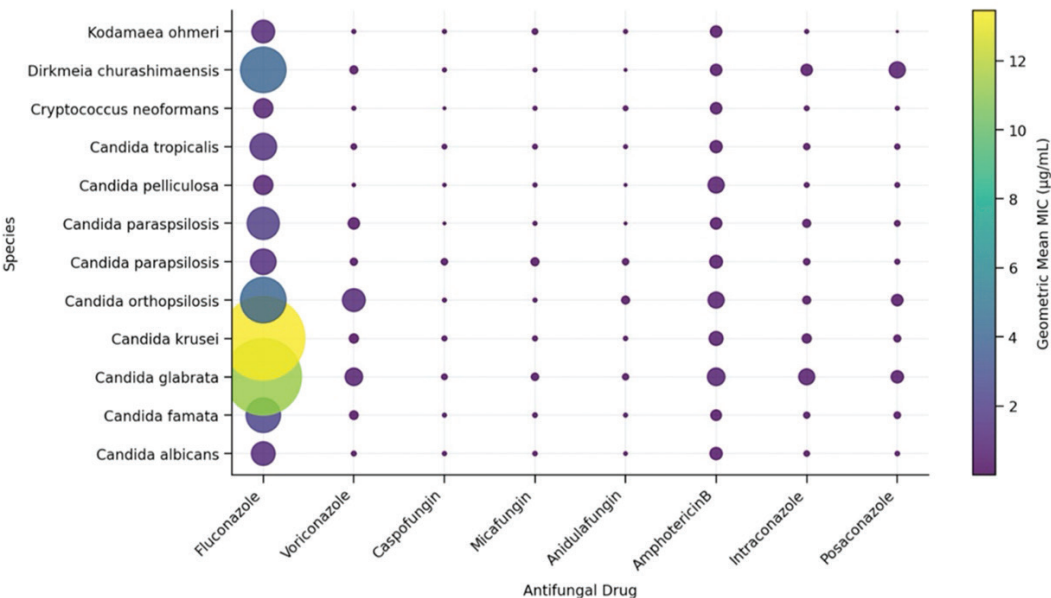


Figure 2. Species-wise antifungal susceptibility patterns based on geometric mean minimum inhibitory concentration values.

Table 3. Species-specific antifungal susceptibility categories among *Candida* isolates.

Species (n)	Fluconazole, S/SDD/R, n (%)	Voriconazole, S/I/R, n (%)	Anidulafungin, S/I/R, n (%)	Caspofungin, S/I/R, n (%)	Micafungin, S/I/R, n (%)
<i>Candida albicans</i> (n = 37)	29 (78.4) / 7 (18.9) / 1 (2.7)	26 (70.3) / 11 (29.7) / 0	32 (86.5) / 5 (13.5) / 0	33 (89.2) / 4 (10.8) / 0	33 (89.2) / 4 (10.8) / 0
<i>Candida tropicalis</i> (n = 32)	27 (84.4) / 3 (9.4) / 2 (6.2)	22 (68.8) / 10 (31.2) / 0	29 (90.6) / 3 (9.4) / 0	30 (93.8) / 2 (6.2) / 0	29 (90.6) / 3 (9.4) / 0
<i>Candida glabrata</i> (n = 4)	0 / 3 (75.0) / 1 (25.0)	NA	3 (75.0) / 1 (25.0) / 0	3 (75.0) / 1 (25.0) / 0	3 (75.0) / 1 (25.0) / 0
<i>Candida krusei</i> (n = 6)	IR: 6 (100)	4 (66.7) / 2 (33.3) / 0	6 (100) / 0 / 0	6 (100) / 0 / 0	6 (100) / 0 / 0
<i>Candida parapsilosis</i> (n = 5)	5 (100) / 0 / 0	3 (60.0) / 2 (40.0) / 0	5 (100) / 0 / 0	5 (100) / 0 / 0	5 (100) / 0 / 0
Other/rare yeasts	NA	NA	NA	NA	NA

S, susceptible; SDD, susceptible-dose dependent; I, intermediate; R, resistant; IR, intrinsic resistance; NA: indicates that no categorical interpretation was assigned because no applicable CLSI breakpoint. *Candida krusei* is intrinsically resistant to fluconazole (IR).

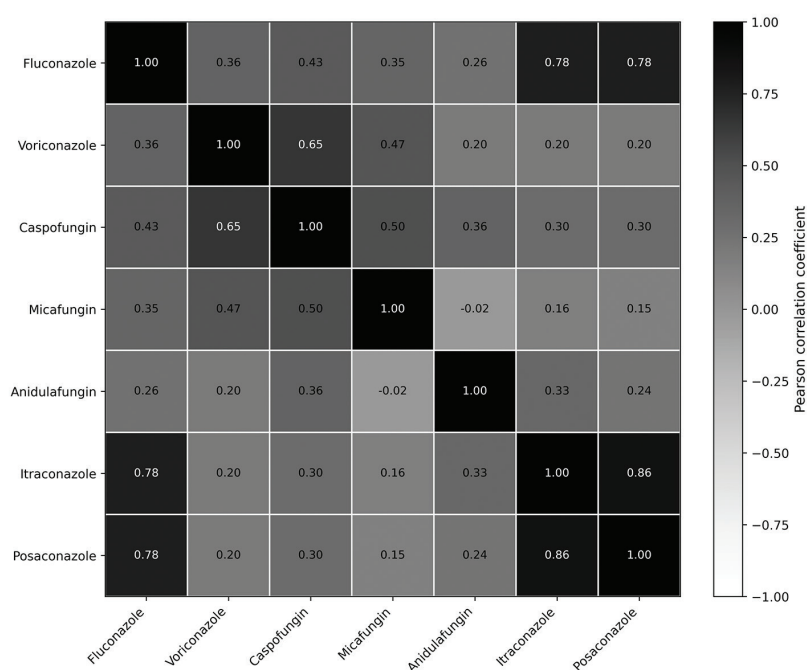


Figure 3. Correlation heat map showing pairwise relationships between minimum inhibitory concentration values of antifungal agents.

and the need for continued surveillance^[14]. Amphotericin B maintained stable *in vitro* activity across most isolates in this study, consistent with previous reports indicating preserved susceptibility^[15].

Overall, these findings are consistent with the role of echinocandins in empirical therapy for high-risk cancer patients, with subsequent therapy guided by species-level identification and local susceptibility patterns^[16].

The antifungal MIC correlation analysis further supported these findings, with strong associations only between certain azole pairs (fluconazole–itraconazole, fluconazole–posaconazole, itraconazole–posaconazole), while other azole pairs were weakly correlated. *Echinocandin correlations* ranged from weak to moderate ($r = -0.02$ to 0.65). Amphotericin B was excluded from the correlation analysis because cross-class correlations with azoles have limited clinical interpretability in the absence of validated clinical breakpoints and treatment-outcome data. These findings highlight the value of considering species-specific susceptibility patterns when interpreting antifungal MIC data^[17,18].

Clinical manifestations and treatment outcomes of candidemia vary according to the infecting *Candida* species, emphasizing the importance of species-level identification in guiding appropriate clinical management^[19].

Consistently low MIC values across most *Candida* species indicate preserved *in vitro* activity of echinocandins, with no

substantial evidence of reduced susceptibility in the isolates tested^[20].

Azole antifungals, particularly fluconazole, demonstrated considerable variability in MIC values. Recent multicenter studies have reported increasing azole resistance among *Candida* species, particularly *Candida parapsilosis*^[21]. Species-specific differences in efflux pump activity and alterations in ergosterol biosynthesis may contribute to reduced azole susceptibility, further emphasizing the importance of early species identification and susceptibility-guided antifungal therapy^[22]. The high frequency of *Candida tropicalis* in our cohort is consistent with reports from Pakistan and India, where *Candida tropicalis* has been identified as a leading *Candida* species, including among patients with hematologic malignancies^[23,24].

It is also important to note that *Candida glabrata* exhibits intrinsically reduced susceptibility to fluconazole, which should be considered when interpreting its elevated MICs. Its occurrence in oncology populations may be associated with factors such as neutropenia, prolonged hospitalization, broad-spectrum antibiotic exposure, and intensive healthcare exposure, although these factors were not specifically evaluated in our study.

From a clinical perspective, the susceptibility findings support the use of an echinocandin as appropriate empirical therapy for high-risk oncology patients with suspected invasive candidiasis, particularly given the consistently low

echinocandin MICs observed among the major *Candida* species. Subsequent therapy should be individualized based on species identification, susceptibility results, and clinical response, with de-escalation considered when clinically appropriate^[16].

Rare yeast species identified in this study, including *Dirkmeia churashimaensis*, *Kodamaea ohmeri*, and *Papiliotrema laurentii*, are uncommon but recognized opportunistic human pathogens, particularly in immunocompromised and oncology patients. Reported infections include fungemia and catheter-related bloodstream infections, while *Dirkmeia churashimaensis* has also been associated with an outbreak of bloodstream infection^[25-27]. The *Dirkmeia churashimaensis* and *Kodamaea ohmeri* isolates identified in our study were confirmed by sequencing. This molecular confirmation reduces the likelihood of species misidentification and supports the reliability of these uncommon findings. However, because these species were represented by single isolates and clinical outcome data were unavailable, their clinical significance in our cohort cannot be determined.

Study Limitations

This study has several limitations. First, it was conducted at a single center and included a relatively small sample size, which may limit the generalizability of the findings. Second, clinical outcome data were unavailable, precluding assessment of the relationship between antifungal susceptibility results and treatment response or patient outcomes. Third, the absence of CLSI clinical breakpoints for several species-drug combinations restricted categorical interpretation for some isolates. The lack of stratification by clinical setting may also limit the interpretation of setting-specific antifungal resistance patterns. Despite these limitations, the study provides valuable local epidemiological and antifungal susceptibility data that may inform clinical practice and future surveillance efforts. The recovery of single isolates of rare yeast species, including *Dirkmeia churashimaensis*, also limited species-specific assessment of their clinical significance and susceptibility patterns.

The taxonomy of *Candida famata* has undergone substantial revision, with isolates historically identified as *Candida famata* now largely assigned to *Debaryomyces hansenii* and related taxa. Therefore, species-level interpretation of the *Candida famata* isolates should be approached with caution^[28].

Conclusion

This study highlights the predominance of non-*albicans* *Candida* species and the variability in antifungal susceptibility patterns in a tertiary care oncology setting. Echinocandins demonstrated consistent *in vitro* activity, whereas azole resistance remains a concern. These findings underscore

the importance of species-level identification and routine antifungal susceptibility testing to guide appropriate antifungal therapy.

Ethics

Ethics Committee Approval: Ethical approval was obtained from Shaukat Khanum Memorial Trust Review Board (protocol number: IRB-23-46, date: 11.12.2023).

Informed Consent: Patient consent was waived by the Institutional Review Board because the patient data were analyzed retrospectively and all identifiers were removed before analysis.

Footnotes

Authorship Contributions

Concept: H.F., A.R., F.S., Design: H.F., S.N., R.Q., Data Collection or Processing: H.F., S.N., N.M., M.M., A.F., M.I., Analysis or Interpretation: H.F., R.Q., S.N., N.M., A.R., M.M., A.F., M.I., F.S., Literature Search: R.Q., F.S., Writing: H.F., R.Q., S.N., N.M., A.R., M.M., A.F., M.I., F.S.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Bays DJ, Jenkins EN, Lyman M, Chiller T, Strong N, Ostrosky-Zeichner L, Hoenigl M, Pappas PG, Thompson Iii GR. Epidemiology of invasive candidiasis. Clin Epidemiol. 2024;16:549-566.
2. Pinho S, Miranda IM, Costa-de-Oliveira S. Global epidemiology of invasive infections by uncommon *Candida* species: A systematic review. J Fungi (Basel). 2024;10(8):558.
3. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007;20:133-163.
4. Guinea J. Global trends in the distribution of *Candida* species causing candidemia. Clin Microbiol Infect. 2014;20 Suppl 6:5-10.
5. Meng L, Li J, Wang D, Han M, Gao S, Zhang Y, Zhu W, Liu C. Epidemiology, risk factors, and antifungal susceptibility analysis of *Candida tropicalis* and non-*C. tropicalis* candidemia. BMC Infect Dis. 2025;25:1089.
6. Tan TY, Hsu LY, Alejandria MM, Chaiwarith R, Chinniah T, Chayakulkeeree M, Choudhury S, Chen YH, Shin JH, Kiratisin P, Mendoza M, Prabhu K, Supparatpinyo K, Tan AL, Phan XT, Tran TT, Nguyen GB, Doan MP, Huynh VA, Nguyen SM, Tran TB, Van Pham H. Antifungal susceptibility of invasive *Candida* bloodstream isolates from the Asia-Pacific region. Med Mycol. 2016;54:471-477.
7. Mirhendi H, Makimura K, Khoramizadeh M, Yamaguchi H. A one-enzyme PCR-RFLP assay for identification of six medically important *Candida* species. Nihon Ishinkin Gakkai Zasshi. 2006;47:225-229.
8. Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W; Fungal Barcoding Consortium; Fungal Barcoding Consortium Author List. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Natl Acad Sci U S A. 2012;109:6241-6246.

9. Pfaller MA, Messer SA, Boyken L, Hollis RJ, Diekema DJ. Evaluation of the Sensititre YeastOne antifungal susceptibility testing system. *J Clin Microbiol*. 2007;45(9):2869–73.
10. Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of yeasts. 4th ed. CLSI standard M27. Wayne (PA): Clinical and Laboratory Standards Institute; 2017.
11. Clinical and Laboratory Standards Institute. Performance standards for antifungal susceptibility testing of yeasts. CLSI supplement M60. Wayne (PA): Clinical and Laboratory Standards Institute.
12. Perlin DS. Echinocandin resistance in *Candida*. *Clin Infect Dis*. 2015;61 Suppl 6:S612–S617.
13. Pfaller MA, Castanheira M. Antifungal susceptibility testing and epidemiology of *Candida* species. *Clin Microbiol Rev*. 2016;29(3):566–91.
14. Wiederhold NP. Antifungal resistance in pathogenic fungi. *J Fungi (Basel)*. 2017;3(4):41.
15. Mesa-Arango AC, Scorzoni L, Zaragoza O. It only takes one to do many jobs: Amphotericin B as antifungal and immunomodulatory drug. *Front Microbiol*. 2012;3:286.
16. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, Reboli AC, Schuster MG, Vazquez JA, Walsh TJ, Zaoutis TE, Sobel JD. Clinical Practice Guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. *Clin Infect Dis*. 2016;62:e1–50.
17. 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.
18. Pfaller MA, Castanheira M, Messer SA, et al. Trends in antifungal susceptibilities of *Candida* spp. to fluconazole and voriconazole: global surveillance results. *J Clin Microbiol*. 2019;57(1):e01136–19.
19. Zhang C, Wu S, Chen X, Yang H, Feng W, Yuan T, Wang Y. Clinical manifestations and treatment of candidemia caused by different *Candida* species: a retrospective study. *BMC Infect Dis*. 2024;24:1234.
20. Odoj K, Garlasco J, Pezzani MD, Magnabosco C, Ortiz D, Manco F, Galia L, Foster SK, Arieti F, Tacconelli E. Tracking candidemia trends and antifungal resistance patterns across Europe: An in-depth analysis of surveillance systems and Surveillance studies. *J Fungi (Basel)*. 2024;10:685.
21. Díaz-García J, Machado M, Alcalá L, Reigadas E, Pérez-Ayala A, Gómez-García de la Pedrosa E, González-Romo F, Cuétara MS, García-Esteban C, Quiles-Melero I, Zurita ND, Muñoz-Algarra M, Durán-Valle MT, Sánchez-García A, Muñoz P, Escibano P, Guinea J; CANDIMAD study group. Trends in antifungal resistance in *Candida* from a multicenter study conducted in Madrid (CANDIMAD study): fluconazole-resistant *C. parapsilosis* spreading has gained traction in 2022. *Antimicrob Agents Chemother*. 2023;67:e0098623.
22. Pfaller MA, Diekema DJ, Turnidge JD, Castanheira M, Jones RN. Twenty Years of the SENTRY antifungal surveillance program: Results for *Candida* species from 1997–2016. *Open Forum Infect Dis*. 2019;6:S79–S94.
23. Khan Z, Ahmad S, Hagen F, et al. Invasive candidiasis in Pakistan: clinical characteristics, species distribution and antifungal susceptibility. *BMC Infect Dis*. 2015;15:356.
24. Kaur R, Dhakad MS, Goyal R, Haque F, Mukhopadhyay AK. Epidemiological and mycological characteristics of candidemia in patients with hematological malignancies attending a tertiary-care center in India. *Indian J Med Microbiol*. 2015;33(2):206–212.
25. Chowdhary A, Sharada K, Singh PK, Bhagwani DK, Kumar N, de Groot T, Meis JF. Outbreak of *Dirkmeia churashimaensis* fungemia in a neonatal intensive care unit, India. *Emerg Infect Dis*. 2020;26:764–768.
26. Zhou M, Li Y, Kudinha T, Xu Y, Liu Z. *Kodamaea ohmeri* as an emerging human pathogen: A review and update. *Front Microbiol*. 2021;12:736582.
27. Londero MR, Zanrosso CD, Corso LL, Michelin L, Soldera J. Catheter-related infection due to *Papiliotrema laurentii* in an oncologic patient: Case report and systematic review. *Braz J Infect Dis*. 2019;23:451–461.
28. Groenewald M, Daniel HM, Robert V, Poot GA, Smith MT. Polyphasic re-examination of *Debaryomyces hansenii* strains and reinstatement of *D. hansenii*, *D. fabryi* and *D. subglobosus*. *Persoonia*. 2008;21:17–27.