

Identification and Susceptibility Pattern of Various *Candida* Species Isolated from Different Clinical Samples in Rahim Yar Khan

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ABSTRACT

Objective: To isolate distinct *Candida* species from various clinical samples and examine their patterns of antifungal susceptibility.

Methodology: This prospective, cross-sectional study was carried out from January 2023 to December 2023 on 210 clinical samples positive for *Candida*, conducted in Pathology Laboratory of Sheikh Zayed Hospital, Rahim Yar Khan. The VITEK 2 compact system was utilized to identify the isolates and determine their antifungal susceptibility pattern.

Results: *Candida* isolates were analyzed, with *Candida albicans* being the most prevalent species at 64.8%. Antifungal sensitivity was highest for amphotericin, followed by caspofungin, fluconazole, flucytosine, and micafungin. Voriconazole was the least sensitive drug.

Conclusion: The study found that *Candida albicans* as most prevalent, followed by *Candida Guilliermondii*. Rare non-*albicans* included *Candida Glabrata*, *Candida Krusei*, *Candida Tropicalis*, *Candida Sake* and *Candida Intermedia*. Amphotericin has a strong antifungal sensitivity.

Key Words: Antifungal sensitivity, *Candida*, VITEK 2

Authors' Contribution:

^{1,2}Conception; Literature research; manuscript design and drafting; ^{3,4} Critical analysis and manuscript review; ⁵Data analysis; Manuscript Editing.

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Introduction

Fungal infections are a major health problem¹ with increasing incidence of sepsis.² Patients with underlying conditions that compromise immunity, such as preterm neonates, elderly, and hospitalized patients, can show up as a variety of clinical symptoms, from mild systemic/invasive infections to serious ones.³ *Candida species*, including *Candida albicans*, are one of the most communal roots of fungal ailments globally.⁴ Over the past 30 to 40

years, From *C. albicans* infections to non *albicans* infections, there has been a progressive shift, accounting for more than 50% of the total infections by *Candida Spp.*⁵ Candidemia is a significant healthcare-associated infection (HCAI) in many countries, with an upsurge in the occurrence of candida infections (0.74 to 6.0/1000 hospital admissions) in underdeveloped countries.⁶ The higher diagnostic cost results in delays in diagnosis and treatment, leading to high mortality rates (30 to 78%) in these countries.⁷ Five species including *C.*

C. albicans, *C. parapsilosis*, *C. krusei*, *C. glabrata*, and *C. tropicalis* are responsible for invasive infections.⁸ In most countries, factors like the use of antifungal drugs, outbreaks, and individual risk factors dictate the incidence of non-*albicans*.⁹ In South America, *C. albicans* is the most isolated *Candida species*, while *C. parapsilosis* is the widespread agent.¹⁰ In recent years, the emerging and spread of new opportunistic pathogen, *C. auris*, has been a concern due to its resistance to common antifungal agents, limited treatment options, and increased mortality rates (30-60%).¹¹ Factors contributing to the overgrowth of yeast biofilms include prolonged hospital stays, total parenteral feeding, and mechanical support.¹² Infections can also be exacerbated by other conditions, such as mucositis, cancer, neutropenia, organ transplants, and chronic diseases. The gastrointestinal and reproductive tracts of humans are home to the commensal opportunistic pathogen known as *Candida albicans*.¹³ It has a balance of growth in the normal flora, controlled by the immune system. Any disturbance can lead to invasive or systemic fungal diseases.¹⁴ Endogenous infections are caused by *Candida*'s intestinal presence, while nosocomial infections are closely related to the disease's ability to make biofilms boosting their innate resistance to immunological responses and antifungal medications.¹⁵ Biofilms can grow on medical devices or host tissues, leading to the dissemination of disease to other tissues.¹⁶ Morbidity and mortality can be reduced by early detection and treatment of *Candida* infection.¹⁷ Due to the high frequency of non-*albicans* infections and increasing resistance against fluconazole, the latest international guidelines strongly suggest preferring echinocandins over fluconazole as first line drugs for candidemia in all adults.¹⁸ However, antifungal susceptibility testing (AFST) of fluconazole is not being done in many patients due to limited resources and unavailability in regional laboratories.¹⁹ Untreated patients are also a major reason for high mortality.²⁰ The increasing diversity of *Candida species* and their changing resistance

patterns require rapid diagnosis and treatment.²¹ The current study designed to identify the distribution of various *Candida species* and their susceptibility pattern in Rahim Yar Khan.

Methodology

This prospective, cross-sectional investigation was conducted at the pathology laboratory of Sheikh Zayed Hospital Rahim Yar Khan, on 210 clinical samples that tested positive for *Candida* between January 2023 and December 2023. The sample size was determined using $n=p(1-p) (Z/E)^2$ with Lusitance (R) as 1.37%, required size of margin of error (E) as 1% and Confidence interval was 99%(Z). The clinical samples include urine, blood, sputum, high vaginal swabs, urinary catheter, oral swabs, broncho-alveolar lavage, nail scrapings, biopsy and corneal scrapings, processed using Bact/Alert 3D and Sabouraud Dextrose Agar (SDA) with Gentamicin. For 72 hours, the samples were inoculated and incubated at 35°C. Gram staining was performed using standard operating procedures. Using Sabouraud Dextrose Agar and colony shape and yeasty smell, 210 isolates were first recognized as fungi. The recognition of *Candida species* was done using germ tube tests and modern instruments to save time and cost.

The VITEK 2 compact system was used for identification of colonies from growth on SDA and AFST. Densi-Chek meters were used for inoculum suspensions and AFST. The VITEK 2 YST and AST-YS08 cards were automatically filled and incubated for at least 18 hours at 37°C. With the VITEK-2 compact system, the likelihood of accurately identifying every isolate to the species level was 90%. A standard strain of *Candida albicans* (ATCC 10231) was used to guarantee quality assurance.⁵ Patients with metabolic abnormalities and those who refused to participate were eliminated from the study, whereas male and female patients of all ages who showed signs and symptoms of fungal infections were included in the research.

Results

This study investigated the antifungal susceptibility of various *Candida species*. 210 samples were isolated from clinical specimens and tested for drug sensitivity using VITEK 2 AST cards. Results showed that 67% of the cases were male and 33% female. Pediatric medical wards received the majority of positive instances, with urology, nephrology, chest illness unit, dermatology outside, and main ICU coming in second and third, respectively. The most prevalent *Candida species* were *C. albicans*, *C. guilliermondii*, *C. ciferii*, and *C. parapsilosis* (table I).

Name of the <i>Candida</i> Isolate	No of Isolate Identified	Percentage Distribution
<i>C. albicans</i>	134	63.8%
<i>C. guilliermondii</i>	37	17.6%
<i>C. ciferii</i>	16	7.6%
<i>C. parapsilosis</i>	14	6.67%
*Others	5	2.3%
Unspecified/invalid	4	1.9%

Antifungal Drug	MICs	Sensitivity
Amphotericin B	≤0.25 >16 μg/ml	92.77%
Caspofungin	≤0.125 >8 μg/ml	90%
Fluconazole	≤0.5 >64 μg/ml	89%
Flucytosine	≤1 >64 μg/ml	83.11%
Micafungin	≤0.06 >8 μg/ml	81.67%
Voriconazole	≤0.125 >8 μg/ml	67.6%

Caspofungin had the best susceptibility against *C. albicans* and *C. glabrata*, followed by

Amphotericin B, Fluconazole, and Micafungin (chart 1 & 2). Overall, Amphotericin had the highest sensitivity to all *Candida species*, followed by Caspofungin, Fluconazole, and Micafungin. Voriconazole was the least sensitive drug (table II).

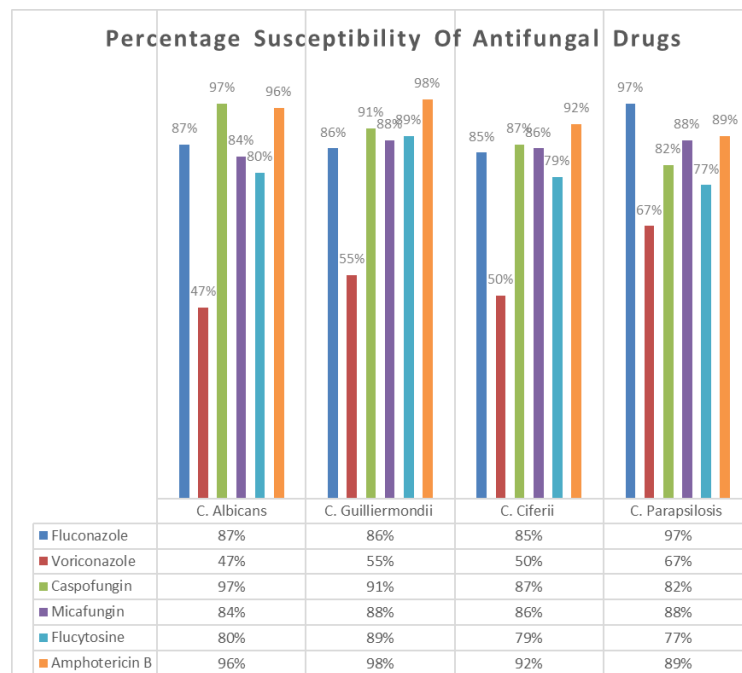


Chart 1: Candida Isolates' Antifungal Susceptibility Pattern (A)

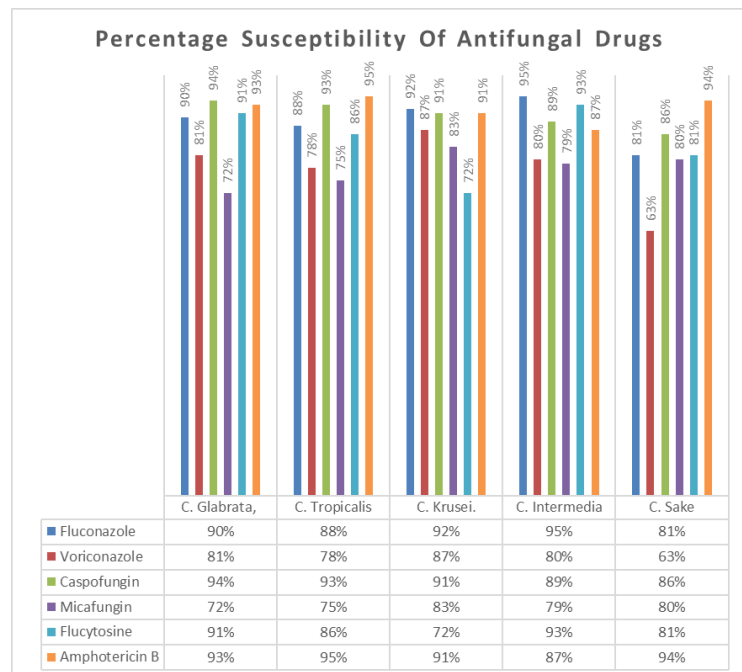


Chart 2: Pattern of Candida Isolate Susceptibility to Antifungals (B)

Discussion

Fungal illnesses resulting from *Candida albicans* and non-*albicans* have emerged as a significant public health issue, impacting 75% of females at some point in their lives.²² Antifungal resistance due to comorbid and immune suppression is increasing.²³ The 210 samples were isolated from clinical samples, and *C. albicans* were identified using germ tube tests, while non-*albicans* were tested using VITEK 2 compact systems and the results are discussed in relation to other researchers' work. This study analyzed 210 clinical samples from various departments of SZH RYK, focusing on the distribution of *Candida* isolates. Most *Candida* isolates were found in urine samples (49.52%), blood samples (24.76%), sputum (10.48%), high vaginal swabs (5.71%), urinary catheter (3.33%), and oral swabs (2.38%). The frequency distribution of patients of candidiasis was mainly male (67%), with females (57%) more affected than males (43%). The frequency of *Candida* species decreased with age, with more chances of candidiasis in older patients by non-*C. albicans*. Among the 210 *Candida* isolates, *C. albicans* was the most prevalent species, followed by *C. guilliermondii*, *C. ciferii*, and *C. parapsilosis* (14, 6.67%). Other distinguished species were *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. intermedia* and *C. sake*. Similar results were observed in a study, which was done by Khairat et al in 2021.²⁴ The most common non-*albicans* were *C. parapsilosis* (21.7%), *C. tropicalis* (15.5%), and *C. glabrata* (13.9%). Few other studies suggest that *C. parapsilosis* was (28.6%) the most prevalent non *albicans*.¹⁰ Caspofungin had the best susceptibility against *C. albicans* (97%), followed by Amphotericine B (98%), Fluconazole (97%). Voriconazole (67.6%) was the least effective in vitro activity. Rodrigues et al. reported *C. glabrata* emergence with high MICs to fluconazole and voriconazole,² in another study while non-*albicans* showed higher resistance to fluconazole and voriconazole, while Amphotericin B was sensitive to non-*albicans*.²⁵ 96% of the *Candida*

isolates were susceptible to both Micafungin and Caspofungin, however Micafungin worked better than Caspofungin. Similar to the findings of this investigation, 86.2% of yeasts were found to be susceptible to flucytosine in other related studies.⁵

Conclusion

The study found *C. albicans* to be the most prevalent species, followed by *C. guilliermondii* (17.6%). Rare non-*albicans* included *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. intermedia* and *C. sake*. The VITEK 2 compact system showed minimal invalid results, but insufficient resistance isolates. Amphotericin had the highest antifungal sensitivity, with low drug resistance.

Recommendation: A multidimensional strategy for controlling emerging fungal infections involves early identification, surveillance, registration, strict SOPs, training, capacity building, centralized networking, and modern technology for effective treatment.

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