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Speciation and Antifungal Susceptibility Testing of *Candida* Isolates from Urine Samples of Patients Admitted in ICU's

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Abstract: ***Background: The presence of Candida species in urine is rarely encountered in healthy people. However, it is of common occurrence in hospitalized patients and in critically ill patients and may also be life-threatening. The immune status of ICU patients is always on the weaker side which is beneficial for the Candida species to cause infections in these patients. Objective: The aim of the study is to identify and speciate Candida species in urine of patients admitted in Intensive Care Units, and determine the antifungal susceptibility testing for the different species identified. Results: Among the 82 samples, C. tropicalis was the most prevalent species (n=30), followed by C. glabrata (n=19) and C. albicans (n=16). Notably, C. krusei exhibited 100% resistance to fluconazole. While C. tropicalis and C. glabrata showed moderate resistance, C. guilliermondii and C. parapsilosis remained highly susceptible to most antifungals. Conclusion: The growing trend towards NAC species among ICU patients with candiduria is a pointer towards the need for improved diagnosis and treatment protocols. An effective antifungal stewardship program and de-escalating therapy are absolutely necessary in the management of resistant Candida infection, preventing hospital stay, and enhancing patient outcomes.***

Keywords: *Candia spp.* Urinary tract infections (UTIs), Antifungal resistance, ICUs, Urinary catheter

1. Introduction

Candida-associated urinary tract infections (UTIs) have become a notable concern since they now represent about 10-15% of all nosocomial urinary infections, especially in critically ill and immunocompromised patients *(Berrouane et al., 1999)*. The urinary tract is a common site of Candida colonization, and long-term catheterization, immunosuppressive treatment, and use of broad-spectrum antibiotics greatly increase the risk of infection. Traditionally, *Candida albicans* was the major fungal pathogen, but recent reports indicate a dramatic epidemiological transition to non-albicans Candida (NAC) species, such as *C. glabrata, C. tropicalis,* and *C. auris,* most of which have decreased susceptibility to fluconazole, the most widely employed antifungal drug in clinical practice *(Juan Camilo Hernández-Pabón et al., 2024)*. This development poses a critical clinical problem because NAC species usually harbour intrinsic mechanism of resistance, rendering standard antifungal therapy ineffective and the risk of treatment failure, increased hospital stay, and increased mortality in ICU patients higher *(Vermitsky & Edlind, 2004)*.

In spite of the clinical importance of Candida UTIs, identification of Candida species in the urine by the laboratory is poor in most healthcare facilities. A majority of health facilities use only culture-based diagnostic methods, which are not good enough to identify closely related species or antifungal resistance patterns, thereby causing suboptimal management of the patient *(Azim & Ahmed, 2024)*

Candida infections among ICU patients are a growing problem because of their increasing incidence, growing antifungal resistance, and diagnostic difficulty. Of these, *Candida*-associated urinary tract infections (UTIs) have become a notable concern since they now represent about 10-15% of all nosocomial urinary infections, especially in critically ill and immunocompromised patients *(Berrouane et al., 1999)*.

Traditionally, *Candida albicans* was the major fungal pathogen, but recent reports indicate a dramatic epidemiological transition to non-albicans Candida (NAC) species, such as *C. glabrata C. tropicalis,* and *C. auris,* most of which have decreased susceptibility to fluconazole, the most widely employed antifungal drug in clinical practice *(Juan Camilo Hernández-Pabón et al., 2024)*. This development poses a critical clinical problem because NAC species usually harbour intrinsic mechanism of resistance, rendering standard antifungal therapy ineffective and the risk of treatment failure, increased hospital stay, and increased mortality in ICU patients higher *(Vermitsky & Edlind, 2004)*.

A majority of health facilities use only culture-based diagnostic methods, which are not good enough to identify closely related species or antifungal resistance patterns, thereby causing suboptimal management of the patient *(Azim & Ahmed, 2024)*. The recent development of multidrug-resistant *C. auris,* in fact, introduced a new dimension of complexity, as it has been linked to ICU outbreaks, typically with resistance to fluconazole, echinocandins, and amphotericin B *(Piatti et al., 2021)*.. Considering the existing gaps in knowledge, most notably concerning rising rates of drug-resistant NAC species among urinary tract infections, this research is not only timely but also of utmost importance to maximizing antifungal stewardship in ICUs. The findings of this study will not only be helpful in the understanding of *Candida* epidemiology but also serve as a foundation for developing new clinical recommendations for effective management of *Candida*-related UTIs in ICU units *(Nascimento et al., 2024)*.

2. Materials and Method

Study Setting

Present prospective study was conducted over a period of four month, from January 2024 to April 2024, at a tertiary care hospital located in a central India. The study is designed to identify and speciate *Candida* species isolated from urine samples of patients admitted to Intensive Care Units (ICUs)

and to perform antifungal susceptibility testing of these isolates.

Study Population and Criteria Used Inclusion Criteria
This study includes all urine samples collected from patients admitted to the Intensive Care Unit (ICU). Only samples where microscopy showed more than seven leucocytes cells per high-power field (HPF) on direct examination and where yeast is isolated as a pure growth will be processed further for *Candida* species identification.

Exclusion Criteria
Urine samples that do not contain pus cells upon microscopic examination are excluded from the study. Additionally, samples that show mixed microbial growth, rather than a pure Candida isolate, are not considered for further analysis.

Sample Collection and Processing
A total of 1294 urine samples collected from patients admitted to various ICU setting suspected of having urinary tract infections (UTIs) or those with risk factors for Candiduria, out of which 82 urine samples were shown to have presence of *Candida* species. Midstream clean-catch urine was collected in sterile containers for catheterized patients; urine samples were obtained aseptically from the catheter collection port using a sterile syringe. Each sample was labelled with the patient's registration number and transported to the microbiology laboratory within one hour of collection to ensure sample integrity.

Processing and Identification of Candida Species
Briefly, Urine samples were subjected to routine culture and sensitivity testing as per standard microbiological protocols. Samples were inoculated on CLED agar and incubated at 37°C for 24-48 hours. Colonies exhibiting yeast-like morphology were subjected to Gram staining and confirmed via germ tube test, Chromogenic Candida Agar and Corn meal agar.

Further speciation was performed using biochemical tests, including sugar assimilation and fermentation tests, as well as automated identification systems such as VITEK-2. Additionally, CHROM Agar for Candida differentiation (HiMedia, Mumbai) was utilized to identify non-albicans *Candida* species.

Antifungal Susceptibility Testing
Antifungal susceptibility was determined using by disc diffusion for fluconazole, itraconazole and amphotericin B was assessed to guide appropriate therapy as per CLSI M44.

All procedures were carried out in a biosafety level II laboratory following strict aseptic techniques.

3. Results

A total of 1294 urine samples collected from patients admitted to various ICU setting out of which 82 urine samples showed presence of budding yeast cell s.

1) Gender distribution- Study population comprised 47 females and 35 males, indicating a slightly higher prevalence of candiduria among female ICU patients.
2) ICU distribution-Trauma ICU (TICU) (n=29), Surgical ICU (SICU) (n=16), and Medical ICU (MICU) (n=37).

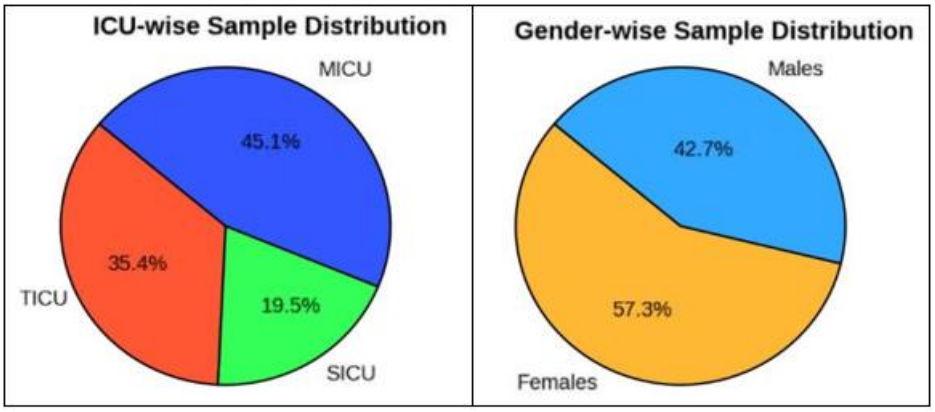

Figure 1: Overview of sample distribution used in this study

3) Candida Species Distribution
Among the 82 urine samples, six different *Candida* species were identified, with *Candida tropicalis* being the most prevalent (n=30), followed by *Candida glabrata* (n=19), *Candida albicans* (n=16), *Candida krusei* (n=7), *Candida guilliemondii* (n=6), and *Candida parapsilosis* (n=4).

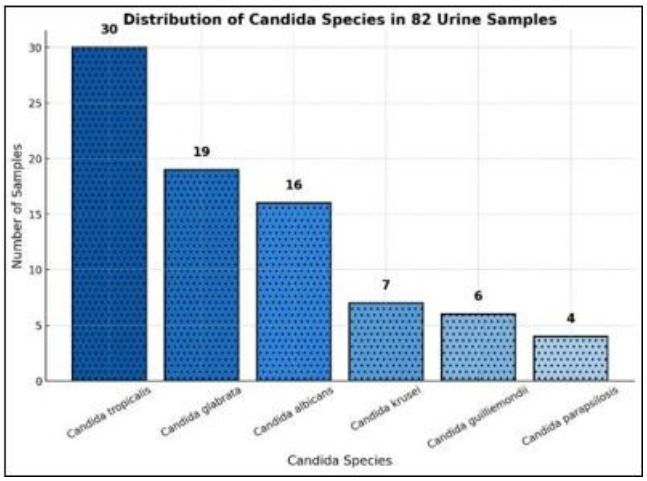

Figure 2: Candida species prevalence in collected urine specimen

4) Risk Factors Associated-

Risk Factors	Number (n=82) (%)
Duration of ICU stay (>48 hrs)	32(39%)
Urinary Catheter	54(66%)
Diabetes Mellitus	72(88%)
Antibiotic Use	63(77%)
Steroid Use	12(15%)

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1) Antifungal Susceptibility Testing Patterns

Susceptibility testing was conducted for Fluconazole, Itraconazole, and Amphotericin B, revealing varied resistance patterns among the isolates. *Candida tropicalis* demonstrated a high susceptibility to Fluconazole (85%), Itraconazole (81%), and Amphotericin B (88%), with resistance rates of 15%, 19%, and 12%, respectively. *Candida albicans* exhibited similar susceptibility rates across all three antifungals: Fluconazole (86%), Itraconazole (86%), and Amphotericin B (94%), with low resistance rates of 14%, 14%, and 6%.

Candida parapsilosis was entirely susceptible to Fluconazole and Itraconazole (100%) but showed some resistance (25%) to amphotericin B. *Candida glabrata* showed lower susceptibility to Fluconazole (78%) and Itraconazole (81%) but had a high susceptibility rate to Amphotericin B (91%). *Candida krusei* displayed intrinsic resistance to fluconazole (100%) and moderate susceptibility to Itraconazole (33%) and amphotericin B (83%). *Candida guilliermondii* exhibited complete susceptibility to fluconazole and amphotericin B but moderate resistance to itraconazole (22%).

The study results show that ICU patients with candiduria mostly harbor *C. tropicalis* and *C. glabrata* despite the historical *C. albicans* dominance. The study reveals *C. krusei* has the strongest resistance to antifungal drugs while demonstrating highest resistance specifically to fluconazole drug. Additionally, *C. guilliermondii* and *C. parapsilosis* show high sensitivity to multiple antifungal treatments. This study demonstrates how regular susceptibility assessments will help doctors select appropriate therapies because non-albicans *Candida* species continue displaying increasing fluconazole resistance. The analysis signals the essential function of ICU antifungal management systems for delivering optimal patient treatment results.

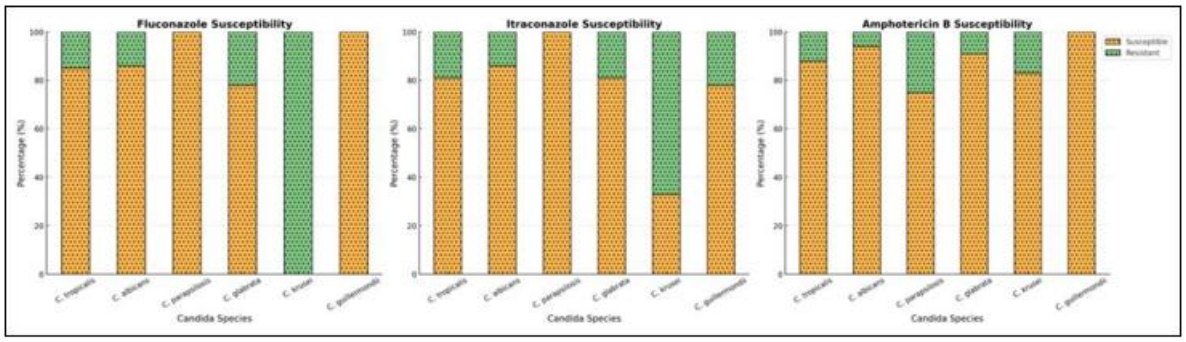

Figure 3: Antifungal Susceptibility Pattern of Candida SPP.

4. Discussion

Intensive care units now face a major care challenge with candiduria cases because the spread of non-albicans Candida species introduces complexity in treatment through their different antifungal drug responses. Our findings matched recent research which shows that hospital patients in intensive care units display emerging NAC species dominance over C. albicans through the prevalence of Candida tropicalis and Candida glabrata *(Hernández-Pabón et al., 2024; Nascimento et al., 2024).* Epidemiological research showing NAC species resist fluconazole treatment requires healthcare institutions to develop new treatment plans *(Singhal et al., 2024).*

North India has previously shown that catheter-associated urinary tract infections mostly involve NAC species including C. tropicalis which constitutes 36.5% of the isolates *(Singhal et al., 2024).* Data reveals C. glabrata occupies the second place among isolated species (23.1%) while exhibiting increased resistance to fluconazole primarily due to elevated expression of multidrug transporters and efflux pumps *(Vermitsky & Edlind, 2004).* Antifungal testing results showed C. glabrata had reduced fluconazole susceptibility at 78% while still demonstrating high susceptibility to amphotericin B at 91% when compared to Portugal's ICU surveillance data *(Nascimento et al., 2024).*

The study findings demonstrated complete resistance of *C. krusei* to fluconazole treatment due to its inherent drug

tolerance. Early identification of pathogenic fungi becomes essential because their decreased sensitivity to itraconazole at 33% compounds treatment limitations *(Piatti et al., 2021).* C. auris has been detected more frequently in ICUs because of its multidrug-resistance pattern despite not being present in our patient sample *(Piatti et al., 2021).* The complete susceptibility of *C. parapsilosis* and *C. guilliermondii* to fluconazole and amphotericin B treatment introduces successful treatment possibilities. The resistance patterns of C. parapsilosis highlight the importance of continuous healthcare surveillance because it demonstrates resistance to amphotericin B treatment in 25% of cases *(Azim & Ahmed, 2024).* Resistant patterns of the NAC species toward fluconazole have been escalating while resistance rates span from 15% (C. tropicalis) to 100% *(C. krusei)* essentially following worldwide patterns *(Berrouane et al., 1999; Hernández-Pabón et al., 2024).* Prior administration of fluconazole in ICU patients on prolonged antifungal prevention creates resistance in the hospital environment. The antifungal properties of itraconazole against NAC species were moderate as *C. tropicalis* (81%) and *C. glabrata* (81%) demonstrated peak susceptibility. The antifungal agent amphotericin B demonstrated maximum effectiveness against all species of yeast with the exception of *C. parapsilosis* where its efficacy reached 75% (Nascimento et al., 2024). The observation of these results should prompt hospitals to reevaluate the choice of fluconazole as an empirical treatment in ICUs which predominantly harbour NAC species.

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The research data revealed that extended catheterization duration combined with previous antibiotic consumption and active suppressive therapy directly contributed to candiduria development according to Kaur et al. (2024). Biofilm formation played an important role in the growth of the *C. tropicalis* and *C. glabrata* strains as researcher *Malinovská et al., 2023* previously demonstrated biofilm-associated resistance mechanisms that make therapy challenging. The MIC values of fluconazole were higher in biofilm-producing isolates which reinforces the necessity for serious catheter management strategies that include antifungal lock therapy for infections related to catheters. ICU surveillance studies by *Lamoth et al., 2018* demonstrate the increase of echinocandin resistance in *C. glabrata* and *C. krusei* found among critically ill patients. The data shows that many ICU patients with candiduria have no apparent symptoms which necessitates proper distinction between skin colonization and actual infection. Antifungal treatment of asymptomatic cases leads to waste of medication and promotes fungal resistance *(Jacobs et al., 2018).* Molecular testing through PCR and MALDI-TOF facilitates doctors to distinguish between invasive pathogenic infections and harmless colonizations so they can intervene at the appropriate time (*Delavy et al., 2019).*

Antifungal stewardship programs prove essential for immediate implementation throughout intensive care units. Surveillance of candiduria must become mandatory for nosocomial infection control programs since laboratory testing of detected species helps healthcare providers select appropriate antifungal medication *(Singhal et al., 2024).* Culture-based methods show their limitations to correctly identify Candida species according to this research which makes molecular diagnostics necessary for resistant detection and exact species identification *(Azim & Ahmed, 2024).* ICUs should conduct ongoing tests for emerging resistant strains of C. auris because of its worldwide rising multidrug resistance *(Piatti et al., 2021).*

The findings of antifungal susceptibility testing of Candida species in urine specimens from ICU patients highlight critical risk factors such as prolonged ICU stay, urinary catheterization, diabetes mellitus, antibiotic use, and steroid administration. In the present study, 39% of patients had ICU stays exceeding 48 hours, a significant risk factor for nosocomial fungal infections, as prolonged hospitalization increases exposure to resistant pathogens *(Nascimento et al., 2024)*. Urinary catheterization was prevalent in 66% of patients, a well-known contributor to Candida colonization and infection in ICU settings, as biofilm formation on catheters enhances antifungal resistance *(Zareshahrabadi et al., 2024).* Diabetes mellitus, observed in 88% of cases, is another major predisposing factor due to hyperglycemia-induced immune dysfunction, which facilitates fungal growth and persistence. Additionally, 77% of patients had prior antibiotic exposure, which disrupts normal flora and promotes fungal overgrowth, a trend consistent with global reports emphasizing the role of broad-spectrum antibiotics in Candida infections. Notably, steroid usage, though less frequent (15%), remains a concerning factor, as it suppresses immune responses and facilitates fungal dissemination, particularly in critically ill patients *(Loh & Lam, 2023* Given these risk factors, antifungal susceptibility testing is crucial for guiding targeted therapy, reducing resistance development, and improving patient outcomes. The increasing prevalence of non-albicans Candida species with reduced susceptibility to fluconazole underscores the necessity of continuous surveillance and tailored antifungal strategies in ICU settings.

5. Conclusion

The increase of *Candida* infections in intensive care unit patients who develop urinary tract infections (UTIs) presents an expanding incidence of non-albicans Candida (NAC) species rather than Candida albicans. The data demonstrates that *Candida tropicalis* and *Candida glabrata* control the infection landscape alongside *Candida krusei* which shows full resistance to fluconazole. The observed resistance patterns demonstrate how regular antifungal treatments lose their effectiveness which requires immediate adoption of well-established antifungal stewardship systems. ICU patients face augmented risks of death combined with longer hospital stays through inaccurate species diagnosis coupled with improper medical treatment. The worldwide pattern of developing antifungal resistance requires healthcare professionals to implement a collaborative approach in the management of *Candida* UTIs. Within critical care areas the prevention of resistant *Candida* strain spread requires prompt species identification and susceptibility testing along with strict infection prevention strategies. New research must investigate innovative therapeutic methods that pair antifungal treatment with catheter infection control measures to combat developing fungal resistance patterns. Improving patient results and controlling the escalating *Candida*

infection threat requires addressing all current challenges within ICU settings.

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