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Candiduria: Analysis of Changing Trends and Antifungal Dynamics in Acute Healthcare Settings with Special Reference to Rare *Candida* Species

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Background: Candiduria is a common phenomenon among hospitalized patients, particularly those in intensive care units (ICUs), wherein multiple predisposing factors such as comorbidities, indwelling urinary catheters, and antimicrobial exposure contribute to its prevalence.

Objective: This study aims to investigate the epidemiological trends and antifungal susceptibility patterns of *Candida* spp. isolated from urine samples of patients at a tertiary care center in Western Maharashtra, India.

Methods: Of the 7,642 urine samples received between 01 June 2022 to 31 May 2023, 142 samples (9%) displayed the growth of *Candida* species. Upon retesting samples from these patients, only 70 true positive results were identified. All the isolates were detected via VITEK MS (bioMerieux, France), while antifungal susceptibility testing (AFST) was conducted using the VITEK 2 Compact with an AST-YS08 card.

Results: The most common isolate was *Candida tropicalis* (50%), followed by *Candida albicans* (25.7%). Rare *Candida* species (e.g., *Candida ciferrii*, *Candida kefyr*, *Candida metapsilosis*, *Candida utilis*) collectively contributed to 7.1% of all *Candida* species. The results of the AFST revealed a high sensitivity to amphotericin B and echinocandins. Fluconazole resistance was observed in 6% of the isolates. *Candida ciferrii* demonstrated a distinct pattern, indicating a statistically significant association ($p = 0.015$) when the patient had two or more comorbidities and resistance to azoles, which was statistically significant (p-values for fluconazole ($p = 0.015$), voriconazole ($p = 0.004$), and flucytosine ($p = 0.002$)) when compared with other *Candida* species.

Conclusion: Resistance trends in India have been changing with increased fluconazole-resistant *Candida* strains being frequently isolated, emphasizing the importance of customized therapeutic strategies based on culture and sensitivity reports.

Key Words: *Candida ciferrii*, *Candida ciferrii* resistance, Candiduria, Fluconazole resistance, Rare *Candida* isolates, UTI

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INTRODUCTION

Candiduria, a relatively rare occurrence in healthy individuals, has become a common phenomenon among hospitalized patients, particularly those in ICUs, wherein multiple predisposing factors, such as diabetes mellitus, indwelling urinary catheters, and antimicrobial exposure, contribute to its prevalence. Asymptomatic candiduria does not require treatment, except in patients with specific conditions that may predispose them to candidemia or disseminated infections[1,2].

Urinary tract infections (UTIs) are classified anatomically as upper UTI (e.g., pyelonephritis) or lower UTI (e.g., cystitis, urethritis). Although *Candida* cystitis presents as an increased frequency of urination, hematuria, and dysuria, most cases of *Candida* pyelonephritis present only as fever and candiduria[1]. In acute healthcare settings, diagnosing and managing candiduria present challenges due to the lack of reliable tests to differentiate between infection and colonization. The Infectious Diseases Society of America provides guidelines for the management of candiduria, based primarily on expert opinions and anecdotal reports. Fluconazole is recommended as the preferred treatment unless resistance is present or the patient has renal impairments[2,3]. However, the emergence of several rare non-albicans *Candida* (NAC) species, such as *Candida auris*, *Candida lusitaniae*, *C. kefyr*, *C. metapsilosis*, *C. ciferrii*, and *C. utilis* in urine samples, poses a challenge to the efficacy of empirical fluconazole treatment, given their intrinsic resistance or susceptibility only to high doses. *Candida ciferrii*, though isolated in small numbers in various human infections, has consistently shown resistance to fluconazole as per the literature worldwide. *Candida auris* is a multidrug-resistant pathogen with limited treatment options[4-8].

This study aims to assess the prevalence of UTIs caused by *Candida* spp. in a tertiary care center in Western Maharashtra. Additionally, it aims to identify any shifts in epidemiological trends among patients in acute healthcare settings, focusing on susceptibility patterns, with a special emphasis on UTIs caused by rare *Candida* uropathogens.

MATERIALS AND METHODS

This observational, cross-sectional study was conducted at the Department of Microbiology in a tertiary care center located in Pune, India, from 01 Jun 2022 to 31 May 2023. The inclusion criteria encompassed all urine samples exhibiting *Candida* spp. growth, as shown in Fig. 1. To eliminate the possibility of contamination and colonization, fresh samples were obtained and processed on the same day. For the catheterized patients, repeat samples were obtained post-catheter change. All patients were suspected cases of urinary tract infections.

Suspected UTI: defined as any patient admitted in acute healthcare settings.

1. With symptoms of increased frequency of urination, dysuria, cloudy urine, and abdominal pain **or**
2. Unexplained fever in an already catheterized patient with/without increased urine output and raised WBC count

Exclusion criteria: asymptomatic candiduria patients.

Sample collection: as per the standard protocol, urine specimens were collected in 50 mL sterile universal containers from both catheterized and non-catheterized patients. All samples were promptly transported to the laboratory, and processing commenced within one hour of collection[9].

Sample processing: all samples were inoculated on Cysteine Lactose Electrolyte Deficient (CLED) agar and incubated at 37℃ for 14~16 h. Following culture inoculation, a urine wet mount preparation was prepared after centrifugation at 3,000 rpm for 15 min to check for pus cells, RBCs, casts, and fungal elements. The growth on CLED was confirmed via gram staining (budding yeast-like cells) and subjected to further analysis using VITEK MS and VITEK 2 Compact. AFST was performed in accordance with the Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI M27M44S 3rd edition)[10].

1. VITEK MS (MALDI-TOF MS) identification[11]

The isolates were identified using VITEK MS (bioMerieux SA, France) as per the MALDI-TOF MS principle. The isolates were smeared onto the sample spots on the target slide, and 1 µL VITEK MS-CHCA matrix was applied over the sample and air-dried until both the matrix and sample co-crystallized. This was followed by the addition of 1 µL formic acid and air-drying. The target slide was loaded into the VITEK MS system to acquire the mass spectra, and data were compared with known mass spectra contained in the comprehensive database.

2. VITEK 2 susceptibility testing[11]

The isolates were subjected to AFST using VITEK 2 Compact (bioMerieux SA, France). From the overnight growth, a

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suspension of 1.8~2.2 McFarland was made, and 20 μL of the suspension was loaded onto the VITEK 2 AST-YS08 card. The sealed cards were incubated at 35℃ for a duration of 18 h. During the entire incubation period, data were collected at 15 min intervals, and the break point minimum inhibitory concentration values were used to categorize the *Candida* species into susceptible, intermediate, or resistant organisms. A total of six antifungals were included in the card, namely, fluconazole, caspofungin, voriconazole, micafungin, amphotericin B, and flucytosine. The CLSI M27M44S guidelines do not recommend the routine reporting of amphotericin B and echinocandins in urine samples; however, they were included in this study for statistical purposes. Quality control was done on a weekly cycle with a known standard strain of *Candida*.

1) Definition of rare isolates

Individually, the *Candida* species contributed to less than 3% of the total prevalence of *Candida* UTI globally. These include *C. auris*, *Candida ciferrii*, *C. kefyr*, *C. metapsilosis*, and *C. utilis*.

3. Statistical analysis

The data collected were entered into an MS Excel master sheet. The data were tabulated and analyzed using the OpenEpi software version 3.01 and Statistical Package for Social Sciences version 22. The categorical data were presented as numbers and percentages (%), whereas the quantitative data were presented in terms of mean and standard deviation. The categorical variables were analyzed using Pearson's chi-square test and Fisher's exact tests (when the expected count of 20% of cells is less than 5). Statistical significance was set at a p-value of < 0.05.

RESULTS

In a year-long observational and cross-sectional study conducted at the Department of Microbiology in a tertiary care center in Pune, India, from June 1, 2022, to May 31, 2023, a total of 7,642 urine samples were obtained, of which 1,576 exhibited growth. Of these, 142 samples tested positive for *Candida* spp., and a repeat sample was requested. Out of a total of 90 repeat samples obtained for repeat testing, only 70 were confirmed as true positive for *Candida* based on urine direct microscopy and cultural characteristics (re-isolation of the same species) (Fig. 1).

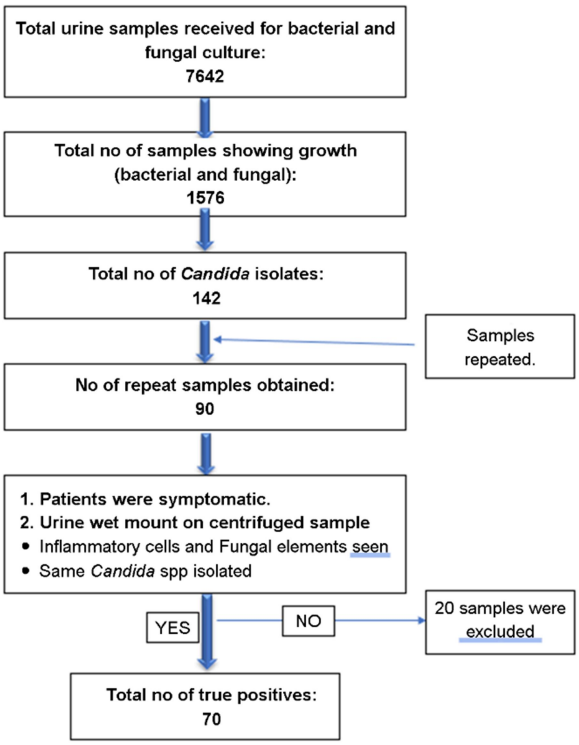

Fig. 1. Study enrolment and sample size

The mean age of the study participants was 54.9±23.6 years, with half of the participants (35 cases, 50%) being over 60 years old. More than two-thirds (48 cases, 68.6%) of the study participants were male and the majority of the isolates originated from medical ICU (44 cases, 62.9%), followed by medical wards (14 cases, 20%). More than half of the study participants (37 cases, 52.9%) had an indwelling urinary catheter, while 42.9% (30 cases) had one or more comorbidities (e.g., diabetes mellitus, hypertension, chronic liver disease, chronic kidney disease), of which 21 cases (30%) had a single comorbidity and the remaining 9 cases (12.9%) had two or more comorbidities.

Candida tropicalis (50%) was the most common type of *Candida* spp., followed by *C. albicans*, which constituted 25.7% of all cases. Other notable species include *Candida parapsilosis* (8.6%), *Candida guilliermondii* (4.3%), and *C. lusitaniae* (4.3%). Moreover, less common *Candida* spp., namely, *C. ciferrii*, *C. kefyr*, *C. metapsilosis*, and *C. utilis*, contributed to 2.9%, 1.4%, 1.4%, and 1.4% of all cases, respectively.

All the rare *Candida* isolates had no gender predilection, affecting males and females equally. The location of admission revealed no significant differences between rare *Candida* spp. and other species across various units, including acute

medical ward, medical ICU, neonatal ICU, pediatric ICU, and surgical ICU, but all patients had at least one comorbidity. *Candida ciferrii* demonstrated a distinct pattern, showing a statistically significant association ($p = 0.015$) when patients had two or more comorbidities (Table 1) and pan-resistance to azoles, which was statistically significant (*p*-values for fluconazole ($p = 0.015$), voriconazole ($p = 0.004$), and flucytosine ($p = 0.002$)) when compared with other *Candida* spp. (Table 2).

Table 1. Comparison of *Candida ciferrii* and other *Candida* species

Parameter	*Candida* species		*p*-value
	Candida ciferrii	Other *Candida* species	
Gender			
Female	1	21	0.533
	50.0%	30.9%	
Male	1	47	
	50.0%	69.1%	
Location of admission			
Acute medical ward	1	17	0.809
	50.0%	25.0%	
Medical ICU	0	21	
	0.0%	30.9%	
Neonatal ICU	0	2	
	0.0%	2.9%	
Paediatric ICU	0	7	
	0.0%	10.3%	
Surgical ICU	1	21	
	50.0%	30.9%	
Number of comorbidities			
None	0	40	**0.015**
	0.0%	58.8%	
One	0	21	
	0.0%	30.9%	
Two or more	2	7	
	100.0%	10.3%	
Total	2	68	
	100.0%	100.0%	

AFST was performed on all isolates with fluconazole, voriconazole, and flucytosine. AFST with amphotericin B was conducted on 68 isolates reported by Vitek, and with caspofungin and micafungin on 66 isolates each. It was observed that the highest drug sensitivity was observed with amphotericin B (66/68 cases, 97.1%), caspofungin and micafungin (62/64 cases, 96.9%), and flucytosine (66/70 cases, 94.3%), followed by voriconazole (66/70 cases, 92.9%) and fluconazole (87.1%). For a better representation, the six drugs have been split into commonly used antifungals, namely, azoles and flucytosine (Fig. 2), and higher antifungals, namely, echinocandins and amphotericin B.

Candida albicans has exhibited notable sensitivity, with percentages of 94.4%, 94.4%, and 100.0% for fluconazole, voriconazole, and flucytosine, respectively. Conversely, *C. ciferrii* showed complete resistance to all three antifungals. *Candida guilliermondii* showed sensitivity rates of 100.0%, 66.7%, and 66.7% for fluconazole, voriconazole, and flucytosine. *Candida kefyr* and *C. utilis* were 100% fully sensitive (100.0%) to all drugs. *Candida parapsilosis* demonstrated

Table 2. Comparison of *Candida ciferrii* and other *Candida* species with drug susceptibility

Drug susceptibility	*Candida* species		*p*-value
	Candida ciferrii	Other *Candida* species	
Fluconazole			
Resistant	2	7	**0.015**
	100.0%	10.3%	
Sensitive	0	61	
	0.0%	89.7%	
Voriconazole			
Resistant	2	3	**0.004**
	100.0%	4.4%	
Sensitive	0	65	
	0.0%	95.6%	
Flucytosine			
Resistant	2	2	**0.002**
	100.0%	2.9%	
Sensitive	0	66	
	0.0%	97.1%	

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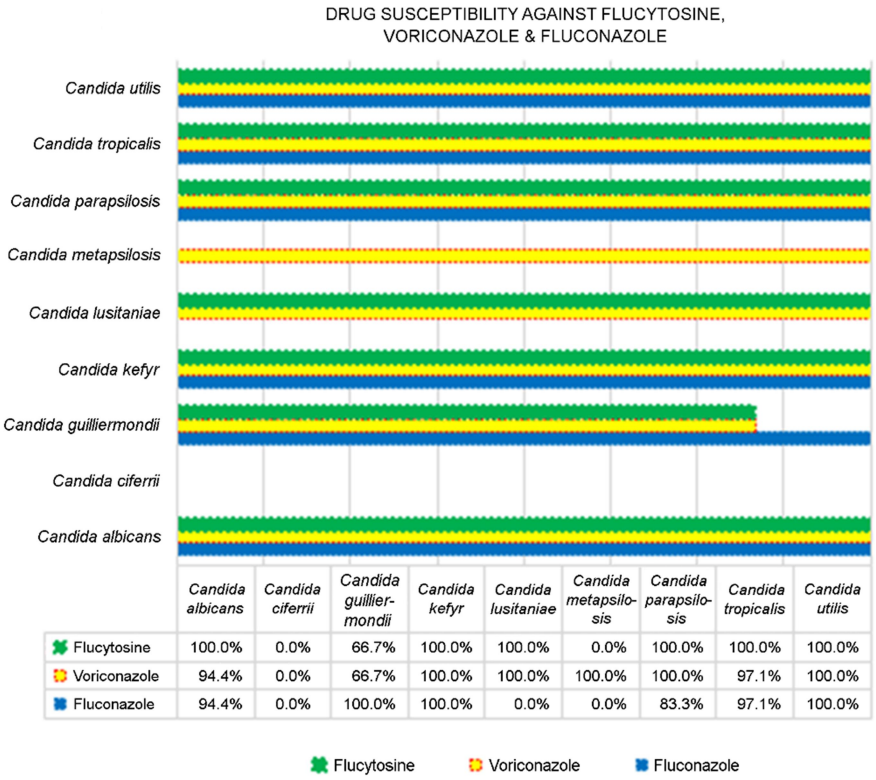
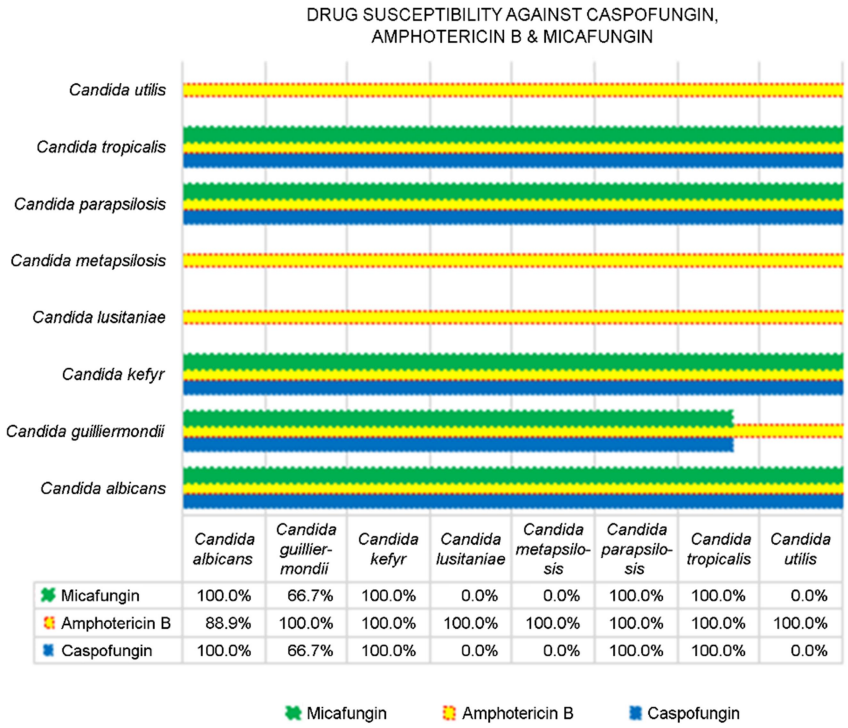


	Candida albicans	Candida ciferrii	Candida guillier-mondii	Candida kefyr	Candida lusitaniae	Candida metapsilo-sis	Candida parapsilo-sis	Candida tropicalis	Candida utilis
Flucytosine	100.0%	0.0%	66.7%	100.0%	100.0%	0.0%	100.0%	100.0%	100.0%
Voriconazole	94.4%	0.0%	66.7%	100.0%	100.0%	100.0%	100.0%	97.1%	100.0%
Fluconazole	94.4%	0.0%	100.0%	100.0%	0.0%	0.0%	83.3%	97.1%	100.0%

Fig. 2. Drug susceptibility of *Candida* species against flucytosine, voriconazole & fluconazole



	Candida albicans	Candida guillier-mondii	Candida kefyr	Candida lusitaniae	Candida metapsilo-sis	Candida parapsilo-sis	Candida tropicalis	Candida utilis
Micafungin	100.0%	66.7%	100.0%	0.0%	0.0%	100.0%	100.0%	0.0%
Amphotericin B	88.9%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Caspofungin	100.0%	66.7%	100.0%	0.0%	0.0%	100.0%	100.0%	0.0%

Fig. 3. Drug susceptibility of *Candida* species (except *Candida ciferri*) against caspofungin, amphotericin B & micafungin

100% sensitivity to flucytosine and voriconazole but susceptibility (83.3%) to fluconazole. *Candida lusitaniae* showed susceptibility to voriconazole and flucytosine but resistance to fluconazole. *Candida metapsilosis* was susceptible to voriconazole and resistant to fluconazole and flucytosine. *Candida tropicalis* showed high susceptibility, with percentages of 97.1%, 97.1%, and 100.0% for fluconazole, voriconazole, and flucytosine, respectively (Fig. 2).

Candida albicans exhibited high susceptibility to all six antifungals, with complete susceptibility of 100.0% to caspofungin and micafungin, and 88.9% to amphotericin B. *Candida guilliermondii* exhibited variable susceptibility rates, with 66.7% for caspofungin and micafungin, as well as complete susceptibility to amphotericin B. *Candida kefyr*, *C. parapsilosis*, and *C. tropicalis* demonstrated full susceptibility (100.0%) to all three antifungals, while *C. lusitaniae* and *C. metapsilosis* exhibited no susceptibility (0.0%) to caspofungin and micafungin. Similarly, *C. utilis* also showed susceptibility only to amphotericin B (Fig. 3). Furthermore, though prevalent globally, the *Candida auris* and *Candida glabrata* species were not isolated in our study.

DISCUSSION

The Catch-22 in diagnosis

Diagnosing bacterial UTIs in symptomatic patients relies on key factors, such as pyuria and quantitative measures (CFUs /mL of urine). However, the same criteria do not apply to *Candida* infections. Studies have not conclusively determined the significance of pyuria or quantitative urine cultures in *Candida*-related UTIs. Attempting to rely on the presence of pseudohyphae in the urine proved ineffective as few *Candida* spp. do not produce pseudohyphae (2). The most effective approach currently available to distinguish between colonization and infection involves obtaining a follow-up urine sample and examining for the continued presence of yeasts. Therefore, we implemented this strategy in our study by obtaining repeat urine samples from all *Candida*-positive patients, coupled with assessing the presence of pus cells in urine (1 pus cell / 7 hpf), to discern between infection and colonization; all patients were symptomatic.

The overall prevalence of candiduria in acute healthcare settings worldwide

Candiduria is a concern in acute healthcare settings globally, impacting diverse patient populations, but the true global ubiquity might be subject to regional variations and healthcare practices. This study was conducted across various acute healthcare settings, i.e., acute medical ward, medical ICU, surgical ICU, pediatric ICU. Factors, such as catheterization duration, comorbidities, and healthcare facility types, contribute to its prevalence, emphasizing the need for continuous monitoring and effective management strategies. The prevalence of various *Candida* species worldwide and in India is depicted in Table 3[12-18].

Table 3. Prevalence of various *Candida* species worldwide and in India

Study conducted by	Year	Country	Percentage			
			Candida albicans	*Candida glabrata*	*Candida tropicalis*	Other NAC*
Alvarez-Lerma et al.[12]	2003	Spain	68	8	4	20
Paul et al.[13]	2007	CMC Vellore India	24	21	31	24
Achkar and Fries[14]	2010	USA, New York	54	36	10	Nil
Yashavanth et al.[15]	2010~2012	Mysore India	30.3	9	45.45	15.25
Singla et al.[16]	2012	Chandigarh India	27.27	50	18.6	4
Pramodhini et al.[17]	2021	Puducherry India	14.3	7.1	65.7	12.9
Vedachalam et al.[18]	2017~2022	ICMR, India	36	6	37	21
Our study	**2023**	**Pune India**	**25.7**	**Nil**	**50**	**24.3**

*NAC - Non albicans *Candida*
ICMR - Indian Council of Medical Research, CMC - Christian Medical College

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Table 4. Comparison of resistance patterns of *Candida* species in India

Candia albicans					
Study	Year	Azoles (Percentage)	Echinocandins (Percentage)	Amphotericin B (Percentage)	Flucytosine (Percentage)
Our study	2023	6	Nil	11.2	Nil
Vedachalam et al.[18]	2017~2022 Multicentric, Pan India study	7	6.5	Not reported	Not reported
Candida tropicalis					
Study	Year	Azoles (Percentage)	Echinocandins (Percentage)	Amphotericin B (Percentage)	Flucytosine (Percentage)
Our study	2023	Nil	Nil	Nil	Nil
Vedachalam et al.[18]	2017~2022 Multicentric, Pan India study	6.9	2	Not reported	Not reported

ICMR - Indian Council of Medical Research

Achkar and Fries[14] meta-analysis revealed that globally, *C. albicans* consistently dominates *Candida*-related urinary isolates (50~70%), followed by *Candida glabrata*, with *C. tropicalis* ranking third. They observed a rising trend in nosocomial infections associated with NAC strains. Among neonates, *C. parapsilosis* was the most prevalent type and consistently isolated. A multicenter study conducted in Spain revealed *C. albicans* as the most frequently recovered species at 68%, trailed by *Candida glabrata* at 8% and *C. tropicalis* at 4%. Interestingly, in their study, *C. albicans* accounted for 54% of all cases, with *Candida glabrata* at 36% and *C. tropicalis* at 10%. In contrast, their meta-analysis using Indian data revealed a shift in prevalence, with *C. tropicalis* (31%) surpassing *C. albicans* (24%) and *Candida glabrata* (21%) as the most common species. This discrepancy underscores regional variations in *Candida* epidemiology, emphasizing the need for nuanced interpretations based on geographical contexts.

Changing trends in epidemiology over the last decade in India

In our study, the prevalence of candiduria was 9%, which is not consistent with the Indian Council of Medical Research (ICMR) data. According to the ICMR's surveillance study across 39 tertiary care hospitals from 2017 to 2022, *Candida* spp. constituted 20.5% of all UTIs[18].

In India, studies conducted by ICMR and CMC Vellore have identified *C. tropicalis* as the primary cause of candiduria, which is consistent with our findings. However, in our study, a notable increase in the prevalence of *C. tropicalis* was observed (from 31% to 50%), with no instances of *Candida glabrata* detected.

Susceptibility patterns of different *Candida* species

In our study, the antifungal susceptibility profile of *Candida* isolates indicated sensitivity to all tested antifungals for *C. tropicalis*, *C. kefyr*, and *C. utilis*. *Candida albicans* demonstrated low resistance levels, with no resistance observed to echinocandins and flucytosine, 6% resistance to azoles, and 11% resistance to amphotericin B, which is consistent with the ICMR pan India report (Table 4). The ICMR report mentions *Candida glabrata* and *C. auris* had high rates of resistance (48% and 44% for fluconazole and 44% and 100% for caspofungin, respectively), but the same were not isolated in our study[18].

Candida ciferrii and resistance: the impact on treatment

Candida ciferrii (*Stephanoascus ciferrii*) has been identified in various human infections, including endophthalmitis, intra-orbital abscess, systemic mycosis, and otitis media, in global research studies[4-6]. Through the sequencing of the 18S rRNA

gene, umiko Ueda-Nishimura and Kozaburo Mikata categorized *S. ciferrii* into the "*S. ciferrii* complex", encompassing *C. ciferrii*, *Candida allociferrii*, and *Candida mucifera*[5]. The advent of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) has significantly improved clinical diagnosis by facilitating the identification of these rare species and guiding appropriate treatment[5]. All three have similar morphological and microscopic characteristics, but the antifungal susceptibility of these species varies. Moreover, echinocandins are the preferred treatment option for all three species. Among the azoles, no resistance against posaconazole, voriconazole, and itraconazole was reported, but fluconazole was considered resistant. Resistance is observed across the board for flucytosine[5,6,19].

In our study *C. ciferrii* showed a distinct pattern, revealing a statistically significant association (p = 0.015) with cases having two or more comorbidities, indicating a 100% occurrence rate. This contrasts with other species, which exhibited different distributions of comorbidity rates (Table 1). It demonstrated a distinctive drug resistance profile compared with other *Candida* species. *Candida ciferrii* showed a 100% resistance to fluconazole, voriconazole, and flucytosine, whereas other *Candida* species showed lower resistance rates (10.3%, 4.4%, and 2.9%, respectively). The p-values for fluconazole (p = 0.015), voriconazole (p = 0.004), and flucytosine (p = 0.002) indicate statistically significant differences in drug resistance between *C. ciferrii* and other species (Table 2). Drug susceptibility testing against caspofungin, amphotericin B, and micafungin was not reported by VITEK 2 Compact[19].

CONCLUSION

Candida UTIs are on the rise in acute healthcare settings and pose challenges due to the absence of standardized criteria for diagnosis and management. Resistance trends in India have been changing with more fluconazole-resistant *Candida* strains being frequently isolated, emphasizing the importance of specific therapeutic strategies based on culture and sensitivity reports. *Candida ciferrii* exhibits distinctive resistance patterns, requiring vigilant management, particularly due to its association with comorbidities.

Regular monitoring and adjustment of empirical therapy based on ongoing surveillance are essential to adapt to the changing epidemiological trends of *Candida* and ensure effective management.

CONFLICT OF INTEREST

In relation to this article, we declare that there is no conflict of interest.

DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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ETHICAL APPROVAL STATEMENT

The study was approved by the Institutional Review Board of (IRB No. IRB Number: 7215/Micro/2024). This study was conducted in accordance with the principles of the Declaration of Helsinki.

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