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Distribution and antifungal susceptibility profile of *Candida* species from candiduria cases at a tertiary care hospital

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ABSTRACT

The present study was conducted to assess the distribution of *Candida* species in candiduria and to evaluate their antifungal susceptibility pattern. Urine samples were subjected to microscopy and screened for the growth. *Candida* isolates were identified and speciated by colour on HiCHROM *Candida* agar, germ tube test, growth in cornmeal agar to study micromorphology, carbohydrate assimilation and fermentation tests according to standard protocols. All isolates were investigated for antifungal susceptibility testing. During the study period, a total number of 2560 urine samples were reported. Out of them 94/2560 (3.7%) urine samples showed significant candiduria. 60 from catheterised patients admitted to the ICU and 34 from patients admitted in different wards. The species-wise distribution among *Candida* isolates are *C.tropicalis* 53 (56.4%), *C.albicans* 21(22.4%), *C.glabrata* 8 (8.5%), *C. krusei* 7 (7.4%) and *C.parapsilosis* 5 (5.3%). *Candida* species exhibited resistance to Amphotericin B 6 (6.4%), Fluconazole 23 (24.5%) Voriconazole 8 (8.5%), Flucytosine 5 (5.3%) and 4 (4.3%) to Caspofungin. *Candida* previously presumed as non-pathogenic has attained an important clinical role in nowadays circumstances of growing risk factors and developing antifungal resistance. The frequency of isolation of non-*albicans Candida* was more when compared to *Candida albicans* and they showed more resistance to antifungal drugs when compared to *C.albicans.* Caution must be taken in reporting *Candida* from urine, but it is recommended not to mistreat candiduria. The *Candida* species identification along with their antifungal susceptibility profile can assist clinicians in choosing better antifungal drugs for the treatment of patients with candiduria.

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INTRODUCTION

The existence of *Candida* in urine is stated as candiduria. Many patients with candiduria suffer a benign course. *Candida* species are rare organisms of urinary tract infection (UTI) in healthy persons, common in the hospitalised patients, particularly those admitted to the intensive care unit (ICU) with predisposing conditions (Fisher *et al.,* 2011). Candiduria is one of the most perplexing forms of candidiasis since the distinction between colonisation and actual infection is challenging to find out. Urinary candidiasis can also be an indication of candidemia or intrusive renal

candidiasis, can become a source of candidemia during intrusive urologic processes. Candiduria can lead to morbidity and mortality if not detected correctly (Hollenbach *et al.,* 2008; Singhi and Deep, 2009).

The clinicians continuously face an investigative problem as to whether the existence of candiduria in a patient denotes contamination, colonisation or real infection. The prevalence of real infection has augmented considerably over the previous few years due to the existence of several predisposing conditions in hospitalised patients. The predisposing conditions commonly related with candiduria are urinary tract catheterisation, previous antibiotic use, extended hospitalised stay, and extremes of age, diabetes mellitus, female gender and use of immunosuppressive medications (Hollenbach *et al.,* 2008; Jain *et al.,* 2011).

In spite of the dominance of *Candida albicans*, there has been upsurge in the incidence of non-*albicans Candida* (NAC) species such as *C. tropicalis, C. glabrata*, *C. parapsilosis, C. krusei, C. lusitaniae*, and *C. guilliermondii* as causative organisms of infections, including UTI (Jain *et al.,* 2011; Mahmoudabadi *et al.,* 2015). Previous studies have well recognised the inherent resistance of non-*albicans Candida* to Fluconazole, requiring speciation of *Candida* in patients with candiduria for the commencement of suitable treatment. Even though the quantification and identification of *Candida* in urine are not regularly done, it is crucial to identify the causative organism because the pathogenicity and the susceptibility profile to antifungal differ between the species (Mahmoudabadi *et al.,* 2015; Joshi *et al.,* 2015).

Candiduria should be identified and documented for epidemiologic data, such as the native prevalence of the candiduria, species causing infection. Therefore, the current study may gather data on the prevalence of candiduria caused by diverse species of *Candida* and their antifungal susceptibility profile. These documents may contribute to the laboratory practice, presenting the implication of carrying out fungal identification, and also to resident clinicians, has susceptibility pattern to antifungal may differ between the species causing the infection, especially in treating patients with opportunistic infections.

The current study was planned to know the distribution of *Candida* species in candiduria, risk factors of the study population and susceptibility pattern of isolates to antifungals used in treatment, as it helps native clinicians concerning epidemiology and antifungal susceptibility pattern of *Candida* isolates.

MATERIAL & METHODS

This prospective study was carried out over a period of one and a half year from June 2015 to December 2016. After obtaining Institutional Ethical Committee (IEC) clearance, Urine samples were collected according to standard protocols. Ninety-four yeast isolates were involved in the study. After taking written informed consent of patients or their legal guardians, patient's demographic particulars such as age, sex, a period of hospital stay, duration of catheterisation and other accompanying risk factors like diabetes mellitus, past antibiotic use, any invasive procedure carried out on the patient were recorded.

Inclusion criteria: The urine samples yielding real growth of yeast cells, a significant colony count with more than 10000 colony forming units(CFU)/ml and direct microscopy displaying evidence of pus cells, budding yeast cells and with or without pseudohyphae were involved in the current study.

Exclusion criteria: Urine specimens where *Candida* species isolated without pyuria, with a mixed growth were omitted from the study.

Urine sample processing and identification: In case of non-catheterized patients a clean-catch midstream urine specimens were collected, whereas in catheterised patients urine specimens were obtained via syringes after cleaning the catheters with an antiseptic solution. The urine specimens were collected in a sterile leak-proof universal container with screw capped lids and transported without delay to the microbiology laboratory. Direct microscopic investigation of urine specimen was performed to find out the occurrence of pus cells, red blood cells, casts, crystals or any bacterial or fungal element (Chander, 2009).

The urine specimens were processed as per standard protocol and cultured on Cysteine Lactose Electrolyte Deficient (CLED) medium by calibrated wire loop method delivering 0.001ml of urine. Moreover, the specimens showing budding yeast-like cells on direct examination were cultured on Sabouraud Dextrose Agar (SDA). Plates were incubated at 37°C for 24 hours or more if essential, aerobically. *Candida* species grown on culture plates with colony count more than 10000 CFU/ml were considered significant (Yashavanth *et al.,* 2013). Gram stained smear was performed from colonies isolated on agar to check the colony as yeast. Additional identification and speciation were performed studying colony morphology on Sabouraud dextrose agar, Hi-CHROM *candida* agar (HiMedia Laboratories, Mumbai), germ tube test

and micro-morphology on cornmeal agar, sugar fermentation, and sugar assimilation tests as per standard protocols (Chander,2009). Anti-fungal susceptibility testing was done by the agar-based E-test method (HiMedia Laboratories, Mumbai). Amphotericin B (0.002-32 µg/mL), Fluconazole (0.016-256 µg/mL), Voriconazole (0.002-32 µg/mL) and Flucytosine (0.002-32 µg/mL), Caspofungin (0.002-32 µg/mL), were used. Standard strains *C. albicans* ATCC 90028 and *C. parapsilosis* ATCC 22019, were included as the quality control.

A suspension of the *Candida* species was prepared in normal saline, adjusted to 0.5 McFarland turbidity standards and inoculated on RPMI-1640 agar (HiMedia Laboratories Mumbai) medium with a swab. Later E-test strips were kept on dried agar plate surfaces. The plates were incubated at 37°C for 24 hours. Susceptibility interpretation was made as per manufacturer's instructions.

RESULTS

During the study period total of 2560 culture, positive urine samples were reported. Among them, 94 (3.7%) urine samples revealed significant candiduria. 60 *Candida* isolates were obtained from urine samples collected from catheterised patients from ICU and 34 *Candida* isolates were obtained from urine samples collected from patients admitted to various wards during the study period. Predominantly non-*albicans Candida* was isolated**.** Microbiological characterisation of yeast was done and the distribution of various *Candida* species presented in (Table 1).

Table 1: Distribution of Candida species

Sl.No.	Isolates	Number (%)
1.	*C. albicans*	21 (22.4)
2.	Non *Albicans candida*	73 (77.6)
3.	*C. tropicalis*	53 (56.4)
4.	*C. glabrata*	8 (8.5)
5.	*C. krusei*	7 (7.4)
6.	*C. parapsilosis*	5 (5.3)

Non-*albicans Candida* species emerged as leading pathogens and accounted for 77.6% for candiduria. *Candida tropicalis* was major species accounting for 56.4% of the cases, whereas *C. albicans* was noted in 22.4% cases.

When risk factors related to candiduria were assed, we noted that 58.5% males and 41.5% were females, a male preponderance was detected in the current study. Maximum *Candida* was isolated from extremes of age groups patients 60.6%, antibiotic therapy in 79.7%, catheterisation in 63.8% and diabetes in 26.6%, were the commonly associated risk conditions leading candiduria. Presented in (Table 2 and 3).

Antifungal susceptibility test results, determined by E-test of *Candida* isolates are presented in TableNo. 4, accordingly 6.4% *Candida* isolates were resistant to Amphotericin B, 24.5% to Fluconazole, 8.5% to Voriconazole, 5.3% to Flucytosine and 4.3% to Caspofungin.

Table 2: Risk factors associated with *candiduria*

S.No.	Risk factors	Number (%)
1.	Male	55(58.5)
2.	Female	39 (41.5)
3.	Antibiotics	75 (79.7)
4.	Catheterization	60 (63.8)
5.	Diabetes mellitus	25 (26.6)
6.	Use of steroids	27 (28. 7)
7.	Previous surgery	21 (22.3)
8.	Underlying comorbid conditions	16 (17.0)

Table 3: Age wise distribution of Candida isolates

Sl. No.	Age	Number (%)
1.	1 -10	5 (5.3)
2.	11 - 20	4 (4.2)
3.	21 - 30	9 (9.5)
4.	31 - 40	10 (10.6)
5.	41 - 50	18 (19.1)
6.	51- 60	26 (27.7)
7.	61 - 70	16 (17.1)
8.	71 - 80	6 (6.3)
Total		94

DISCUSSION

Candiduria is reported from many tertiary care hospitals. The isolation of *Candida* species in urine samples may infer that the patient has cystitis or pyelonephritis, or reflect colonisation of the perineum, indwelling catheters or bladder. Differentiating contamination from real infection is difficult, despite the presence of reliable diagnostic criteria for significant candiduria. In the current study, significant candiduria was noted in 3.7%. Studies from several centres have revealed different prevalence rates of 2.2% and 5.3% candiduria their patients respectively (Yashavanth *et al.,* 2013; Izabela *et al.,*2014). Our result is slightly higher and little lower than previous reports it depends upon hospital setting and patient group studied.

Candida albicans has historically been stated as the main cause of funguria, however, in recent decades a shift occurred towards NAC, particularly *C. glabrata*, *C. tropicalis* and *C. krusei* (Kauffman, 2005). *Candida albicans* was the common species isolated from nosocomial UTI (Kauffman, 2005). In some reports, *C. tropicalis* was ranked as the most

Table 4: Antifungal susceptibility of Candida isolates

Antifungal Agent	*C.albicans* N (%) 21 (22.4)	*C.tropicalis* N (%) 53 (56.4)	*C. glabrata* N (%) 8 (8.5)	*C. krusei* N (%) 7 (7.4)	*C. parapsilosis* N (%) 5 (5.3)	Total 94
Amphotericin B						
Sensitive	20 (95.2)	50 (94.4)	7 (87.5)	6 (85.7)	5 (100)	88 (93.6)
Resistant	1 (4.8)	3 (5.6)	1 (12.5)	1 (14.3)	--	6 (6.4)
Fluconazole						
Sensitive	16 (76.2)	38 (71.7)	--	--	4 (80)	58 (61.7)
S D D	2 (9.5)	5 (9.4)	6 (75)	--	--	13 (13.8)
Resistant	3 (14.3)	10 (18.9)	2 (25)	7 (100)	1 (20)	23 (24.5)
Voriconazole						
Sensitive	18 (85.6)	45 (84.9)	6 (75)	5 (71.4)	5 (100)	79 (84.1)
S D D	2 (9.6)	3 (5.6)	1 (12.5)	1 (14.3)	--	7 (7.4)
Resistant	1 (4.8)	5 (9.5)	1 (12.5)	1 (14.3)	--	8 (8.5)
Flucytosine						
Sensitive	20 (95.3)	46 (86.8)	8 (100)	7 (100)	5(100)	86 (91.5)
S D D	--	3(5.7)	--	--	--	3 (3.2)
Resistant	1(4.7)	4 (7.5)	--	--	--	5 (5.3)
Caspofungin						
Sensitive	21(100)	50 (94.4)	7 (87.5)	7(100)	5 (100)	90 (95.7)
S D D	--	--	--	--	--	--
Resistant	--	3(5.6)	1(12.5)	--	--	4(4.3)

SDD: susceptible dose dependent

important NAC species (Binesh and Kalyani, 2015; Achkar and Fries, 2010).

In the present study, major species isolated in candiduria were *Candida tropicalis* (56.4%), and *Candida albicans* was isolated in 22.4%. Binesh and Kalyani also reported a high rate of *C.tropicalis* isolation (63.9%) in candiduria. Medical practices, such as catheterisation of the bladder, abnormalities of the urinary tract, antibiotics use and corticosteroids use, may provoke urinary tract infection by non-*albicans Candida*. The exact identification of yeast helps in guiding the clinicians for proper management of candiduria (Binesh and Kalyani, 2015 Kumari *et al.,* 2015). *Candida* species were isolated maximum from critical care units patients. Patients who required a ventilator, critically ill and debilitated are admitted to the critical care unit.

UTI owing to *Candida* species was quite common during extremes of age (60.6%). This might be due to diminished host defences at extremes of age. This observation is sustained by other investigators also (Jain *et al.,* 2011; Achkar and Fries, 2010).

Meanwhile, colonisation of the vulvar vestibular region by *Candida* species is common in females. They are at an additional risk of developing candiduria owing to ascending infection (Bukhary, 2008). However, in our study, we found that candiduria was more common in males (58.5%) as compared to the females (41.5%). This might be due to the preponderance of other associated risk factors in our study group.

Prior history of antibiotics use (79.7%) was a risk factor in our patients. Antibiotics intensify the risk of colonisation of *Candida* species by disturbing endogenous flora and the threat of candiduria increases with extended antibiotic use (Jain *et al.,* 2011; Achkar and Fries, 2010; Kumari *et al.,* 2015).

Diabetes mellitus was perceived in 26.5% of patients. Diabetes is a renowned risk factor for developing nosocomial UTI due to *Candida* species. This is because diabetes depresses host resistance to invasion by fungi and also endorses stasis of urine in the neurogenic bladder, thus further increasing the chances of colonisation of *Candida* species (Achkar and Fries, 2010).

Urinary catheters aid in entry of organisms and become colonised if left for an extended duration (Jain *et al.,* 2011; Kauffman, 2005). There is a strong relation between days of catheterisation and risk of developing candiduria. In the present study, catheterisation was seen with 63.8% and the mean duration was 11.5 ± 6 days which validated with the studies done by Jain *et al.,*. Binesh and Kalyani reported one of the principal risk factors in patients with candiduria was urinary catheterisation 89.7 % (Binesh and Kalyani 2015). All these studies favour urinary catheterisation as one of the risk factors in

candiduria. This is because *Candida* species are commensal in the genitourinary tract of humans can turn into pathogenic as and when the ecological niche gets imbalanced, becomes virulent due to adherence, biofilm formation (Rajeswari *et al.,*2018). We noted *Candida* species causing candiduria might be shifting to NAC species (77.6%). The variation in aetiology towards non-*albicans Candida* species has been perceived by other Indian authors also (Jain *et al.,* 2011; Yashavanth *et al.,* 2013).

ANTIFUNGAL SUSCEPTIBILITY

Antifungal susceptibility is a relatively fresh concept not performed routinely in the microbiology laboratory. Due to easy availability of oral azoles, clinicians are treating the patient empirically and indiscriminately with azoles which have led to the development of antifungal resistance and shifting of classical *C. albicans* to NAC species. Antifungal susceptibility profile displayed *Candida* isolates were most susceptible to Amphotericin B, Flucytosine, and Caspofungin when compared to azoles. Fluconazole resistance was observed with *C. albicans* (67.5%) and (57.14%) of *C. tropicalis* (Ayseaynali *et al.,* 2014). Voriconazole has greater intrinsic activity and sensitivity than other azole drugs against *C. glabrata* and *C. krusei* (Kauffman *et al.,* 2000).

Fluconazole is the most frequently used azoles for the therapy of candiduria, development of increased resistance to it is distressing (Yashavanth *et al.,* 2013). In the current study, *C. albicans* strains were more susceptible to azoles than NAC species. This is comparable to the outcomes of other authors (Yashavanth *et al.,* 2013; Shivanand *et al.,* 2012).In the current study, we found *Candida* isolates resistant to Amphotericin B 6 (6.4%) Fluconazole 23 (24.5%), Voriconazole 8 (8.5%) Flucytosine 5 (5.3%) and Caspofungin (4.3%) highest resistance was noted with Fluconazole. All *C. albicans, C. krusei and C. parapsilosis* isolates were susceptible to Caspofungin. Our results confirmed the previous results that have shown Caspofungin is an active antifungal drug (Mahmoudabadi *et al.,* 2012). *C.glabrata* displayed high resistance to Fluconazole (46.8%) and Ketoconazole (46.8%), Itraconazole (45.7%) and (33.1%) to Amphotericin B (Deorukhkar and Saini, 2014).

CONCLUSION

To conclude, the upsurge in the predisposing illnesses in recent years has led to a rising prevalence of candiduria. Prevalence of NAC species was more than *C.albicans*. NAC species exhibited more resistance to antifungal drugs than *C.albicans*. Hence, *Candida* isolates should be identified to species level along with their antifungal susceptibility patterns. Has it influence patient care by choosing better antifungal drug by the clinicians.

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CONFLICT OF INTEREST

Authors declared no conflicts of interest.

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