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Characterization and Antifungal Susceptibility Profile of *Candida* Species Isolated from Candiduria Cases in a Tertiary Care Hospital, Kolkata

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A B S T R A C T

Candida species are unusual cause of urinary tract infections in healthy individuals, but common in hospital setting or among patients with predisposing factors. The isolation of *Candida* from urine is challenging both for clinician and microbiologist as to whether the candiduria represents colonization or urinary tract infection. Species identification of *Candida* is also important as non albicans *Candida* are on rise in recent years which are more resistant to antifungal drugs and as a result morbidity and mortality is also increasing. The objective of this study was to determine the prevalence of *Candida* species causing UTI, analyze various risk factors associated with candiduria and their antifungal susceptibility pattern. A total of 1860 urine samples from clinically suspected cases of urinary tract infection were analysed in a tertiary care Hospital during a period of one year. 105 *Candida* isolates were obtained from these urine samples and species identification was done following standard laboratory protocol. Antifungal susceptibility testing was performed by disc diffusion method following CLSI guideline M-44A. Among all suspected cases of urinary tract infection, candiduria was diagnosed in 5.64% cases. *Candida tropicalis* 44(42%) was the predominant isolate followed by *Candida albicans*-38(36%), *Candida glabrata*-14(13%), *Candida parapsilosis*-07(07%) and *Candida krusei-02*(02%). The antifungal susceptibility pattern showed that *Candida* isolates were more resistant to Fluconazole-39(37%), followed by Itraconazole-30 (28.5%). Fluconazole resistance was more common among non *albicans Candida* (41%) than *Candida albicans* (29%). Our study reflects a change in trend with shift towards non-albicans candida replacing *Candida albicans* as the predominant cause of candiduria. Non *albicans Candida* species are more resistant to antifungal drugs compared to *C. albicans*. Therefore species identification of *Candida* isolates along with their antifungal susceptibility pattern is important to guide clinicians for better therapeutic options which will reduce both morbidity as well as financial burden on patients.

Introduction

Fungal infections of the urinary tract encompass a broad variety of fungi, However, the overwhelming majority of fungal infections of the urinary tract are caused by *Candida* species. *Candida* species account for almost 10-15% nosocomial UTI[1]. The

important risk factors associated with candiduria are anatomic defects of urinary tract, indwelling urinary drainage devices, abdominal surgery, ICU stay, broad spectrum antibiotic therapy, diabetes mellitus, increased age and female sex [2].

Isolation of *Candida* species from urine samples moves both the Microbiologist and the physician with a challenge as to whether the candiduria represents colonization or, lower or upper urinary tract infection and renal candidiasis with sepsis.[3]

Most observational studies of candiduria have been reported predominance of *C. albicans*[3]. But in recent years with the advent and increasing use of fluconazole, the emergence of non-*albicans Candida* (NAC) spp. is noted[4]. The NAC spp. are not only well adapted to the urinary tract but also are difficult to eradicate than *C. albicans*.[2] Therefore, Species identification of *Candida* is important as non *albicans Candida* species are increasing in number, more resistant to antifungal drugs and some non-albicans *Candida* spp. are inherently resistant to treatment with fluconazole.[5,6]

The present study was carried out to determine the prevalence of candiduria, phenotypic characterization of the isolated *Candida* species with their antifungal susceptibility pattern and also to analyse various risk factors associated with candiduria in a tertiary care hospital.

Materials and Methods

The present study was carried out in the department of Microbiology, in a tertiary care Hospital, Kolkata from over a period of one year. A Total of 1860 urine samples were analyzed. Permission from the institutional ethical committee was taken.

Inclusion criteria

Both indoor and outdoor patients who had presented with signs and symptoms of urinary tract infection (bladder discomfort, low grade fever, and leucocytosis) were included in the study. Pure growth of yeast isolates with significant colony count was included in the study.

Exclusion criteria

The urine samples where *Candida* species was isolated in the absence of pyuria, *Candida* with colony count ≤ 1000 CFU/ml and mixed growth were excluded from analysis.

The mid-stream clean-catch urine samples were collected aseptically in a sterile container and transported immediately to microbiology laboratory. The urine samples were inoculated on Blood agar and Cysteine Lactose Electrolyte Deficient media (CLED) by semi-quantitative method as per standard protocol for urine culture. The culture plates were incubated aerobically at 37°C for 24 to 48 hours. *Candida* species isolated on culture plates with colony count >10000 CFU/ml were considered significant. Contamination was differentiated from infection by obtaining second urine sample.

The 105 *Candida* isolates were further speciated by conventional phenotypic methods i.e. Gram staining, culture on Sabouraud's dextrose agar, germ tube test, growth on corn meal agar, sugar fermentation, sugar assimilation test and colony colour in Chrome agar. Antifungal susceptibility testing was performed by disc diffusion method according to CLSI guideline M44A.The antifungal drugs tested were AmphotericinB, Ketoconazole, Fluconazole, Itraconazole, and Voriconazole.

The antifungal discs were procured from Himedia Laboratories Pvt. Limited, Mumbai. Muller-Hinton agar supplemented with 2% glucose and 0.5µg/ml methylene blue was used for the sensitivity testing. *C. albicans* (ATCC 90028) and *C. krusei* (ATCC 6258) were used as control strains.

Patient's demographic and clinical data were recorded in a proforma.

Results and Discussion

A total of 105 (5.6%) samples showed the growth of *Candida* species out of 1860 urine samples.

In the study candiduria was found to be more common in females- 56 (53%) than males- 49 (47%). In both the sexes maximum patients belong to age group >60 years

In most cases association of more than one risk factor was observed. Broad spectrum antibiotics use was found to be the most important risk factor. Out of 105 patients 82(78%) were on broad spectrum antibiotics. Urinary catheterization 60 (57%) and diabetes mellitus -44 (42%), pregnancy (23%) were the other major risk factor noted for the development of candiduria.

Out of 105 isolates, *Candida tropicalis* 44(42%) were found to be the most common isolate followed by *Candida albicans* 38(36%), *Candida glabrata* 14(13%), *Candida parapsilosis* 7(07%) and *Candida krusei*-2(02%)

Table 1 shows antifungal sensitivity pattern of *Candida* isolates. Maximum resistance was seen against fluconazole-39 out of 105 isolates (37%). Fluconazole resistance was more in *Non albicans Candida* i.e. *Candida krusei*-2(100%), *Candida glabrata* 10 (72%), *Candida tropicalis* -14(32%) than *C. albicans* 11(29%). Amphotericin B & Voriconazole resistance was seen only in 5.7% and 3.8% isolates respectively. Hence irrespective of any species of *Candida* isolate, the most effective drug can be considered as voriconazole (98%) followed by Amphotericin-B (96%) and Ketoconazole (77%).

Candida species is considered as the fifth most common nosocomial pathogen in India[7]. In our study the prevalence of candiduria is 5.64% while Anju *et al.,* documented candiduria cases as 1.3% among all patients with urinary tract infection[8]. However in the literature it has been documented as 11-52% cases of nosocomial urinary tract infections are caused by *Candida* species[9-11].

In the present study, a shift towards NAC (Non-albicans *Candida*) spp. replacing *Candida albicans* as a causative agent of candiduria was noted. *Candida tropicalis* (42%) was the major isolate in our study. Contributory factors in the emergence of *C. tropicalis* includes injudicious use of antifungal drugs, increase in the population of immunocompromised hosts, use of broad spectrum antibiotics and long term use of catheters[12].

Study of Prasad *et al.,* also showed that isolation of non- *Candida albicans spp*. was higher (52.4%) as compared to *C. albicans* (47.6%)[13]. Chakrabarthi A et al found *C. tropicalis* (58.7%) was the commonest species isolated from urine followed by *C. albicans*[14].

In our study Candiduria was found to be more common in female (53%) than male (47%) patients. Similar observation was seen in the study done by Payam Behzadi *et al.,*[15]. Since colonization of vulvo vestibular area with *Candida* spp. is frequent in females, they are more at risk of developing candiduria due to

ascending infection[1]. In the present study, we also found that UTI due to *Candida* spp. was more common in old age (>60 yrs). This could be due to lowered host defence at extremes of age.

In the present study more than one risk factor was identified in patient with candiduria. The most common associated risk factor was long term antibiotic therapy (78%). Similar finding was observed by study of Weinberger *et al.*,[16]. It is likely that antibiotics contribute to colonization by *Candida* species by suppressing endogenous bacterial flora[17]. Urinary catheterization was found to be the second most important risk factor in our study. Guler *et al.*,[18] reported that the risk of development of candiduria is increased 12-fold after use of urinary catheter. This may be due to the fact that Catheters placed into the bladder serve as a portal of entry for microorganisms into the urinary drainage system and become colonized if left in place long enough.[19] Sebastian *et al.*, also studied an association of candiduria with disseminated candidiasis, diabetes mellitus, pregnancy, long term use of broad spectrum antibiotics and the use of catheters[8.]

Table.1 Antifungal susceptibility pattern of different *Candida* species

Antifungal drugs	*Candida tropicalis* (n-44)	*Candida albicans* (n-38)	*Candida glabrata* (n-14)	*Candida parapsilosis* (n-7)	*Candida krusei* (n-2)	
Amphotericin B						
Sensitive	40 (91%)	34(89%)	13(93%)	7(100%)	2(100%)	96(91.2%)
Dose-dependent Sensitive	1(2%)	2 (5%)	_	_	_	3(2.8%)
Resistant	3(7%)	2(5%)	1(7%)	_	_	6(5.7%)
Fluconazole						
Sensitive	24(54%)	25(66%)	2(14%)	5(71%)	_	56(53.3%)
Dose-dependent Sensitive	6(14%)	2(5%)	2(14%)	_	_	10(9.5%)
Resistant	14(32%)	11(29%)	10(72%)	2(29%)	2(100%)	39(37%)
Itraconazole						
Sensitive	27(61%)	26(68%)	3(21%)	5(71%)	1(50%)	62(59%)
Dose-dependent Sensitive	5(11%)	3(8%)	4(29%)	1(14%)	_	13(12%)
Resistant	12(27%)	9(24%)	7(50%)	1(14%)	1(50%)	30(28.5%)
ketoconazole						
Sensitive	32(73%)	27(71%)	11(79%)	5(71%)	2(100%)	77(73%)
Dose-dependent Sensitive	3(7%)	2((5%)	1(7%)	1(14%)	_	7(6.6%)
Resistant	9(20%)	9(24%)	2(14%)	1(14%)	_	21(20%)
Voriconazole						
Sensitive	40(91%)	36(95%)	13(93%)	7(100%)	2(100%)	98(93.3%)
Dose-dependent Sensitive	2(4.5%)	1(2.5%)	_	_	_	3(2.8%)
Resistant	2(4.5%)	1(2.5%)	1(7%)	_	_	4(3.8%)

Fig.1 Age and sex distribution of patients with candiduria

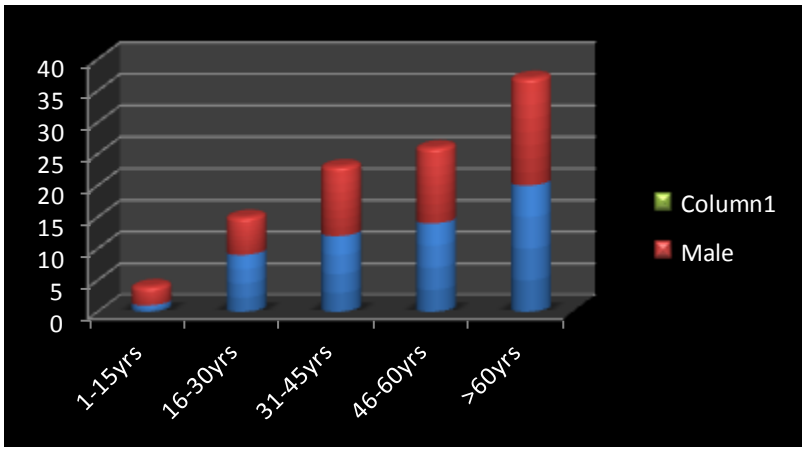


Fig.2 Associated risk factors

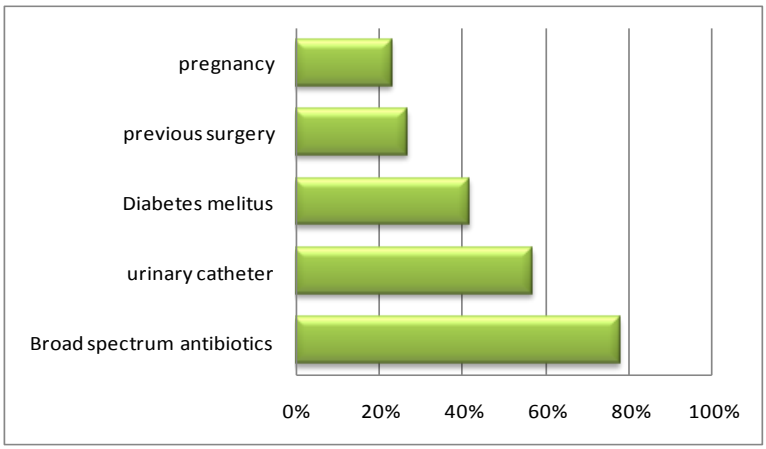
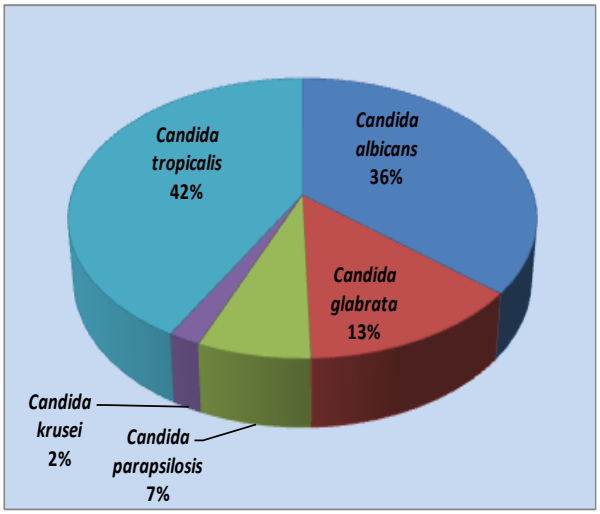


Fig.3 Distribution of different *Candida* species



It has been studied and documented in the literature as the risk of candiduria increases by twelve fold following use of urinary catheters, while six, four, two and one fold rise is observed after the use of broad spectrum antibiotics and urinary abnormalities, following abdominal surgeries, in presence of diabetes mellitus and in association with corticosteroid administration respectively[3].

Antifungal susceptibility pattern in our study showed that *Candida* isolates developed more resistance against Fluconazole (37%) as compared to other antifungal agents. Resistance to Fluconazole was more in the non-*Candida albicans* group ($p<0.05$) when compared to *C. albicans*. Similar results have been observed in the study conducted by Malhotra Aanchal[20] *et al.,* & by Omprakash Bobade *et al.,*[21] from India.

In conclusion, isolation of *Candida* from urine can no longer be ignored as contamination or commensal. But as the line between colonization, contamination and infection is not very well demarcated the approach to patient with candiduria needs to be individualized. Traditionally Fluconazole is preferred by clinicians to combat any type of fungal infection because of its wider spectrum, affordability, easy to administer and less toxicity. The emergence of non-albicans *Candida* as a causative agent of Candiduria and its resistance to Fluconazole has generated great concern. Although in this study *Candida* species are found to be highly susceptible to Amphotericin and Voriconazole, the situation should be properly weighed before administering these drugs considering its adverse effects and high cost which might put financial burden on the patient. Therefore speciation of *Candida* and their antifungal susceptibility pattern is crucial for the treatment and to prevent the emergence of drug resistance.

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