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Clinico-laboratory profile of Candiduria isolates-special reference to speciation and antifungal susceptibility pattern

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Abstract

Background: Candida causes urinary tract infections in persons with conditions such as diabetes, prolonged hospitalization, instrumentation, prior antibiotic therapy etc. Mere presence of Candida in urine is not indicative of true infection and it requires other evidence such as pyuria, symptoms suggestive of urinary tract infection, repeated isolation of same organism etc.
Objective: The study was done to estimate prevalence of Candiduria in the hospital and to know the predominant species causing the same. The study also determined antifungal susceptibility pattern of the isolates
Materials and methods: Routine urine samples suspected of urinary tract infection sent to laboratory for culture and antibiotic sensitivity testing were analyzed. Culture on routine media were done, followed by speciation using Auxanography method of sugar assimilation. Antifungal susceptibility testing was done following CLSI guidelines using fluconazole and voriconazole discs.
Results: Prevalence of Candiduria was estimated to be around 2.2% amongst urine samples. *C. tropicalis* was the predominant species (51%), followed by *C. albicans* (26%). Other Candida species isolated were *C. glabrata* (11%), *C. guelliermondi* (6%), *C. parapsilosis* (4%) & *C. krusei* (2%). All isolates were susceptible to voriconazole. Two isolates of C. tropicalis was intermediately sensitive to Fluconazole, whereas one isolate of *C. krusei* was resistant to fluconazole.
Conclusion: The prevalence rate of 2.2% of Candiduria was similar to few studies observed. Pyuria was noted in 80% isolates highlighting the non-reliance of pyuria in cases of Candiduria. Majority of the isolates had colony count of $>10^4$cfu/ml, which is significant in diagnosis of urinary tract infection. Non-albicans Candida (NAC) were the predominant group (74%), which is in consonant with global trend of increasing prevalence of NAC species in Candida infection. Certain species are developing resistance to routinely used antifungals like Fluconazole, notably *C. krusei*. It was also noted in the study. The study highlights the importance of knowledge of various Candida species causing Candiduria and their antifungal resistance pattern.

Keywords: Candiduria, speciation of candida, antifungal susceptibility testing, candida tropicalis

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Introduction

Presence of Candida in urine (Candiduria) is not commonly seen in otherwise healthy individuals. Candida is known to be inhabitant of genital region and is also known to cause oro-genital infections in individuals who are hospitalized and /or have certain comorbid conditions. Important risk factors for Candiduria includes, extremities of age, diabetes, instrumentations (especially urinary catheter), extremities of age, prior antibiotic treatment etc. [1].

Though Candida is known to cause oro-genital infections, mere presence of Candida in urine is not an indicator of infection. Candida can be present in urine during instrumentation where candida can get temporarily relocated deeper into urethra or urinary bladder or during sample collection when candida present in urogenital tract can get along with urine when it is collected [2].

It is often difficult for the diagnostic laboratories to identify true infection. One of the indicators of active urinary infection is presence of pyuria [3].

Since last few years there has been steady increase in presence of Non-Albicans Candida (NAC) in urine [4]. Presence of NAC in urine has consequences both for diagnostic laboratories and in choice of antifungal therapy.

In this context, the present study was done to identify prevalence of Candiduria in our hospital and its characterization in terms of speciation and antifungal susceptibility.

Materials and Methods

The study was conducted for a period of six months in a tertiary care teaching hospital. All urine samples collected in a wide mouth sterile universal container and sent routinely to microbiology laboratory for culture and antibiotic sensitivity testing were evaluated. The samples were first plated on blood agar and Cysteine lactose electrolyte deficient agar (CLED) with Andrade's indicator agar with a calibrated wired loop which would hold 0.001ml of urine as standard protocols suggest (semi quantitative technique). The plates were then incubated aerobically for 24 hours at 37 ^{o}C. The samples showing presence of Candida colonies were noted and colonies were counted manually. Colony count of > 1000 cfu/ml were considered as significant and were further evaluated.

Preliminary tests like gram staining and germ tube identification were done on the isolates. A subculture of Candida isolates from blood agar was done on glucose free nutrient agar slant. For species identification, two methods were followed.

1. Morphological identification by Delmau's method using Corn meal agar.
2. Auxanography method of sugar assimilation by Hazen and Howell [5].

Whenever a discordant observation was made between these two methods, the Auxanography method was considered and standard and the result were interpreted accordingly.

Antifungal susceptibility testing was done by Kirby-Bauer disk diffusion method following the CLSI (M44-A2) guidelines using modified Muller Hinton agar (MHA with 5% glucose and Methylene blue indicator) and commercially available antifungal disks (Fluconazole-25 µg & Voriconazole-1 µg).

The demographic data was obtained retrospectively from medical records. The data was analysed using standard statistical methods.

Inclusion criteria: All urine samples sent for routine culture and antibiotic sensitivity testing to the laboratory from in-patients which showed presence of Candida spp. (>10^3 cfu/ml) in routine culture media were included in the study

Exclusion criteria: Presence of mixed growth (polymicrobial) and samples sent from OPD of the hospital were excluded from the study.

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Results

A total of 2725 urine samples were evaluated for presence of Candiduria. Candida were isolated from 60 (2.2%) samples. 47 (78.3%) of the samples with Candiduria had significant growth (>1000 CFU/ml) as per the inclusion criteria. Only these 47 were further evaluated. 27/47 (57.4%) samples were from males and remaining 20/47 (42.6%) were from females. The age distribution is highlighted in table-1. Most of our isolates 28/47 (59.6%) were predominantly from paediatric age group. 41/47 (87.2%) isolates were obtained from persons who were catheterized when the sample was collected (Table-2). The remaining 6/47 (12.7%) had been catheterized during their stay in the hospital and catheter was removed few days ago. 36/47 (76.5%) isolates had colony count of $>10^5$ cfu/ml (Table-3). Of the isolates obtained from individuals who had no urinary catheter in-situ, 5/6 isolates had colony count of $>10^5$cfu/ml. 38/47 (80.8%) isolates had pyuria (presence of >10 polymorphonuclear leucocytes/mm^3).

Table1: Age distribution amongst Cases

Age range (in years)	Cases (n=47)
<1	20
1-15	8
16-40	4
41-59	7
>60	8

Table 2: Risk factors amongst cases

Sl. No.	Risk factor	Cases
1.	Catheterisation	41/47
2.	Diabetes mellitus	11/47
3.	Prolonged hospitalisation	39/47

Table 3: Colony count among Candida isolates from urine

Colony count	Isolates (n=47)	
	Catheterized (n=41)	Non-catheterized (n=6)
10^3-10^4cfu/ml	4	0
$>10^4$cfu/ml & $<10^5$ cfu/ml	6	1
$>10^5$cfu/ml	31	5

Species distribution is highlighted in the figure-1. A total of six different Candida species were isolated. *C. tropicalis* 24/47 (51%) was the most predominant, followed by C. albicans 12/47 (25.5%). Other Candida species isolated include *C. glabrata* 5/47 (10.6%), *C. guelliermondi*
3/47 (6.3%), *C. parapsilosis* 2/47 (4.25%) and *C. krusei* 1/47 (2.12%).
Antifungal susceptibility pattern revealed that all isolates across species were sensitive to Voriconazole. The only isolate of *C. krusei* was resistant to fluconazole, whereas 2/24 (4.2%) isolates of *C. tropicalis* showed intermediated sensitivity against fluconazole. Rest all Candida isolates were sensitive to fluconazole.

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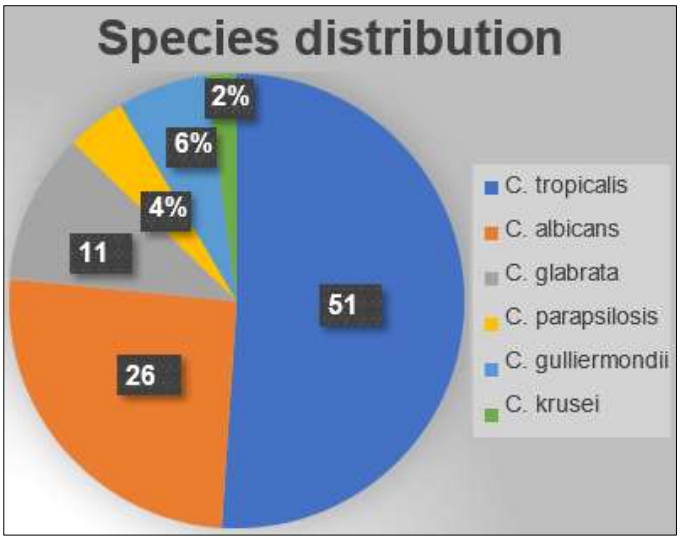

Fig 1: Species distribution amongst Candida isolates from urine

Table 4: Antifungal susceptibility pattern of various isolates of Candida from urine

Candida species	Fluconazole			Voriconazole		
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
C. tropicalis	22	2	0	24	0	0
C. albicans	12	0	0	12	0	0
C. glabrata	5	0	0	5	0	0
C. gulliermondii	3	0	0	3	0	0
C. parapsilosis	2	0	0	2	0	0
C. krusei	0	0	1	1	0	0

Discussion

The study demonstrated that the prevalence of Candiduria for six months period was around 2.2%. This is similar to another single center study done by Yashvanth *et al* [6]. (2013) and other studies such as Rishi *et al* [7]. (2020) showed higher prevalence (4.4%). Certain multicentric studies in India have found the prevalence of Candiduria to be around 8-9% which is significantly higher than our study [8, 9].

The isolates were predominantly from male. Nearly 76% of our isolates were from extremities of age group (<1 & > 60 years). Extremities of age is known to be a risk factor for Candiduria [10].

In the study, majority of the isolates were from catheterized individuals. A study highlighted that instrumentation in urogenital area is one of the major risk factors for acquiring Candiduria [11]. It is often difficult to differentiate true pathogens from colonizers. It becomes very important to be stringent on sample collection especially in catheterized individuals. Another option would be repeating the sample once there is growth of Candida in the urine to identify the temporary colonizers from others. In our study most of the isolates had colony count of more than 10^5cfu/ml. Though the significance of colony count in bacteriuria has been demonstrated by several studies [3], in case of Candiduria, there have been conflicting reports. It is one of the limitations of the study that we could not ascertain if all these cases were true infections and not mere colonization. But co-relation with pyuria was noted in about 80% of the cases. Several studies have demonstrated that pyuria can be an indicator for urinary tract infection, but there have been conflicting reports regarding usefulness of pyuria as an indicator for urinary tract infections due to *Candida* spp. Some studies have found the usefulness of colony count as a marker of Candiduria versus true infection [12], several other studies have found it to be useful only in limited settings such long

term catheterized individuals [3,13,14]. Though significance of single type of isolate from sample indicates towards infection, some studies have demonstrated that there can be Candiduria even in presence of other isolates, notably bacteria.

Speciation of the isolates in our study revealed that 6 different species of Candida were responsible for Candiduria. This high diversity amongst Candida isolated from urine requires special mention. In consonant with global trend, Non-albicans Candida (NAC) species were predominant species isolated in our study and C. albicans was isolated only in about 25% cases. Several studies have shown that [15,16, 6] the NAC are the most predominant group causing Candiduria. Further, studies [17] have shown that C. tropicalis to be the most predominant species isolated from Urine. The same was observed in our study.

Antifungal susceptibility testing revealed that sensitivity to voriconazole was present in across all isolates belonging to different species. Voriconazole has been advocated by some studies to be drug of choice for treatment of infections due to Candida, especially amongst those isolates which are resistant to fluconazole. It is a known fact that some of the NAC species are known to be resistant to fluconazole, namely *C. krusei*, which is intrinsically resistant and *C. tropicalis* which shows dose dependent resistance. The later has been highlighted in few studies [18, 19]. Therefore, speciation becomes important for all diagnostic microbiology laboratories and so is the knowledge regarding prevalence antifungal resistance amongst different species.

One of the strengths of the study was that we could ascertain prevalence of Candiduria amongst urine samples sent to laboratory for culture. We could also know predominant species and their antifungal susceptibility pattern. In future, the studies (preferably large multi centric studies) correlating Candiduria with true infection would make the picture clearer.

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