Urinary Candida isolates from a tertiary care hospital: Speciation and resistance patterns

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ABSTRACT

Background: Candida species (spp.) is an integral member of the human microbiota and is an opportunistic pathogen. This pathogen infects the urinary tract both by ascending the urinary tract and by haematogenous spread. **Aim:** Speciation and antifungal susceptibility patterns of Candida isolates from urine specimens. **Materials and Methods:** Yeast-like isolates from urine culture were identified using a Mini-API® (bioMérieux, Marcy-l'Etoile, France) tool with ID 32 C strips (bioMérieux, Marcy-l'Etoile, France) for species identification and ATB Fungus 3 strips (bioMérieux, Marcy-l'Etoile, France) for susceptibility testing to five antifungal agents and results interpreted as per Centers for Disease Control and Prevention (CDC) guidelines. The significance of data was analysed using the chi-square test. **Results:** Candida spp. isolation was 10.2% (112/1092) and the commonest was *Candida tropicalis* [54.5% (61/112)], followed by *C. glabrata* 25% (28/112), and *C. albicans*, 11.6% (13/112). *C. albicans* showed good susceptibility to Flucytosine (100%) and Amphotericin B (84.6%) while non-albicans Candida susceptibility was only 82.9% and 60.6%, respectively. **Conclusion:** Non-albicans Candida have replaced *Candida albicans* as the more common causative agent. Resistance to both Amphotericin B and Fluconazole is on the rise, requiring the judicial use of antifungals in the immunocompetent and immunocompromised host as therapeutic and prophylactic therapy.

Key words: Antifungal susceptibility, *Candida albicans*, candiduria, non-albicans Candida

INTRODUCTION

Urinary tract is one of the most conducive of anatomical sites for the development of infection especially in hospitalized patients. Even though many factors are responsible for the rise in fungal infections, the important ones are the increase in medical device usage, immunosuppressant drugs, and interventional procedures. The incidence of Candida isolation from blood (candidaemia) and urine (candiduria) is rising and posing an important public health problem.[1]

Although rare in healthy adults, candiduria is common in hospitalised patients and may involve all anatomic levels of the urinary tract, resulting in a spectrum of diseases varying from asymptomatic candiduria to clinical sepsis.[2,3] Candiduria is usually common in the elderly and neonates, especially those on prolonged antibiotic therapy.[4] The isolation rate of Candida is as high as 10-15% of all positive urine cultures in tertiary care centres.[2,5]

Earlier, *Candida albicans* (*C. albicans*) accounted for the majority of candiduria reported, about 50-70% but at present non-albicans Candida are emerging pathogens, especially *C. glabrata and C. tropicalis*.[3-6] Decades ago, *Candida* species (spp.) were regarded as merely culture contaminants.[7] Today, Candida spp. have emerged as the most common cause of fungal infections ranging from mucocutaneous infections to life-threatening invasive infections.[4,8]

Several studies state that candiduria can be an early marker of disseminated fungal infection in critically ill patients and is associated with higher mortality.[3,5,7,9,10]

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Many studies suggest Fluconazole as effective in the short-term eradication of candiduria. The optimal therapy for candiduria is becoming complicated by the emerging resistance to antifungal agents such as newer azoles and echinocandins.[11,12] It is disconcerting to see the increasing rate at which drug-resistant Candida spp. are being reported. It is important for clinicians to be aware of these trends of resistance for which continuous evaluation is required from time to time.

The present study was undertaken to determine the species commonly causing candiduria at this tertiary care centre and to evaluate the resistance pattern to the commonly used antifungal agents.

MATERIALS AND METHODS

The present study was conducted in the Bacteriology Laboratory of the Department of Microbiology in a teaching hospital in Northwest India. This tertiary care hospital, attending to all income groups, has 1,953 beds. Urine samples were received from the various Outpatient Departments (OPDs) and Inpatient Departments (IPDs) of the attached tertiary care hospital. Samples from all age groups and from both the genders were included in the study.

Inclusion criteria

The isolate was considered urinary Candida infection if recovered from a second sample too and collected using aseptic precautions.

Exclusion criteria

Isolates from mixed growth and those not fulfilling the inclusion criteria were excluded from this study.

Routine culture of urine sample was done on blood agar and MacConkey agar and incubated at 37°C overnight. The second samples were processed for all yeast-like isolates. All isolates fulfilling the inclusion criteria were processed for species identification based on Gram staining, colony morphology, germ tube production, and microscopic appearance on corn meal agar with 1% Tween 80. *C. albicans* ATCC 10231 and *C. parapsilosis* 22019 were used as controls.

A Mini-API® (bioMérieux, Marcy-l'Etoile, France) tool in the microbiology laboratory was used for confirmation of the species and for antifungal susceptibility testing. ID 32 C strips (bioMérieux, Marcy-l'Etoile, France) were used for species identification. ATB Fungus 3 strips (bioMérieux, Marcy-l'Etoile, France) were used for susceptibility testing to five antifungal agents, namely, Flucytosine, Amphotericin B, Fluconazole, Itraconazole, and Voriconazole. This strip has 16 pairs of cupules with the first pair acting as growth control. The other 15 pairs contain the five antifungal agents in varying concentrations to enable MIC determination or susceptibility categorization (Flucytosine — 4 mg/L and 16 mg/L, Amphotericin B — 0.5-16mg/L, Fluconazole— 1-128 mg/L, itraconazole — 0.125-4 mg/L, and Voriconazole — 0.06-8 mg/L in doubling concentrations). The software installed in the Mini-API® (bioMérieux, Marcy-l'Etoile, France) allows the susceptibility testing of the Candida isolates to the antifungal agents in a semi-solid medium under conditions, which is similar to the European Committee on Antibiotic Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) recommendations for determining susceptibility and resistance.

A retrospective analysis of case files was done to note the demographic profile of the patient and to assess the association with commonly implicated risk factors.

Statistical analysis

The descriptive statistics was used to characterise the study group. Continuous data were summarised as mean and standard deviation while categorical data was summarised as percentage. Chi-square test was applied for the analysis of categorical data. All statistical calculations were done by using Medcalc 14.0.0 version (MedCalc software, Belgium). P value < 0.05 was taken as statistically significant for interpretation.

RESULTS

A total of 2,817 urine samples were collected, out of which 1,248 (44.3%) were from the OPD and 1,569 (55.7%) from the IPD. Of these 2,817 samples, 1,603 (56.9%) were sterile and 1,092 (38.8%) had growth on culture. Candida spp. (including *Candida albicans*) accounted for 112 (10.2%) of the 1,092 culture isolates.

Characteristics of patients with candiduria

The patients' age ranged from 5 months to 80 years, with a mean age of 46.4 (±21.1) years. The rate of candiduria was highest in patients above 60 years of age, being 40 (35.7%) out of 112.

A retrospective chart review determined that the patients exhibited the expected standard risk factors associated with candiduria including female sex, old age, diabetes, prior antibiotic use, and indwelling urinary tract catheters [Table 1]. Four patients were found to be seropositive for human immunodeficiency virus (HIV).

The rate of Candida spp. isolation was higher in inpatients, being 99 out of 112 (88.4%) compared to outpatients, where it was 13 out of 112 (11.6%) and this was statistically significant ($P < 0.0001$).

Characterisation of Candida isolates

A total of 112 Candida isolates were obtained from urine culture in the study period. The microbiological assay for species identification showed a predominance of non-albicans Candida in the isolates, i.e, 99 (88.4%) while *C. albicans* accounted for only 13 (11.6%) cases. The non albicans isolates comprised of 61 (54.5%) *C. tropicalis*, 28 (25%) *C. glabrata*, 5 (4%) *C. pseudotropicalis*, 2 (2%) each of *C.crusei* and *C. Gullermondii* and only one (1%) of *C. rugosa*.

Age-wise analysis of data showed even distribution of *C. albicans* in all the age groups while non-albicans Candida were predominant in the elderly patients [Table 2]. There was no difference between the genders, for any *Candida* spp.

Sensitivity of Candida isolates to antifungal agents

Antifungal susceptibility testing was done for all the 112 isolates [Table 3]. *C. albicans* showed 100% susceptibility to Flucytosine and 84.6% to Amphotericin B. However, the non-albicans group showed a lower susceptibility to both Flucytosine (82.9%) and Amphotericin B (60.6%). The difference in susceptibility to Flucytosine and Amphotericin B between the albicans and non-albicans groups was not statistically significant ($P = 0.10$ and $P = 0.09$, respectively). The sensitivity of *C. albicans* was low to all the azoles tested [Table 3]. Sensitivity to both Fluconazole and Voriconazole was seen in 6/13 (46.2%) *C. albicans* isolates while only 2/13 (15.4%) isolates were sensitive to itraconazole.

DISCUSSION

The recovery of Candida spp. in urine samples exposes clinicians to a great challenge due to the magnitude of clinical possibilities such as pyelonephritis, cystitis, occurrence of haematogenous dissemination from renal cortex or colonization of anatomical sites such as bladder and perineum.[9] The pathogenesis and prognosis of candidial infections are affected by the host immune status and also differ greatly according to disease presentations.[4]

Candida isolation rate in the present study was 10.2% and is much higher than 2.27% and 1. 37% as reported by two studies from Southern India by Yashavanth *et al.* and Ragini *et al.* respectively.[13,14] This shows a marked geographical variation in the rate of infection by Candida in urinary tract infections warranting a multi-centric study to establish an overall pattern of isolation.

Urinary catheterization increases chances of catheter-associated urinary tract infection (CA-UTI) by allowing migration of the organisms into the bladder from the periurethral surface. In our study, a retrospective analysis of records showed catheterization to be the commonest risk factor associated with candiduria, with 59 (52.7%) out of the total 112 cases of candiduria being catheterised. Diabetes mellitus has been reported as the most frequent risk factor, associated with 20-30% of cases and in the present study the association was found in 40 (35.7%) out of 112 cases.[3,14,15]

Over the past decade, a dramatic shift from *C. albicans* to non-albicans Candida as the prime aetiology of candiduria has taken place. *C. albicans* was responsible for about 50-70% cases of candiduria reported but today the scenario has changed.[1,5,7,16,17] Non-albicans Candida have become the major pathogens, especially in hospital settings; as yet

Table 1: Demographic profile of patients with candiduria

Category	Male No. (%)	Female No. (%)	Total No. (%)
Age			
0-15 years	7 (14)	4 (6.5)	11 (9.8)
16-30 years	9 (18)	9 (14.5)	18 (16.1)
31-45 years	7 (14)	13 (21)	20 (17.8)
45-60 years	8 (16)	15 (17.7)	23 (20.5)
>60 years	19 (38)	21 (33.9)	40 (35.7)
Total	50 (44.6)	62 (55.4)	112 (100)
OPD/IPD			
OPD	7 (14)	6 (9.7)	13 (11.6)
ICU	15 (30)	26 (41.9)	41 (36.6)
Wards	28 (56)	30 (48.4)	58 (51.8)
Risk factors			
Foley's catheter	27 (54)	32 (51.6)	59 (52.7)
Diabetes	19 (38)	21 (33.9)	40 (35.7)
Immunosuppressants	18 (36)	18 (29)	36 (32.1)
Antibiotic therapy	11 (22)	22 (35.5)	33 (29.5)
Renal pathology	4 (8)	5 (8.1)	9 (8)
None	1 (2)	5 (8.1)	6 (5.4)

OPD: Outpatient department; ICU: Intensive care unit; IPD: Inpatient department

Table 2: Age and gender-wise distribution of *Candida* species

Age (years)	*C. albicans*		C. tropicalis		*C. glabrata*		Others	
	Male (No.)	Female (No.)	Male (No.)	Female (No.)	Male (No.)	Female (No.)	Male (No.)	Female (No.)
0-15 years	0	0	4	3	2	0	1	1
16-30 years	2	1	4	6	3	0	0	2
31-45 years	1	2	3	6	2	3	1	2
45-60 years	0	3	6	5	2	6	0	1
>60 years	2	2	11	13	6	4	0	2

the reason for this inversion of species distribution has not been completely clarified but it could be related to their virulence potential and resistance to antifungals.[1] The *non-albicans* group now accounts for more than 65-70% isolates from urine.[13,18-21] The high rate of candiduria caused by non-albicans Candida in the present study, i.e, 99/112 (88.4%) correlates with this.

The correct diagnosis of the species involved in *Candida* infection is not only of epidemiological interest but also of clinical significance and the choice of antifungal is dependent on the species of Candida.[22]

In the present study, the rate of isolation of *C. tropicalis* (54.5%) followed by *C. glabrata* (25%) and *C. albicans* (11.6%) is similar to some studies in a similar period in India but differ from some others, emphasising that every centre needs to evaluate local patterns from time to time.[13-15,20,21] [Table 4]. Significant geographic variations in the aetiological pattern of invasive Candida spp. infections have been reported in various countries. In North America, there is a predominance of *C. glabrata* among the non-albicans speciesl in South America, however, *C. parapsilosis* and *C. tropicalis* are the predominant ones.[1] Thus, the speciation of Candida is important to provide a database for a given geographical area. The indiscriminate antifungal drug usage at inadequate doses, especially azoles, has caused an increase in the resistant strains of *Candida*. In the present study, antifungal susceptibility testing showed the highest susceptibility to Flucytosine followed by Amphotericin B and least susceptibility to azoles. Studies report an intrinsic resistance to Flucytosine in up to 10% of clinical isolates of *C. albicans* but *C. albicans* showed no resistance to Flucytosine in the present study.[7,13,14]

C. albicans in the present study showed only 84.6% susceptibility to Amphotericin B while various studies show 100% susceptibility.[13,14]

Furthermore, *C. albicans* and non-albicans Candida showed a similar susceptibility to Fluconazole (46.2% and 51.2%, respectively) in the present study, which again is in contrast to the results of studies from South India[13,15] [Table 4]. The increase in resistance to Fluconazole is a matter of great concern as it is the most commonly used azole for

Table 3: Pattern of antifungal susceptibility of the isolates

Candida spp./ Antifungal agent	*C. albicans* (*n* = 13) (%)	non albicans Candida (*n* = 99) (%)	*C. tropicalis* (*n* = 61) (%)	*C. glabrata* (*n* = 28) (%)	Others (*n* = 10) (%)
Flucytosine					
S	13 (100)	82 (82.9)	55 (90.2)	20 (71.4)	7 (70)
I	0	12	3	7	2
R	0	5	3	1	1
Amphotericin B					
S	11 (84.6)	60 (60.6)	41 (67.2)	12 (42.9)	7 (70)
R	2	39	20	16	3
Fluconazole					
S	6 (46.2)	42 (42.4)	26 (42.6)	11 (39.3)	5 (50)
I	0	5	3	2	0
R	7	52	32	15	5
Itraconazole					
S	2 (15.4)	25 (25.2)	15 (24.6)	6 (21.4)	4 (40)
I	10	32	22	8	2
R	1	42	24	14	4
Voriconazole					
S	6 (46.2)	37 (37.4)	22 (36.1)	10 (35.7)	5 (50)
I	0	9	3	5	1
R	7	53	36	13	4

S: Sensitive; I: Intermediate; R: Resistant

Table 4: Comparison of isolation percentage and antifungal susceptibility of Candida spp. in various studies

Studies	*C. albicans*				non albicans Candida			
	Isolation rate (%)	Flucyto-sine (%)	Amphot-ericin B (%)	Flucon-azole (%)	Isolation rate (%)	Flucyto-sine (%)	Amphot-ericin B (%)	Flucona-zole (%)
Shivananda *et al.* 2011	47	77.8	66.7	33.3	53	50	50	31.2
Ragini *et al.* 2012	13.3	—	100	62.5	86.6	—	100	59.6
Yashwanth *et al.* 2013	30.3	90	100	80	69.7	78.2	86.9	60.8
Present study	11.6	100	84.6	46.2	88.4	82.9	60.6	42.4

the treatment of candiduria.[13,23] The mechanism of azole resistance could be a structural or functional change at the azole-binding site, expression or over-expression of efflux pumps or change in biosynthetic pathway of ergosterols.[7] Azole resistance develops secondary to the accumulation of many resistance factors over time.

Among the azoles tested, the susceptibility to Itraconazole was the lowest irrespective of species. Pfaller *et al*. in 2012 stated that the clinical break points for Itraconazole are flawed.[24] They proposed epidemiological cut-off values of Itraconazole for eight candida species. Susceptibility to Itraconazole with MIC being ≤ 0.12 ug/mL was observed in 11% while 83% of *Candida albicans* had an MIC of 0.25-0.5 ug/mL in the study by Pfaller.[24] Mahmoudabadi *et al*. reported similar Itraconazole susceptibility and dose-dependent susceptibility rate — 6.5% and 55.9%, respectively, for *C. albicans*, which in the present study too was 15.4% and 77%, respectively.[25] Pfaller in 2003 also showed similar observations for Itraconazole susceptibility of Candida spp.[26] Thus, there is a need for a multi-centric study to establish cut-off values of Itraconazole for Candida spp.

CONCLUSION

Non-albicans Candida species have replaced *Candida albicans* as the most common causative agents. This study shows the species and antifungal susceptibility pattern in this region and highlights the variation from other regions. Therefore, an ongoing surveillance of the different species of *Candida* and their susceptibility patterns should be in place to help in early and effective treatment. Resistance to both Amphotericin B and Fluconazole is on the rise requiring the judicial use of antifungals in the immunocompetent and immunocompromised host both for therapeutic and prophylactic therapy.

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Conflicts of interest
There are no conflicts of interest.

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