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CANDIDURIA- PREVALENCE, SPECIATION AND INTERPRETATION OF SUSCEPTIBILITY PATTERN: A STUDY FROM PUDUCHERRY

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ABSTRACT

Candiduria is a common finding in hospitalized patients, Non-albicans *Candida species* (NAC) have emerged as a pathogenic fungus group, where speciation is important, due to intrinsic resistance to commonly used azoles. In this study, 5180 urine samples were processed to determine prevalence of candiduria, to characterize and determine their antifungal susceptibility pattern by disk diffusion and E-test method. Out of 5180 urine samples, 2092 grew pathogens. Candida accounted for 2.58% (n=54) of these uropathogens. *C.albicans* was the commonest urinary pathogen (46.3%, n=25). NAC accounted for 53.7% (n=29) of Candida isolates. *C.albicans* showed 88% susceptibility to fluconazole and 96% susceptibility to voriconazole. *C.tropicalis* showed 82.4% sensitivity to fluconazole and 88.2% susceptibility to voriconazole. The results indicate that NAC is emerging and replacing *C. albicans* as a cause for candiduria. Most of the Candida isolates remained susceptible to commonly used antifungal agents like fluconazole and voriconazole.

KEYWORDS: ***Candida albicans*; non-albicans Candida; Candiduria; antifungal susceptibility.**

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INTRODUCTION

Infections caused by *Candida* species are becoming increasingly common in recent years due to substantial increase in immunocompromised patients as a consequence of global HIV epidemic, immunosuppressive therapy for organ transplantation and cancer chemotherapy. [1, 2] *Candida albicans* constitutes the most common *Candida* species associated with human infections. [1] Although urinary tract infection due to *Candida* is uncommon in community setup, candiduria is not uncommon in hospital patients, especially in presence of diabetes, prolonged antibiotic usage and corticosteroid therapy and urine flow obstruction.[3] In recent years, Non-albicans *Candida* (NAC) species have emerged as a potentially pathogenic fungus group. Several NAC species are isolated in increasing numbers from urine samples. [4, 5] *C. glabrata* has been frequently found to be the second or third most common pathogenic species after *C. albicans*. Owing to inherent resistance to fluconazole, *C. glabrata* infections are often difficult to treat and have a high mortality.[2] *C. parapsilosis* infections are reported to be associated with parenteral nutrition, prosthetic devices, and indwelling catheters, as well as the nosocomial spread in neonatal and intensive care units through the hands of health care workers.[6] Current research reflects the ongoing potential for these NAC isolates to develop resistance to azoles.[2, 7] Furthermore, potentially fatal systemic and blood stream infections caused by NAC are not uncommon in critically ill patients with candiduria.[2, 8] Consequently, speciation and antifungal susceptibility testing of *Candida* isolates has gained greater recognition. Antifungal susceptibility pattern of these *Candida* isolates is essential for monitoring the changing trend in susceptibility of different *Candida spp.* [9] Though there are numerous studies on speciation and antifungal susceptibility of *Candida* species in HIV patients,[10] diabetics, and vulvovaginal candidiasis,[11, 12] there are inadequate data from our region on prevalence of candiduria . This is important in order to rationalize selection of antifungals for empirical treatment, when necessary. Hence, this study was undertaken with an objective to identify prevalence of candiduria, to characterize and determine the antifungal susceptibility pattern of *Candida* isolates recovered from urine samples and to see if there was any variation in interpretation of results as per CLSI and EUCAST guidelines.

METHODOLOGY

A prospective study was carried out in Microbiology laboratory of a tertiary care hospital in south India from January to December 2013. All urine samples received during the study period were processed as per standard microbiology procedures.[13] Colonies with characteristics suggestive of yeast were subjected to gram staining and were further identified up to species level by microscopic morphology, germ tube test, carbohydrate fermentation test, growth characteristics on Sabouraud dextrose agar (SDA), Cornmeal agar and Candida CHROM agar. The *Candida* isolates recovered during the study period were subjected to antifungal susceptibility testing by disc diffusion and E-test method. Antifungal susceptibility testing by disc diffusion for *Candida* isolates were carried out as per Clinical Laboratory Standards Institute (CLSI) guidelines.[14] Mueller–Hinton agar plates supplemented with 2% dextrose and 0.5 mg/L methylene blue were inoculated by dipping sterile swabs into the 0.5 McFarland standard inoculum (1 x 10^6 cells/ml) of yeast suspension and evenly streaking it over the surface of agar. After drying, fluconazole (25µg), voriconazole (1µg), itraconazole (10µg) and amphotericin B (20µg) discs (Himedia, Mumbai, India) were applied onto the agar surface. Zone of inhibition were measured after 24 and 48 hours of incubation at 35^{O}C.Minimum inhibitory concentration (MIC) for fluconazole and voriconazole were determined by E-test (Himedia, Mumbai, India) according to the manufacturer's instructions. The inoculum of *Candida* strains was adjusted to 0.5 McFarland and spread over agar surface using a cotton

swab. The MIC endpoint was read after an incubation period of 24 and 48 hours at 35^{O}C. The antifungal concentration associated with 80% inhibition of growth around fluconazole and voriconazole strips, was considered as the MIC. Quality control of disc diffusion and E-test methods was ensured by using *Candida albicans* ATCC 90029 and *C. parapsilosis* ATCC 22019 strains. All data were analyzed by SPSS software (version 17, Chicago). Statistical analysis involved calculation of percentages and proportions. Fischer's exact test was used to calculate the p values. A P value of 0.05 or below was considered significant and a P value of 0.05 < P < 0.10 was considered marginally significant. A P value of > 0.10 was considered not significant.[15]

RESULTS

Out of total 5180 urine samples processed, 2092 yielded growth of pathogens. *Candida* accounted for 54 (2.581%) of these pathogens isolated. The commonest *Candida* isolates were - *Candida albicans* (n=25, 46.3%), followed by *Candida tropicalis* (n=17, 31.5%), *Candida krusei* (n=8, 14.8%), *Candida glabrata* (n=3, 5.6%) and *Candida parapsilosis* (n=1, 1.9%). The results are depicted in Table 1. NAC accounted for 53.7% of the isolates, while *C. albicans* accounted for the rest (46.3%).

Table 1
Commonest Candida species (n=54) isolated

Candida species	Frequency (n)	Percentage (%)
C. albicans	25	46.3
C. tropicalis	17	31.5
C. krusei	8	14.8
C. glabrata	3	5.6
C. parapsilosis	1	1.9

A total of 54 urinary Candida isolates were tested. The resistance patterns of different species of Candida- i.e., C. albicans, C. tropicalis and C. krusei are given in tables 2, 3 and 4 respectively.

Table 2
Resistance pattern of Candida albicans (n=25) isolates to different antifungal drugs tested

Antimicrobial	Sensitive		SDD*		Resistant	
	N	%	N	%	N	%
Fluconazole (E-test)	22	88	2	8	1	4
Fluconazole (disc diffusion)	23	92	2	8	0	0
Voriconazole (E- test)	24	96	0	0	1	4
Voriconazole (disc diffusion)	24	96	0	0	1	4
Itraconazole (disc diffusion)	10	40	15	60	0	0
Amphotericin-B (disc diffusion)	21	84	2	8	2	8

SDD, sensitive dose dependent.

Table 3
Resistance pattern of Candida tropicalis (n= 17) isolates to different antifungal drugs

Antimicrobial	Sensitive		SDD*		Resistant	
	N	%	N	%	N	%
Fluconazole (E-test)	14	82.4	2	11.8	1	5.8
Fluconazole (disc diffusion)	16	94.1	0	0	1	5.9
Voriconazole (E- test)	15	88.2	0	0	2	11.8
Voriconazole (disc diffusion)	16	94.1	0	0	1	5.9
Itraconazole (disc diffusion)	1	5.9	6	35.3	10	58.8
Amphotericin-B (disc diffusion)	10	58.8	7	41.2	0	0

***SDD, sensitive dose dependent*.**

Table 4
Resistance pattern of Candida krusei (n=8) isolates to different antifungal drugs tested

Antimicrobial	Sensitive N	%	Intermediate N	%	Resistant N	%
Fluconazole (E-test)	*Candida krusei* is intrinsically resistant to Fluconazole, hence no values given.					
Fluconazole (disc diffusion)						
Voriconazole (E- test)	8	100	0	0	0	0
Voriconazole (disc diffusion)	8	100	0	0	0	0
Itraconazole (disc diffusion)	6	75	0	0	2	25
Amphotericin-B (disc diffusion)	4	50	3	37.5	1	12.5

All urinary Candida glabrata (n=3, 100%) isolates from this study were sensitive to fluconazole and voriconazole by both E-test and disc diffusion method (Figure 1, 2a and 2b). However, testing for itraconazole by disc diffusion showed all isolates (n=3,100%) were SDD sensitive dose dependent, while amphotericin-B testing showed 2 isolates as sensitive (66.67%) and 1 resistant (33.3%) to amphotericin-B.

Figure 1
Antifungal susceptibility of Candida isolates by disc diffusion method

Figure 2a
Antifungal susceptibility of Candida isolates by E-test for fluconazole

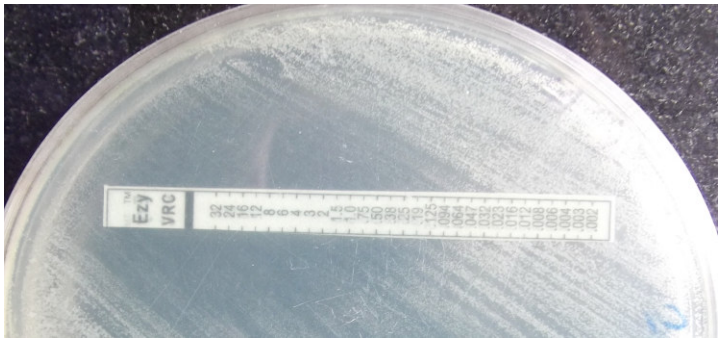

Figure 2b
Antifungal susceptibility of Candida isolates by E-test for voriconazole

We compared the difference between EUCAST and CLSI criteria for candida isolates. EUCAST criteria for fluconazole MIC, when applied for *C. albicans* isolates showed 64% of isolates (n=16) were sensitive to fluconazole and 36% (n=9) isolates were resistant. In contrast, CLSI criteria when applied showed 88% (n=22) sensitive, 8% (n=2) SDD and 4% (n=1) resistant for the same ($p < 0.1$, marginally significant).

EUCAST criteria for fluconazole MIC, when applied for *C. tropicalis* isolates showed 70.6% of isolates (n=12) were sensitive to fluconazole and 29.4% (n=5) isolates were resistant. In contrast, CLSI criteria when applied showed 82.4% (n=14) sensitive, 11.8% (n=2) SDD and 5.8% (n=1) resistant for the same ($p=0.68$, Not Significant).

Likewise, EUCAST criteria for voriconazole MIC, when applied for *C. albicans* showed 76% (n=19) sensitive and 24% (n=6) resistant strains. In contrast, CLSI criteria when applied showed 96% (n=24) sensitive and 4% (n=1) resistant strains ($p<0.1$, marginally significant). Similarly, EUCAST criteria for voriconazole MIC, when applied for *C. tropicalis* showed 52.9% sensitive (n=9) and 47.1% resistant (n=8). However, CLSI when applied for the same showed 88.2% (n=15) sensitive and 11.8% (n=2) resistant ($p=0.06$, marginally significant).

DISCUSSION

In our study, *Candida* accounted for 2.58% of the total uropathogens isolated during the study period and 1.043% of all samples. In a European study, *Candida* accounted for 1% of all positive and 0.2% of all urine samples (n=100,522) received.[16] This higher percentage could be due to two reasons- firstly, the lower number of samples processed in our study and secondly, most of the samples could be from inpatients with various risk factors for candida infection.

Non-albicans Candida versus C. albicans

In our study, though *C. albicans* was the commonest urinary pathogen (46.3%, n=25), the higher isolation rates of NAC species (53.7%, n=29) of which, *C. tropicalis* (31.5%, n=17), (was the commonest), followed by *C. krusei* (14.8%, 8) and *C. glabrata* (5.6%, n=3) respectively, point to the importance of these species in causing UTI. This is in agreement with other Indian and European studies.[17, 18] However, the Indian studies have reported much higher rates of NAC isolation rates of 89.5-89.8% from candidaemia patients.[17, 19] In contrast, we have isolated only 53.7% of NAC isolates from urine samples. Nevertheless, it is evident that NAC has overtaken *C. albicans* as a pathogen in large parts of India.

Susceptibility pattern of various Candida isolates

While the susceptibility of *Candida* to currently available antifungal agents is generally predictable if the species of the infecting isolate is known, some individual isolates do not follow this general pattern. For this reason, susceptibility testing is increasingly used to guide the management of candidiasis, especially when the patient fails to respond to initial antifungal therapy.[20] Susceptibility testing for itraconazole by disc diffusion method (non-CLSI) for *C. albicans* revealed susceptibility to only 40% of the isolates (n=10), while 60% (n=15) of the isolates were SDD. Itraconazole testing for *C. tropicalis* revealed only 1(5.9%) isolate as susceptible, 6 (35.3%) were SDD and majority of isolates (58.8%, n=10) were resistant. However, majority of *C. krusei* isolates were susceptible to itraconazole (n=6, 75%).Resistance to amphotericin-B (non-CLSI) was observed in 8% of *C. albicans*, 12.5% of *C. krusei* isolates. The worrying factor was development of SDD (Sensitive dose dependent) susceptibility to amphotericin-B in 37.5% of *C. krusei* isolates. However, all the *C. tropicalis* isolates were susceptible to amphotericin-B.MIC testing of fluconazole by E-test revealed 1 isolate of *C. albicans* to be resistant, while disc diffusion for the same could not identify this resistant strain. Similarly, fluconazole MIC determination for *C. tropicalis* picked up 2 SDD strains, while disc diffusion failed to identify it. It should be noted that *Candida krusei* is intrinsically resistant to fluconazole, hence susceptibility is not tested. Susceptibility testing for voriconazole by both MIC and disc diffusion method was found to be similar in results in case of *C. albicans*, i.e., 1 isolate (4%) was resistant by both methods. However, for *C. tropicalis*, 1 more isolate (total 2 resistant isolates, 11.8%) was identified as resistant by MIC method as against disc diffusion. All *C. krusei* isolates were susceptible to voriconazole by both MIC and disc methods. However, the variations observed in interpretation of results by both disc diffusion and MIC testing was not statistically significant. Hence, both MIC testing and disc diffusion method are equally good in providing susceptibility results for *Candida* isolates.

Variation in MIC interpretation for fluconazole and voriconazole according to EUCAST and CLSI guidelines

The interpretations of MIC values vary when CLSI [21] or EUCAST [22] guidelines are applied for fluconazole and voriconazole. This is depicted in Table 5.

Table 5

MIC interpretation for fluconazole and voriconazole in EUCAST and CLSI guidelines

MIC	EUCAST-fluconazole		CLSI - fluconazole			EUCAST-voriconazole		CLSI- voriconazole		
	S	R	S	SDD	R	S	R	S	SDD	R
C. albicans	≤2	>4	<_2	4	>_8	≤0.12	>0.12	<_0.12	0.25-0.5	>_1
C. glabrata	≤0.002	>32	X	<_32	>_64	X	X	X	X	X
C. tropicalis	≤2	>4	<_2	4	>_8	≤0.12	>0.12	<_0.12	0.25-0.5	>_1
C. parapsilosis	≤2	>4	<_2	4	>_8	≤0.12	>0.12	<_0.12	0.25-0.5	>_1
C. krusei	X	X	IR	IR	IR	X	X	<_0.5	1	>_2

S, sensitive; R, resistant; SDD, sensitive dose dependent.

Several studies have compared susceptibility profile of Candida species by EUCAST and CLSI guidelines. Orasch *et al.* studied the susceptibility profile of blood stream isolates of Candida species. *C. albicans* was mostly susceptible to fluconazole, voriconazole and caspofungin without significant inconsistency between CLSI and EUCAST.[23] However, a

greater number of *C. tropicalis/C. parapsilosis* isolates were non-susceptibile to the azoles when applying EUCAST and new CLSI breakpoints, compared with old CLSI breakpoints. In another study, no difference was observed between EUCAST and revised CLSI clinical breakpoints for fluconazole in *C. albicans* isolates.[24] We found a marginally significant variation in the interpretation of MIC results by CLSI and EUCAST, when testing for both fluconazole and voriconazole for *C. albicans*. Also, significant variation is observed in interpretation of MIC results for voriconazole, when testing for *C. tropicalis* isolates. There are no criteria in EUCAST for voriconazole MIC testing in *C. krusei*, while CLSI recommends testing for these isolates. In our study, as per CLSI, *C.krusei* was fully sensitive (100%) to voriconazole by MIC testing. Thus, there is a need for international guideline agencies to provide uniform criteria for better interpretation of results.Cost constraints prevented further testing for other important antifungal agents like caspofungin, micafungin, anidulafungin, posaconazole and 5-flucytosine. Also, due to the lower number of isolates tested, the results of this study will have to be validated by conducting more multicentric studies.

CONCLUSION

Prevalence of candiduria was 1.04%. Non-albicans *Candida* is emerging and replacing *C. albicans* as a urinary isolate. Speciation is important in this group as intrinsic resistance to commonly used azoles is well known. There is a marginally significant variation in the interpretation of MIC results by CLSI and EUCAST when testing for both fluconazole and voriconazole for *C. albicans* and for voriconazole testing in *C. tropicalis* isolates. Both MIC testing and disc diffusion method are equally good at providing susceptibility results for *Candida* isolates.

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