Isolation of Candida species from urine samples along with its antifungal susceptibility pattern in Paediatric patients attending Tertiary Care Hospital of Baroda

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Abstract

Background: The presence of yeast cells in urine is called Candiduria. It is an increasingly common finding in hospitalized patients.Candida albican is the most common causative agent of fungal infections. However, in recent years there has been an increasing trend in the emergence of non albicans candida as a potential pathogen which is found to be more resistant to antifungal drugs than Candida albicans. The main aim of this study is to identify & differentiate the Candida spp isolated from urine samples of Peadiatric patients along with their antifungal susceptibility pattern.

Material and Method: This study was conducted from April 2013 to September 2013 in Department of Microbiology, Medical College Baroda. A total 1202 urine samples were collected from Paediatric patients suspected of having urinary tract infections. Urine samples were processed by semi quantative culture method. Antifungal susceptibility of all the isolates was performed by the Modified Kirby Baur disc diffusion method with fluconazole, voriconazole.

Result: A total 57 Candida species were isolated from 427 culture positive urine samples. Candida albicans was most commonly isolated (24.50%) followed by Candida tropicalis (22.80%). Overall, 75% cases were due to non albican candidas species. All candida isolates were found to be susceptible to voriconazole but higher degree of resistance was seen with fluconazole.

Conclusion: Candida spp were the pathogens identified in 13% of hospital-acquired urinary tract infections in Peaditric patients. Non albicans candida species are replacing to Candida albicans as predominant pathogen causing nosocomial urinary tract infections. Candida glabrata and Candida krusei were more resistant to fluconazole, so it is necessary to use other antifungal drug to combat the infection caused by these species.

Keywords: Candiduria, Voriconazole, Fluconazole**,** Candida albican

Introduction

The natural history of urinary tract infection (UTI) in children has changed due to the introduction of antibiotics and improvements in healthcare. This change has contributed to uncertainty about the most appropriate and effective way to manage urinary tract infection in children whether investigations and follow-up are justified or not. It may be difficult to recognise urinary tract infection in children because the presenting symptoms and signs are non-specific, particularly in infants and children younger than 3 years. Collecting urine and interpreting results are not easy in this age group, so it may not always be possible to unequivocally confirm the diagnosis.(1)

Candiduria is the presence of yeast cells in urine and it is an increasingly common finding in hospitalized patients. Most of patients with candiduria are asymptomatic as there are no associated signs and symptoms. However quantitative cultures with colony count of > 10^5/ml of urine are associated with infection in patients without indwelling catheters in contrast, clinically significant renal Candidiasis has been reported even with low colony counts of 10^3/ml of urine. Moreover pyuria usually supports diagnosis of Candida infection. It involves vast majority of patients, however, Candiduria most likely reflects colonization or infection of lower urinary tract or collecting system of kidneys.(2)

Candida spp is one of the most common causes of nosocomial urinary tract infections.(3) Prolonged hospitalizations, long stay in NICU, urinary tract abnormality, immunocompromised patients, broad spectrum antibacterial therapy for long time and prophylaxis by antifungal agents are presented as important risk factors for urinary tract infections.(4-7) Candiduria in hospitalized patients in intensive care unit (ICU)/NICU can be a relevant marker for systemic candidiasis.(8)

It has been reported that 11to 52% of nosocomial urinary tract infections are caused by Candida spp.(9-13) Candida albicans were recovered from 97% of urine cultures in children.(14) However, during last two decades incidence of non albicans species was increased.(15) C.tropicalis, C. glabrata, C. parapsilosis, C. lusitaniae, C.guilliermondii and C. krusei are non albicans species that cause candiduria.(16)

The susceptibility range of Candida spp varies to antifungal drugs. Several reports show that non albicans spp are more resistant to antifungals, especially fluconazole.(15,17) The recognition of differences in incidence, populations at greater risk, species distribution is important in order to establish appropriate measures of infection control and the management of this disease.

Generally, the routine laboratory practice is limited to report only the presence of fungal structure in urine. The complete identification and antifungal susceptibility test is not performed. This fact may endanger the effectiveness of antimicrobial therapy and patient's health, since different species have a different virulence as well as different susceptibility to differentiated antifungal drugs. The aim of this study was to identify and differentiate the Candida species isolated from urine samples along with their antifungal susceptibility pattern in Peadiatric patients attending tertiary care hospital Baroda.

Material and Method

This retrospective study was conducted at the Department of Microbiology in a SSG hospital, Baroda. It is tertiary care centre, referral and teaching hospital. This study was conducted from April 2013 to September 2013. During this period total 1202 urine samples were collected from Paediatric patients suspected of having urinary tract infection. This study comprises 57 Candida species isolated from urine sample of Paediatric patient.

Sample processing: A midstream clean catch or catheterized specimens were selected, as these minimize the presence of genital flora. The urine sample is inoculated on Sabouraud's dextrose agar and blood agar and incubated at 37°C for 24-48 hours. The samples were selected on the basis of their growth on routine Sabouraud's dextrose agar and on blood agar media.

Confirmation of Candida spp and Species level identification: On Sabouraud's dextrose agar Candida produce creamy moist colony and on blood agar the colonies of Candida were large creamy moist & white in color when incubated for 24-48 hrs at 37°C.

Identification of Candida species were further speciated by a panel of tests like Gram stain, Germ tube formation test, Colour on CHROM agar Candida medium, Chamydospore formation on Corn meal agar, Sugar fermentation test and Sugar assimilation test on Yeast Nitrogen Base agar and Urease test.[2]

Antifungal Susceptibility Testing: Antifungal susceptibility of all the isolates was performed by the Modified-Kirby Baur disc diffusion method according to the Clinical and Laboratory Standard Institute M 44A standard. Mueller-Hinton agar supplemented with 2% glucose and 0.5 ug/ml methylene blue dye medium was used. Inoculum was prepared in 5 ml of Normal Saline to match the turbidity with 0.5 Mc Ferland standard opacity tube. Lawn culture of inoculums was done on Muller Hinton agar and then commercially available HIMEDIA susceptibility test disc fluconazole (25µg), voriconazole (1 µg) were placed on it. These plates were incubated at 37°C for 48 hrs. The diameters of inhibition zone were measured by zone measuring scale. Result were expressed as "Susceptible", "Susceptible dose dependent "and "Resistant".[18,19]

Table 1: The interpretive criteria for fluconazole and voriconazole disk diffusion testing were those published by the CLSI and were as follows

Antifungal disc	Susceptible	Susceptible dose dependent	Resistant
Fluconazole (25µg)	≥19 mm	15-18 mm	≤14 mm
Voriconazole (1µg)	≥17 mm	14-16 mm	≤13 mm

Result

In the present study a total number of 57 Candida spp were isolated from urine samples of Paediatric patient during year 2013(April- September). Out of 57 patients with candiduria, 40 (70.17%) were male and 17 (29.83%) were female. Among them majority of the patients 40 (70.17%) were less than 5 years. Most commonly involved age group was 1 year -5 year.(29.83%).

Table 2: Age and Sex distribution of the study group in Candida spp

Sr. No	Candida spp	Age groups					Sex		Total
		Neonates	1month -1 year	1-5 year	5-10 year	10-13 year	Male	Female	
1	C.albican	2	2	5	3	2	13	1	14 (24.56%)
2	C.dublinensis	1	4	4	0	0	7	2	9 (15.78%)
3	C.tropicalis	1	4	3	3	2	10	3	13 (22.80%)
4	C.glabarata	0	1	4	2	2	4	5	9 (15.78%)
5	C.krusei	1	1	0	0	1	1	2	3 (5.26%)
6	C.parapsilosis	2	4	1	2	0	5	4	9 (15.78%)
Total		7 (12.28%)	16 (28.07%)	17 (29.83%)	10 (17.54%)	7 (12.28%)	40 (70.17%)	17 (29.83%)	57 (100%)

Among 57 patients with candiduria, 44 (77.19%) were hospitalized in ICU and in Paediatric wards. The majority of children with candiduria were hospitalized in ICUs (40.35%).

Table 3: Distribution of Candida species among in patients and Outpatients

	Candida spp	In patients		Out patients	Total
		ICU	ward	OPD	
1	C.albican	7	6	1	14(24.56%)
2	C.dublinensis	4	4	1	9(15.78%)
3	C.tropicalis	3	3	7	13(22.80%)
4	C.glabarata	3	5	1	9(15.78%)
5	C.krusei	2	1	0	3(5.26%)
6	C.parapsilosis	4	2	3	9(15.78%)
Total		23(40.35%)	21(36.84%)	13 (22.80%)	57(100%)

Most of the candiduria have been caused by non albicans Candida (75.43%).C. albicans accounted for 24.56% followed by C.tropicalis(22.80%),C. glabrata (15.78%),C. parapsilosis(15.78%), C.dublinensis(15.78%) and less common isolates was C.krusei(5.26%).

In the present study all the Candida species were found to be susceptible to voriconazole(100%). Higher degree of resistance against fluconazole was seen among C.krusei(100%) followed by C.glabarata(20%). C.glabarata were also found to be susceptible dose dependent to fluconazole(60%).

Table 4: Antifungal susceptibility of candida spp to fluconazole and voriconazole

Candida spp	Fluconazole			Voriconazole		
	S	S-DD	R	S	S-DD	R
C.albican (N=14)	14(100%)	0	0	14(100%)	0	0
C.dublinensis(N=9)	9 (100%)	0	0	9(100%)	0	0
C.tropicalis(N=13)	13(100%)	0	0	13(100%)	0	0
C.glabarata(N=9)	2(20%)	5(60%)	2(20%)	9(100%)	0	0
C.krusei(N=3)	0	0	3(100%)	3(100%)	0	0
C.parapsilosis(N=9)	9(100%)	0	0	9 (100%)	0	0

Note: *S- Susceptible, *S-DD- Susceptible dose dependent, *R-Resistant

Discussion

Over the last two decades, fungal infections have increased at an alarming rate. Candida spp are commensals and act as opportunistic pathogen only on interruption of normal host defense.

In the present study, candiduria was diagnosed in 13% of the patients with urinary tract infection. This finding is well correlated with other studies. It has been reported that 11to 52% of nosocomial urinary tract infections are caused by Candida spp.[9-13] Increased age, female sex, antibiotic use, urinary drainage devices and prior surgical procedures are considered as risk factors for candiduria.[20]

Although females have higher risk for developing candiduria, in our study, similar to Seifi Z et al and Yashavanth R reports, candiduria was more common in males (70.17%) than females (29.83%).[4,21]

In the present study the age range of the most of the patients with candiduria was 1-5 year old(30%) followed by 1month to 1 year old (28%).These observations are different from the findings of Seifi Z. et al in which the age range of the most of the patients with candiduria was less than 1 year(42.9%).[4]

Philips et al. reported that Candida spp were responsible for 42% UTI in infants admitted to a neonatal intensive care unit.[20,22] The prevalence of candiduria in the ICU population is increasing ranges from 19 to 44% of urine specimens has been reported among different population.[4,9] In this study, the majority of children with candiduria were hospitalized in ICUs (40.35%). The higher number of candiduria cases in patients of ICUs might be due to concurrent factors that contribute to the selection of these pathogens such as underlying diseases, immunodeficiency, and multiple manipulations by health care personnel and broad spectrum antibacterial therapy for long time.[9]

All Candida species are capable of causing urinary tract infections. Although, 50-70% of candiduria are caused by C. albicans.[4,20,23,24] However, during last two decades incidence of non albicans species was also increased.[6,7,14] In the present study, most of the candiduria have been caused by non albicans Candida (75.43%).Which correlates with the study by Yashavantha R.et al.[21]

Seifi, Z et al, Azam N et al, Izabela A et al. and Ozhak-Baysan et al reported that C.albicans was the dominant species.[4,24,25,26] In this study C.albicans

accounted for (24.56%) followed by C.tropicalis(22.8%), C.glabrata(15.78%), C.parapsilosis (15.78%), C.dublinensis(15.78%)and C. krusei(5.26%).

Table 5: Distribution of Candida spp isolated from urine samples in different studies

Candida spp	Present study	Seifi, Z et al[4]	Yashavantha R. et al[21]	Ozhak-Baysan et al[26]	Izabela A et al[25]
C.albican	24.56%	65.5%	30.3%	44%	69.3%
C.dublinensis	15.78%	----	----	-----	----
C.tropicalis	22.80%	----	45.45%	20%	1.8%
C.glabarata	15.78%	31.0%	9.09%	18%	12.9%
C.krusei	5.26%	3.5%	15.15%	6%	8.1.%
C.parapsilosis	15.78%	-----	----	4%	----
Other Candida spp.	----	-----	-----	8%	1.8%

Some of the clinicians have believed that the presence of Candida spp in urine samples is marked as harmless colonization, or lower tract infection. On the other hand, candiduria is well- known as an important risk factor for invasive candidiasis with considerable morbidity and mortality.[27]

In the present study Candida isolates showed highest susceptibility to voriconazole (100%). The finding of present study correlates with the study R. Adhikary et al in which all candida isolates were susceptible to voriconazole.[28]

Higher degree of resistance against fluconazole was seen among C.krusei (100%) followed by C.glabarata (20%). The findings of the present study correlated with those of a study by Jin-Sol Lee et al in which C. krusei resistant to fluconazole(100%)followed by C. glabrata (38%).[29] C.glabarata were also found to be susceptible dose dependent to fluconazole(60%) Overall dose dependent susceptibility was observed in 9% of Candida isolates. The findings of the present study are lower than a study by R. Adhikary et al in which Dose dependent susceptibility was observed in 25% Candida isolates.[28] C. krusei and C. glabrata showed high resistance to fluconazole, probably due to their innate resistance to these drugs.

Conclusion

Candida species were the pathogens identified in 13% of hospital-acquired urinary tract infections in Paediatric patient. The majority of children with candiduria were hospitalized in ICU. Candida glabrata and Candida krusei were more resistant to fluconazole. Candiduria in critically ill newborn very often reflects to Candidemia and disseminated candidiasis. As fluconazole is the first choice of drug for treatment as well as for prophylaxis, it is necessary to use other antifungal drug to combat the infection caused by these species. These data demonstrate the importance of conducting full identification of candida spp and susceptibility to antifungal agents in urine sample especially when patient have high risk factors for developing fungal infections.

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