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Speciation and antifungal susceptibility profile of candida isolates from urine samples in a tertiary care hospital

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Abstract
Background: Candiduria is one of the most common symptoms of urinary tract infections caused by several species of Candida. Several antifungal drugs are available to treat such candida infections. During the last decade, resistance to antifungals especially in non-albicans species has increased.
Objectives: The present study aimed to evaluate the speciation and antifungal susceptibility profile of Candida isolates from urine samples.
Materials and Methods: A total of 100 Candida spp. isolated from urine samples were included in the study. Speciation of Candida was done by conventional methods and colony color on CHROM agar. Antifungal susceptibility testing of the isolates was performed by disc diffusion method on Glucose Methylene Mueller - Hinton agar (GM-MH)
Results: Candiduria was more common in females. In both the sexes maximum patients belong to age group 51-60 years. Urinary catheterization followed by the use of broad spectrum antibiotics and diabetes mellitus were the major risks for the development of candiduria. Out of 100 isolates, 36(36%) were *C. albicans* whereas 64 (64%) belonged to Non albicans Candida (NAC) spp. Among NAC spp. *C. tropicalis* was the major isolate. Candida species showed maximum sensitivity to Amphotericin B 95%, followed by Ketoconazole 69%,
Conclusions: Candiduria is becoming an important nosocomial infection. Incidence of non Candida albicans was more than *Candida albicans.* NAC species are more resistance to antifungal as compared to *C albicans*

Keywords: candiduria, non albicans candida, antifungal susceptibility testing

Introduction

Candida species are true opportunistic pathogens. The presence of Candida in urine is referred to as candiduria. It is one of the most common symptoms of urinary tract infections caused by several species of Candida [1]. Among the hospital acquired urinary tract infections, 80% are caused by urinary catheters. Surveillance data (1986 to 1996) from the United States National Nosocomial infection Surveillance System, has shown that *Candida albicans* is the fourth most common cause of urinary tract infections [2].

The predisposing factors causing Candiduria are urinary tract instrumentation, Diabetes mellitus, prior antibiotic use, prolonged hospital stay, extremes of ages, immunosuppressive therapy and female sex [3, 4]. The isolation rate of Candida is as high as 10-15% of all positive urine cultures in tertiary care centres [5, 6]. 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* [7, 8].

The increased incidence of fungal infections in patients suffering from urinary tract infections associated with obstructive uropathy as well as the emergence of azole antifungal drug resistance [9]. The growing trend in antifungal drug resistance and emergence of new species of Candida, poses a need for regional surveillance of antifungal drug susceptibility profiles, since in vitro drug susceptibility patterns are associated with therapeutic outcome [10].

The present study was undertaken to determine the incidence of candiduria, the distribution of species and the accompanying antifungal susceptibility profile which may be used to monitor the efficacy of current antifungal treatment guidelines for candiduria.

Material and Methods

Study Design: This study was carried out in the Department of Microbiology, SMS Medical College Jaipur from April 2016 to March 2017 to determine the speciation and antifungal susceptibility profile of Candida isolates from urine samples. Permission from institutional ethical committee was obtained for study. A total of 100 Candida species was isolated from urine. Quantitative cultures with Candida colony counts of > 10^4 colony forming unit (CFU)/ ml inpatients without indwelling urinary catheter and of ≥ 10^3 CFU/ ml of urine in patients with indwelling urinary catheter were considered significant.

Sample Collection and Methods: Species identification of Candida isolates was done by conventional techniques and a colony color on HiChrome Candida agar [11]. Antifungal susceptibility of the isolates for fluconazole, ketoconazole, itraconazole and amphotericin B was done by disc diffusion method on glucose methylene Mueller- Hinton agar (GM-MH) [12-14]. The zone diameters were interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines. *C. albicans* (ATCC 90028) and *C. krusei* (ATCC 6258) were used as control strains.

Statistical analyses were done using computer software primer. The qualitative data were expressed in proportion and percentages and the quantitative data expressed as mean and standard deviations. The difference in proportion was analyzed by using chi square test. Significance level for tests was determined as 95% ($P< 0.05$)

Results

In the present study, candiduria was more common in females. In both the sexes maximum patients belong to age group 51-60 years (27%) followed by 41-50 years of age group (16%) Urinary catheterization followed by the use of broad spectrum antibiotics and diabetes mellitus were the major risks for the development of candiduria. Out of 100 isolates, 36(36%) were *C. albicans* whereas 64 (64%) belonged to Non albicans Candida (NAC) spp. Among NAC spp. C. tropicalis was the major isolates followed by C. parapsilosis (Table 1). In this study Candida species showed maximum 95% sensitivity to Amphotericin B, followed by Ketoconazole 69%, Itraconazole 66% while in case of Fluconazole sensitivity was 63%.(Table 2) Statistically it was significant.

Table 1: Distribution of the *Candida* species on CHROM agar

Candida species	Number	Percentage%
Candida albicans	36	36
Candida tropicalis	37	37
Candida glabrata	6	6
Candida krusei	3	3
Candida parapsilosis	18	18
Total	100	100

Table 2: Antifungal Susceptibility Pattern of *Candida* Species

Fluconazole	Number	Percentage%
Sensitive	63	63
Resistant	37	37
Ketoconazole		
Sensitive	69	69
Resistant	31	31
Itraconazole		
Sensitive	66	66
Resistant	34	34
Amphotricin – B		
Sensitive	95	95
Resistant	05	05

Discussion

Candiduria is the presence of yeast cells in urine and is an increasingly common finding in hospital patients [14]. *Candida* species is often isolated from urine samples and has been estimated to account for as many as 21% of urinary yeast isolates [15]. In India *Candida* species is the fifth most common nosocomial urinary pathogen [16]. and the age range for funguria has been documented 1-75 years [17].

In this study, the highest incidence of candiduria was seen in the age group 51 to 60 years of age (27%), followed by 41 to 50 years of age (16%) This could be due to elderly people are at higher risk of funguria because of decreased immunity in advance age. Our results are similar to those of Yashavanth *et al*, [18] Goyal RK *et al*. [19] and Naseema Shaik *et al*. [20] who also observed similar findings.

Candiduria is seen in association with catheterization, administration of antibiotics, diabetes mellitus and use of immunosuppressive drugs [21]. The risk to develop candiduria increases by 12 fold after urinary catheterization, 6 fold after the use of broad spectrum antibiotics and urinary tract abnormalities, 4 fold following abdominal surgeries, 2 fold in the presence of diabetes mellitus and 1 fold in association with corticosteroid administration [22]. In this study we found that risk factor was more (30%) in catheterized patients, followed by prolonged antibiotics (21%), diabetes mellitus (17%) and the least was Carcinoma and Chemotherapy (3%). Our results are in accordance with various authors [23-25] who also reported the same findings. Catheterization process increases infection by introducing organisms during catheterization process or by allowing migration of the organisms into the bladder along the surface of the catheter from the external periurethral surfaces [26].

Over the past decade, a dramatic shift from *C. albicans* to Non albicans Candida as the prime etiology of candiduria has taken place. *C. albicans* was responsible for about 50- 70% cases of candiduria reported but today the scenario has changed [27-29] Non-albicans Candida have become the major pathogens, especially in hospital settings; as yet 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.[28] The Non albicans group now accounts for more than 65-70% isolates from urine [30, 31] In this study, we observed that Non-albicans *Candida* species had predominance over *C. albicans*, i.e. 64% which is consistent with the studies done by others from different parts of the world [8, 32-34].

In this study CHROM agar was used for the identification of Candida species based on the color of the colonies; *Candida albicans* produces green colonies, *Candida tropicalis* yields metallic blue colonies, *Candida parapsilosis* creamy white to pale pink colonies and *Candida glabrata* grow as light pink colonies, and *Candida krusei* appears as pink and fuzzy colonies. Thus, CHROM agar is useful for differentiating *Candida albicans* from Non-albicans Candida.

We found five species of Candida by using standard conventional method. *Candida tropicalis* was the most isolated species 37% followed by *C. albicans* 36%, *C. parapsilosis* 18%, *C. glabrata* 6% and the least was *C. kruesi* 3%. A geographical variation in the etiological pattern of invasive *Candida* species infections have been reported in various countries. In North America, there is a predominance of *C. glabrata* among the non-albicans species. However, in South America *C. parapsilosis* and *C. tropicalis* are the predominant ones.[28] Thus, the speciation of Candida is necessary to provide a database for a given geographical area.

The in vitro susceptibility testing of antifungal agents is increasingly becoming important because of the introduction of new antifungal agents and the recovery of clinical isolates that exhibit inherent or acquired resistance during chemotherapy. In this study *Candida* species showed maximum sensitivity (95%) to Amphotericin-B, followed by Ketoconazole 69% Itraconazole 66% while *Candida* species showed only 63% sensitivity to Fluconazole. Our results are in accordance with various authors [20, 24, 35] Candida isolates showed maximum resistance to fluconazole as compared to

other antifungal agents. The resistance to fluconazole in Candida is of significant concern as it is a useful drug because of high concentration active drug in urine and better tolerability.

Conclusion

Candiduria is becoming an important nosocomial infection. Incidence of Non albicans Candida was more than *Candida albicans.* This shift towards NAC species as a causative agent has generated the concern. NAC species are more resistance to antifungal as compared to *C albicans.* Therefore species identification of Candida isolates along with their antifungal susceptibility pattern can help the clinicians better in treating candiduria.

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