Speciation, Characterization and Antifungal Susceptibility Pattern of Candida Species

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ABSTRACT

Introduction: The incidence of *Candida* has been on the rise worldwide. Urinary tract infections as a result of *Candida* species are becoming common in hospitalized patients. The species identification of *Candida* is important, as non-albicans *Candida* species are increasing in number and more resistant to antifungal drugs. The present study was aimed to speciate, characterize and perform antifungal susceptibility testing of the yeast isolates from the urine samples.

Material and methods: The study was conducted in the Department of Microbiology, Adesh Institute of Medical Sciences and Research, Bathinda over 1 year period. Atotal of 1732 urine samples were analysed and isolated Candida species were subjected to speciation and antifungal susceptibility was performed according to standard procedures.

Results: A total of 100 *Candida* species were isolated from 1732 urine samples, incidence of candiduria was 5.77%. *Candida tropicalis* (*C. tropicalis*) was the commonest species (45%). Females in the age group >60 years (73%) were predominantly affected. Isolated species were more susceptible to Amphotericin (97%) and itraconazole (60%) followed by fluconazole (36%).

Conclusion: Non-albicans *Candida* (NAC) species have emerged as important cause of urinary tract infections. Non-albicans *Candida* (NAC) show is more resistant to antifungal drugs than Candida albicans (C.albicans). Therefore, the species identification of *Candida* isolates along with their antifungal susceptibility pattern can help the clinician in better treatment of patients with candiduria.

Keywords: Candiduria, non-albicans *candida*, Antifungal susceptibility.

INRODUCTION

Urinary tract infections (UTIs) are amongst the most common infection in both outpatients as well as hospitalized patients.[1] Majority of the fungal infections of urinary tract are caused by *Candida* species and they usually present as complicated nosocomial infections. Rarely does one encounter candiduria as a community acquired infection in a structurally normal urinary tract. *Candida* species account for almost 10-15% nosocomial UTIs.[2]

Yeast belonging to the genus *Candida* as exists as saprophytes, colonizing mucosal surfaces and external genitalia of humans of either gender, but especially near urethralmeatus of healthy, premenopausal women.[3] Risk factors for candiduria include extremes of age, female sex, use of immunosuppressive agents, use of IV catheters, interruption of the flow of urine, radiation therapy and genitourinary tuberculosis.

The shift of *Candida* spp from commensal to potent pathogen is facilitated by a number of virulence factors such as adherence to host tissue and medical devices, biofilm formation and secretion of extracellular hydrolytic enzymes.[4] Earlier *C. albicans* accounted for the majority of candiduria reported about 50-70% but at present non-albicans *Candida* especially *C.glabrata*, *C.krusei* and *C.tropicalis*.[5] The clinical manifestations of infection caused by different members of NAC spp. are usually indistinguishable but several NAC species are inherently resistant or acquire resistant to commonly used antifungal drugs.[6]

Moreover drug resistance is a major cause of treatment failure in these patents. Among the different antifungal agents, resistance to the polyene compounds has remained an uncommon problem. But resistance to flucytosine and azoles now appear to be increasingly important in some group of patients, especially after the widespread use of fluconazole for extended periods.[7]

The present study was conducted to speciate, characterize and perform antifungal susceptibility of yeast isolates obtained from urine samples.

MATERIAL AND METHODS

The study was conducted in the Department of Microbiology, Adesh Institute of Medical Sciences and Research, Bathinda, (Punjab) from January-2016 to December 2016 during which 100 cases of candiduria were isolated.

Inclusion criteria

Male and female patients of all age groups were considered in present study. Both outpatients and inpatients who presented with signs and symptoms of urinary tract infection were included. Pure growth of yeast isolates with significant colony count was included in the study.

Exclusive criteria

The urine samples from where *candida* species were isolated in the absence of pyuria, *Candida* with colony count ≤1000 cfu/ml and mixed growth (polymicrobial growth) were excluded in analysis.

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Collection and procesing of samples

Urine samples from patients admitted in various wards and intensive care units were collected and inoculated by calibrated loop (0.01 ml) onto Blood agar and Mac Conkey agar medium, incubated at 37^0 C and read at 24 hours and 48 hours interval. Dry creamy white opaque colonies on blood agar and tiny dry lactose fermenting pink colonies on Mac Conkey agar medium that resemble *Candida* were confirmed by gram stain.[8,9] *Candida* isolates were then subcultured on Sabouraoud's Dextrose Agar and CHROM agar candida medium for speciation.

Colour pattern of various *Candida* species were noted on CHROM agar medium. C.allbicans isolates impart distinctive light green colonies. *C.tropicalis* produce blue violet smooth colonies with halo diffusing gar. *C.krusei* ioslates produce rough,fuzzy spreading big pink colonies with pale edges. *C. glabrata* imparts small pink coloured colonies.[10] Germ tube test was performed for preliminary identification of *C.albicans* and *C.dublinesis*, further confirmation was done by following tests:

Carbohydrate fermentation test

An inoculum pool was prepared by emulsifying a heavily loaded loop full of the strain to be identified in 5ml of sterile saline. The test organism was inoculated by adding one drop of the inoculum suspension into each sugar fermentation tube. It was incubated for 48-72 hours at 30^o C. The ability to ferment sugar was shown by the presence of acid and gas in the Durham's tube.

C. albicans ferments glucose and maltose with gas production. *C. tropicalis* ferments glucose, sucrose and maltose with gas production and *C. krusei* and *C. glabrata* ferments glucose with gas production.[11]

Carbohydrate assimilation test

The organism was inoculated on a carbohydrate free medium. Carbohydrate containing filter paper disks were added and utilization was determined by the presence of growth round the disc. It consist sugar disk of 4% concentration.[12]

C. albicans assimilate glucose, maltose, trehalose, sucrose, lactose and cellobiose. *C. krusei* assimilates glucose only. *C. tropicalis* assimilates glucose, sucrose, maltose, trehalose and cellobiose and *C. glabrata* assimilate glucose and trehalose only.[8]

Antifungal drug susceptibility testing

Media used for antifungal drug susceptibility

For antifungal drug susceptibility testing, disk diffusion method was used. A disk contained the antifungal agent as routinely done in antibacterial sensitivity testing, which diffuses in the surrounding medium inhibiting the growth of fungi and measurements of zone of inhibition were taken accordingly. For antifungal susceptibility testing of azoles, yeast nitrogen base with glucose and asparagine was used and for amphotericin B, yeast nitrogen base with glucose and without asparagine was used.[7,13]

Method for antifungal susceptibility testing

A suspension of an isolated colony of *Candida* was made in sterile saline (NaCl 0.9%w/v in water) that did not exceed the turbidity of McFarland //Stanford 1 (prepared by mixing 0.1ml of 1% barium chloride with 9.9 ml of 1% sulphuric acid) in the similar way inoculums preparations was also done for the control strain.

The swabs soaked in the inoculum was inoculated in one-half of the Petri dish from periphery to the centre. One-half the plate was inoculated with control strain and the other half with the test strain in such a manner which were unable to produced confluent growth.[13]

Antifungal discs

Commercially available [Hi-media] discs of amphotericin B,fluconazole and itraconazole were used. Antifungal disks were placed in the centre of control as well as the test strains with the help of forceps. The plates were incubated at 35^{o}C for 48 hours and measurements of zone of inhabitation were taken.[9]

Result and interpretation

After the measurement of zone of inhibition the result of antifungal susceptibility testing were interpreted accordingly.[7, 9,13]

RESULTS

A total of 1732 urine samples were screened and 100 *Candida* isolates were identified on the basis of microscopic and stained smear examination, cultural characteristics and biochemical tests. The incidence of Candiduria in our study was 5.77%. Female predominance (73%) was noted in the present study. In cases of females, the maximum number of patients was in the age group of > 60 years. The most common predisposing factors responsible for candiduria was Foley's catheter (90%) followed by diabetes (75%), IV catheter (65%), frequent use of antibiotics (55%), surgical procedures (34%). *C. tropicalis* was the most common species isolated (45%) followed by *C. albicans* (32%), *C.krusei* (15%) and *C.glabrata* (8%).

Antifungal susceptibility of *Candida* isolates is presented in following table. It was observed that all species showed maximum susceptibility to Amphotericin (97%) followed

Candida species	Total no.	Amphotericin		Itraconazole		Fluconazole	
		S	R	S	R	S	R
C. tropicalis	45	42(93.3%)	3(6.7%)	30(66.7%)	15(33.3%)	17(37.8%)	28(62.2%)
C. albicans	32	32(100%)	0(0%)	20(62.5%)	12(37.5%)	19(59.4%)	13(40.6%)
C. krusei	15	15(100%)	0(0%)	8(53.3%)	7(46.7%)	0(0%)	15(100%)
C. glabrata	8	8(100%)	0(0%)	2(25%)	6(75%)	0(0%)	8(100%)
Total	100	97%	3%	60%	40%	36%	64%

Antifungal susceptibility pattern of Candida species

by itraconazole (60%) and fluconazole (36%). *C. albicans*, *C. krusei* and *C. glabrata* species showed 100% sensitivity to Amphotericin. Maximum resistance to Fluconazole was shown by *C. tropicalis* (62.2%) species. Sensitivity to itraconazole was maximally shown by *C.tropicalis* (66.7%) and least by *C. glabrata* (25%) species.

DISCUSSION

In the present study the incidence of candiduria was found to be 5.77%. Manikandan et al[14], Goyal et al[15] and Yashavanth et al[16] obtained lower incidence rate than our study (3.4%, 2.36% and 2.27%). However, Singhal et al[5] and Kobayashi, Claudia et al[17] obtained higher incidence rate of 10.2% and 22%, therefore the prevalence of candiduria varies considerably in the hospital setting.[2]

In this study observed that females were predominantly affected (73%) as in the study of Manikandan et al[14], N. Jain et al[18] and N. Safdar et al[19] most probably due to short urethera in females. Most common age group affected with candiduria was > 60 years which was similar to as stated by Yashavanth et al[16], Kobayashi et al.[17]

Urinary catherization increases chances of UTI and the most common predisposing factor in present study is the Foley's catheter (90%) Above finding is in accordance with Francisco et al[20] (97.9%), Kobayashi, Claudia et al[17] (84.4%) and Navin Paul et al[21] (66%).

Isolation of non-albicans *Candida* (68%) was more than *C.albicans* (32%) as with studies done by Iman et al[22], Yashavanth et al[16] and Singhal et al.[5] This is consistent with emergence of predominance of non-albicans *Candida* species all over world (Pfaller et al., 1999).[23] Identification of *Candida* species is important as non-albicans is more resistant to azoles as compared to *C. albicans*. *C. krusei* and *C. glabrata* is intrinsically resistant to fluconazole.

Antifungal susceptibility pattern showed that *Candida* isolates were more susceptible to Amphotericin (97%) than azoles (Itraconazole 60% and fluconazole 36%) as in the study of Manikandan et al[14], Yashavanth et al.[16] *C.albicans* is usually sensitive to Amphotericin where as non-albicans are more resistant to antifungal drugs especially fluconazole (Saha et al., 2008).[24] The increase in resistance to fluconazole is a matter of concern as it is most common antifungal drug used for treatment of candiduria.[25,26]

CONCLUSION

From above study, it is concluded that NAC species have emerged as common cause of UTI. UTI is more common in catherterized patients, diabetes and systemic antibiotic use. Females in >60 years age group are more commonly affected. For treatment of candiduria, antifungal drugs like Amphotericin B and itraconazole can be given. Therefore, early identification of *Candida* species in patients with UTI will help clinician in selecting appropriate antifungal agent and it will lead to reduction to cost of treatment and duration of hospital stay.

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