

Occurrence And Characterization Of *Candida* Species Isolated From Symptomatic Cases Of Urinary Tract Infection

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Abstract

Background and Objective: Candidiasis is the most common fungal infection in the human population. The increase in incidence of *Candida* species over the past two decades is significant and non-*albicans* *Candida* species continue to replace *Candida albicans* at most of the clinical sites. The aim of the present study was to determine the incidence of *Candida* infection in symptomatic Urinary Tract Infection (UTI), to assess the occurrence of non-*albicans* *Candida* infection and to investigate the risk factors associated with candiduria.

Methods: The study was carried out in the department of Microbiology, Navodaya Medical College Hospital and Research Centre, Raichur, Karnataka over a period of 9 months. The samples were screened to find out suitability by wet mount and Gram stain microscopy, cultured on Sabouraud's Dextrose Agar and also on MacConkey's Agar and CLED Agar to identify the potential urinary pathogen. For the identification of *Candida*, colonies were processed for Gram stain, Germ tube test, Production of chlamydo-spore, sugar fermentation and assimilation test.

Results: A total of 1600 consecutive urine samples were screened from symptomatic cases of urinary tract infection, of which, 312 (19.5%) showed growth in significant number. Occurrence of candiduria in females was 57.1% and in males 42.8% with a male to female ratio 1:1.3. The incidence of *Candida* species in symptomatic UTI was 6.7%. Among *Candida* species isolated, *Candida albicans* accounted for 61.9% and Non-*albicans* *Candida* 38.1%. Of Non-*albicans* *Candida* isolates predominant species was *C. tropicalis* 4 (19%), followed by *C. krusei* 2 (9.5%), *C. glabrata* 1 (4.8%) and *C. parapsilosis* 1 (4.8%). The most frequent risk factor observed in patients with candiduria was age above 61 years, Diabetes mellitus, Catheterized patients, and Pregnancy with an incidence of 52.4%, 42.8%, 14.2%, and 9.5% respectively.

Conclusion: *Candida* species was fourth predominant organism encountered in urinary tract infection. Although the majority of candiduria are caused by *Candida albicans* (61.9%), non-*albicans* species especially *C. tropicalis* (19.0%) emerged as second common causative agent of candiduria. The important risk factors associated with candiduria was age group above 61 years (52.4%) and Diabetes mellitus (42.8%).

Key words: Candiduria, *Candida albicans*, Non-*albicans* *Candida*.

Introduction:

Candidiasis is the most common fungal infection in the human population. *Candida albicans*, the major human pathogen of the genus *Candida*, a commensal yeast of the oral, gastrointestinal, and vaginal mucosa of the healthy individuals and seems to be almost universally present^{1,2,3}. The use of broad-spectrum

antibiotics, steroids or other immunosuppressive agents; diabetes mellitus; AIDS; cancer chemotherapy and radiotherapy; and organ transplantation can increase the risk for opportunistic bacterial as well as fungal infections⁴. The candidiasis has increased significantly in recent times due to advent of new diagnostic and therapeutic procedures to patients, such as urinary indwelling catheters, nephrostomy tubes, immunosuppressive therapy, hemodialysis, previous surgery, long term and broad spectrum antimicrobial usage and renal

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transplantation.^{5,6,7} The increase in incidence of Candida species over the past two decades is significant and non albicans candida species continue to replace Candida albicans at most of the clinical sites like blood stream infections⁸. Due to the commensal nature of the Candida species, most of the times it is found to be a cause of endogenous infection⁸. According to the United States of America National Nosocomial Infection Surveillance Systems, Candida species are the 7th most common nosocomial pathogens and the 4th most common organism responsible for blood stream infection⁹. The aim of the present study was to determine the incidence of Candida infection in symptomatic UTI, to assess the occurrence of non albicans Candida infection and to investigate the risk factors associated with candiduria.

Material And Methods:

Clean -catch mid stream urine samples collected in (uricol, Hi-Media) sterile disposable container were immediately transported to the service laboratory of Microbiology department and processed within one hour.

1. Gram staining of uncentrifuged urine:

A drop of uncentrifuged well mixed urine was taken on clean grease free glass slide, air dried and stained by Gram staining method and examined under oil immersion field of microscope (examining 20 fields). Presence of = 01 bacteria per oil immersion field correlates with significant bacteriuria¹⁰.

2. Wet examination for leucocytes count:

Well mixed 0.05 ml of uncentrifuged urine was transferred on to the slide. A cover slip having 22 x 22 mm dimension was placed on the slide avoiding trapped bubbles and observed with the high power (40x) field dry objective of the microscope. Finding of 01 leukocyte per 7 high power fields corresponds with 104 leucocytes per ml and the finding of clearly larger number than this indicates significant pyuria¹⁰.

3. Culture:

Samples showing Gram positive yeast like budding cells were inoculated on Sabouraud's Dextrose Agar (SDA) containing chloramphenicol and cyclohexamide. SDA slants were incubated at 37oC and examined twice a week to look for the growth to cream coloured pasty colonies suggestive of Candida species. The slants were incubated for one week and discarded if no growth occurred. Alternatively Direct

Gram stain smears which are negative for yeast like budding cells but creamy colonies resembling Candida species in routine Mac Conkey agar and CLED agar were also processed further for identification and speciation of Candida isolates. For the identification of Candida, colonies were processed for Gram stain, Germ tube test, Production of chlamyospore, sugar fermentation and assimilation test⁸. For the identification of bacteria, Gram stain, culture, and biochemical tests were performed by standardized procedures¹¹.

Germ tube test:

A single colony was inoculated in human serum and inoculated at 37oC. After 2-4 hours, wet mount was prepared and examined under the microscope to look for the presence of germ tube.

Chlamyospore formation:

All candida isolates were tested for the production of chlamyospores in corn meal agar with Tween 80. After 72 hours, the plates were examined under the microscope for the presence of chlamyospores.

Sugar fermentation test:

All Candida isolates were subjected to carbohydrate fermentation test. Carbohydrate solutions used were 6% solutions of dextrose, maltose, lactose, and sucrose with basal media.

Sugar Assimilation test:

The assessment of the ability of the yeast to utilize carbohydrates is based on the use of carbohydrate free yeast nitrogen base agar and observing for the presence of growth around carbohydrate impregnated filter paper disks after an appropriate period of incubation. Carbohydrates used were glucose, lactose, maltose, sucrose and galactose.

Results:

A total of 1600 consecutive urine samples were screened over a period of 9 months from October 2011 to July 2012 from symptomatic cases of urinary tract infection from Navodaya Medical College Hospital and Research Centre, Raichur, Karnataka. Of 1600 samples screened, 312 (19.5%) samples showed growth in significant number. Occurrence of UTI in females was 67% (132/197) and in males 33% (65/197). However, candiduria in females was 57.1% and in males 42.8% with a male to female ratio 1:1.3.

The predominant organism isolated in significant number from UTI cases was E.coli 197 (63.1%) followed by Klebsiella pneumoniae 41 (13.1%),

Proteus sps. 23 (7.3%), *Candida* sps. 21 (6.7%), *Staphylococcus aureus* 12 (3.8%) and *Pseudomonas aeruginosa* 8 (2.5%) as shown in the table-1. In our study, *Candida* species was fourth predominant organism encountered in urinary tract infection.

Among *Candida* species isolated, *Candida albicans* accounted for 61.9% and Non albicans *Candida* 38.1%. Of Non albicans *Candida* isolates predominant species was *C. tropicalis* 4 (19%), followed by *C. krusei* 2 (9.5%), *C. glabrata* 1(4.8%) and *C.parapsilosis* 1(4.8%) as depicted in table-2.

In our study, *Candida* species was isolated in the age group above 21 years and the isolation rate was high in age group above 61 (52.4%) years followed by 51-60 years (23.8%), and 31-40 years (14.3%) of age group as mentioned in table-3.

The most frequent predisposing factor observed in patients with candiduria was age above 61 years, Diabetes mellitus, Catheterized patients, Pregnancy and ureteral stent with an incidence of 52.4%, 42.8%, 14.2%, 9.5% and 4.7% respectively (Table-4).

Discussion:

Candiduria accounted for 10% of UTI and has resulted in increased rate of mortality during the last decade due to use of new medical instruments, new treatments, surgery and transplantation¹². In the present study, candiduria was the 4th most common causative agent of symptomatic UTI, and 6.7% of urine samples were positive for *Candida* species. A study in Brazil, reported 22% of urine samples from hospitalized patients were positive for *Candida* species¹³. In our study, male to female ratio was 1:1.3 (42.8% male; 57.1% female), contrary to the male predominance reported in the study by Paul et al.¹⁴ In a similar study conducted by Kandhari et al¹⁵ at AIIMS, New Delhi, the ratio was 1:1.57 (M: F) and Rizvi MW et al,¹⁶ also reported female preponderance with a ratio of 0.85:1 (M:F).

We observed high prevalence of candiduria in age group above 61 years (52.4%) followed by 51-60 years (23.8%), and 31-40 years (14.3%) of age group. Zarei-Mahmoudabadi et al observed high prevalence of candiduria of 44.7% in age ranging between 36 and 65 years followed by; 41.5% >35 years, and 13.8% <66 years.¹⁷

Jaylaxmi et al reported maximum cases in the age group of 21-40 years.¹⁸ Clayton et al observed that majority of *Candida* infections were in the age group

of 20-60 years, with mean age of 43.4 years.¹⁹ *Candida albicans* had remained the major agent of candiduria until recently.²⁰ However several reports shown that non-albicans species, especially *C.tropicalis* and *C.glabrata* are now predominate in may regions.²¹ Non albicans species accounted for 71% and 64.4% of isolates in Paul et al¹⁴ and Kobayashi et al¹³ reports, respectively. Although the majority of candiduria in the present study are caused by *Candida albicans* (61.9%), non albicans species especially *C.tropicalis* (19.0%) emerged as second common causative agent of candiduria. Similarly Yang et al in Taiwan reported that *C. albicans* was the commonest species followed by *C. tropicalis* (12.9%) and *C. krusei* (0.6%).²²

Zarei-Mahmoudabadi et al reported strong correlation between the incidence of candiduria in hospitalized patients and broad spectrum antibiotics therapy (69.1%).¹⁷ However in the present study,

Table 1: Organisms isolated from symptomatic Urinary Tract Infection

Sl.No	Organism isolated	No. of isolates (n=312)	% of isolates
1	<i>Escherichia coli</i>	197	63.1
2	<i>Klebsiella pneumoniae</i>	41	13.1
3	<i>Proteus species</i>	23	7.3
4	<i>Candida species</i>	21	6.7
5	<i>Staphylococcus aureus</i>	12	3.8
6	<i>Pseudomonas aeruginosa</i>	8	2.5
7	Coagulase Negative <i>Staphylococcus species</i>	4	1.2
8	<i>Enterococcus faecalis</i>	2	0.6
9	<i>Acenotobacter species</i>	2	0.6
10	<i>Enterobacter cloacae</i>	1	0.3
11	Beta-haemolytic <i>Streptococci</i>	1	0.3

Table 2: Different species of *Candida* isolated from symptomatic UTI

Sl.No	Organism isolated	No. of isolates (n=312)	% of isolates
1	<i>C. albicans</i>	13	61.9
2	<i>C.tropicalis</i>	4	19
3	<i>C.krusei</i>	2	9.5
4	<i>C.glabrata</i>	1	4.8
5	<i>C.parapsilosis</i>	1	4.8

Table 3: Age distribution among candiduria cases

Sl.No.	Age group	No.of isolates	% of isolates
1	21-30	1	4.8
2	31-40	3	14.3
3	41-50	1	4.8
4	51-60	5	23.8
5	≥61	11	52.4

Table 4: Predisposing factors among UTI cases with candiduria

Sl.No	Predisposing factors	No. of isolates	% of isolates
1	Age >60 years	11	52.4
2	Diabetes mellitus	9	42.8
3	Catheterization	3	14.2
4	Pregnancy	2	9.5
5	Ureteral stent	1	4.7

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