

ISOLATION AND IDENTIFICATION OF CANDIDA SPECIES IN THE PATIENTS OF UTI

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ABSTRACT

Fungal infections of the urinary tract especially those caused by *Candida* species are becoming increasingly common due to prolonged antibiotic use, indwelling urinary catheters, and increase in the number of immunocompromised individuals. Symptoms of *Candida* pyelonephritis, cystitis, prostatitis, or epididymo-orchitis are little different from those of the same infections produced by other pathogens. Candiduria occurring in critically ill patients should initially be regarded as a marker for the possibility of invasive candidiasis. The first step in further evaluation is to verify funguria by repeating the urinalysis and urine culture.

MATERIALS AND METHODS

A total of 3381 clinically diagnosed cases of UTI from OPD and IPD of JAH and KRH Hospitals, G. R. Medical College, Gwalior were studied for one year from 1st Jan, 2015, to 31st Dec, 2015. Urine samples were aseptically collected. Cultures were done on blood agar, MacConkey agar, CLED agar, and Sabouraud Dextrose Agar. Both bacteria and yeast were isolated. Both bacteria and yeast were identified further as per standard protocol. The isolated yeasts were included in our study for evaluation.

RESULTS

Total of 87 (2.57%) cases were found positive for yeast growth. Among these, 4 species are identified with predominance of *C. albicans* 31.03% (n=27), *C. krusei* 29.89% (n=26), *C. glabrata* 24.14% (n=21), *C. tropicalis* 14.94% (n=13).

CONCLUSION

The finding of candiduria in a patient with or without symptoms should be neither dismissed nor hastily treated, but requires a careful evaluation, which should proceed in a logical fashion.

KEYWORDS

Candiduria, UTI, Risk Factors, Germ Tube Test, HiCrome *Candida* Differential Agar, Growth at 45°C.

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INTRODUCTION

Candida is a normal inhabitant in the skin, mucous membrane of the mouth, respiratory track or vagina, but may invade the other parts of the body especially in immunocompromised individuals.¹ They exist predominantly in the unicellular form with both sexual and asexual forms and show thin walled ovoid cells (blastospores) that reproduce by budding.² There are more than 150 species of *Candida*, but most important pathogenic species are *C. albicans*, *C. tropicalis*, *C. krusei*, *C. glabrata*.¹ They may cause simple lesions to life threatening systemic infections. The most common species is *C. albicans* even though there has been a striking increase in the frequency of non-*albicans* candida in the last few years. At present, there is increase in treatment failure maybe because of drug resistance mainly in non-*albicans* candida.^{1,3,4} Thus, *Candida* is the 6th most common isolated nosocomial pathogen especially from urinary tract.²

There is also important emerging evidence that *Candida* infection can be acquired from hospital environment. Among

the hospitalised population, the following categories pose a great risk in acquiring candidal infection viz., transplant patients, haematological malignancies, prolonged antibiotic therapy, catheterisation of urinary tract or vascular system, renal failure, hepatic failure, prolonged hospital stay and inter hospital transfer,⁵ use of immunosuppressive agents,⁶ extremes of age and female sex⁷ etc. The generous use of broad-spectrum antibiotics and other molecules have paved way for new opportunistic pathogen like *Candida*. In addition, diabetes mellitus and HIV infection provides a soil for candidal infection.⁸

Guze and Harley found funguria in only 15 of 1500 patients; more than half of these 15 patients had diabetes mellitus and were receiving antibiotics.⁹ A study performed by Platt et al¹⁰ showed that 26.5% of all urinary infections related to indwelling catheters were caused by fungi. Rivett et al¹¹ found that 2% of urine specimens submitted to a hospital microbiology laboratory tested positive for yeast versus 11% of the urine samples obtained from patients in the leukaemia and bone marrow transplantation unit in the same hospital. Therefore, the prevalence of candiduria varies considerably in the hospital setting and is most prevalent among patients in the Intensive Care Unit (ICU).^{11,12}

Candiduria is a relatively rare finding in otherwise healthy people.¹³ When yeast like organisms are discovered in the urine, the major decision that must be made is whether or not this signifies infection of either the upper or lower urinary tracts, colonisation of the bladder, or contamination

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of the urine sample. Most of the time, these yeasts are *Candida* species.¹⁴ Contamination can be differentiated from colonisation or infection by obtaining a new urine sample to verify funguria.^{6,15,16} Most patients with candiduria are asymptomatic and yeasts are noted in the urine as a serendipitous finding on a routine urinalysis or urine culture.¹⁷ In another study, the results of urine cultures were positive for 10 of 440 healthy adults, but these culture results reverted to negative when clean-catch techniques were used.¹⁸

Most of these patients do not have a *Candida* Urinary Tract Infection (UTI). However, in those individuals who do have symptomatic *Candida* infection, the symptoms are indistinguishable from those caused by bacterial infections. Cystitis is associated with dysuria, urgency, suprapubic discomfort, and rarely fever; pyelonephritis leads to fever, chills, and flank pain with or without lower tract symptoms. Oliguria, stranguria (difficult and painful urination), the passage of particulate matter, and/or pneumaturia suggest a complication such as the presence of a fungus ball.^{17,19} Presently, 10%-15% of nosocomial UTIs are caused by *Candida* species.²⁰⁻²³

The aim of our study was to isolate *Candida* species in culture in clinically diagnosed cases of UTI, speciate them using various methodology, and determine the prevalence of various species of *Candida* causing UTI in our region.

MATERIAL AND METHODS

The study was conducted after obtaining approval from institutional ethical committee and fully informed and voluntary consent were obtained from the patient and/or their attendants.

Place of Study

Mycology section, Department of Microbiology, G. R. Medical College, Gwalior, Madhya Pradesh.

Type of Study

Prospective cross-sectional study during the period of one year from 1st Jan, 2015 to 31st Dec, 2015.

Inclusion Criteria

1. All urine specimens of clinically diagnosed urinary tract infection patients attending OPD or admitted in various wards and ICU.
2. All urine samples, which are culture positive for fungal growth (Yeast only).

Exclusion Criteria

1. Those urine sample, which are culture negative (No growth).
2. Bacterial growth.

Specimen Collection

Clean catch mid-stream urine samples were collected in a sterile wide mouth leak-proof container and in catheterised patients' catheter clamp technique were used for sample collection.

Transportation

All samples were transported to the laboratory as soon as possible with mean transport time of one hour, if delay of 2 to

4 hours samples were refrigerated and more than 4 hour, then samples discarded and fresh sample were collected along with their proper requisition form.

Isolation and Identification of *Candida* Spp.

All urine samples were inoculated following semi-quantitative technique by calibrated loop (0.01 mL) onto blood agar (blood agar base, HiMedia Laboratories Pvt. Ltd. Mumbai, India), MacConkey agar (HiMedia Laboratories Pvt. Ltd. Mumbai, India), and CLED agar (Cystine-Lactose-Electrolyte-Deficient, HiMedia Laboratories Pvt. Ltd. Mumbai, India) medium and incubated at 37°C and read twice at 24 hours and 48 hours of incubation. Both bacteria and yeast were isolated. Dry creamy white opaque colonies on blood agar and tiny dry lactose fermenting pink colonies on MacConkey agar medium that resembled *Candida* colony were confirmed by Gram Stain. These *Candida* isolates were subcultured on Sabouraud's Dextrose Agar (HiMedia Laboratories Pvt. Ltd. Mumbai, India) (Fig. 1. showing cream-coloured colony of *Candida* species on Sabouraud's Dextrose Agar media.), Corn meal agar (HiMedia Laboratories Pvt. Ltd. Mumbai, India), and HiCrome *Candida* differential agar medium (HiMedia Laboratories Pvt. Ltd. Mumbai, India) for further identification.

Identification was carried out by performing Gram stain, germ tube test²⁴ (Fig. 2. showing germ tube test under 400x.), chlamydospore production test²⁵ (Fig. 3. showing chlamydospore under 400x.), growth at 45°C²⁴ and HiCrome *Candida* differential agar medium²⁶ (Fig. 4. Showing Growth of *Candida* species on HiCrome *Candida* differential agar. {1 and 3-*C. albicans*, 2-*C. tropicalis*, 4-*C. krusei*, 5-*C. glabrata*}) as per the CLSI guidelines.



Fig. 1: Showing Cream Coloured Colony of *Candida* Species on Sabouraud's Dextrose Agar Media

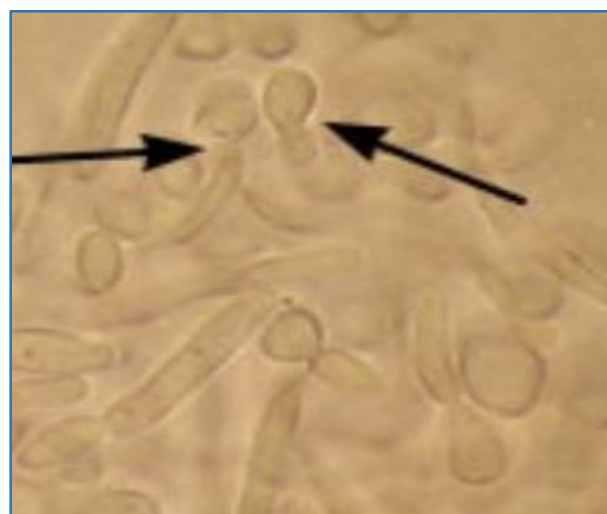


Fig. 2: Showing Germ Tube Test Under 400x.

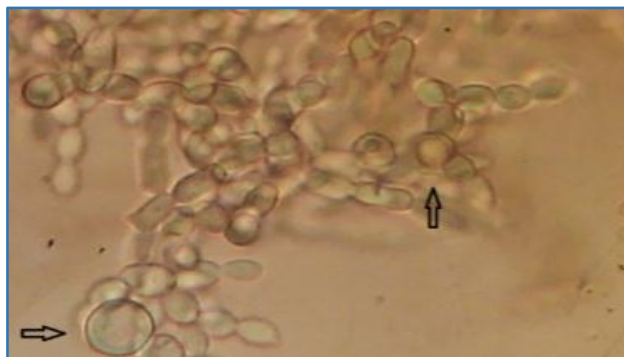


Fig. 3: Showing Chlamydo-spore under 400x.

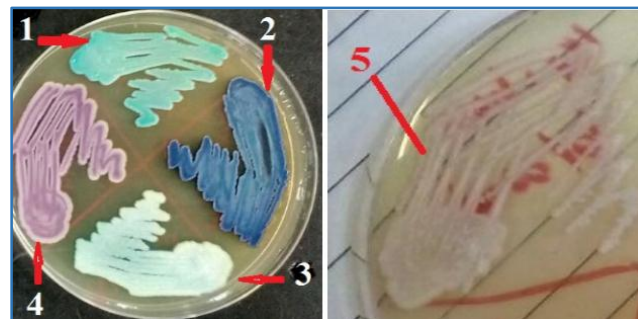
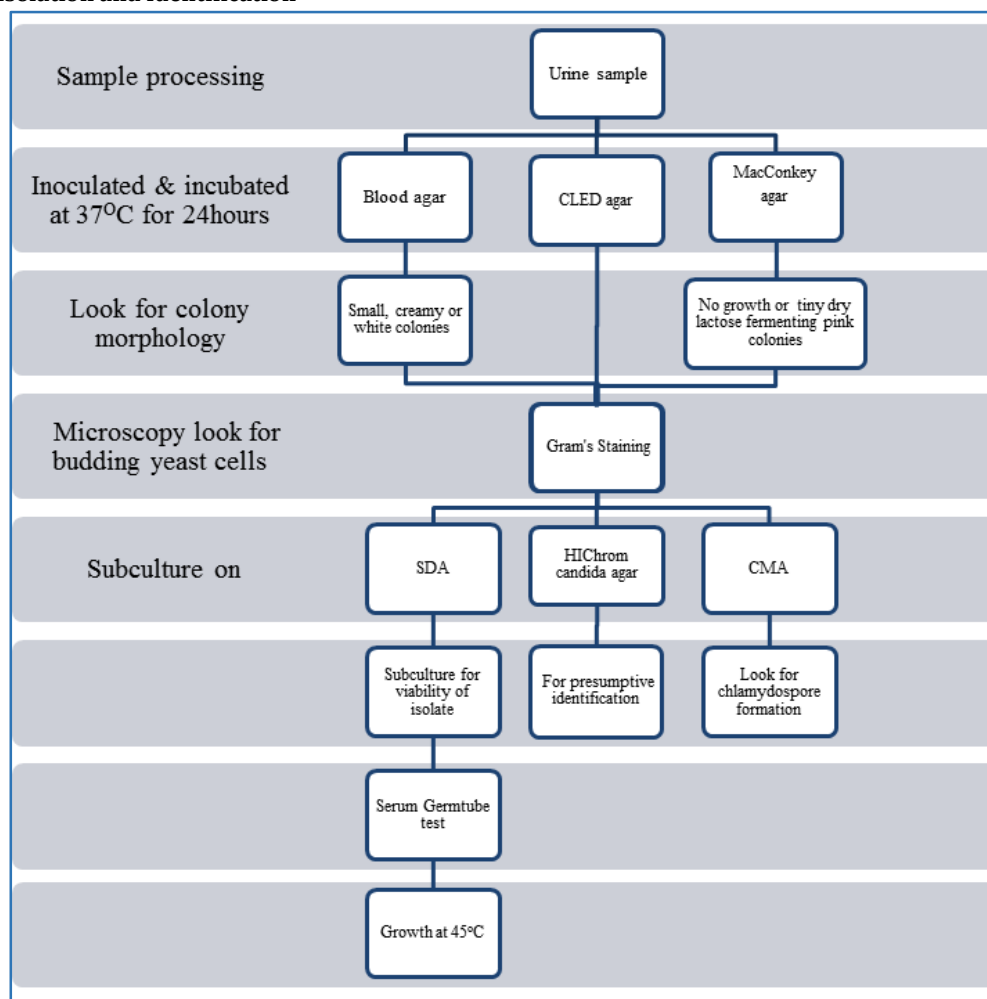


Fig. 4: Showing Growth of Candida Species on HiCrome Candida Differential Agar. (1 and 3-C. Albicans, 2-C. Tropicalis, 4-C. Krusei, 5-C. Glabrata)

Flow Chart for Isolation and Identification



RESULTS

Total of 3381 urine sample for culture were received in the department. 2417 were culture negative, 877 were bacteriologically positive, and only 87 samples were showing yeast growth. These 87 samples were included in our study.

Sl. No.	Results	No. of Samples	Percentages
1	No Growth	2417	71.49
2	Bacterial Growth	877	25.94
3	Yeast Growth	87	2.57
Total		3381	100

Table 1: Results of Urine Culture

Sex and age wise distribution of cases under study depicted in Table 2 and Fig. 5 shows that there was a predominance of females reported with candiduria. In case of females, the maximum numbers of cases were in the age group of 31-60 years. Similarly, the majority of the case in males also fell in the age group of 31-60 years.

Out of 87 isolates, 58 (66.67%) were isolated from female patients and 29 (33.33%) from male. Male-to-female sex ratio was found to be 1:2. (Table 2).

Sex	No. of Cases	Percentage
Male	29	33.33
Female	58	66.67

Table 2: Sex-Wise Distribution of Fungal Isolates

Out of 87 isolates, 69 (79.31%) were recovered among the 31 to 60 years of age. (Fig. 5).

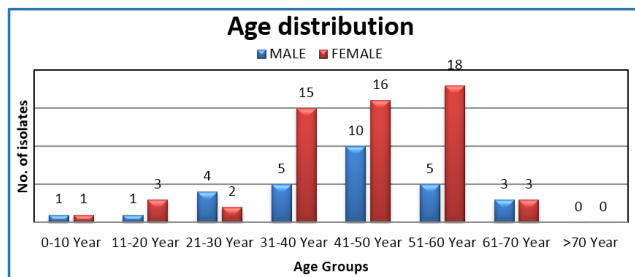


Fig. 5: Bar Diagram Showing Age-Wise Distribution of Candida Species in Male and Female.

Out of 87 isolates, 27 isolates were positive for germ tube test and growth at 45°C on SDA media indicating all 27 isolates were *C. albicans*.

Out of 87 isolates, 27 (31.03%) were *C. albicans* and 60 (68.97%) were non-*albicans candida* (NAC). Common predisposing condition included urinary catheter 61.8% patients using antibiotics 54.4%, diabetes in 44.5%, ICU stay in 26.4%, age group of 31 to 60 year (79.31%) were more commonly affected. (Table 3).

	Sex	Male		Female		Total
	Type of Isolate	<i>C. albicans</i>	Non- <i>Albicans Candida</i>	<i>C. albicans</i>	Non- <i>Albicans Candida</i>	
	No. of Isolates	11 (12.64%)	18 (20.69%)	16 (18.39%)	42 (48.28%)	87
Sl. No.	Risk Factors					
1.	Urinary Catheter	7 (12.96%)	12 (22.22%)	10 (18.52%)	25 (46.30%)	54 (61.8%)
2.	Antibiotic Use	9 (19.15%)	14 (29.79%)	11 (23.40%)	13 (27.66%)	47 (54.4%)
3.	Diabetes	5 (12.82%)	7 (17.95%)	10 (25.64%)	17 (43.59%)	39 (44.4%)
4.	ICU Stay	5 (21.73%)	6 (26.09%)	6 (26.09%)	6 (26.09%)	23 (26.4%)
5.	Age Between 31-60 Years	7 (10.14%)	13 (18.84%)	12 (17.39%)	37 (53.62%)	69 (79.31%)

Table 3: Sex-Wise Distribution of *C. Albicans* and Non-*Albicans Candida* and their Associated Risk Factors

Total of 87 candida isolates, 4 species are identified with predominance of *C. albicans* 31.03% (n=27), *C. krusei* 29.89% (n=26), *C. glabrata* 24.14% (n=21), *C. tropicalis* 14.94% (n=13). (Table 4, Fig. 6).

Fungal Isolates	No. of Isolates	Percentage
<i>C. Albicans</i>	27	31.03
<i>C. Krusei</i>	26	29.89
<i>C. Glabrata</i>	21	24.14
<i>C. Tropicalis</i>	13	14.94
Total	87	100

Table 4: Percentage of Various Candida Species Isolated from Urine

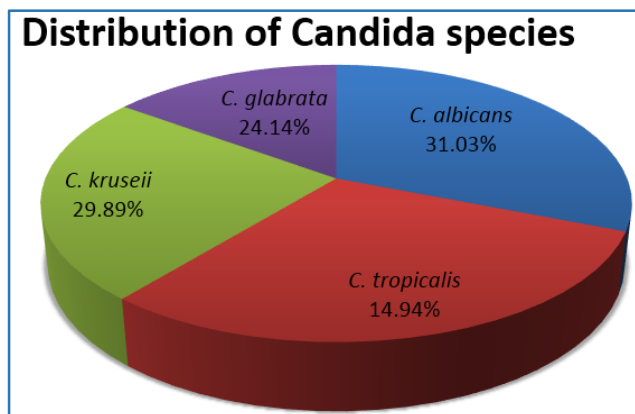


Fig. 6: Pie Diagram of Distribution of Candida Species

DISCUSSION

In the present study, the incidence of candiduria was found to be 2.57%. The incidence of candiduria in patients admitted in various ward and ICU of tertiary care hospital in Bathinda,

Punjab was 5.3% as studied by Mahajan A. et al.²⁷ In a study by Rivett et al, the incidence of candiduria among the urine

specimens submitted to a hospital microbiology laboratory was 2%.¹¹ However, studies conducted by Kobayashi, Claudia et al.²⁸ and N. Febre V. Silva et al.²³ higher incidence of candiduria were reported 22% and 18.6% respectively. Our study is in accordance with the study of Rivett et al¹¹ where incidence of candiduria was 2%. Therefore, the prevalence of candiduria varies considerably in the hospital setting.

In the present study, it was observed that incidence of candiduria was reported higher among the females (66.67%) than males (33.33%). Mahajan A. et al²⁷ reported 74% females and 26% males had Candiduria. In a study, N. Safdar et al²⁹ found that 77% females had candiduria. N. Jain et al³⁰ observed that 77.4% females had candiduria. However, Kobayashi et al²⁸ reported female incidence to be 57.8%. Kauffman CA et al⁷ reported 59.9% females with candiduria. Hence, all studies done in different parts of the world show that females have more predilections towards candiduria most probably due to short urethra in females.

In the present study, out of 87 Candida isolates isolated from urine specimens, *C. albicans* predominated 31.03% followed by *C. krusei* 29.89%, *C. glabrata* 24.14%, and *C. tropicalis* 14.94%. Similarly, Mahajan A. et al²⁷ reported 34% of *C. albicans* followed by *C. dubliniensis* (31%), *C. krusei* (19%), *C. tropicalis* (15%), and *C. glabrata* (1%). Kobayashi et al²⁸ reported incidence of *C. albicans* to be 35.6%, *C. tropicalis* 22%. N. Safdar et al²⁹ in their study reported incidence of *C. albicans* to be 35%, *C. tropicalis* 1%, *C. glabrata* 53%, *C. krusei* 1%, and *C. parapsilosis* to be 4%. So, it is fair to assume that *Candida albicans* is the commonest species isolated.

In the present study, common predisposing condition included urinary catheter 61.8%, patients using antibiotics 54.4%, diabetes in 44.5%, ICU stay in 26.4%, age between 31

to 60 year and sex that was affected more is female that is 66.67%. According to Navin Paul et al,³¹ incidence of various predisposing factors was catheterisation 66.6%, intake of antibiotics 47.61%, diabetes 38.09%, and surgery in 38.09%. That is in accordance to the present study. Kobayashi et al²⁸ reported incidence of various predisposing factors was: intake of antibiotics 100%, urinary catheter was present in 84.4%, surgical procedure in 66.7%.

CONCLUSION

The present study and several similar studies show that *Candida albicans* was the predominant isolate. Over the last three decades, an increase in the prevalence of candiduria as well as in the incidence of candida UTI are associated with certain risk factors, which are use of antibiotics, urinary catheterisation, diabetes mellitus, patients undergoing surgery, and female sex. In this study, a fair idea is obtained about the predisposing factors and epidemiological data of our study can serve as a template for the development of local guideline for making diagnosis of Candiduria.

The finding of candiduria in a patient with or without symptoms should be neither dismissed nor hastily treated, but requires a careful evaluation, which should proceed in a logical fashion.

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