International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Microbiological and Clinico-Demographic Correlation of *Candida* Isolates from Health Care Associated Urinary Tract Infections: A Study from a COVID Dedicated Tertiary Health Care Centre

Bineeta Kashyap[1], Kirti Nirmal[2], Kapil Singh[3], N. P. Singh[4]

[1]MBBS, MD, Professor, Department of Microbiology, University College of Medical Sciences and Guru Tag Bahadur Hospital, Dilshad Garden, Delhi. 110095, India
Corresponding author Email: *dr_bineetakashyap[at]yahoo.co.in*

[2]MBBS, MD, DNB, Assistant Professor, Department of Microbiology, University College of Medical Sciences and Guru Tag Bahadur Hospital, Dilshad Garden, Delhi. 110095, India
Email: *doctorkirtinirmal[at]gmail.com*

[3]MBBS, Post Graduate Student, Department of Microbiology, University College of Medical Sciences and Guru Tag Bahadur Hospital, Dilshad Garden, Delhi. 110095, India
Email: *kapilchoudhary03[at]gmail.com*

[4]Director Professor and Head, Department of Microbiology, University College of Medical Sciences and Guru Tag Bahadur Hospital, Dilshad Garden, Delhi. 110095, India
Email id: *singhnanjna[at]yahoo.co.in*

Running title: *Candida* species identification and clinico-demographic profile in Candidiuria patients.

Abstract: ***Microbiological and Clinico-demographic correlation of Candida isolates from health care associated urinary tract infections: A study from a COVID dedicated tertiary health care centre. Introduction: Candida species are ubiquitous yeasts, found as normal flora of gastrointestinal tract in healthy individuals. This study was undertaken with the aim to identify Candida species using Chromagar from isolates showing significant counts from urine samples of admitted patients in a COVID dedicated tertiary care centre. We also aim to analyze the clinic-demographic correlation of Candida species from urinary isolates. Material and Method: This prospective study was conducted at the Bacteriology laboratory in the Department of Microbiology at a tertiary care centre, referral and teaching hospital. A total of 30 isolates of Candida species from the patients with significant Candidiuria (i.e. >10 ^{5} CFU of Candida per ml of urine) were included. Result: Out of 30 samples (isolates of Candida) received male to female distribution ratio of 1: 1.7. Candida isolates were identified by using colour and colony morphology on Candida Chromagar. After speciation of Candida, 4 species were found to be prevalent- C. albicans (56%), C. tropicalis (16.6%), C. glabrata (10.5%) and C. krusei (6.6%). Conclusion: Health care associated UTI resulting in Candidiuria are more common in patients with indwelling urinary catheters, systemic antibiotic use, underlying genitourinary abnormality, previous surgery and diabetes mellitus. Identification of Candida species with Chromagar Candida is a rapid, reliable, affordable method to identify various commonly isolated Candida species.***

Keywords: Clinico-demographic, Chrome agar, *Candida* species, COVID dedicated hospital

1. Introduction

Candida species are ubiquitous yeasts, found as normal flora of gastrointestinal tract in healthy individuals. They are opportunistic pathogens in immunocompromised patients.[1] *Candida* species account for almost 9 to 40% of nosocomial urinary tract infections.[2] *Candida albicans* and non–*C.albicans Candida* (NACA) species are considered important among the microbial normal flora in the oral cavity, alimentary canal and vagina in a vast range of the healthy people. Furthermore, they colonize the urethral opening in premenopausal and healthy females.[3] Although there are approximately more than 150 species of *Candida*, it is well established that only a small number are human pathogens. *C. albicans* has been the most frequently cultured species from clinical specimens.[4] The most frequently isolated species is *Candida albicans*, but *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, and *Candida parapsilosis* are also emerging as important etiologic agents of *Candida* infection.[5] Candidiuria, the presence of *C.albicans* and NACA species in urine, may occur in both asymptomatic and symptomatic urinary tract infection (UTI).[3] *Candida* species and in particular, *Candida albicans* (*C.albicans*) are the most remarkable opportunistic pathogenic fungi causing nosocomial UTIs.[6] Several reports show that the frequency of UTI due to yeasts has increased during the last decades.[7, 8]

Fungal urinary tract infections have become more frequent as a result of the risk factors that includes long Intensive care unit (ICU) stay, structural abnormality of the urinary tract, immunocompromised status, long duration antibacterial therapy with broad spectrum antibiotics, antifungal prophylaxis, instrumentation, prior surgical procedures, recent use of antibiotics, advanced age, female gender, disturbance of urine flow, diabetes mellitus, AIDS, corticosteroids, total parental nutrition intake and prolonged hospital stay are presented as more important risk factors for

UTI and also have been associated with Candidiuria.[9, 10 11,12] Nosocomial pathogens causing UTIs which has higher antibiotic resistance as compare to simple UTIs. In every hospital Infection control policy has to available in terms of limiting the number of hospital-acquired UTIs. Other important points include catheterization using an aseptic technique and sterile equipment and the use of closed drainage systems. UTIs should be treated only after a urine sample has been sent and the advice of a microbiologist sought.[12,13]

Hence, identification of *Candida* species among hospitalized patients is very important it has therapeutic significance in allowing the use of appropriate antifungal agents and preventing emergence of drug resistance.

Routine identification of *Candida* species requires germ tube test, urease test, growth on cornmeal agar, fermentation and assimilation tests, and growth at 45°C which are cumbersome and time consuming. Chromogenic media can be used for the rapid identification of various *Candida* species. *Candida* Chromagar is a selective and differential medium, which helps in rapid isolation of yeasts from mixed cultures and also allows the differentiation of *Candida* species namely *C.albicans, C.krusei, C.tropicalis, C.parapsilosis and C.glabrata* on the basis of colouration and colony morphology.[13] On these media results are obtained within, 48 hours which facilitates rapid and presumptive identification of common yeasts. Interpretation of the results is easy and the whole procedure requires less time and is cost effective.[14] Chromagar culture will also enable the clinician to choose appropriate antifungal drugs and thereby decrease patients' mortality and morbidity.[15]

This study was undertaken with the aim to identify *Candida* species using Chromagar from isolates showing significant counts from urine samples of admitted patients in a COVID dedicated tertiary care centre. We also aim to analyze the clinic-demographic correlation of *Candida* species from urinary isolates.

2. Material and Method

This prospective study was conducted in the Bacteriology laboratory, Department of Microbiology at a tertiary care centre, referral and teaching hospital. This study was conducted from March 2021 to May 2021. A total of 30 isolates of *Candida* species from the patients with significant Candidiuria (i.e. $>10^5$ CFU of *Candida* per ml of urine) were included. Clinical and demographic data was collected in Google format.

Sample processing: All the samples were received in sterile containers with a request for routine bacterial culture. Immediately after collection, the urine samples were processed according to the recommended procedures.[16] All the samples were inoculated on Cysteine Lactose Electrolyte-Deficient (CLED) medium by using calibrated inoculating loop of 0.01 mm and 0.001 mm diameter. Those plates were incubated at 37°C and examined after 24 hrs of incubation. Only pure isolates of *Candida* on CLED media with significant count i.e. $>10^5$ CFU were included in this study. For the identification of *Candida species*, the colonies from CLED agar media were processed for Gram stain and further species confirmation as per the standard protocol.[16]

Germ tube formation: A single colony was inoculated in human serum and incubated at 37˚C. After 2 - 4 hours, a wet mount was prepared and examined under the microscope to look for the presence of germ tube. The germ tube appeared as short lateral hyphae filament with no point of constriction at the origin, classically described as hand mirror appearance.[16]

Confirmation of *Candida* species on *Candida* Chromagar: All these urinary *Candida* isolates were inoculated on Chromagar *Candida* (Chrome agar, Himedia, Mumbai, India). The Chromagar plates were incubated at 37ºC for 48 hrs. Culture plates were observed after 48hrs of incubation. There was luxuriant growth of *Candida* isolates on Chromagar *Candida*. Different isolates produced distinctive coloured colonies. The size, colour, texture of colonies were noted and depending upon that identification of various *Candida* species were done. The interpretation of colony morphology was done using standard references.

Colony morphology of *Candida* species on *Candida* Chromagar.

1) Candida albicans: light green coloured smooth colonies
2) Candida tropicalis: blue to metallic blue coloured raised colonies
3) Candida glabrata: cream to white smooth colonies
4) Candida krusei: purple fuzzy colonies

We used ATCC strains of *Candida albicans ATCC 10231*, *Candida glabrata ATCC 15126, Candida krusei ATCC 14243* and *Candida tropicalis ATCC 750* as control.

Statistical Analysis: The data was obtained and entered using SPSS software 18.0 version for statistical analysis. Descriptive statistics was used to characterize the study group.

3. Result

Out of 30 samples (isolates of *Candida*) received 36.7% were males and the remaining 63.3% females, with a male to female ratio of 1:1.7. Majority of the patients with Candidiuria belonged to the age group of 30-50 years (43%). The median range of age was 35 years. The Interquartile range at 25th and 75th percentile was 1, 2.5 respectively. Candidiuria was found to be low (6.6%) among the children less than 12 years of age. Age wise distribution of Candidiuria isolates in the hospital acquired UTIs is shown in Figure 1.

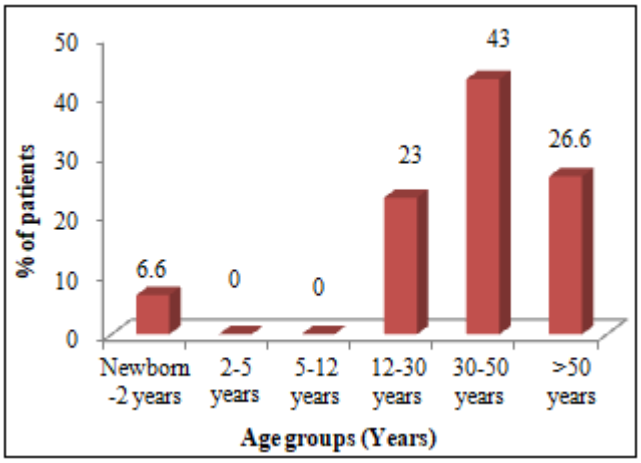

Figure 1: Age wise distribution of Candidiuria isolates in the hospital acquired UTI (N=30)

The risk of developing Candidiuria was high in patients who were admitted in the ICU's and had a history of urinary catheterization. Antibiotic intake was a universal risk factor seen in all the patients. Imipenem/Meropenem group of drugs were the most vulnerable, while those who were on Vancomycin had the least risk. Of the 30 patients in the study, 6 patients (16.7%) were taking more than 3 groups of antibiotics. 13 patients (41%) had history of diabetes mellitus, while none of the female patients were pregnant. Renal disease was not found in any patient of the study group. The mean total leukocyte count (TLC) was 8850± 3747. The Interquartile range at 25th percentile and 75th percentile was 1,3 respectively. The associated risk factors and clinic-demographic profile of the study group are shown in (Table 1).

Table 1: Clinico-Demographic profile and various risk factors in patients with Candidiuria, (N=30)

Sociodemographic and clinical parameters		N=30 (%)
Suprapubic aspirate		2 (6.6)
Catheterization		8 (27)
Midstream urine sample (MSU) collection		25 (83)
Males		11 (36.7)
Females		19 (63.3)
Pregnant females		Nil
Mean duration of Intensive care unit (ICU) stay		13.27 ±6
Previous surgery		7 (23.3)
Previous hospitalization		12 (40)
History of Diabetes		13 (41)
History of organ transplantation		Nil
History of Renal disease		Nil
History of smoking		7 (17)
History of drinking		7 (17)
History of tobacco chewing		3 (10)
Total parental nutrition (TPN)		3 (10)
Mean Total leukocytes count (TLC)/ mm^3		8850± 3747
Symptoms & Sign of UTIs	Fever	20 (66)
	Burning micurition	25 (83)
	Lower abdomen pain	22 (73)
	Suprapubic pain	25 (83)
	Hematuria	6 (20)
	Frequency & urgency of urine	6 (20)
	Others	9 (30)
Antibiotics intake	Imipenem/ Meropenem	20 (66)
	Fluoroquinolones	8 (27)
	Cephalosporin	16 (53)
	Aminoglycosides	11 (36)
	Vancomycin/Teicoplanin	5 (16)
	More than 3 antibiotics	6 (16.8)

Out of total 30 isolates *Candida* isolates were identified by using colour and colony morphology on *Candida* Chromagar. The prevalence of various *Candida* species among the study isolates were *C. albicans* (56%), *C. tropicalis* (16.6%), *C. glabrata* (10.5%) and *C. krusei* (6.6%). (Fig 2)

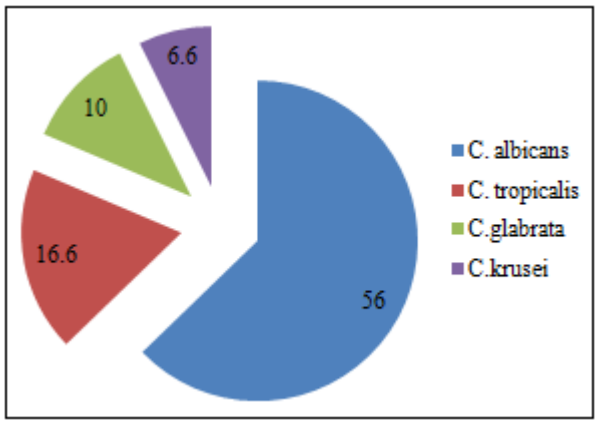

Figure 2: Distribution of various *Candida* species in the study group (N=30).

The morphology of *Candida albicans*, *Candida tropicalis*, *Candida krusei* and *Candida glabrata* on *Candida* Chrome agar is shown in Figure 3.

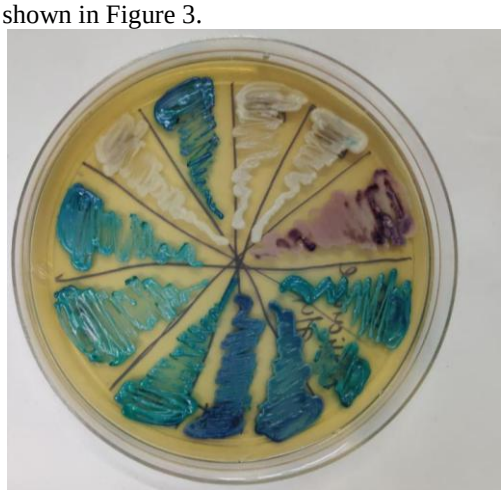

Figure 3: Morphology of *Candida* species on *Candida* Chrome agar.

4. Discussion

Hospital care associated urinary tract infection is the most common nosocomial UTI. Fungal infections of the urinary tract, especially those caused by *Candida* species, are becoming progressively more commonly. [17] *Candida* species are increasingly becoming important causative agent of nosocomial UTI. *Candida* species account for almost 10 - 15% of hospital acquired UTIs.[18, 19] In the present study, we observed that nosocomial UTI due to *Candida* spp. was more common in the age group of 30-50 years (43%). This could be due to the lowered host defense at this age. This finding is supported by many other researchers.[20,21] Our study group comprised of 11 (36.3%) males and 19 (63.7%) females with the male to female ratio 1:1.7 which was

similar to the findings of Anandkumar *et al* [22] with male to female ratio of 1:1.65 (42.8% males; 57.1% females) and Kandhari *et al* [23] with the ratio of 1:1.57. In another study by Rizvi MW etal[24] females (63.7%) outnumbered males (36.3%) in Candidiuria. The colonization of vulvo vestibular area with *Candida* spp. is frequent in females, they are more at risk of developing Candidiuria due to the ascending infection. Among other risk factors that were present, diabetes mellitus was seen in 41% of our patients. Diabetes is a well-known risk factor for developing nosocomial UTI due to *Candida* species.[18, 19] This is because diabetes lowers host resistance to invasion by fungi and also promotes stasis of urine in neurogenic bladder, thus further increasing the chances of colonization by *Candida* species.[25] 17 % of our patients had history of smoking and drinking while, 10% patients had habits of chewing tobacco. None of the patients was pregnant or had any other significant renal disease. Intake of antibiotic was a universal risk factor in our patients. The risk was highest after treatment with Imipenem/ Meropenem group of drugs (66%), followed by cephalosporin (53%). Antibiotics increase the risk of colonization of *Candida* spp. by suppressing endogenous flora and the risk of Candidiuria increases with prolonged antibiotic use.[21] There is definite risk of developing invasive candidiasis in the presence of associated risk factors, and these patients are more prone to higher mortality. Hence, in critically ill patients having other associated risk factors and admitted in the ICUs, Candidiuria should not be ignored. Urinary catheters serve as a portal of entry and most catheters become colonized if left for longer duration.[25] In our study 27% patients had catheterization history which has direct relationship between the duration of catheterization, candidal colonization and nosocomial Candidiuria. Thus, as the duration of catheterization increases, the risk of developing Candidiuria also increases. This point strongly favors the weaning of urinary catheters as soon as possible in ICU patients.

The *Candida* species identification helps in the clinicians for proper treatment and management of Candidiuria. Chromagar *Candida* is a chromogenic medium which gives rapid presumptive identification of some clinically important *Candida* species. This medium contains substrates with which the different enzymes produced by *Candida* species react to form a specific colour. This medium easily and accurately identifies the important *Candida* species namely *C. albicans*, *C. tropicalis*, *C. glabrata* and *C. krusei* based on the colour and morphological features. Many studies have reported *C. albicans* to be the most frequent species isolated from nosocomial UTI.[12, 26, 27] The majority of Candidiuria in the present study were caused by *C.albicans* (56%) followed by the non-*albicans* species among which, *C. glabrata* (22.7%) emerged as a significant nosocomial pathogen. Similar study by Zarei *et al.* from Iran showed the most common isolates to be *C. albicans* (53.3%), followed by *C. tropicalis* (16%), *C. glabrata* (10%) and *C. krusei* (6.6%).[28] *C. albicans* has remained the major agent of Candidiuria until recently. Several reports, however, show that non-*albicans Candida* species; especially *C. tropicalis* and *C. glabrata* now predominate in many regions. [29, 30]

The impact of rapid identification of the *Candida* species in patients with candidal urinary tract infection will help the clinician in selecting the appropriate antifungal agent timely, and thus contributing to overall reduction in the cost of treatment and the duration of hospital stay. Large-scale surveys of Candidiuria are needed in certain populations at risk to find the true incidence of the disease and compare the microbiology pattern, which will help in further understanding of the problem in our population.

5. Conclusion

Health care associated UTI resulting in Candidiuria are more common in patients with indwelling urinary catheters, systemic antibiotic use, underlying genitourinary abnormality, previous surgery and diabetes mellitus. Identification of *Candida* species with Chromagar is a rapid, reliable, affordable method to identify various commonly isolated *Candida* species from urine samples. Rapid identification reduces patient mortality and morbidity that helps in controlling the rise in antifungal drug resistance due to proper treatment.

Conflict of interest: Nil
Sources of support: Nil
Acknowledgement: Nil

References

[1] Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH. *Manual of Clinical Microbiology*. 8th ed. 2003; 2:1702-1704.
[2] Kauffman CA, Vazquez JA, Sobel JD, Gallis HA, McKinsey DS, Karchmer AW *et al*. Prospective multicenter surveillance study of funguria in hospitalized patients. *Clin Infect Dis*. 2000; 30: 14-8.
[3] Voltan AR, Fusco–Almeida AM, Mendes–Giannini MJS. Candiduria: Epidemiology, Resistance, Classical and Alternative Antifungals Drugs. SOJ Microbiol Infect Dis. 2014; 2: 1–7.
[4] Jacqueline, M., Achkar and Bettina, C., 2010. Candidal infections of the genitourinary tract. *Clin. Microbiol. Rev.*, 23(2): 253-273.
[5] Krcmery, V. and Barnes, A.J., 2002. Non-*albicans Candida spp*. causing fungaemia: pathogenicity and antifungal resistance. *J. Hosp. Infect*., 50: 243-260.
[6] Calderone RA. Taxonomy and Biology of *Candida*. *Candida* and Candidiasis. Washington, D.C. ASM Press.2002; 15-27.
[7] KHorvath, L.L., Hospenthal, D.R., Murray, C.K. and Dooley, D.P., 2003. Direct isolation of *Candida* spp., from blood cultures on the chromogenic medium CHROMagar *Candida*. *J. Clin. Microbiol.*, 41: 2629-2632.
[8] Laverdiere, M., Labba, A.C., Restieri, C., Rotstein, C., Heyland, D. and Madger, S, *et al.*, 2007. Susceptibility patterns of *Candida* species recovered from Canadian intensive care units. *J Crit. Care*., 22(3): 245-250.
[9] Saha, R., Dasm S., Kumar, A. and Kaur, I.R., 2008. Pattern of *Candida* isolates in hospitalised children. *Indian J. Pediatr.*, 75: 858-860.
[10] Nayman, A.S., Ozgunes, I., Ertem, O.T., Erben, N., Doyuk, K.E. and Tozun, M., *et al.*, 2011. Evaluation of risk factors in patients with candiduria. *Microbiol. Bul*., 45(2): 318-324.

[11] Dalen, D.M., Zvonar, R.K. and Jessamine, P.G., 2005. An evaluation of the management of asymptomatic catheter-associated bacteriuria and candiduria at The Ottawa Hospital. *Can. J. Infect. Dis. Med. Microbiol.*, 16(3):166-170.
[12] Kobayashi, C.C., de Fernandes, O.F, Miranda, K.C., de Sousa, E.D. and Silva, M. R., 2004. Candiduria in hospital patients: A study prospective. *Mycopathologia*, 158: 49-52.
[13] Baradkar VP, Mathur M, Kumar S. Hichrome Candida agar for identification of Candida species. Indian Journal Of Pathology and Microbiology, 2010;53(1):93-95.
[14] Sanjeev Kumar et.al. Application of CHROMagar Candida for identification of clinically important candida species and their antifungal susceptibility pattern. Internation Journal of Biological and Medical Research. 2013; 4(4): 3600-3606.
[15] Akpan A, Morgan R. Oral candidiasis. A review. *Postgrad Med J*. 2002; 78: 455-459.
[16] Forbes S, Betty A, Sahm DF, Weissfeld A. Laboratory methods in basic mycology. Bailey and Scott's Diagnostic Microbiology, Missourn Mosby. 1998: 871-961.
[17] Prasad KN, Agarwal J, Dixit AK, Tiwari DP. Role of yeast as Nosocomial pathogens and their susceptibility to Fluconazole and Amphotericin B. *Ind J of Med Res*. 1999;110: 11-17.
[18] Kauffman CA. Candiduria. *Clin Infect Dis*. 2005; 41: S371-6.
[19] Lundstrom T, Sobel J. Nosocomial candiduria: A review. *Clin Infect Dis*. 2001; 32: 1602-7.
[20] Passos XS, Sales WS, Maciel PJ, Costa CR, Miranda KC, Lemos Jde A, *et al*. Candida colonization in intensive care unit patients' urine. Mem Inst Oswaldo Cruz 2005;100:925-8.
[21] Fisher JF, Newman CL, Sobel JD. Yeast in the urine: Solution for a budding problem. Clin Infect Dis 1995;20:183-9.
[22] Anand Kumar H, Ramakrishna, Srinivas T, Rao KS, Bhat KH, Rao A. Occurrence and characterization of *Candida* species isolated from symptomatic cases of urinary tract infection. *J Pub Health Med Res*. 2013; 1(1): 28-31.
[23] Khandari KC, Rama Rao KM. Clinical and laboratory studies on cutaneous candidisis. *Ind J Der Ven Lep.* 1969; 35: 102-107.
[24] Rizvi MW, Malik A, Shahid M, Singhal S. Candida albicans infections in a north Indian tertiary care Hospital: Antifungal resistance pattern and role of SDS-PAGE for characterization. *Biology and Medicine*. 2011; 3: 176-181.
[25] Goeke TM. Infectious complications of diabetes mellitus. In: Grieco MH, editor. Infections in the abnormal host.New York: Yorke Medical Books. 1980; 585-600.
[26] Stamm WE. Catheter associated urinary tract infections: Epidemiology, pathogenesis and prevention. Am J Med 1991; 91: 65S-71S.
[27] Febré N, Silva V, Medeiros EA, Wey SB, Colombo AL, Fischman O. Microbiological characteristics of yeasts isolated from urinary tracts of intensive care unit patients undergoing urinary catheterization. *J Clin Microbiol*. 1999; 37: 1584-86.
[28] Zarei, MA, Zarrin M, Ghanatir, F. and Vazirianzadeh, B., 2012. Candiduria in hospitalised patients in teaching hospitals of Ahvaz. *Iranian J. Microbiol.*, 4 (4): 198-203.
[29] Guler S, Ural O, Findik D, Arslan U. Risk factors for nosocomial candiduria. *Saudi Med J*. 2006; 27: 1706-10.
[30] Jain N, Kohli R, Cook E, Gialanella P, Chang T, Fries BC. Biofilm formation by and antifungal susceptibility of *Candida* isolates from urine. *Appl Environ Microbiol.* 2007; 73: 1697-1703.