

## **Isolation of *Candida spp* . locally from urine**

### **عزل انواع المبيضات *Candida spp* . عملياً من الأدرار .**

Asst. Lecturer Maysaa Taqe Abdul hasan  
College of Science , University of Karbala

#### **Abstract**

In this study 226 isolates of *Candida spp*. from urine specimens were identified and their association with various clinical factors such as age , sex , antibiotic therapy , anemia , diabetes mellitus , indwelling catheter , pregnancy and burns were analysed . *C. albicans* (62.7%) , *C. tropicalis* (23.5%) and *C. parapsilosis* (7.1%) were most commonly isolated from urine specimens . There was a higher prevalence of *Candida* species in female (70.8%) than in male (29.2%) and in age group 30-39 about (24.0%) . Associated factors frequently encountered were antibiotic therapy (84.5%) , anemia (18.6%) and diabetes mellitus . (11.1%).

#### **الخلاصة**

في هذه الدراسة عزلت من عينات الأدرار 226 عزلة من خميرة *Candida* و شخّصت مخبرياً و درست علاقتها مع العديد من العوامل السريرية كالعمر ، الجنس ، العلاج بالمضادات الحيوية ، الحمل ، القسطرة ، الإصابة بفقر الدم ، داء السكري و الحروق . كانت *C.albicans* بنسبة (62.7%) و تليها *C. tropicalis* بنسبة (23.5%) و *C. parapsilosis* بنسبة (7.1%) من أكثر العزلات شيوعاً في عينات الأدرار. كما وجد انها أكثر انتشاراً لدى الإناث (70.8%) مقارنة بالذكور (29.2%) و عند المصابين من الفئة العمرية (30-39) و تمثل (24.0%) كما وجد ان من أكثر العوامل السريرية المساعدة لحدوث الإصابة هي العلاج بالمضادات الحيوية بنسبة (84.5%) و يليها فقر الدم (18.6%) و داء السكري بنسبة (11.1%)

#### **Introduction**

*Candida* species are ubiquitous fungi and are the most common fungal pathogens that affect humans. The growing problem of muscosal and systemic candidiasis reflects the enormous increase in the pool of patients at risk and the increased opportunity that exists for *Candida* species to invade tissues normally resistant to invasion . *Candida* species are true opportunistic pathogens that exploit recent technological advances to gain access to the circulation and deep tissues [1] . The presence of *Candida* in the urine may reflect a variety of clinical conditions [2]. Asymptomatic candiduria, lower tract infections in the urethra or bladder infection limited to the kidneys or systemic infection also involving the kidney . Asymptomatic candiduria implies colonization of the lower urinary tract without tissue invasion [3]. It usually occurs in a setting with one or more predisposing factors , such as use of indwelling urinary devices , diabetes mellitus , antibiotic use , immunosuppressive therapy , extended hospitalization , extreme of age and sex [4].

Although ,non-albicans species as *C. tropicalis* and *C. glabrata* have been frequently found in this clinical specimens , *C. albicans* is still the most frequent yeast recovered from urine [5,6].

The aim of this study was to identify *Candida* species isolated from the candiduria of hospitalized and non hospitalized patients admitted to Al-Hussain general hospital in Karbala and investigate the possible risk factors associated with infection in both groups .

## **Materials and methods**

### **Samples**

During March to August 2009 , urine samples were obtained from 935(408 males and 527 females) patients attending to Al-Hussain general hospital in Karbala . Data such as sex , age and variables as possible predisposing factors were obtained from these patients .

### **Laboratory tests**

Urine samples were collected in Al-Hussain general hospital laboratory by cleaning catch and then according to [7] the urine samples were centrifuged for 15minute at 2000 rpm. The supernatant was decant and pool the sediment.

The smear of sediment were prepared directly by 10% KOH for direct microscopic examination , and then 1 ml of the sediment e was inoculated onto Sabouraud Dextrose Agar with gentamicin and chloramphenicol at 28 c for 2-7 days . Finally the *Candida* growth were identified according to [8,9]

### **Germ tube test**

This test is performed by inoculating a small inoculum of yeast cells obtained from an isolated colony in 0.5 ml of human serum ,and incubated the tubes at 37 c for 3 hours. A drop of the suspension was removed and placed on a slide , then examined under microscope for the presence of germ tube [8,9 ] .

### **Chlamydospores formation test**

A single colony of *Candida* was picked from the a pure culture medium and inoculated on a plate of Cornmeal agar containing 1% Tween 80 and trypan blue by making three parallel cuts to the culture medium ,then a coverslip was added and incubated at 30c for 48 hours .After 48 hours , the cover slip was removed and placed on a slide contain a drop of lactophenol blue stain and then examined under a microscope for the presence of chlamydospores [8,9 ] .

### **Pellicle in broth test**

A drop of inoculum suspension was inoculated on the a Sabouraud broth and incubated at 30 c for 48 hours. The presence of a pellicle on the surface growth indicate the positive result [8,9 ] .

### **Sugar fermentation test**

Fermentation medium was Prepared according to [7], which composed of 0.25ml of sugar solution 2% and the yeast was inoculated by adding one drop of inoculum suspension into each tube and incubated at 30 c for 48 hours. The presence of acid (indicator become red) and gas trapped in the Durham tube indicate to the ability of *Candida* to ferment sugar [8,9 ] .

### **Sugar Assimilation test**

A suspension of *Candida* was Prepared at a density equivalent to a McFarland tube . A plate of Nitrogen Base Agar containing bromocresol purple were inoculated with the suspension of *Candida* and by using a sterile forceps a disk of carbohydrate were put on the surface of the agar. The plates were incubated at 30c for 48 hours. Observation of a color change around the carbohydrate disks indicate the positive result [8,9 ] .

### **Urea test**

Aslant of a Christensen Urea agar were inoculated with a single colony of *Candida* and incubated at 30 c for 48 hours. The Conversion of phenolphthalein indicator from yellow to red indicate the positive result [8,9 ] .

### **Statistical analysis**

Data in this study were statistically analyzed by Statistical Analytic System (SAS) Duncan Multiple Range test [10]. Appropriate P values of  $\leq 0.01$  were considered significant .

## **Results**

Among the 935 urine samples cultured , *Candida* was recovered in 226(24.2%) . Five species of *Candida* were identify according to the results of cultural and biochemical tests as shown in the table (1)

The predominant species were *C. albicans* isolated from urine of 62.4% of the patients , followed by *C. tropicalis* isolated in 23.5% , *C. parapsilosis* in 7.1% , *C. krusei* in 4.4% and *C. kefyr* in 2.6% as is evident in the figure (1).

Concerning the distribution of *Candida* species to the sex of patients , *Candida* was higher significantly presence in females ( 70.8% ) than in males ( 29.2% ) (  $p \leq 0.01$  ) as show in table (2).

The present study showed statistical significant association between the age of patients and *Candida* species . The examined patients were 10-59 years group . However , patients of 30-39 group were the most prevalent significantly for *Candida* ( 42% ) (  $P \leq 0.01$  ) then followed by patients of 20-29 years group (26.5% ) ( Table 3 ).

Regarding to the relationship between the risk factors in patients and *Candida* species . The common coexisting exposures in patients with candiduria were antibiotic therapy (84.5%) and anemia ( 18.6% ) ( $P \leq 0.01$ ) as show in table (4) .

## **Discussion**

Fungal infection has become an important problem on the past decade[11]. In the present study candiduria was detected in 24.2% . The same observation was demonstrated by [12] in Delhi where they found these cases in a range of 19 % ,but [13] were recorded the proportion 4.7% which much less than ours . According to [11] presence of funguria in those patients might be indicative of urinary tract or systemic infection , and these infection might be related with several factors such as increased of human immunodeficiency syndrome, neutropenic persons due to anticancer treatment , the abusive use of extended spectrum antibiotics and metabolic disorders such as diabetes mellitus [14] .

The commonest *Candida* species found in urine specimens was *C.albicans* (62.4%) followed by *C.tropicalis* (23.5%) and then *C.parapsilosis* (7.1%) . Our finding seems to be agree able with that of other workers . In the study [15] *C.albicans* was isolated from 66.6% , followed by *C. tropicalis* from 14.3% and *C. parapsilosis* from 6.3 % , and [16] recorded *C.albicans* in 49% followed by *C.tropicalis* in 22% , where [12] found *C.albicans* in 45.8% followed by *C.tropicalis* in 24.7% and *C.parapsilosis* in 10.5% .

According to [17] *C.albicans* was accounted for 70 to 90 % of *Candida spp.* that founded in human body while *C.glabrata* and *C.tropicalis* were accounted for approximately 5% . Other *Candida spp.* were only rarely isolated from clinical specimens .

Our recorded incidence of *C.krusei* was 4.4% and *C.kefyr* was 2.6% .In contrast ,the workers [15] were recorded *C.krusei* and *C.kefyr* in 3.2% , where [12] observed an incidence of *C.krusei* and *C.kefyr* in 9% .

Interestingly, candiduria was higher in females (70.8%) than in males (29.2%) . This incidence in females may reflect vaginal candidiasis . Yeast may ascend from the genital tract to the urinary tract , explaining a higher candiduria incidence in females , This hypothesis was suggested by [18], whom found five of eight patients with positive vaginal secretion and later showed the presence of the same yeast species in their urine . However , the result in this study consistent with the observations of other investigators[19, 20,11] .

The age of the patients showed an asymmetric distribution with more patients included in the age group of 30-39 years (42%), this might be related with fact that this the age group with higher hormonal variations , sexual activity and rates of pregnancy [11, 14].

When associated factors were analyzed, it was found that in most cases of candiduria had antibiotic therapy 84.5% followed by anemia 18.6% and diabetes mellitus 11.1% . The reason the *Candida* was an opportunistic yeasts caused infection in immunocompromised , surgery

patients and in those on long term intravenous therapy [21] . This result was differented with [16] and [ 22] whom observated that the most patients with candiduria had acatheter .

Table (1): Results of cultural and biochemical tests of *Candida spp* . were isolated from urine samples .

<i>Candida spp.</i>	Germ tube test	Chlamyospores formation test	Pillecl in broth test	Sugar fermentation test				Sugar assimilation test					Urea test
				M	S	L	G	M	S	L	R	X	
<i>C.albicans</i>	+	+	-	+	+	+	+	+	+	-	-	+	-
<i>C.tropicalis</i>	-	-	+	+	-	+	+	+	+	-	-	+	-
<i>C.parapsilosis</i>	-	-	-	-	-	-	-	+	+	-	-	+	-
<i>C.krusei</i>	-	-	+	-	-	-	-	-	-	-	-	-	+
<i>C.kefyr</i>	-	-	-	-	+	+	+	-	+	+	+	+	-

+ :positive reaction , \_ :negative reaction , M : Maltose ,S: Sucrose ,L: Lactose ,G: Glucose , R: Rafinose , X: Xylose .

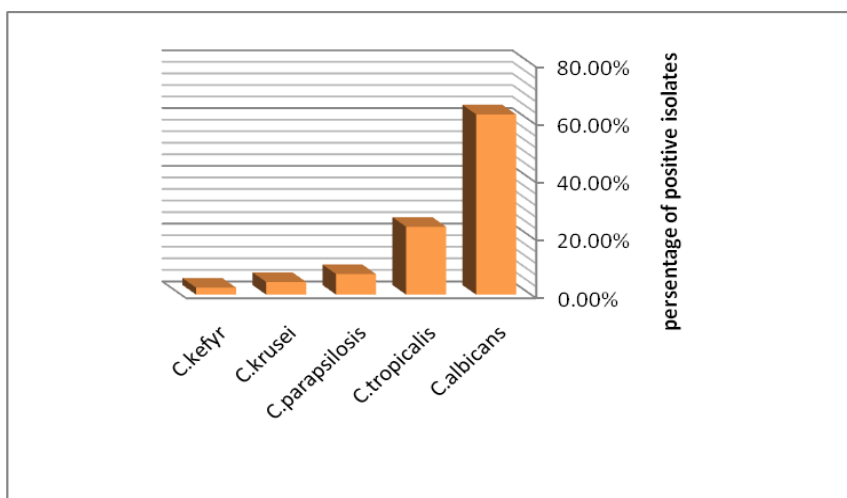


Figure (1) : the percentage of species isolated from 226 positive cases of candiduria .

Table ( 2 ) : Distribution of *Candida spp.* according to sex of patients .

<i>Candida spp.</i>	Female		Male		Total		Duncan Multiple range test value
	No.	%	No.	%	No.	%	
<i>C.albicans</i>	98	61.3	43	65.2	141	62.4	70.50*
<i>C.tropicalis</i>	37	23.1	16	24.2	53	23.5	26.50*
<i>C.parapsilosis</i>	13	8.1	3	4.5	16	7.1	8.0*
<i>C.krusie</i>	7	4.4	3	4.5	10	4.4	5.0*
<i>C.kefyr</i>	5	3.1	1	1.5	6	2.6	3.0*
Total	160	70.8	66	29.2	226	100	
Duncan Multiple range test value	32.0*		13.2*				

\*Significant  $p \leq 0.01$

Table ( 3 ) : Distribution of *Candida spp.* according to age of patients .

<i>Candida spp.</i>	Age of groups										Total		Duncan Multiple range test value
	10-19		20-29		30-39		40-49		50-59		No.	%	
	No.	%	No.	%	No.	%	No.	%	No.	%			
<i>C.albicans</i>	13	65	35	58.3	62	65.3	25	67.6	6	42.9	141	62.4	27.8*
<i>C.tropicalis</i>	7	35	16	26.7	22	23.2	6	16.2	2	14.3	53	23.5	10.6*
<i>C.parapsilosis</i>	-	-	4	6.7	5	5.3	3	8.1	4	28.6	16	7.1	3.2*
<i>C.krusie</i>	-	-	2	3.3	4	4.2	3	8.1	1	7.1	10	4.4	2.0*
<i>C.kefyr</i>	-	-	3	5.0	2	2.1	-	-	1	7.1	6	2.6	1.2*
Total	20	8.8	60	26.5	95	42.0	37	16.4	14	6.3	226	100	
Duncan Multiple range test value	4.0*		11.6*		19.0*		7.4*		2.8*				

\*Significant  $p \leq 0.01$

<i>Candida spp.</i>	Diabetes mellitus		Indewelling catheter		Antibiotic therapy		Anemia		Pregnancy		Burns		Total		Duncan Multiple range test value
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
<i>C.albicans</i>	19	76	18	100	122	63.9	21	50	7	46.7	3	42.9	141	62.4	70.5*
<i>C.tropicalis</i>	3	12	-	-	43	22.5	9	41	4	26.7	2	28.6	53	23.5	26.5*
<i>C.parapsilosis</i>	2	8	-	-	10	5.2	7	16.7	13	20	1	14.3	16	7.1	8.0*
<i>C.krusie</i>	1	4	-	-	10	5.2	5	11.9	1	6.7	1	14.3	10	4.4	5.0*
<i>C.kefyr</i>	-	-	-	-	6	3.1	-	-	-	-	-	-	6	2.6	3.0*
Total	25	11.1	18	8.0	191	84.5	42	18.6	15	6.6	7	3.1	226	100	
Duncan Multiple range test value	5.0*		3.6*		38.2*		8.4*		3.0*		1.4*				

\*Significant  $p \leq 0.01$

Table (4) : The relationship between *Candida spp.* and risk factors of patients .

## References

1. Alexandr , B.D. and pfaller, MA.(2006) Contemporary tools for the diagnosis and management of invasive mycoses . Clin.Infect Dis.43:15-27.
2. Crislip , M.A. and Edwards , J.E. (1989) . Candidiasis. Infect Dis. Clin. North Am. 3(1): 103 - 133 .
3. Cantillep , A. O. ; Julius , M. D. ; Lecciones , A. ; Jose , M. D ; , Almario , S. ; Madel , M.D. Thelma , M.D. ; Tupasi , M.D. and Eileen M.D. (1995) . Positive urine cultures for *Candida albicans* in 55 patients at Makati medical center , Implications for management . Phil . J. Microbiol . Infect Dis. 24(2):47-53 .
4. Sobel , J.D. (2002) . Controversies in the diagnosis of candiduria , what is the critical colony count . Infect Dis. 4 : 81-83 .
5. Hazen , K. C. , Gordan , W. T. and Susan , A. H. (1999) . Chronic urinary tract infection due to *Candida utilis* . J. Clin. Microbiol . 37(3): 824 – 827 .
6. Nucci , M. (2000) . Candiduria in hospitalized patients . review Bras . J . Infect Dis . 4 : – 172 .
7. Ellis , D. H. (1994) . Clinical mycology , opportunistic mycoses . Gillingham printers Ltd. , Australia.
8. Kurtzman , C. p. and fell , J. W. (1998) . The yeasts , taxonomic study . 4<sup>th</sup> ed. ; Elsevir ; New york . p. 891 – 894.
9. Warren , N. G. and Hazen , K. C. (1999) . *Candida* , *Cryptococcus* and other yeasts of medical importance 7<sup>th</sup> ed. P. 1186 .
10. SAS. (2001) . SAS/STAT 'user' Guide for personal computers release 6.12. SAS Institute , Inc. Cary , N.C.V.S.A.
11. Sena , X.P. ; Wether , S.S. ; Patricia , J.M. ; Carolina , R.C. ; Karla , C.M. ; Janine , D.L. and Maria , R.S. ( 2005 ) . *Candida* colonization in intensive care unit patient's urine . Mem . Inst. Osvaldo Cruz . Rio. De . Janeiro . 100 (8) : 925 – 928 .
12. Basu , S. ; Gugnani , H. C. ; Sangeeta , J. and Nerra , G. (2003) . Distribution of *Candida* species in Delhi , India and proteinase and phospholipase activity of *Candida albicans* isolated. Rev. Ibero .am . Microl . 20:137-170 .
13. Chen,S.C.; Tung , Z. S. ; Lee , O. C. ; Halliday , C. ; Play for , E. G ; Widmer , E. ; Kong , E. R. ; Wang , C. and Sorrelt , T. C. (2008) . Clinician response to *Candida* organisms in the urine of patients attending hospital .Eur . J. Clin . Microbiol .infect Dis. 27 : 201 – 208 .
14. Fernandes , R. ; Ana , V. and Fatima , C. (2009) . *Candida* species distribution in clinical samples . 6 : 264 – 271 .
15. Sengupta p.c. (1999).Study of yeast species isolated from clinical specimens .Med.J.Armed forces Ind.55 :319 -312 .
16. Yasin , M. S. ; Jamal , F. ; Samed , S.A. ; Zainal Z. and Misngi , S. (1986) . The presence yeast in urine . J. of singapove med. ; 4(27) : 326 – 328 .
17. Virella,g. (1997). Microbiology and infection diseases . 3ed edn .,Williams and Wilkins comp.,U.S.A.343 pp.
18. Febre , N. ; Silva , V. ; Mederro , E.A. ; Wey , S.B. ; Colombo , A.L. and Fischman , O. (1999) . Microbiological characteristics of yeasts isolated from urinary tract of intensive care unit patients undergoing urinary catheterization . J. Clin. Microbiol. 37: 1584 – 1586 .
19. Ahmad , S ; Khan , Z. and Mustafa , S. A. (2003) Epidemiology of *Candida* colonization In an intensive care unit of a teaching hospital in Kuwait. Med. Mycol . 41 :in 487 an 493 .
20. Alvarez – Ierma , F. ; Nolla – Sallas , J. ; Palomar , M. ; Jorda , R. ; Carrasco , N. and Bobillo , F. (2003) . Candiduria in critically ill patients admitted to intensive care medical units . Inten . Care Med. 29 : 1069 – 1076 .
21. Forbes ,B.A . ;Sohm ,D .F.and Weissfeld ,A.S.(1998) .Diagnostic Microbiology . 10 th .edn.Mosby Inc.
22. Kobayashi , C. B. ; Fernandes , O. F. ; Miranda , K. C. ; Souza , E.D. and Silva , M. R. (2004) Candiduria in hospital patients , a study prospective . Mycopathologia. 158: 49 – 52.