Candiduria and Urinary Candidiasis in Basrah, Iraq

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Abstract:

Out of 2000 patients with candiduria , 43 patients (20 males and 23 females) showed significant candiduria (with about $1x10^3$ - $\geq 1x10^5$ cfu /ml) and with significant pyuria. These cases were followed and mid-stream urine samples were examined by direct microscopy and by culturing on Sabouraud's Dextrose Agar(SDA) with the antibacterial antibiotic chloramphenicol. The underlying diseases, sex and age were also recorded . Five *Candida* species were isolated and identified , *C. albicans* constituted 51% of the examined cases , followed by *C. glabrata* (26%), *C. tropicalis* and *C. parapsilosis* each constituted 9.3% while *Candida* sp. constituted 4.65%. Five cases were associated with bacterial pathogens (two with *Escherichia coli,* two with *Pseudomonas aeruginosa* and one with *Streptococcus viridans*). Diabetes mellitus was the most affecting factor (35%), followed by multiple antibiotic users (11.6%), renal stones, renal surgery, cancer and Foley's catheters each constituted 9.3%. Amphotericin-B (AMP-B), Fluconazole and Ketoconazole showed good activity against *Candida* isolates. AMP-B showed a wider range of fungistatic levels and 80% (20/25)of the tested strains were inhibited at a concentration of 3.13 µg/ml and 100% inhibition obtained at 12.5 µg/ml. Both fluconazole and ketoconazole showed 100% inhibition at 25 µg/ml, while clotrimazole reach 100% of inhibition at 50 µg/ml. The polyene antibiotic, nystatin showed 80% inhibition at 50 µg/ml. The results of the minimal fungicidal concentration (MFC) are either the same of the minimal inhibitory concentration (MIC) results or of two to four folds or greater.

Key words: Candiduria, urinary candidiasis, *Candida*, antifungal susceptibility.

Introduction

Infections due to *Candida* species are the most common of the fungal infections. *Candida* species produce a broad range of infections, ranging from non- life threatening mucocutaneous illness to invasive process that may involve virtually any organ. Such a broad range of infections requires an equally broad range of diagnostic and therapeutic strategies [1]. Although fungal urinary tract infections occur less frequently than bacterial infections, their incidence has been increased during the last decades. Diabetes mellitus, parenteral nutrition, corticosteroids, immunosuppressive drugs, and prolonged antibiotic therapy are often predisposing factors[1,2,3].

Candiduria is for the most part a benign process associated with the use of urinary catheters and antimicrobial therapy. Nevertheless , candiduria may be one of the most common challenging of the candidal infections. The challenge comes from the fact that finding *Candida* spp. in the urine can be either completely insignificant (e.g., due to contamination or asymptomatic colonization) or be a marker of a very serious entity such as invasive renal parenchymal disease related to disseminated candidiasis or postlaparatomy peritonitis [3-6]. *Candida* cystitis or bladder colonization may be caused by prolonged catheterization with concomitant antibiotic treatment, diabetes and glycosuria, anatomical uropathy, previous bladder endoscope or surgery, diabetic neurogenic bladder, chronic obstruction from prostatic hypertrophy, or pelvic irradiation for cervical cancer [7].

Renal candidiasis is usually due to hemato-genous seeding to the kidneys, and associated with candidemia, which may present with sepsis, hemodynamic instability and renal insufficiency

[1,8,9]. Patients with a fungus ball may have almost any presentation. Asymptomatic fungus balls are seen when the obstruction is incomplete. Complete obstruction is often associated with a urosepsis-like picture that includes fever, chills, and flank pain. Some patients will report having spontaneously passed whitish debris ("fungus balls") in their urine[10].

In Iraq, studies on candiduria and urinary candidiasis are not available and this study is the first and was conducted to evaluate the role of *Candida* spp. in the urinary tract infection in Basrah, Iraq.

Materials and methods:

Patients: 43 patients (20 males and 23 females) with significant candiduria (urine containing $1x10^3$ to $\geq$ $1x\ 10^5$ CFU/ml with pyuria) were selected from 2000 patients with candiduria, they were out of 17,000 patients admitted for urinalysis at Al-Jazira Clinical Laboratory, Al-Zubair district, west of Basrah during the period of June 2004 to May 2008. The underlying diseases, age and sex were recorded.

Methods: Mid-stream urine samples were recollected for:

a. **Direct microscopy**: Mid-stream urine samples were centrifuged and the deposits were examined for the presence of yeast cells and slide smears were prepared, fixed by flaming and stained by Gram's and methylene blue stains for microscopy and for microphotogarphy. Nikon microscope was used for examination and imaging.

b. **Culture**: Using pour plate method, 0.1 ml of mid-stream urine samples were transferred to about 25 ml of unsolidified Sabouraud's Dextrose Agar(SDA) + chloramphenicol (250 mg/L) at around 45°C, mixed gently by rotating the Petri dishes horizontally, left to solidify at room temperature and incubated at 35°C for 2-3 days. The isolated colonies were then counted and processed for identification, following the criteria described by Cruckshank *et.al*,1975(11), McGinnis,1980(12), Kreger –Van Rig ,1984(13),Finegold& Barron 1986(14), Buckley,1989(15) , Ellis,1994(16), Hoog& Guarro,1995(17).

c. **Antifungal susceptibility testing**:

a. ***Isolates*** : 25 isolates of the five identified *Candida* species: *C. albicans*. 11 ; *C. glabrata*,7 ; *C. parapsilosis*,3 : *C. tropicalis*, 3; *Candida* sp.,1 were tested against five antifungal drugs.

b. ***Medium***: Sabouraud's Dextrose Broth (SDB) and double strenghth Sabouraud's Dextrose Broth supplemented with chloramphenicol (250 mg/L) were used.

c.Antifungals: Commercial samples of Amphotericin-B (Abbot France,S.A.), Clotrimazole (SDI, Sammara-Iraq), Fluconazole (Pfizer, Madrid, Spain) and Ketoconazole (Roig-Farma, Madrid, Spain) were used . Fungizone, the commercial preparation of amphotericin-B(AMP-B) and fluconazole were dissolved in sterilized distilled water and the three other antifungal compounds were dissolved with 100% dimethylsulphoxide (DMSO) and prepared as initial concentration of 10,000 µg/ml (by dissolving 50 mg of each drug in 5 ml of solvents). All drugs then diluted in sterile distilled water to a working concentration of 800 µg/ml.

d. ***Preparation of inocula:*** From the maintained stock culture, the tested strains were subcultured on SDA at 30°C for two days, then the inocula were prepared by transferring parts of several colonies of each strain with a loop and suspended in SDB. The suspensions were adjusted to match the turbidity of McFarland standard No. 0.5 [18] to give a final conc. of $1x10^6$ cfu/ml which then processed directly or kept at a refrigerator (to prevent multiplication) until used within few hours.

E. ***Determination of Minimal Inhibitory Concentration(MIC):*** A serial two fold dilution was carried out in plastic microdilution trays containing 98 cupped button wells (Nunclon, Denmark) according to the method described by Schaude *et.al*., 1987[19] with little modification. Briefly 50 µl of the double-strengthed SDB were dispensed in all wells with a micropipette. Then 50 µl of the working drug solutions were dispensed into the first well of the appropriate row (each drug was tested in two parallel horizontal rows). Twelve doubling dilutions were obtained by transferring 50 µl from well to well in each row. The pipette tips were changed after each dilution step. The addition of 150 µl of yeast

suspension ($1x10^6$ CFU/ml) to each well created a final serial of antifungal concentration ranging from 100 to 0.05 µg/ml. The final cells count per well was $7.5 x10^5$ CFU/ml. The trays then were covered with loose fitting lids and incubated at 35°C for 48 hr. The MIC was considered as the last broth cup remaining clear (free from visible yeast growth).

F. ***Determination of Minimal Fungicidal Concentration(MFC):*** After the MIC has been determined , a known quantity (0.01 ml) of inoculum from each of the wells of broth that showed no visible turbidity after 48 hr incubation was subcultured to SDA plates, and incubated at 35°C for 48 hr. The lowest concentration of antifungal that allowed less than 0.1% (< 75 colonies) of the original inoculum to survive is the MFC.

Results:

Out of 2000 patients with candiduria , 43 cases of significant candiduria (20 males and 23 females) were studied and diagnosed through the repeated direct examination and culture of the midstream urine samples.

Wet preparation and stained slide smears of urine deposits showed pus cells with or without red cells, fungal hyphae and or blastospores, and in five cases were associated with bacterial pathogens . (Figs. 1-3)

A. *DIRECT MICROSCOPY:*

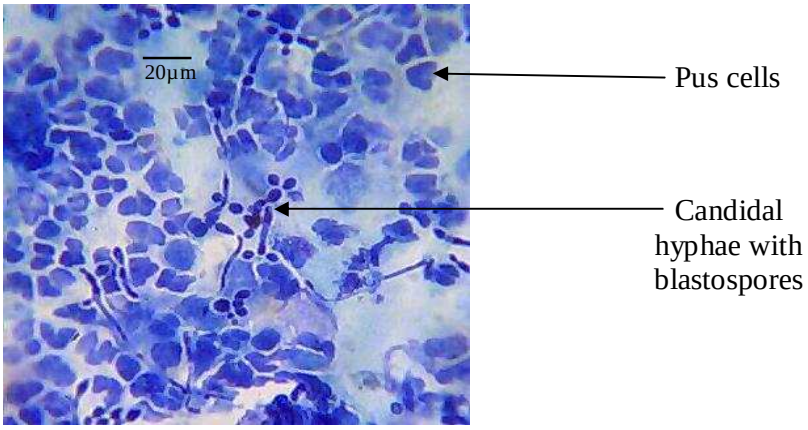

Fig 1.*Candida albicans* in urine deposit. Slide smear stained with methylene blue. .

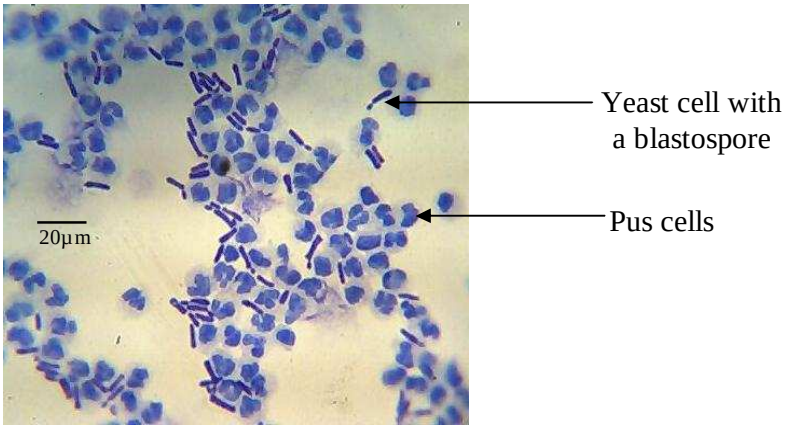

Fig 2.*Candida* sp. with pus cells in urine deposit.Slide smear stained with methylene blue.

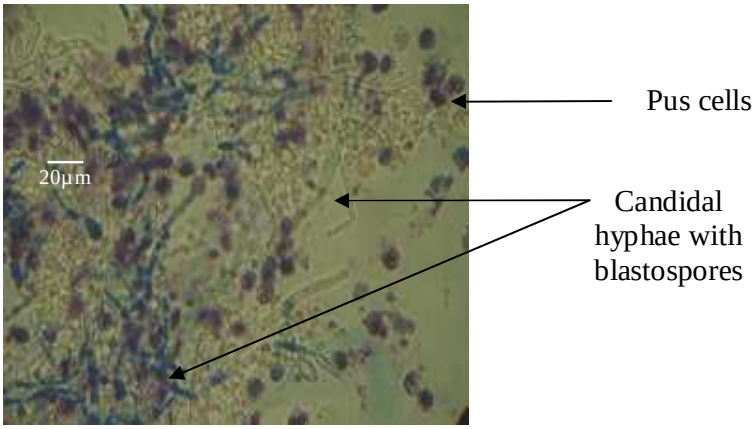

Fig 3.*Candida albicans* in urine deposit, fungus ball (wet preparation, methylene blue stained).

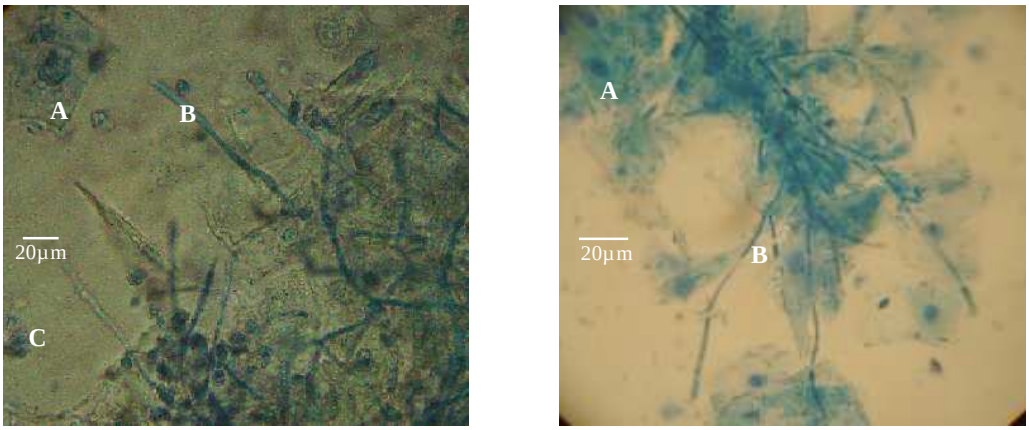

Fig 4.*Candida albicans* in urine deposit - vaginal candidiasis(wet preparation, methylene blue stained). A,vaginal epithelial cells.B, candidal hyphae with blastospores . C,pus cell.

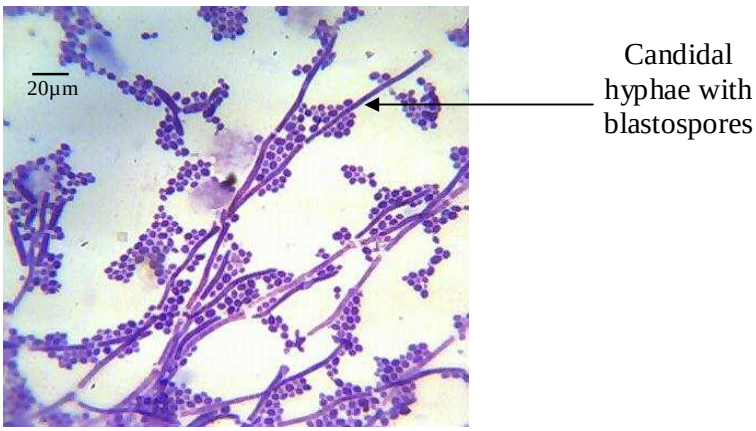

Fig 5. Urine contaminated with *Candida* sp.(Foley's catheter), Gram's stained.

B. CULTURE:

Forty three isolates belonged to five *Candida* species were identified, namely: *Candida albicans*, 22 isolates; *C. glabrata*, 11 isolates; *C. parapsilosis* and *C . tropicalis*, 4 isolates for each, and *Candida* sp., 2 isolates. Among the cases studied, *C. albicans* caused 51.2 % of the disease, followed by *C. glabrata*, 26 %; *C. parapsilosis* and *C. tropicalis*, each responsible for 9.3% and *Candida* sp. 4.7% (which was isolated from two female patients

only) (Table 1 and Figures 1-3). The bacterial pathogens; *Escherichia coli* was associated with *C. albicans* in two male patients (one diabetic and the other had chronic bacterial UTI), *Pseudomonas aeruginosa* with *C. albicans* were isolated from a female diabetic patient and with *C. glabrata* from a female with previous renal stone surgery , while *Streptococcus viridans* was isolated together with *C. albicans* from one female diabetic patient. The candidal cell counts were ranged from $1x10^3 - \geq 1x10^5$ CFU/ ml of urine.

Among all the cases studied, elderly (61-80 yrs) were the largest age groups infected constituted 35% (Table 1) and *C. albicans* was the most prevalent yeast species responsible for 60% (9/15) of candiduria among the diabetes mellitus patients (Table 2).

Table(3) showed that diabetes mellitus was the most common predisposing factor seen in 35% of the patients followed by those with prolonged use of multi-antibiotics (11.6%), while 7% had no underlying disease recorded.

Table.1. Distribution of *Candida* spp. according to age groups and sex.

Candida species	No. of isolates (%)	Age groups (years)								Sex	
		0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	♂	♀
C. albicans	22(51.2)	1		1	3	2	6	6	3	14	8
C. glabrata	11(25.6)		1	1	2	1	3	2	1	2	9
C. parapsilosis	4(9.3)				2		1	1		2	2
C. tropicalis	4(9. 3)		1	2				1		2	2
Candida sp.	2(4.65)					1		1		0	2
Total No.	43	`1	2	4	7	4	10	11	4	20	23

Table 2. Distribution of *Candida* spp. according to the underlying diseases.

Underlying Diseases	*Candida* species					Sex	
	C. albicans	*C. glabrata*	*C. parapsilosis*	*C. tropicalis*	*Candida* sp.	♂	♀
Diabetes mellitus	9	3	1	1	1	6	9
Renal stone	3	1				2	2
Renal surgery	2	2				2	2
Prostatic hypertrophy	2	1	1			4	0
Multiple antibiotics	2		2	1		1	4
Cancer	1	2		1		1	3
Foley's catheter	2	1		1		3	1
None	1	1			1	1	2
Total	22	11	4	4	2	20	23

Table. 3. Distribution of patients with candiduria according to underlying diseases , age groups and sex.

Underlying diseases	No. of Patients (%)	Age groups (years)								Sex	
		0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	♂	♀
Diabetes mellitus	15(35)				2	1	5	7		6	9
Renal stone	4(9.3)	1		1		2				1	3
Renal surgery											
Prostatic hypertrophy	4(9.3)		1	1	1		1			2	2
Multiple antibiotics	4(9.3)						1	2	`1	4	0
Cancer	5(11.6)			1	1		2	1		1	4
Foley's catheter											
None	4(9.3)		1			2			1	2	2
	4(9 .3)			1		1			2	3	1
	3(7)				1		1		1	1	2
Total	43	1	2	4	7	4	10	11	4	20	23

Antifungal susceptibility testing:

The results of MICs and MFCs are shown in Table 4. AMP-B, Fluconazole and Ketoconazole showed better activity against *Candida* isolates. AMP-B showed wider range of fungistatic levels and 80% (20/25) of the tested strains were inhibited at 3.13 µg/ml and 100% inhibition obtained at 12.5 µg/ml. Both fluconazole and ketoconazole showed 100% inhibition at 25 µg/ml, while clotrimazole reach 100% of inhibition at 50 µg/ml. The polyene antibiotic, nystatin, which has a local action, showed 80% inhibition at 50 µg/ml. The results of the MFC are either the same of the MIC results or of two –four folds or greater.

Table 4. Antifungal activity of Amphotericin-B, Clotrimazole, Fluconazole, Ketoconazole, Miconazole, and Nystatin against *Candida* spp.

Fungal species (No. of isolates)	Anti-fungals	M IC range (µg/ ml)	No. of strains inhibited at the indicated conc. (µg/ml)											MFC range (µg/ ml)
			0.05	0.1	0.2	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	
Candida albicans (11)	AMP	0.05 – 6.25	2	1	3	2		1	1	1					0.1 – 25
	C	0.39 – 50				3		4		1	1		2		1.56 – >100
	F	0.2 – 12.5			3	2		1	2	1	2				0.2 – 50
	K	0.1 – 25		1	3		1		2	1	2	1			0.2 – 50
	N	6.25 – >100								1	3	4	1	1	25 – > 100
C. glabrata (7)	AMP	0.1 – 12.5		1	3					1	2				0.2 – 50
	C	0.78 – 50					2	1		2			2		1.56 – >100
	F	0.1 – 25		2				1	2	1		1			0.39 – 100
	K	0.05 – 12.5	2		1	2					2				0.2 – 50
	N	6.25 – 100								2	1		1	3	12.5 – >100
C. parapsilosis (3)	AMP	0.1 – 12.5		1			1				1				0.39 – 25
	C	1.56 – 12.5						1		1	1				3.13 – 50
	F	0.2 – 12.5			2						1				0.39 – 25
	K	1.56 – 12.5						1	1		1				3.13 – 100
	N	3.13 – 25							1		1	1			12.5 – 100
C. tropicalis (3)	AMP	0.78 – 3.13					1		2						1.56 – 12.5
	C	1.56 – 25						1			1	1			3.13 – 50
	F	0.2 – 6.25			1					2					0.78 – 12.5
	K	0.2 – 12.5			1						2				0.39 – 25
	N	6.25 – 50								1	1		1		25 – 100
Candida sp. (1)	AMP	0.39				1									1.56
	C	3.13							1						12.5
	F	0.2			1										0.39
	K	6.25								1					25
	N	12.5									1				50
Total No. of isolates	AMP= Amphotericin-B		2	5	11	14	16	17	20	22	25				
	C= Clotrimazole					3	5	12	13	17	20	21	25		
	F= Fluconazole			2	9	11	11	13	17	21	24	25			
	K= Ketoconazole		2	3	8	10	11	12	15	17	24	25			
	N= Nystatin								1	5	12	17	20	24	

Discussion:

The presence of fungal hyphae in clinical specimens means colonization or infection. The practical problem in a patient with candiduria is to distinguish between colonization and/or contamination and infection and between lower urinary tract colonization and upper urinary tract infection . Therefore, it is important to determine whether renal infection is present or whether infection is confined to the bladder. Mycological findings are usually inconclusive which makes the clinical parameters important [7,20]. Because these organisms commonly colonize skin and mucous membranes, isolating them in culture specimens from these sites is not a proof of invasion [21]. Some authors consider that 1000 CFU/ ml constitutes a significant level of infection [22], while others have established a much higher limits , $>10^5$ CFU/ml [23]. Odds [21] referred to values of 10^4 CFU/ml as indicative of Candiduria. According to Michigan [22] the growth of yeast in urine cultures that have been collected in sterile conditions should always be considered of pathological significance, independent of the number of colonies, particularly because no correlation has been found between the degree of infection caused by yeasts and the site or severity of infection. However, in the present study, all the cases selected have symptoms of UTI, with pyuria and high candidal colony count , with midstream urine samples containing $1x\ 10^3$ - $\geq$ $1x\ 10^5$ CFU (Fig.1-3) and this could mean that the patient has cystis or pyelonephritis. These cases have been differentiated from vaginal candidiasis patients whose urine samples containing *Candida* structures plus large number of epithelial cells with or without mucus threads and pus cells and usually observed in firstly voided urine samples (Fig.4), or from those urine samples of patients with indwelling urinary catheters (Fig.5). Moreover most of the candiduria cases (38/43) examined showed significant pyuria(usually supports the diagnosis of infection) with the absence of the bacterial pathogens and the absence of antibiotic treatment are suggestive of urinary *Candida* infections, and systemic antifungals should be given . Cases of candiduria without significant number of *Candida* cells and without pyuria were not included. In contrast, clinically significant renal candidiasis has been reported even with low colony counts of 1000 cfu/ml of urine(21). The incidence of asymptomatic candiduria is higher in women than in men, which is probably due to vaginal colonization [24,25]. Pregnancy also increases the rate of colonization, but again this may be related to vaginal colonization [26,27] and again such cases were excluded in this study. However, the presence of *Candida* species in urine samples presents the physician with a challenge as to whether the candiduria represents colonization or, lower or upper urinary tract infection including ascending pyelonephritis and renal candidiasis with sepsis. [8,9,21,28].

The most important risk factors for developing a significant candiduria are the presence of a urinary catheter, antimicrobial therapy, age, and diabetes [3.4,10,24,25,29,30] . In the present study, diabetes mellitus was the most common underling disease seen in 35% of the patients studied. It was the most common underlying disease seen in other studies of candiduria [3,8,23,24]. Other predisposing factors were antibiotics (11.6%), renal surgery, renal stones, prostatic hypertrophy and malignancy each constituted (9.3%), while only 7% had no underling diseases (Table 3).

Considering antifungal susceptibility testing, and according to the interpretative criteria for resistance published by Sutton *et al*. [31] who considered the following breakpoints: $\geq$ 2 µg/ml for AMP-B; $\geq$ 16 µg/ml for miconazole. ; $\geq$16 µg/ml for ketoconazole and for fluconazole: $\geq$µg/ml ; the antifungal susceptibility testing showed that AMP-B, fluconazole 32 and ketoconazole revealed a very good activity against all the tested strains of the *Candida* spp. (Table 4), in concomitant with other studies [(32,33] . However , the toxicological side effect of AMP-B limits its use in debilitated patients [34].

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تواجد المبيضات في الإدرار ومرض ابيضاض المجاري البولية في البصرة / العراق

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الخلاصة

من مجموع 2000 مريض تواجدت خميرة المبيضات في الإدرار كان 43 مريض (20 ذكراً و23 انثى) اظهروا تواجد الخميرة باعداد ملفته للنظر في الإدرار (ما يقارب 1×10^3 – $\leq 1\times10^5$ وحدة تكوين مستعمرة / مل) مع تبول قيحي. هذه الحالات المرضية تم متابعتها وأعيد جمع نماذج من وسط تيار البول حيث تم اعادة فحصها مجهريا وزرعت على وسط السابروید دكستروز اكارالمضاف اليه المضاد البكتيري الكلورامافينيكول. كما سجلت الامراض المرافقة وجنس المرضى واعمارهم. خمسة انواع من المبيضات قد تم عزلها وتصنيفها وكان النوع *C. albicans* مسبباً 51% من مجموع الحالات المفحوصة , تلاه النوع *C.glabrata* (26%) و النوعان *C.parapsilosis* و *C.tropicalis* كل منهما كون 9.3% .اما النوع *Candida* sp. فظهر في 4.65% من مجموع الحالات المرضية المدروسة . خمس حالات مرضية ارتبط تواجد الخميرة في بولهم مع بعض الممرضات البكتيرية (حالتين مع بكتريا القولون *Escherichia coli* وحالتين مع *Pseudomonas aeruginosa* وفي حالة واحدة مع المكورات المسبحية *Streptococcus viridans*). كان مرض التبول السكري العامل المؤثر الرئيسي في ظهور الحالات المدروسة (35%) , تلاه مرضى متناولي المضادات الحيوية (11.6%) ومرضى حصى الكلى , مرضى جراحة الكلى , مرضى السرطان والمرضى مستعملو انبوب التبول كل منهم شكل نسبة 9.3% من مجموع الحالات المرضية المدروسة . كانت المضادات الفطرية :Amphotericin-B (AMP-B) و Fluconazole و Ketonazole الاكثر فعالية تجاه انواع خميرة الـ *Candida* , اذ اظهر المضاد AMP-B مدى تثبيطي واسع قدره 80% (20\25) من العزلات المختبرة تحت التركيز 3.13% مايكروغرام \مل وثبط 100% من العزلات تحت التركيز 12.5 مايكرو غرام \مل. اظهر كل من المضادين الفطريين Fluconazole و Ketonazole نسبة تثبيط 100% تحت تركيز 25 مايكرو غرام / مل , بينما المضاد Clotrimazole ثبط جميع العزلات تحت تركيز 50 مايكرو غرام / مل. المضاد الحيوي الفطري Nystatim اظهر نسبة تثبيط بلغت 80% تحت تركيز 50 مايكرو غرام / مل. كانت نتائج التركيز القاتل الفطري الأدنى (MFC) أما مساويا لنتائج التركيز المثبط الادنى (MIC) او زادت عليه بضعفين الى اربعة اضعاف او اكثر.