

Isolation and Identification of *Candida albicans* in different clinical samples

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Abstract

The prevalence of candidacies caused by *Candida albicans* has increased years ago. The samples (Vaginal swabs; urethral swabs; urine; and ear swabs) were cultured on Sabouraud's Dextrose Agar and CHROM agarTM *Candida* to detect *Candida albicans* in patients clinically suffered from candidiasis.

Six hundred and five clinical samples were collected from patients whose ages ranged from (20 – 69) years. All *Candida albicans* isolates were identified by using microscopic examination in wet smear and stained smear; in addition to germ tube test and API *Candida*. The highest rate of *Candida albicans* infections were 73.3% in pregnant women in age group (20 – 43) years; 43.7 % in men of (30 – 39) years old and (33.3) % in men and (37.9) % in female with urinary tract infection. Susceptibility testing of *Candida albicans* to six antifungal drugs was carried out by disc diffusion method; They were Amphotericin B, Nystatin, Fluconazole, Ketoconazole, Cotrimazol and Itraconazole. Resistant *Candida albicans* isolates resistance were found against Fluconazole (45.45) %; Ketoconazole and Itraconazole (13.6) % respectively. 100% of susceptibility was observed for Cotrimazol, Amphotericin B and Nystatin.

Keywords: Candidiasis, anti-fungi, Chrom medium agar

عزل وتشخيص المبيضات من نماذج سريرية مختلفة

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المخلص

أن أنتشار داء المبيضات الذي تسببه المبيضات قد أزداد منذ سنوات. في هذه الدراسة تم زرع العينات (مسحات المهبل ، مسحات الإحليل ، البول ، ومسحات الأذن) على الوسط الزرعي دكستروز سابوراود أكار ووسط كروم أكار المبيضات للكشف عن تواجد المبيضات في المرضى الذين يعانون سريرياً من داء المبيضات.

جمعت وطبقت الدراسة على ستمائتوخمس عينات سريرية من مرضى تراوحت أعمارهم بين (20 - 69) سنة. تم التعرف على جميع عزلات المبيضات باستخدام الفحص المجهرى في المسحة الرطبة والمسحات المصبوغة ؛ بالإضافة إلى اختبار الأنثوب الجرثومي و API Candida. كان أعلى معدل لعدوى المبيضات (73.3) % في النساء الحوامل في الفئة العمرية (20 - 43) سنة ؛ (43.7) % من الرجال بعمر (30-39) سنة و (33.3) % في الرجال و (37.9) % في الإناث المصابات بعدوى المسالك البولية. تم إجراء اختبار حساسية المبيضات إلى ستة مضادات للفطريات من خلال طريقة انتشار القرص. كانوا أمفوتيريسين B، نيساتين، فلوكونازول ، كيتوكونازول ، كوترمازول و أيتراكونازول. تم العثور على مقاومة لعزلات المبيضات ضد فلوكونازول (45.45) % . كيتوكونازول و أيتراكونازول (13.6) % على التوالي. في حين لوحظ (100) % من الحساسية لكوترمازول ، أمفوتيريسين B ونيساتين.

الكلمات المفتاحية: المبيضات ، مضادات للفطريات ، وسط كروم أكار المبيضات

1.Introduction

Candidiasis one type of fungal infections caused by *Candida albicans*. It is normally present in the gastrointestinal tract; genital tract on mucous membranes and skin without causing infection; but it can cause infection in human .Most candidiasis is mild; however, some patients can suffer from severe infections especially in diabetes,cancer,immuno-compromise, transplant recipients and pregnant women .Clinical samples can be examined under light microscope and cultured on special culture media especially Sabouraud's Dextrose agar and chromogenic media for rapid identification of *Candida albicans* [1,2].

The treatment is an antifungal drug like Amphotericin B or Fluconazole for systemic infections and urinary tract infection; Nystatin for oral and esophageal candidiasis and Clotrimazole for skin and genital tract infections [3]. Otomycosis is fungal infection of the external auditory canal. The incidence of otomycosis is more common in cities with hot climates. The prevalence rate of *Candida albicans* has been (10) % of patients with otitis. [4]. *Candida albicans* rarely lead to death [5].

The presence of *Candida albicans* in urinary tract is called candiduria which is more prevalent among the hospitalized patients and in both sexes (males and females). Many physicians do not describe the systemic antifungal drugs for candiduria, while symptomatic candiduria may result from invasion of the blood with candidemia [6].

Because *Candida albicans* spread spreads in all ages and gender of human and is resistant to most anti – fungal drugs;So, the aims of study are:

1. Isolation and identification of *Candida albicans* in pregnant women with vulvovaginitis.

2. Determination of *Candida albicans* distribution among patients with otitis; urinary tract infection; and urethritis.
3. Testing anti- fungal sensitivity among *Candida aalbicans* isolates.

2. Materials and Methods

Six hundreds and five (605) samples were collected from patients attended to AL-Yarmouk Teaching Hospital/Baghdad, during the period from January /2016 to November /2017. The ages of the patients under study ranged from 20 to 69 years [Table 1].

Table 1: The number of clinical samples and sex under study

Clinical samples	Female	Male	Total
Vaginal swabs	105	-----	105
Urethral swabs	-----	100	100
Urine samples	100	100	200
Ear swabs	110	90	200
Total	315	290	605

2.1. Detection and Identification of *Candida albicans* in Vaginal swabs

One hundred and five vaginal swabs were collected from pregnant women age group between (20 - 43) year. Two high vaginal swabs were taken from each woman. Microscopical examination for direct examination was done under the light microscope by using Gram stain for the first high vaginal swab. Also wet smear was done to detect *Candida albicans*. The second high vaginal swab was cultured on the Sabouraud's dextrose agar (Oxoid) with the addition of 0.05 g/L chloramphenicol [11] and CHROMagar™ *Candida* (Rambach™, Paris, France) [12]. Then all plates were incubated at 30C° and 37C° for 24 – 48 hr.

After incubation, the isolated colonies were inoculated in tubes containing 0.5 ml human serum, and the tubes were incubated at 37C° for (2 – 3)hr. Germ tube formation was examined by using light microscope [13].

2.2. Detection and Identification of *Candida albicans* in Urethral swabs

One hundred urethral swabs were taken from patients suffered from urethritis and increasing in urethral discharge. All urethral swabs were directly examined under light microscope (wet smear); stained with Gram stain [14], and cultured on Sabouraud's dextrose agar (Oxoid) with the addition of 0.05 g/L chloramphenicol [11] ; Nutrient agar (Oxoid); and CHROMagar™ *Candida* (Rambach™, Paris, France) [12]. Then all plates were incubated at

30C° and 37C° for 24 – 48 hr. After incubation, Germ tube formation was examined by using light microscope [13].

2.3. Detection and Identification of *Candida albicans* in urine samples

Two hundred of midstream urine samples were collected from patients, clinically suffering from severe urinary tract infection. They were one hundred urine samples from female and one hundred urine samples from male. All samples were examined directly under light microscope by centrifuged the urine at 3000 r.p.m. for 5 min. and the deposits examined for the presence of *Candida* [15]. After that, slide smears from each deposit were fixed and stained with Gram stain, and from each sample that had ≥ 5 pus cells/high power field (H.P.F.) with or without of *Candida*, one loop full cultured on Sabouraud's dextrose agar (Oxoid) with the addition of 0.05 g/L chloramphenicol [11]; Nutrient agar (Oxoid); and CHROMagar™ *Candida* (Rambach™, Paris, France) [12]. Then all plates were incubated at 30C° and 37C° for 24 – 48 hr. After incubation, Germ tube formation was examined by using light microscope [13].

2.4. Detection and Identification of *Candida albicans* in ear swabs samples

Two hundred ear swabs (110 females; 90 male) were collected from patients clinically suffered from otitis externa with heavy discharge and pus. Two swabs were collected from each patient. To detect and diagnose *Candida albicans* in swab, Enoz *etal.* reference [16] was followed, and as following:

One swab was used for direct microscopic examination (Gram stain) and the other swab was used for culture examination (Inoculated on surface of Sabouraud's dextrose agar (Oxoid) with the addition of 0.05 g/L chloramphenicol [11]; Nutrient agar (Oxoid); and CHROMagar™ *Candida* (Rambach™, Paris, France) [12]. Then all plates were incubated at 30C° and 37C° for 24 – 48 hr. After incubation, Germ tube formation was examined by using light microscope [13]. The age of the patients ranged from 20 to 65 years.

All *Candida albicans* Isolates were diagnosed by using API® - *Candida* system (BioMerieux ® SA) for biochemical tests.

2.5. Antifungal resistance of *Candida albicans*

Antifungal susceptibility test was performed on all *Candida albicans* isolated from clinical samples using disc diffusion method. Antifungal discs used were 6.5 mm in diameter disc (HiMedia Laboratories, India antibiotic discs) of: Amphotericin-B (AP) 100Unit; Nystatin

(NS) 100Unit; Fluconazole (FLC) 25µg; Ketoconazole (KT) 10µg; Clotrimazole (CC) 10µg and Itraconazole (IT) 10µg. Few colonies of *Candida albicans* isolates (Subcultured on Sabouraud Dextrose Agar at 37 C° for 24 hr) were mixed with sterile (0.85)% NaCl₂. The turbidity adjusted to match a 0.5 MacFarland turbidity standard and then diluted 1:2 with sterile saline solution. Muller Hinton agar (Oxoid) surface was inoculated by streaking with moistened cotton swab, and then allowed to dry for 15 min. Antifungal discs were fixed on the surface of Muller Hinton agar with sterile forceps. All plates were incubated at 37 C° for 24 hr. The inhibition zones were measured in (mm) by using a ruler. The results were compared with table 2. The statistical analysis were done by using Windpipe computer program version 11.65, Pearson's chi-square, to express the probability.

Table 2: Disc diffusion diameter as susceptible, intermediate and resistant according to manufactures instruction

Antifungal agents	Sensitivity (mm)	Intermediate (mm)	Resistant (mm)
Amphotericin-B	> 10	≤ 10	≤ 10
Nystatin	> 10	≤ 10	≤ 10
Fluconazole	≥ 30	10 – 29	≤ 18
Ketoconazole	= 20	10 – 19	< 10
Clotrimazole	= 20	10 – 19	< 10
Itraconazole	= 20	10 – 19	< 10

mm = Millimeter

3.Results and Discussion

The shape of *Candida* under light microscope after staining with Gram stain was Gram positive and oval shape. Microscopic examination of wet smear revealed pseudo – hyphae and budding cells. *Candida albicans* on Sabouraud's Dextrose Agar showed white yellowish creamy colonies and green colour on CHROMagar™ *Candida* after 24 – 48 hr at 37 C° of incubation. Germ tube formation after incubation with human serum was seen in positive samples. The biochemical tests in API *Candida* were positive for sucrose; maltose; glucose and galactose fermentation except Lactose, it which gave negative result. Our results were compatible with other researches [7,8,9 and 10].

The results revealed that the rate of infection in pregnant women (in age ranged from 20 to 43 years) with *Candida albicans* was (73.3) % [Table 3].

Table 3: *Candida albicans* infection in pregnant women

Positive cases (%)	Negative cases (%)	Total (%)
77 (73.3)	28 (26.7)	105 (100)

Table 4: *Candida albicans* infection in pregnant women according to their age

Age group (year)	Positive no. case (%)	Negative no. case (%)	Total (%)	P value
20 – 29	32 (41.55)	12 (42.85)	44 (41.9)	2.0×10^{-5}
30 – 39	45 (58.4)	15 (53.57)	60 (57.1)	4.3×10^{-8}
40 – 49	0 (0)	1 (3.57)	1 (0.95)	0.157
Total (%)	77 (100)	28 (100)	105 (100)	1.3×10^{-11}

The results in table 3 agree with many researchers. Yousif and Hussien found that the percentage of *Candida albicans* infection in 23 pregnant women of 30 years old was (100) %. They attended Baghdad Teaching Hospital in between 2010 [17]. In another study, in Baghdad Teaching Hospital in 2011, danash [18] found that *Candida albicans* infection in 38 pregnant women (18 – 50) years old were (13.4) % and they suffered from abnormal vaginal discharge.

Table 4 shows *Candida albicans* infection in pregnant women according to age groups. The results show that the rate of *Candida albicans* infection in pregnant women whose ages ranged from 30 to 39 years were 45 cases (58.4) %, followed by age group (20 – 29) years old, 32 cases (41.6) %. While the women whose ages ≥ 40 years had not been infected with *Candida albicans*. Our results agree with study of Taher and Ahmed, 2017 [18], who found that the percentage of *Candida albicans* isolates from 100 vaginal swabs of recurrent vulvovaginal candidiasis infected women were (39.6) % with age range (15 – 50) years. In another study, Alwan and Aziz detected *Candida albicans* in vaginal swabs and urine samples in women who attended AL-Ulwyia Hospital. They were (43.7) % and (7.6) % respectively [19].

Table 5 shows *Candida albicans* infection in male with urethritis in ages between 20 to 69 years old. The results show that the rate of infection with *Candida albicans* was (16) %. In table 6 the highest rate of *Candida albicans* infection was in men aged between (30 – 39) years; and it was (43.7) %.

Our results related with *Candida* urethritis in men are agreement with Sabri and Salih results [20]. They found that the highest rate of *Candida albicans* infection in age group (20 – 39) years old; it was (16.6) % but there was no infection in men of (40 – 49) years. Our finding is in agreement with results of many studies in which the researchers reported that *C. albicans* was detected in prostatic fluid samples and urethral catheter of one chronic prostatitis patient [21],

and from urine sample in Iraqi patients with prostatic enlargement; *Candida albicans* isolates represented 6.2% [22].

Table 5: *Candida albicans* infection in patients with urethritis

Positive cases (%)	Negative cases (%)	Total (%)
16 (16)	84 (84)	100 (100)

Table 6: Frequency of *Candida albicans* infection according to the age in patients with urethritis

Age group (year)	Positive cases (%)	Negative cases (%)	Total (%)	P value
20 – 29	5 (31.25)	21(25)	26(26)	9.1×10^{-6}
30 – 39	7 (43.75)	27(32.1)	34(34)	1.2×10^{-6}
40 – 49	2(12.5)	25(29.76)	27(27)	3.9×10^{-10}
50 – 59	2 (12.5)	10(11.9)	12(12)	1.0×10^{-3}
60 – 69	0 (0)	1(1.2)	1(1)	0.157
Total (%)	16 (100)	84(100)	100(100)	6.8×10^{-22}

Of the two hundreds ear swabs obtained from patients clinically diagnosed as cases of otitis. The results showed that (32.8) % of females and (67.1) % of males were infected with *Candida albicans* [Table 7].

The highest percentage of infection in female age group between(30 – 39) years old was (45.5) % while the highest percentage of infection was (37.8) % in male age group between(40 – 49) years [Table 8].However, our results do not agree with results of Khammas *et al.* [23] who found that 75 patients (44 female and 31 male), with otomycosis gave positive culture for *Candida* in (82.2) % female and (17.78) % male, and the highest age group infected with *Candida albicans* in female between (16 – 30) years was (39.6) % while in male in the same range of age it was (6.7) %. In another study [24], in Baghdad city, the highest infection with *Candida albicans* in 9 males in age group (11 – 20) years old, it was (34.61) %, and in 5 females in age group (11 – 20) years, it was (50) %. *Candida albicans* isolated from one female (15 years old) suffered from chronic suppurative otitis media attended to Hilla Teaching Hospital [25], while in Ramadi city [26], the percentage of *Candida albicans* in patients with otitis was (4.6) %.

Table 7: *Candida albicans* infection in patients with otitis

Sex	Positive cases (%)	Negative cases (%)	Total (%)	p value
Female	22 (32.8)	88 (66.17)	110 (55)	7.8×10^{-6}
Male	45 (67.16)	45 (33.8)	90 (45)	
Total	67 (100)	133 (100)	200 (100)	

Table 8: Distribution of *Candida albicans* in patients with otitis according to age group

Age group (year)	Positive no. cases Male (%)	Positive no. cases Female (%)	Negative no. cases Male (%)	Negative no. cases Female (%)	Total (%) (Male)	Total (%) (Femal)	p value (Male)	p value (Female)
20 – 29	9 (20)	6(27.27)	16(35.55)	17(19.3)	25(27.77)	23(20.9)	0.048	1.1×10^{-3}
30 – 39	10(22.2)	10(45.5)	7(15.6)	20(22.7)	17(18.9)	30(27.2)	0.303	9.8×10^{-3}
40 – 49	17(37.8)	3(13.6)	7(15.6)	35(39.77)	24(26.7)	38(34.5)	3.9×10^{-3}	2.1×10^{-13}
50 – 59	6(13.3)	2(9.1)	10(22.2)	9(10.2)	16(17.77)	11(10)	0.157	2.8×10^{-3}
60 - 69	3(6.7)	1(4.5)	5(11.1)	7(7.95)	8(8.9)	8(7.27)	0.317	2.7×10^{-3}
Total (%)	45(100)	22(100)	45(100)	88(100)	90(100)	110(100)	1.0	5.6×10^{-19}

Out of 200 patients with urinary tract infection (UTI), 100 females and 100 males were studied and diagnosed by direct microscopic examination and culture of urine samples on special fungal culture media. In wet smear of urine and stained smear of urine deposits, pus cells; epithelial cells and yeast cells were showed in [Table 9]. All samples that gave ≥ 5 pus cells / H.P.F. and yeast cells, gave positive culture of *Candida albicans* on Sabouraud's Dextrose Agar and CHROMagar™ *Candida*; (45) % for male and (55) % for female. Among all the cases under study, the age group range from 50 to 59 years old were the highest age group infected with *Candida albicans* and gave positive culture results on Sabouraud's Dextrose Agar and CHROMagar™ *Candida*; they were (33.3) % for male and (37.9) % for female [Table 10].

Table 9: results of general urine examination under higher power field (40 X) of light microscope

Sex	Positive no. cases containing yeast cells and ≥ 5 pus cells (%)	Negative no. cases containing yeast cells and ≥ 5 pus cells (%)	Total (%)	p value
Male	54 (45)	46 (57.5)	100 (50)	0.083
Female	66 (55)	34 (42.5)	100 (50)	
Total	120 (100)	80 (100)	200 (100)	

Table 10: Distribution of *Candida albicans* in patients with UTI diagnosed on fungal culture media

Age group (year)	Positive no. cases Male (%)	Positive no. cases Female (%)	Negative no. cases Male (%)	Negative no. cases Female (%)	Total (%) (Male)	Total (%) (Female)	p value (Male)	p value (Female)
20 - 29	5(9.25)	7 (10.6)	12(26.1)	12 (35.3)	17 (17)	19 (19)	0.016	0.105
30 – 39	11 (20.3)	13 (19.7)	18 (39.1)	9 (26.4)	29 (29)	22 (22)	0.066	0.228
40 – 49	7 (12.96)	9 (13.6)	10 (21.7)	7 (20.6)	17 (17)	16 (16)	0.303	0.480
50 – 59	18 (33.3)	25 (37.9)	3 (6.5)	2 (5.9)	21 (21)	27 (27)	3.7×10^{-6}	3.9×10^{-10}
60 – 69	13 (24.1)	12 (18.18)	3 (6.5)	4 (11.76)	16 (16)	16 (16)	4.1×10^{-4}	4.7×10^{-3}
Total (%)	54 (100)	66 (100)	46 (100)	34 (100)	100 (100)	100 (100)	0.258	6.0×10^{-6}

These results were also found by Al- Jammaly and Abdulla [27]. They reported that out of 202 examined high cervical swabs and urine samples in women (aged between 16 – 45years old) with genitourinary tract infections, (10.8) %were cultured positive for *Candida albicans*. The results of our studies related to candiduria are in agreement with AL – Duboon, 2010 [28].

The results revealed that the highest percentage of infection with *Candida albicans* found in age group (55 – 65) years old, and constituted (51) % of the examined urine samples. The most infection occurred in females rather than males.

4. Percentage of antifungal susceptible of *Candida albicans*

Antifungal susceptibility results of (200) *Candida albicans* isolates in both males and females are showed in Table11. All isolates under study were (100) % susceptible for Amphotericin-B (AP), Nystatin (NS) and Clotrimazole (CC). The highest resistant was for Fluconazole (FLC); it was (45.45) % for *Candida albicans* isolated from ear swabs, followed by Ketoconazole (KT) and Itraconazole (IT) (13.6%,13.6 %) respectively.

Table 11: Percentage of antifungal susceptibility of *Candida albicans* isolates

Sample	Sex	No. Of positive samples	AP S (%)	AP R (%)	NS S (%)	NS R (%)	FLC S (%)	FLC R (%)	KT S (%)	KT R (%)	CC S (%)	CC R (%)	IT S (%)	IT R (%)
Urethral swab	M	16	100	0	100	0	81.25	18.7	93.7	6.25	100	0	87.5	12.5
Vaginal swab	F	77	100	0	100	0	81.8	18.2	84.4	6.4	100	0	96.1	3.9
Urine sample	M	45	100	0	100	0	95.6	4.4	95.6	4.4	100	0	95.6	4.4
Urine sample	F	22	100	0	100	0	90.1	9.1	96.3	3.6	100	0	96.3	3.6
Ear swab	M	45	100	0	100	0	86.7	13.3	88.9	11.1	100	0	88.9	11.1
Ear swab	F	22	100	0	100	0	54.5	45.5	86.3	13.6	100	0	86.3	13.6

M = Male, F = Female, S = Sensitive, R = Resistant

The results of antibiotics depend on the measuring of inhibition diameter around the anti – fungal discs. The results of our study agreed with the results of Jabir *et al.* in Al-Habboobi Hospital - Thi-Qar [29]. They revealed that Fluconazole had no activity against *Candida albicans* isolates at concentrations (5, 20 and 100) mg/ml. Also, our results agreed with other studies [24]. They found that *Candida albicans* isolates from urine samples were less sensitive to Clotrimazole, while high sensitivity appeared against to Nystatin and Econazole. Others have established that Amphotericin-B; Fluconazole and Ketoconazole were high active against *Candida albicans* isolates isolated from patients with candiduria in Basrah [28].

According to AL-Maliki and AL-Ani results [30] finding *Candida albicans* isolates were resistant to Fluconazole (21.62) %; Ketoconazole (8.1) % and Econazole (5.4) %, but no resistance was observed to Clotrimazole. Al-Rubayeet *et al.* isolated *Candida albicans* from women with Vaginal Candidiasis in Kut Province [31], and found that the activity of antifungal agents against *Candida albicans* isolates were sensitive to Amphotericin B; Ketoconazole; Fluconazole and Nystatin.

Mohamed and AL. Thwani revealed that *Candida albicans* were sensitive to Ketoconazole and Clotrimazole and resistant to Nystatin. The highest incidence of *Candida albicans* was among female sofoage group between (19-39) years who attended AL-Zahra hospital in Baghdad [32].

References

- [1] Goncalves B, Ferreira C, Alves CT, Henriques M, Azeredo J, Silva S. Vulvovaginal candidiasis: Epidemiology, microbiology and risk factors. *Critical reviews in microbiology*, Vol. (42):905-27. (2016).
- [2] Nadeem S Gh , Hakim Sh T and Kazmi Sh Uj .Use of CHROMagar Candida for the presumptive identification of *Candida* species directly from clinical specimens in resource-limited settings. *Libyan J Med*, Vol. (5) :10. (2015).
- [3] Pappas PG, Kauffman CA, Andes DR, et al. Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*, Vol. (62): e1-50. (2016).
- [4] Herasym K, Bonaparte JP, Kilty S; A comparison of Locacorten-Vioform and clotrimazole in otomycosis: A systematic review and one-way meta-analysis. *Laryngoscope*. Vol. (126), No. (6):1411-9. (2016).
- [5] Elayoubi F, Lachkar A, Aabach A, Chouai M, Ghailan MR. Malignant otitis externa caused by *Candida albicans*. *Pan Afr Med J*, Vol.(22) , No.24:322.(2016).
- [6] Zida A, Bamba S, Yacouba A, Ouedraogo-Traore R, Guiguemdé RT. Anti-*Candida albicans* natural products, sources of new antifungal drugs: A review. *J Mycol Med*. Vol. (27) No. (1):1-19. (2017).
- [7] Baker F. J. and Breach M. R. *Medical Microbiology* Bulterworths, London, Boston. Sydney. Wellington. Durban, Toronto, (1980).
- [8] Brook G. F., Buted J. S. and Morse S. A. , Jawetz McInick and Adelberg's *Medical Microbiology* , 23^{ed} – Lang Medical book , Mograw Hill New York. (2004).
- [9] Saigal S., Bhargava A., Mehra S. K., and Dakwala F. Identification of *Candida albicans* by using different culture medias and its association in potentially malignant and malignant lesions. *Contemp Clin Dent*. 2011 Jul-Sep; 2(3): 188–193.
- [10] Nadeem S. Gh., Hakim SH.T., and Kazmi Sh. U. Use of CHROMagar *Candida* for the presumptive identification of *Candida* species directly from clinical specimens in resource-limited settings. *Libyan J Med*.5: 3402/ljm. v5i0.2144. (2010).
- [11] Koneman, E.M. and Roberts, G.D. (1985). *Practical Laboratory Mycology*. Third ed. London, Williams and Wilkins., pp:27-30.
- [12] Nadeem S. Gh., Hakim Sh. T., and Kazmi Sh. U. Use of CHROMagar *Candida* for the presumptive identification of *Candida* species directly from clinical specimens in resource-limited settings. *Libyan J Med*. 2010; vol.5: 10.

- [13] Mattei AS, Alves SH, Severo CB, Guazzelli Lda S, Oliveira Fde M, Severo LC. Determination of germ tube, phospholipase, and proteinase production by bloodstream isolates of *Candida albicans*. Rev Soc Bras Med Trop. 2013; vol.46, No. (3):340-2.
- [14] Dabke KK, Deodhar II, Gogate AA. Microbiological study of urethritis in males with special reference to *Candida urethritis*. JPGM, (1987), Vol. 33, Issue: 4, Page: 198-200.
- [15] Al-Oebady M.A. H. Isolation and identification of *Candida* species from vaginal, urine and oral swabs by chromagar *Candida*. International Journal of Advanced Research (2015), Vol. 3, Issue 1: 948-956.
- [16] EnozI M.; SevincII I.; Lapeña J. Bacterial and fungal organisms in otitis externa patients without fungal infection risk factors in Erzurum, Turkey. Brazilian Journal of Otorhinolaryngology Braz. j. otorhinolaryngol. vol.75, no.5. (2009).
- [17] Yousif E. E. and Hussien S. A. *Candidaalbicans* vulvovaginitis in pregnant. J. Fac. Med. Baghdad, vol. (52), No. (2): 183. (2010).
- [18] Taher Sh. and Ahmed L. Detection of *Candidaalbicans* responsible for vulvovaginitis women. AL – Kindy College Medical Journal, Vol. (13), No. (1): 82. (2017).
- [19] Alwan M. J. and Aziz M. M. Isolation and identification of *Candida* species from urogenital infections of women in Baghdad city. Proceeding of the Eleventh veterinary scientific conference: 115-119. (2012).
- [20] Sabri M. and Salih M. Investigation of some ethological agent associated with male urethritis. Medical J. Babylon, Vol. (4), No. (1 – 2): 49. (2007).
- [21] Vaidyanathan S., Soni B., Hughes P., Ramage G., Sherry L., Singh G., and Mansour P. *Candidaalbicans* Fungaemia following Traumatic Urethral Catheterisation in a Paraplegic Patient with Diabetes Mellitus and Candiduria Treated by Caspofungin. Case Rep Infect Dis.pp: 693480. (2013).
- [22] Khalil Z K. Uropathogenic Infections among Patients with Diabetes in Baghdad City. Iraqi Journal of Science, Vol. 57, No.1B, pp: 360-366. (2016).
- [23] Khamas A.H., Abbas A. K., Ilabis S. and AL – Najjar A. Isolation and Identification of fungi associated with otomycosis. Iraq J. Comm. Med. Vol. (3): 186. (2010).
- [24] Abdulshaheed D. A. Isolation and diagnosis of some fungal types from external ear canal infection cases with sensitivity test to some antifungal agents. The Iraqi Journal of Veterinary Medicine, Vol. (33) ,No. (1) :82 – 88.(9002).
- [25] Hassan S. H. Identifying bacterial etiology and patterns of drug sensitivity to inflammation of the middle ear, chronic exudative when the kids in the city of Hilla. Journal of Al-Kufa University for Biology, Volume: 4 Issue: 2 Pages: 46-53 27. (2012).
- [26] AL - Juboori A. N. and AL – Ouqaili M. Microbiology aspects of chronic suppurative otitis media in Ramadi city. AL – Anbar medical Journal, Vol. (5), No. (1):49. (2005).

- [27] AL- Jammaly M. M. and Abdulla B A. Prevalence of microorganisms in genitourinary tract infections of females from Mousl city. Tikrit Journal of Pure Science, Vol. (13), No. (1):46 – 51. (2008).
- [28] AL – Duboon A. Candiduria and urinary Candidiasis in Basrah, Iraq. J. Basrah Researchers, Vol. (36), No. (1): 9. (2010).
- [29] Jabir H. B., Abbas F. N. and Khalaf R. M. In vitro assessment of antifungal potential of apple cider vinegar and acetic acid versus fluconazole in clinical isolates of otomycosis. Thi - Qar Medical Journal (TQMJ): Vol (5) No. (1): 126 – 133. (2011).
- [30] AL-Maliki R S and Zouhair I, AL-Ani. Antifungal resistance of Candida species isolated from Iraqi women infected with vulvovaginal Candidiasis. QMJ, Vol. 7, No.11:117. (2011).
- [31] Al-Rubaye N. I., Al-Hamadani A. H. and AL- Hilaly H. Detection of causative agents, antifungal susceptibility profile and cross-resistance of *Candida albicans* isolated from oral and Vaginal Candidiasis. QMJ, Vol. 6 No.9: 197. (2010).
- [32] Mohamed B. J., AL. Thwani A. The susceptibility test of vaginal yeasts and their relationship with the age in Iraqi women. Baghdad Science Journal Vol.7, No. (3): 1174. (2010).