



ISSN: 0067-2904

## Isolation and Identification *Candida* spp from Urine and Antifungal Susceptibility Test

Karwan Ismael Othman\*, Shorish Mustafa Abdullah, Belan Ali, Mjda Majid

Biology department, Faculty of Science, Soran University, Soran- Erbil, Iraq

### Abstract

*Candida* spp. are of medical importance because they are the most common opportunistic mycosis worldwide, a common cause of nosocomial urinary tract infections (UTIs), oral candidiasis and genitourinary candidiasis. The development of *Candida* spp infection depends on several factors such as age, sex, and immunity of the host-pathogen relationship. They are resistance to one or more antifungal. The purpose of these studies to isolation and identification of *Candida* spp from urine sample and investigation of susceptibility of these strains to Amphotericin B and Fluconazole. There are 105 Urine sample of the renal failure were collected using a sterile urinary cap, different diagnostic techniques were used for characterization of *Candida* spp Culture characteristic, gram stain, Germ tube, CHROM agar *Candida* and scanning electronic microscopic. Results showed 40% urine sample positive also Different *Candida* spp isolated, *C. albicans* (20%), *C. parapsilosis* (20%), *C. glabrata* (32.72%), and *C. krusei* (27.27%). The antibiotic susceptibility test of the isolates was determined by the Kirby-Bauer disk diffusion method. *Candida* spp. sensitivity to 6mg/L Amphotericin B but Fluconazole was ineffective against *Candida* spp. The most common pathogen in Urinary tract system is *Candida* spp. both *Albicans* and non-*Albicans* are unusual causes of urinary tract infections in healthy individuals also *Candida* spp resistance to Fluconazole but sensitivity to low concentration Amphotericin B.

**Keywords:** Urinary tract infection; *Candida* spp.; Fluconazole; Amphotericin B

## عزل وتشخيص الفطر *Candida* spp من الإدرار واختبار حساسيته للمضادات الفطرية

كاروان اسماعيل عثمان\*، شورش مصطفى عبدالله، بيلان علي، مزده مجيد

قسم الأحياء، كلية العلوم، جامعة سوران، اربيل، العراق

### الخلاصة

يعد الفطر مهم طبيًا لأنه أكثر الأمراض انتشارًا في العالم، وهو يسبب التهاب المسالك البولية في (*Candida* spp المستشفيات) وأكثرها شيوعًا هو داء المبيضات الفموي وداء المبيضات التناسلي UTIs يعتمد تطور عدوى المبيضات على عدة عوامل منها العمر والجنس ومناعة العلاقة بين العائل الممرض. فهي مقاومة لواحد أو أكثر من مضادات الفطريات. الغرض من هذه الدراسة لعزل وتشخيص *Candida* spp من عينة البول والتأكد في قابلية هذه السلالات إلى Amphotericin B و Fluconazole. تم جمع 105 عينة البول من الفشل الكلوي باستخدام غطاء البول المعقمة، استخدمت تقنيات التشخيص المختلفة لتوصيف *Candida* spp. الأوساط المفرقة مميزة، Germ tube، Gram stain، CHROM agar *Candida*، المجهر المسح الضوئي الإلكتروني. أظهرت النتائج 40% عينة بول موجبة أيضًا. *C. albicans* (20%)، *C. parapsilosis* (20%)، *C. glabrata* (32.72%)، *C. krusei* (27.27%).

\*Email: Karwan.othman@soran.edu.iq

تم تحديد اختبار حساسية المضادات الحيوية من العزلات بواسطة طريقة نشر القرص. حساسية *Candida* spp. إلى Amphotericin B 6 mg / L لكن Fluconazole كان غير فعال ضد *Candida albicans* and *Candida* spp. الممرض الأكثر شيوعاً في جهاز المسالك البولية هو *Candida* spp. كلا من *Candida non-albicans* هي أسباب غير عادية لالتهابات المسالك البولية لدى الأفراد الأصحاء وأيضاً *Candida* spp. مقاومة إلى Fluconazole لكن حساسية للتركيز المنخفضة للـ Amphotericin B.

## 1. Introduction

*Candida* species member of the normal flora of an individual's from the gastrointestinal tract, vagina, oral cavity, skin and mucosal surfaces of human [1]. *Candida* spp are micro biota in healthy humans during in immune compromised situations can cause human infection, The genus *Candida* includes more than 100 different species, however, only a few of them can infection human [1, 2, 3].

The last decade has seen *Candida* spp are continuously medical importance, they could be opportunistic can causes life to threaten systemic infections and chronic mucocutaneous infection in immune compromised patients[1,4]. *Candida* species are present human fungal pathogens can cause genitourinary candidiasis which involves vulvovaginal candidiasis in female and balanoposthitis and balanitis in male, oral candidiasis, and the digestive tract and candiduria in both genders[1-5]. Has several virulence factors causes to increase pathogenicities such as Adherence to various tissues and inanimate surfaces, Biofilm formation, Phenotypic switching, Dimorphism, Production of hydrolytic enzymes [6].

The infections mostly occur in lower urinary tract bladder and urethra [7]. Candiduria is the present of *Candida* spp in urine [8], Vulvovaginal candidiasis is the second most frequent infection of the female genital tract [9]. The most common pathogen in nosocomial UTI are *Candida* spp including those *C. albicans*, *C. glabrata* and *C. tropicalis* respectively [6], also *C. albicans* is the most prevalent species that counted of all *Candida* spp [4, 10]. Females were more infection by *Candida* spp than males [11]. Have the different class of antifungal each of them affects utilizes inhibit growth or kill different fungal pathogens [10]. Antifungal agents are a major part of the treatment for UTI, they are limited but increasing number of antibiotics can be used to treat mycotic infections [2, 9]. The classes of currently available drugs belongs group polyn and Azole; they are due to their actions on the fungal cell membrane, whereas echinocandins act by disrupting the fungal cell wall [12, 13].

Recently recognized worldwide an increase in the number of *Candida* spp that are resistant to antifungal drugs, *C. tropicalis* and *C. parapsilosis* are both generally susceptible to azoles. *C. glabrata* and *C. krusei* are intrinsically more resistant to antifungal agents, particularly to Fluconazole [14].

## 2. Materials and method:

### 2.1. Sample collection

A total of 105 urine sample of the renal failure were collected from both Birth hospital and Ashty hospital in Soran city, belonging to different age groups of females ranging from 15 to 65 ages, using sterile urine cap all specimen was transported immediately to the laboratory and culturing within the 2h the collection.

### 2.2. Isolation *Candida* spp.

Urine Samples were cultured on Sabourauds dextrose agar plates, containing 0.5 mg per 1000ml chloramphenicol and incubated at 37 °C and examined for its growth at 24, 48 and 72h. The culture plates were examined for the appearance, size, color and morphology of the colonies.

### 2.3. Identification

#### 2.3.1 Macroscopically identification *Candida* spp.

The colonies were studied for their morphological characters such as size, color, and morphology of the colonies and production of hyphae.

#### 2.3.2. Microscopically identification of *Candida* spp.

Gram stain to identified *Candida* spp and use lacto phenol cotton blue to stain *Candida* finally examined under 40 x and 100 x with oil emersion.

#### 2.3.3. Germ tube test for identification of *Candida albicans*

Colony inoculums of suspected *Candida* cultures was inoculated into 0.5 ml of human serum in a test tube and incubated at 37 °C for 2-4 hours. After incubation, a loop-full of culture was placed on a glass slide, overlaid with a cover-slip and examined microscopically for the presence or absence of germ-

tubes. Formation of germ tubes was seen as long tube like projections extending from the yeast cells with no constriction or septa at the point of attachment to the yeast cells. The germ tube is indicative of *C. albicans*.

#### 2.3.3.1. CHROM agar *Candida*

Chromo agar *Candida* media a chromogenic medium used for isolation and identification of *Candida* sp. After mixing material distilled water was added, Heat, to boiling, to dissolve the medium completely. After cooling media to 50°C or 54°C poured to sterilized plates, colony appearance of *Candida* spp on CHROM agar *Candida* depend on colony color.

#### 2.3.4. Scanning Electron Microscope:

Using SEM to identified *Candida* spp, samples need to be dehydrated and coated with a gold film under vacuum in an argon atmosphere using a sputter coater according

#### 2.4.1 Antifungal stock solution

##### Serial dilution for Amphotericin B and Fluconazole:

Antifungal were weighted and dissolved in dimethyl sulphoxide. After that prepared serial dilution to Amphotericin B concentration from 0. 1mg/L to 255 mg/L also prepared serial dilution to Fluconazole from 0.21 µg/ml to 2.5 µg/ml

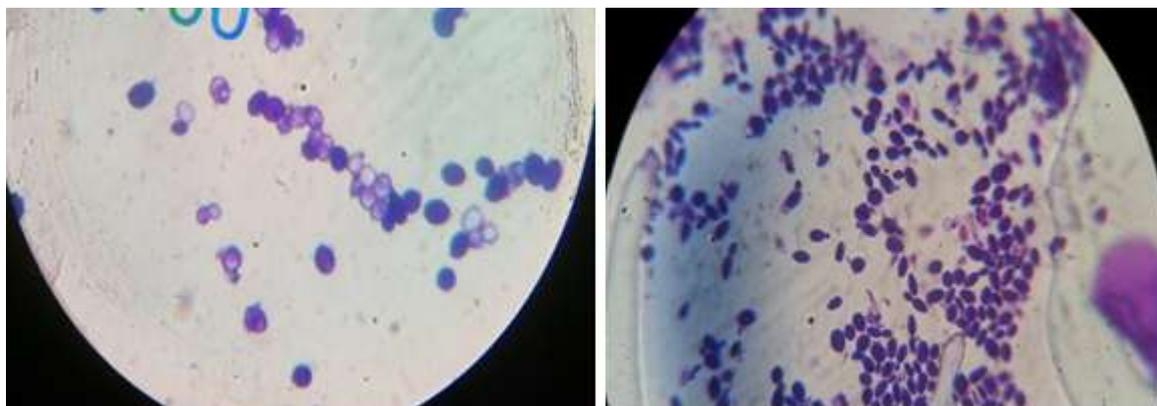
##### Disk Diffusion Assay

The *Candida* spp suspension prepared above was inoculated Into the entire surface of a plate media (inoculation *Candida* sp 24- to 48-h growth at 35°C were suspended in sterile 0.85% saline and then adjusted spectrophotometrically to produce a 0.5 McFarland standard density giving a  $1 \times 10^6$  to  $5 \times 10^6$ -CFU/ml suspension). Sterile paper disks impregnated with the antifungal solution from different diluted was placed on the surface of each plate agar a sterile pair of forceps. The plates were incubated aerobically at 37°C for 24 – 48 h. The diameter of inhibition zone was measured after 24 -48 h incubation using a ruler.

### 3. Result and Discussion:

The sample urine cultured on the SDA (40%) was positive *Candida* sp and (60%) sample urine cultured on the SDA was negative. By culture characteristics on the SDA *Candida albicans* white colored, smooth, and yeast-like in appearance, *C. parapsilosis* colonies on SDA, white to creamy, shiny and smooth, *C. glabrata* colonies on SDA smooth, and cream-colored, *C. krusei* rough colonies on SDA. Microscopically simple stain and gram stain *Candida* spp budding yeast-like cells, spherical to sub spherical, and Hyphae or pseudohyphae Figure-1. Using germ tube test method all *Candida albicans* was positive while *Candida glabrata*, *Candida krusei* and *C. parapsilosis* isolated were negative Figure-2. After culturing samples on Chromo agar *Candida* which is selective media for *Candida* spp and according to the color of colonies identifying the species of *Candida*. *C. albicans* green color, *Candida glabrata*, Pink-purple, *C. parapsilosis* White to pale pink colonies, *C. krusei* pink color. Scanning electron microscope shows *Candida* spp (Figures-3 and 4) show different shapes depend on species of *Candida*.

The present study explained that the fungal isolates include *albicans* and non-*albicans*. *Candida* with the variable percentage also some urine sample isolated more than one species .*C. albicans*: 11 out of 42 (20%), *C. parapsilosis*: 11 out of 42 (20%), *C. glabrata*:18 out of 42(32.72%), *C. krusei*15 out of 42(27.27%) Figure-5.



**Figure 1-** *Candida* spp yeast like cell and budding 40X



Figure 2- Germ tube *Candida albicans* 40X

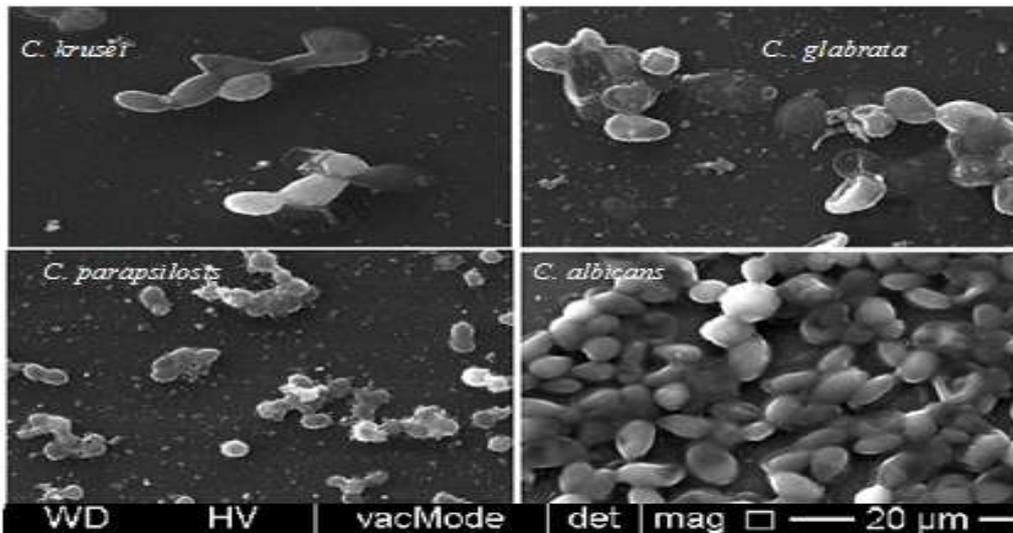


Figure 3- Scanning electron microscope *Candida* spp

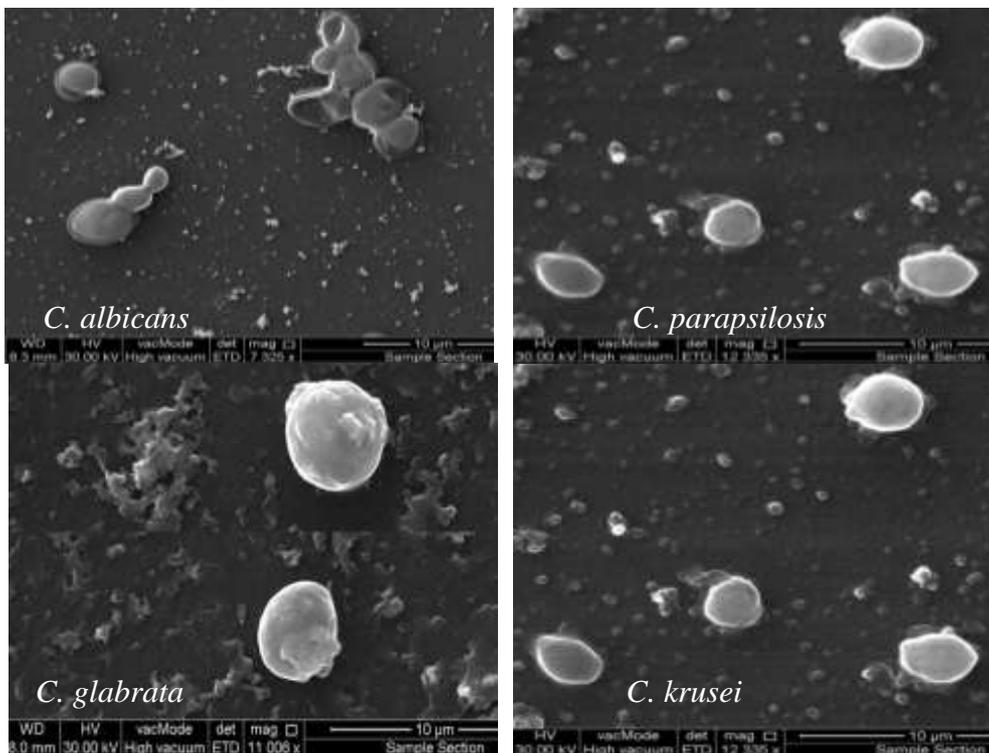
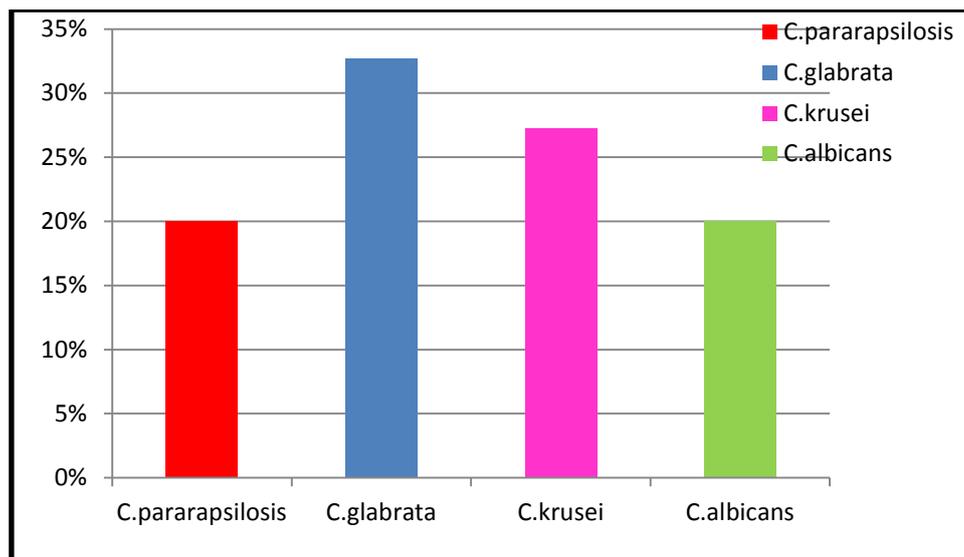


Figure 4- Scanning electron microscope *Candida* spp



**Figure 5-** Variable percentage of *Candida* spp. Isolated from urine

The data presented in Table-1 and Figure- 6 revealed that Amphotericin B was the most active drug against the majority of *Candia* spp tested Amphotericin B were using different concentration by disc diffusion method between 0.1 and 255 mg /L, all isolated *Candida* spp from Urine the inhibition zone to lower concentration between 8 to 15mm but *Candida albicans* more sensitivity to Amphotericin B, in lower concentration compare to another species Figure- 6. But from 7.8 mg/L best concentration to affect on *Candida* spp was isolated from urine the inhibition zone by this concentration 21 mm also all *Candida* spp.

On the other hand, the highest number of resistant *Candida* spp was observed Fluconazole Figure-7 and Table- 2 All *Candida* spp were isolated from urine resistance to Fluconazole were used different concentration between 0.21 to 2.5 µg/ml the inhibition zone to a different concentration between 6 to 9 mm Figure-7 and Table -2 .

**Table 1-**Sensitivity of *Candida* species to Amphotericin B and Zone of activity in mm

Amphotericin B mg/L	<i>C .albicans</i> Inhibition zone mm	<i>C. pararapsilosis</i> Inhibition zone mm	<i>C. glabrata</i> Inhibition zone mm	<i>C .krusei</i> Inhibition zone mm
0.1	9	8	9	9
1	15	13	12	12
3	15.5	14	12	11
6	19	14	13	11
7.8	21	20	20	21
15.7	23	20	21	19
31.5	23	25	25	24
63	24	28	25	23
127	24	28	25	22
255	24	28	30	24

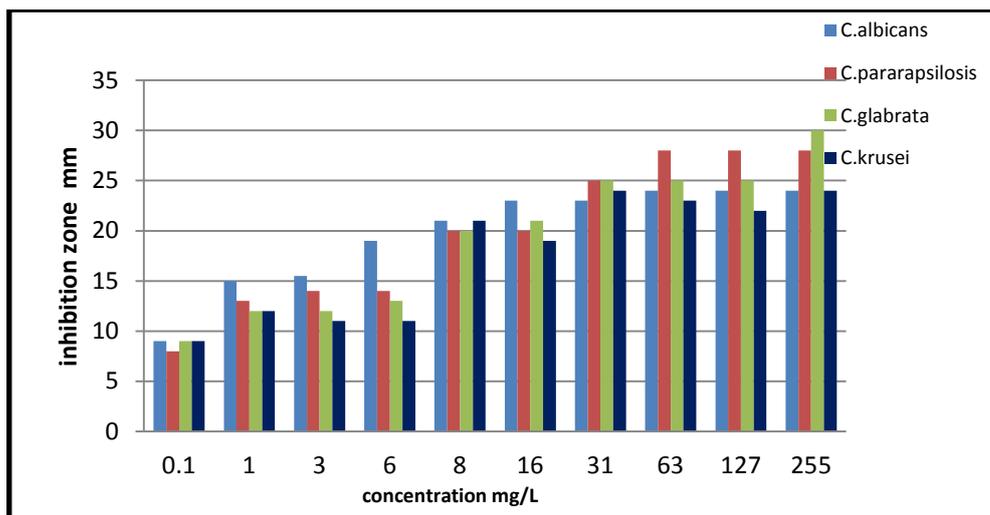


Figure 6- Sensitivity of *Candida* species to Amphotericin B and Zone of activity in mm

Table 2- Sensitivity of *Candida* species to Fluconazole and Zone of activity in mm

Fluconazole $\mu\text{g/ml}$	<i>C. albicans</i> Inhibition zone mm	<i>C. parapsilosis</i> Inhibition zone mm	<i>C. glabrata</i> Inhibition zone mm	<i>C. krusei</i> Inhibition zone mm
0.21	8	7	8	6
0.42	8	8	8	6
0.84	8	8	8	8
1	8	8	8	8
1.5	9	8	8	8
2	9	8	9	8
2.5	9	9	9	8

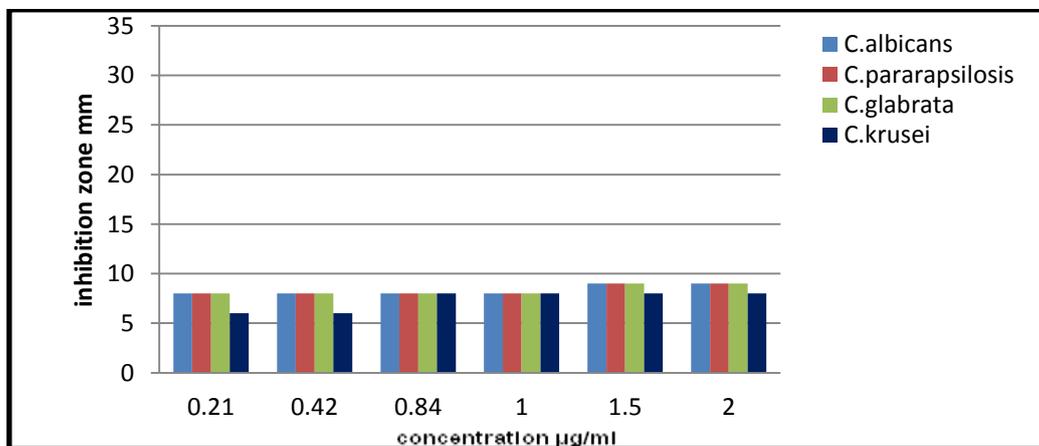


Figure 7- Sensitivity of *Candida* species to Fluconazole B and Zone of activity in mm

The data presented in Table-1 and Figure-6 revealed that Amphotericin B was the most active drug against the majority of *Candida* spp tested. Amphotericin B was used at different concentrations by the disc diffusion method between 0.1 and 255 mg/L. All isolated *Candida* spp from urine showed an inhibition zone that increased with concentration, ranging from 8 to 15 mm at lower concentrations, but *Candida albicans* showed more sensitivity to Amphotericin B at lower concentrations compared to other species in Figure-6. However, from 7.8 mg/L, the best concentration to affect *Candida* spp isolated from urine was 21 mm, and all *Candida* spp were affected.

On the other hand, the highest number of resistant *Candida* spp was observed with Fluconazole in Figure-7 and Table-2. All *Candida* spp isolated from urine were resistant to Fluconazole at different concentrations between 0.21 and 2.5  $\mu\text{g/ml}$ . The inhibition zone varied between 6 and 9 mm in Figure-7 and Table-2.

*Candida* sp causes of urinary tract infections in both female and male [7]. Genital regions from female infected by *Candida* spp are called vulvovaginitis [15]. Our study revealed that out of 105 urine samples 40 % samples were positive for the presence of *Candida* spp. They are major human pathogen [16], *Candida* species in the urine in any concentration reflected renal involvement [5, 6]. Most Scientists are referring to *Candida* species causes urinary tract infections [8]. *Candida* spp was isolated from urine and the main isolate were *C. glabrata* followed by *C. kursii*, *C. albicans* and *C. parapsilosis*, The prevalence of candiduria caused by different species *Candida* [10, 11, 17], which include *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, *C. guilliermondii*, and *C. lusitanae* [4], both *albicans* and non *albicans* ability infection urinary system [3].

However were using different antifungal to detect susceptibility of *Candida* sp including Amphotericin B and Fluconazole, Amphotericin B is a polyene drug, it is decreasing B-1, 3-glucan cause reduces cell wall stability of fungi [13]. Cell death by Amphotericin B due to increased membrane permeability it is bound to sterols and causes pore formation in the fungal cell membrane [18, 19]. It has a broad spectrum of activity [7]. In the resulted disc diffusion assay for Amphotericin B showing strong effect inhibiting *Candida* spp using concentration as low as 0.1mg/L the zone of inhibition was 9 mm to all *Candida* 6 mg/L concentration the inhibition zone was 19 mm to *C. albicans* but 7.8 mg/L to all *Candida* 20 to 21 mm inhibition zone as shown in Figure-5. Amphotericin B was effective against on *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida tropicalis* and *Candida lusitanae* [2]. Isolated *Candida parapsilosis*, *Candida albicans*,

*Candida glabrata*, *Candida krusei* and *Candida famata* from catheters, urine and vaginal swabs, further more all isolated were susceptible to Amphotericin B [17].

Fluconazole drugs affect the production of ergosterol by preventing convert lanosterol to ergosterol [18], inhibit synthesis of fungal sterols and inhibit cell membrane formation [12]. Fluconazole was used widespread use as treat infects by *Candida* [20]. Moreover Species of *Candida* which resist to azole drugs [21]. The resulted about Fluconazole by disc diffusion with different concentration, this antifungal has a little or no effect on *Candida* spp, the zone of inhibition was 9 mm by high concentration which is presented in Figure-7. *C. glabrata* and *C. krusei* are intrinsically more resistant to Fluconazole [22, 23]. *Candida* spp decrease susceptibility to azole compounds [2]. Fluconazole, a fungistatic drugs little or no efficacy against *Candida* spp [14].

More *Candida* spp isolate was susceptible to Amphotericin B and maximum resistance to Fluconazole as compare to other antifungal agents [2, 24]. *Candida* spp are resistant to Fluconazole [14, 22].

#### 4. Conclusion

The most common pathogen in Urinary tract system was *Candida* spp both *albicans* and non-*albicans*, this study has shown non-*albicans* a major cause of candiduria. *C. glabrata* is the most common species in the tested patients.

All the isolates *Candida* spp were susceptible to Amphotericin B, but Fluconazole was ineffective against *Candida* spp.

Isolation and Identification *Candida* spp from urine and with their antifungal susceptibility test can help treating candiduria.

#### 5. Acknowledgment

I would like to pay special thankfulness, warmth and appreciation to the Shadi Kh my research successful and assisted me to identified *Candida* spp.

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