

## A study of anti-Fungal Resistance Strains of *Candida Albicans* Yeast Which Isolated from Clinical Specimens of Women Suffering from Vaginal Infections in Baqubah City

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### ABSTRACT

vaginal swabs were collected from 40 ladies suffering from vaginal infections, their ages ranged between 20-50 years, attending the consulting clinic at Al-Batool Teaching Hospital in Baqubah for the period from December /2020 to February /2021, for married women. The samples that gave a positive test result for *Candida albicans* infection were 20 samples. The remaining 20 were negative. Of the 20 positive cases, 17 were pregnant women, their ages ranged between 20-30 years, and the non-pregnant women were 3 cases infected with *Candida albicans*, and their ages ranged between 31-50 years. Therefore, the infection rate of *Candida albicans* for the sample group was 80%, of which 59.4% were infected with *C. albicans*. Positive samples taken from pregnant women and 40.6% of samples taken from non-pregnant women. By using the Kirby-Bauer standard disk diffusion method, it was possible to assess the sensitivity and resistance of five *C. albicans* isolates to various antifungals. *C. albicans* had the highest level of resistance to various antifungals. Its resistance to Metroconazol (MZ), Fluconazol (FLU), and Ketoconazol (KCT) was all above 90%, but Amphotericin B (AMB) and Caspofungin (CAS) resistance rates were significantly lower at 25% and 45%, respectively. Antibiotic susceptibility testing of *C. albicans* for this study revealed that 12/20 (or 60%) of the isolates were multidrug resistant (MDR).

**Key words:** Azols, albicans, resistance, sensitive

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### INTRODUCTION

Bloodstream infections are often caused either by key pathogenic organism *Candida* (BSIs). In research conducted in the US and Europe over the past 20 years, candidaemia has been listed as the fourth and seventh most frequent healthcare-associated BSI, respectively. One of the most frequent fungi that infect people are candida infections, which can affect the body's mucosa, skin, nails, and internal organs. In patients with impaired immune systems, *Candida* infections are a prevalent opportunistic illness. Mayer, et al. The incidence of candidiasis has been rising over the past ten years as a result of contemporary medical procedures, with fatality rates ranging from 40 to 50% [1].

In U.S. hospitals, *Candida albicans* is now the third most prevalent nosocomial pathogen. Adults commonly get vulvovaginal candidiasis; 70–75 percent of women encounter this infection at least once in their lifetime. VVC patients may experience vaginal discharge, which is typically described as white, creamy, curd-like (like cottage cheese), and adherent to the epithelium. However, the discharge could also be thin and loose, comparable to that seen in other vaginitis types [2].

Since candidiasis normally has a vaginal pH of 4 to 4.5, it can be distinguished from trichomoniasis or bacterial vaginitis, which both have elevated pH levels. *Candida albicans* is responsible for about 90% of vulvovaginal candidiasis and *Candida glabrata* for 5% of cases. Vulvovaginal candidiasis often affects pregnant women or women who use contraceptives because they contain estrogen, which raises glycogen levels, lactic acid bacteria, which breaks down glycogen, and mannoproteins, which enhances the likelihood of yeast adherence to mucous membrane [3-5]. Red ulcers with distinct edges, thick white or yellow discharges, burning, and discomfort in the infection location are all symptoms of infection. Thrush can be categorized as either uncomplicated or complicated, depending on whether the symptoms

are mild to moderate and whether *Candida albicans* is the cause. Thrush may be referred to as complicated if it coexists with pregnancy, poorly managed diabetes, an immunological deficiency, four or more episodes of thrush in a year, or when any of these conditions are present. Additionally, complicated thrush may not be caused by the *Candida albicans*. Generally, allyl amines, azoles, echinocandins, morpholines, and polyenes are used as topical or systemic antifungal medications to treat *C. albicans* infections. Genes that code for enzymes playing significant roles in the pathway for ergosterol biosynthesis are disrupted by allylamines, azoles, and morpholines [5-7]. Fungi static azoles are among the various antifungals that are available, and because of their great efficacy, low cost, and minimal toxicity, they are frequently used to treat *Candida* infections. The widespread and sustained use of azoles, particularly fluconazole (FLU), is linked to the formation of azole-resistant *Candida albicans* strains.

The study's objective was to determine which antifungals from *Candida albicans* yeasts were the most responsive and resistant in women with vaginal infections.

## MATERIAL AND METHODS

### Collection of specimens

For the purpose of isolating candida, 40 samples of high vaginal swab (H.V.S.) were obtained using cotton swap media and loop full in the clinical labs of Al-BatouAl Teaching Hospital and Baquba Teaching Hospital between December 17, 2020, and February 17, 2021.

### Direct examination

The standard procedure for examining all vaginal swab specimens was to mount them on a clean slide with a drop of KOH 10%, cover them with cover-slip, and then gently worm the slide (but not boiling). looked at under a microscope to look for growing *Candida* cells. To determine the isolates' response to stain, morphologies, arrangements, and yeast budding form, Gram stain was used to stain the samples [8-10].

### Identification and Isolation of *Candida albicans* Isolates

The specimens were struck directly on (SDAM) Sabouraud Dextrose Agar Medium and incubated at 37C for 48 hours (Al-Oebady, et al.). Following this, the positive results (*Candida*) were identified based on the morphological features on culture media and germ tub formation, and we then removed attachment from the resulting growth and struck it on (CAC) Chrom Agar *Candida* and incubated at 37Co for 48 hours [11-15]. Yeast isolation was then carried out.

### Germ tub formation

By combining a little amount of an isolated colony with 0.5 ml of human serum, the ability of the yeast isolates to produce germ tubes was examined. For 2.5 hours, this suspension was incubated at 37°C.

As other yeast species may start to develop germ tubes, the incubation duration must not be longer than 3 hours. A cover slip was placed over a drop of the cultured serum and a microscope was used to check for the existence of germ tubes. In this test, serum was made by carefully aspirating blood from healthy human volunteers into vacutainer tubes without anticoagulants, incubating the samples in an upright position at room temperature for 30 minutes, followed by centrifuging the samples at 3000 rpm for 15 minutes [16-20]. The serum was then carefully transferred into sterilized tubes and defrosted at -8°C.

### Susceptibility to Antibiotics *Candida albicans*

#### Setting up yeast suspension

Colony counts from a pure culture should be transferred using a sterile swab and suspended in 3 ml of sterile saline in a transparent test tube. The turbidity was compared to the McFarland tube, which contains to  $1.5 \times 10^8$  CFU per milliliter.

#### Common Disc Diffusion Method

According to Magiorakos, et al. The susceptibility test of 7 antibiotics was conducted using the Kirby-Bauer method as a subordinate. The yeast suspension was made by selecting 4-5 isolated *Candida albicans* colonies from the native culture and putting them in a test tube with 4 ml of normal saline. This produced a fungal suspension with a mild turbidity that was comparable to the standard turbidity solution. A portion of the fungal suspension was carefully transferred and evenly dispersed over the Mueller-Hinton agar medium using a sterile cotton swab, then it was allowed to sit for 10 minutes [21-23].

The antifungal discs were then positioned on the agar and firmly pushed using a sterile forceps to ensure contact with the agar. The plates were then turned over and incubated for 18 to 24 hours at 37 degrees Celsius. With a ruler and the standard dimensions established by CLSI 2019 (Wayne, et al.) for *Candida albicans*, the zones of inhibition were measured [24].

## RESULT READING

Using a metric ruler, the diameters of the entire zone of inhibition were measured in millimeters after incubation. By comparing the diameter of each antibiotic disc's inhibition zone to the standard inhibition zone, sensitive, intermediate, and resistant groups were determined.

## RESULTS AND DISCUSSION

### Gather samples

A total of 40 clinical specimens were gathered from vaginal swabs of a married female of various ages; of these, 40 (100%) tested positive for diverse species of *Candida*, whereas 20 isolates (50%) tested positive for *Candida albicans*. In this study, 40 patients with

Table 1: Lists the 2019 CLSI quality control standards for antifungal drugs.

Antifungal	Disc Concentration µg/disc	Diameter of inhibition zone (mm)		
		Resistant	Intermediate	Sensitive
Amphotericin B	100	<9	7-Oct	>18
Ketoconazole	30	<30	31-35	>37
Metroconazol	100	<15	16-25	>32
Fluconazole	10	<27	28-38	>40
Caspofangin	50	<8	19-Sep	>25

candidiasis between the ages of 21 and 5 years were assessed. The age range was divided into three groups: 21–30, 31–40, and 41–50 years. In the 21–30 age range 5/8 (62.5%) of the 50 patients with fungal infections in that age group had positive results for *Candida albicans*, while 5/10 (50%) of the patients with candidiasis in the age group of 31 to 40 had such results, and 10/22 (50%) of the patients with candidiasis in the age group of 41 to 50 had such results. Table 1 shows that the results obtained don't agree with more recent findings. They discover that patients aged 27 to 32 have the highest infection prevalence, followed by those aged 33 to 38 and those aged 21 to 26. Only 2 of 42 individuals (4.8%) between the ages of 39 and 45 have a yeast infection [25].

#### Recognizing *Candida albicans* from a vaginal swab

*Candida albicans* was identified from a High vaginal swab based on morphological characteristics of the culture media and germ tube production. Although several species were gathered for the current investigation, the most prevalent species will truly be the focus: *Candida albicans*, which appears as oval to spherical budding cells around epithelial cells, was identified using the KOH Direct quantity method by directly examining high vaginal swab specimens using 10% KOH. Numerous budding yeasts are seen on saline and KOH microscopy, however hypha components are not present [26]. The second phase in the identification process for *Candida albicans* was cultural traits. On Sabouraud dextrose agar, *Candida* colonies had the following characteristics: white to cream color; round, curved, soft and smooth to wrinkled shape, characteristic yeast odor; they proliferated quickly in 24 hours; and they matured in 3 days. Bhavan, et al. Concur with these findings.

The third step in the identification process was to examine the microscopic characteristics of *Candida albicans* in gram stain. According to *Candida albicans* isolates were gram positive, round to oval, and had budding present Alshaikh, et al. (Figure 1).

#### *Candida albicans* identification using a Hichrom Agar medium

*Candida* colonies that developed on SDA were sub cultured on Hichrom agar media in order to identify *Candida albicans*. After an overnight incubation, the isolates grew well and produced colonies with different colors. According to the manufacturer's instructions, the colors of the colonies were observed and an assumptive identification was made of *C. albicans*, which was green, *C. tropicalis*, blue, *C. krusei*, pink colonies with a matte

surface, *C. norvegenensis*, cream to pale pink, *C. glabrata*, pale cream, and *C. famata*, a light green Figure 2. With a sensitivity and specificity of 98%, the chromogenic medium Hichrom agar *Candida* provides a quick, easy way to identify common *Candida* species. The current investigation discovered that *C. albicans* could only be identified using Hichrom agar, the form and color of the *Candida* colonies on SDA and Hichromo *Candida* agar are shown in Figure 2.

#### Test for antifungal susceptibility

Based on the diameter of the inhibition zone (mm), which is listed in the materials and method table, the antifungal susceptibility of *Candida albicans* isolates was assessed using the disk diffusion method (Kirby-Bauer) according to the requirements of the Laboratory and Clinical Standards Institute CLSI (2019). Table 2 All 20 *Candida albicans* isolates underwent this test against five antifungal drugs from three classes: Fluconazol (FLU), Ketoconazol (KCT), Metroconazol (MZ), Amphotrecin B (AMB), and Caspofungin are examples of the azole class (CAS) [27]. As shown in Table 3 and Figures 3 for isolate *C. albicans*, the results demonstrated that all *Candida albicans* clinical isolates have a high level of resistance to the antifungal drugs tested. The table of the appendix also showed the various isolates' susceptibilities to these antibiotics.

Antifungal susceptibility test results are shown in Table 3 by the percentage of resistant, moderate cases.

These findings are in agreement with Tamai, et al. Who found that while all *Candida* isolates showed high resistance to ketoconazole, fluconazole, and metroconazol, they were all susceptible to amphotericin B and caspofungin. The isolates (*Candida albicans*) were more responsive to amphotericin B, according to Ashour, et al. Also mentioned that all *Candida* isolates were amphotericin B susceptible. According to Kaur, et al. Out of the 103 *Candida* isolates, 6.7% of the isolates exhibited amphotericin B resistance, while 60 of the isolates (58.25%) exhibited fluconazole resistance (in contrast to 2.3% and 22.7% for Amphotericin B and Fluconazole, respectively, in the present investigation). Typically, Amphotericin B and caspofungin are effective against the majority of *C. albicans* strains. According to Lohse, et al. This medication has good in vitro efficacy against *C. albicans* biofilms. However, many *Candida* species, particularly *C. albicans*, now have higher rates of resistance to these antifungal medications, and the implications of this can be seen in the clinical situation. It is crucial to have antifungal medications

Table 2: Shows the specimen distribution by age group in years

Age group in years	No. total positive specimens	No. positive specimens of <i>Candida albicans</i>	Percentage %
21-30	8	5	62.50%
31-40	10	5	50%
41-50	22	10	50%
Total	40	20	

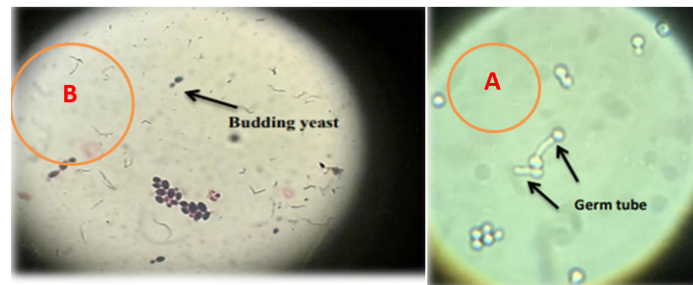


Figure 1: yeast that B-budding, Gram stain of *Candida albicans* (40X) A-germ tube development.

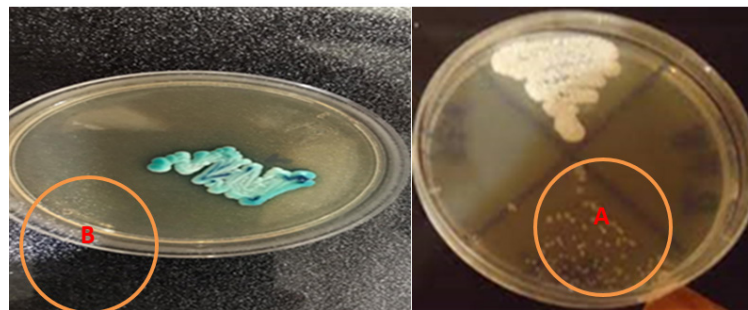
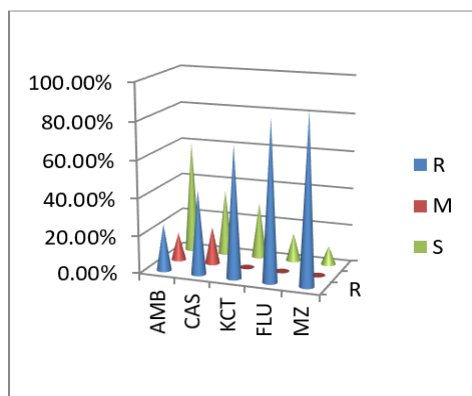


Figure 2: *Candida albicans* on SDA, part A Hichromo candida agar with B-*Candida albicans*.

Table 3: Antifungal Susceptibility

Anti-fungal	Number of <i>Candida albicans</i> (%) total No.=20		
	Resistant (R)	Moderate(M)	Sensitive(S)
Amphotricin B	5(25%)	3(15%)	12(60%)
Caspofangin	9(45%)	4(20%)	7(35%)
Ketoconazol	14(70%)	0(0%)	6(30%)
Fluconazol	17(85%)	0(0%)	3(15%)
Metroconazol	18(90%)	0(0%)	2(10%)



(R) stands for resistant (M) for moderate (S) for sensitive

Figure 3: Patterns of *Candida albicans* antifungal susceptibility.

that can treat these infections without causing greater resistance, which typically results from mutations in the pathway used for sterol biosynthesis, just like with azole

treatments like fluconazole and clotrimazole. Tasca, et al. Although this has happened when azoles antifungal medications have been used against *Candida* species. CLSI breakpoints must change along with changes in the resistance of fungi to antifungal medications [28]. The largest category of antifungal medications, azoles, have been used for many years to treat infections brought on by *Candida* species. *Candida* species have developed more azole resistance recently, both in vitro and in clinical settings.

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