Studying the *candida* resistance and sensitivity for some antifungals

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Abstract

Back ground: The current study included 150 samples from patients with *Candida* from a hospital of Medical City / education labs - Baghdad, and ranged in age from (10 – 65)years for both sexes, all isolates taken from different sources (Lower respiratory tract, Urine, Skin, Vaginal and Oral thrux), where the number of males (55)as a percentage (37%) while the number of females (95) as a percentage (63%).

The objective :The objectives of this study are studying resistance and sensitivity of *candida* spp to some antifungals.

Material and methods: All the required material which used for the study, and the direct examination was conducted using potassium hydroxide KOH 10%, As well as microscopic examination and laboratory transplantation for all samples, for the purpose of investigating the *candidiasis*. Also Vitek System examination was conducted on all positive samples for microscopy and laboratory culturing; and so to be diagnosed on the species level, and this study included using (3) anti-fungal equipped from Himedia (India), an (Fluconazole, Clotrimazole and Nystatin), anti-fungal used by standard disk Diffusion to know the resistance of *Candida* and its sensitivity toward used anti-fungal.

The results: The results of direct examination were vaginal swab (50), Sputum (28), Oral swab (34), Urine (21) and Skin swab (17).

Conclusion: *Candidiasis* was more common in female patients as well as from medical units, where the rate of infection in females more than in males, Candida species were identified by the manual and automated methods and we found that the automated method by using VITEK^2 YST Card was the best for species identification.

Keywords: Antifungal, *Candida Spp,* Resistance, Sensitive, Fluconazole, Clotrimazole , Nystatin.

Introduction

Candidiasis is a fungal infection caused by yeasts that belong to the genus *Candida*. This infections may be primary or secondary fungal infection caused by *Candida species*, And generally all this infections may be acute, Sub acute, or chronic. There are about approximately 200 different species of *Candida species* but there are over twenty species of *Candida* yeasts that can cause infection in humans. It is worth mentioning fungal pathogen found as part of the normal microflora in the human digestive tract. [1, 2]

The most common of which is *Candida albicans* and then followed by other species such as *C.glabrata, C.krusei, C.norvegenesis, C.kefyr, C.parapsilosis, C. metapsilosis, C. orthopsilosis, C.famata, C.sphaerica, C.guilliermondii* and others. [3, 4, 5].

All humans are colonized with *Candida* species, mostly *Candida albicans yeast*s which usually found and that naturally colonizes the in the intestinal or gastrointestinal tract and can be found on mucous membranes, skin and reproductive tract without causing infection; however, overgrowth of these organisms can cause symptoms to develop. Symptoms of *candidiasis* vary depending on the area of the body that is infected. Therefore we all carry this organism on our skin, in our mouth, in our gastrointestinal tract (gut), and, in the case of women, in the vagina. [1, 2, 5, 6, 7, 8].

Candidiasis may also infect the blood stream of human or internal organs such as the spleen or liver. In addition *candidiasis* is the most commonly identified *Candida* species in clinical contexts and is one of the leading causes of hospital-acquired infections. [6, 9, 10].

In recent years, the number of cases of fungal infections has increased. This is due to an increase in the number of diseases that weaken the immune system, and according to recent statistics fungi cause more than billion skin infections, One hundred [100] million mucosal infections, 10 million serious allergies and more than a million deaths each year. Global mortality owing to fungal infections is greater than for breast cancer, malaria, HIV and tuberculosis. [11, 12, 13].

During the last decades was noticed that the extravagant advances in modern therapeutic technologies has not only prolonged the life of critically ill patients but also has led to an enormous increase of fungal infections, This recent upsurge in fungal infections has resulted in high morbidity and mortality rate in the infected patients, and because of the various fungal infections and their spread, drugs and antifungal have

been used to test infections. However, there has been a virulence factors in a used *candida species* resistance to antifungal which used and the failure of some antifungal to inhibition of growth in culture media.

Therefore, fungal infections that are resistant to treatment are a public health challenge. We all should have a role in preventing these infections and reducing antifungal resistance. [2, 14, 15, 16, 17].

The present study is directed to use of some antifungals fuconazole, nystatine and cotrimazole to determine the sensitivity of these species and resistance.

Materials and methods

Samples collection:

The swaps of vaginal and skin, sample of urine, sputum and lower respiratory were collected from 150 patients (10 – 65) years old of both gender suspected have infection with *Candidiasis*, (as clinically identified by a physician), during the period of November/2017 to March/2018 from skin and respiratory diseases specialized center, Ministry of health, Baghdad Province. The samples were examined directly under the microscope using 10% KOH and culturing on the sabouraud dextrose agar [18].

Samples Culturing

Samples were cultured on sabouraud dextrose agar (SDA) supplemented with 0.05 mg/ml chloramphenicol to inhibit the growth of bacteria, then incubated at 25°C and 37 °C for 10 days [19].

Identification of Isolate Using direct microscopic examination [KOH]

Microscopic examination was conducted using potassium hydroxide (KOH) 10%, according to the method that mentioned [18].

Development and diagnose isolates

The isolates were cultured and diagnosis as stated in [20] for examination and diagnosis of the isolates.

Cultivation of samples on the media culture

Swabs were taken cultured on the sabouraud dextrose agar (SDA) in a glass dish, according to the method [18].

Diagnosis of the genus *Candida*

The special examination were Conducted for the diagnosis of *Candida* genus and their types and these examinations include the following:

- Phenotypic examination: phenotypic examination was conducted according to the method [21].
- Microscopic examination: microscopic examination was conducted according to what is stated in [19].
- Isolates development on the media of CHROMO agar: The isolates development on the media of CHROMO agar, according to [22].

- **Surface growth formation examinations**

This examination was conducted inoculating tubes containing liquid (SDA), as stated in [23].

- **Germ tube formation of *Candida albicans***

Candida albicans formed germ tube after three hours of Incubation in human serum are shown in. *Candida albicans* showed a short tube like structure, with no constriction at the attachment point. This extension represents the beginning of true hyphae formation. No germ tube was observed in Candida species other than *C. albicans.*

Diagnosis using biochemical test (Vitek apparatus)

Diagnosed by genus and species using biochemical tests by Vitek System consists of Cassette and Reagent Cards that contained [64] pits every pit represents the substrate to conduct the test, and plastic pipes as well as Densi Chek device and the unity of the input and output of information [24].

Results

The results that showed in (Table 1) showed different of distributed more than one types of sample

Table (1): Distribution of *Candida* infections in affect clinical samples

Sample	No. of isolates	Percentage%
Vaginal swab	**50**	**33**
Sputum	**28**	**19**
Oral swab	**34**	**23**
Urine	**21**	**14**
Skin swab	**17**	**11**

Microscopic Exanimation

The results of direct microscopy were shown in (Table 1) using (10% KOH) and shown in the (Figure 1, 2, 3) [140] positive samples and (93%) of the total number of samples of patients, while the number of negative samples by direct microscopy (10) samples and (7%). And the number of male positive samples [48] isolation by (34%) from total of positive isolations, while the results showed that the number of positive isolates for females (92) (66%) from the positive samples total. In the negative cases, the results of the direct examination showed that the percentage of male negative samples (7) by [70%] and female (3) by (30%) from the negative samples total.

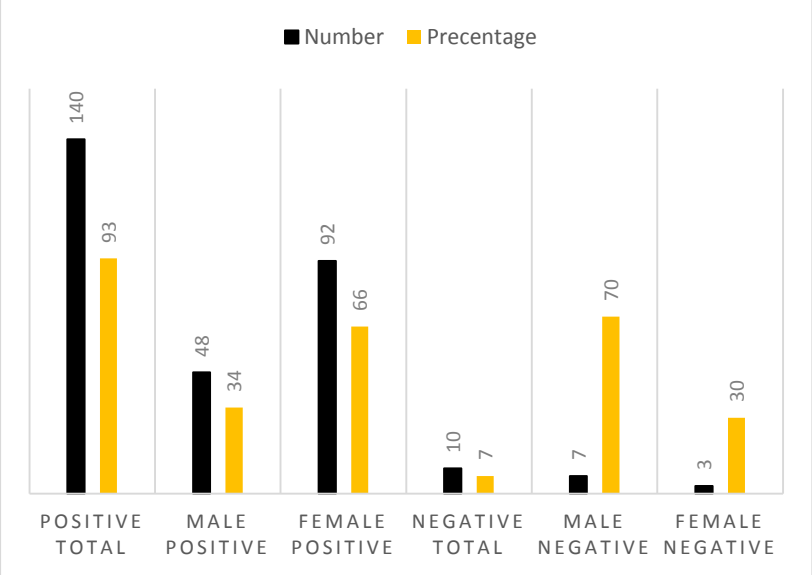
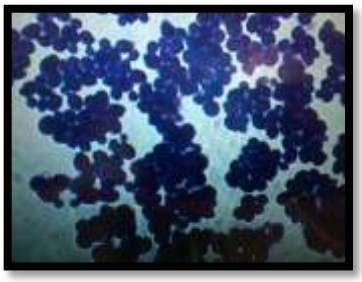
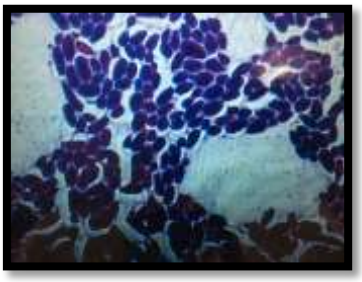


Figure (1): Results of direct microscopy using (10% KOH). Surface growth formation examinations

Figure (2): Growing *Candida* spp on Sabouraud dextrose agar after incubation for 24 hrs and at 35 ºC (±2 ºC)

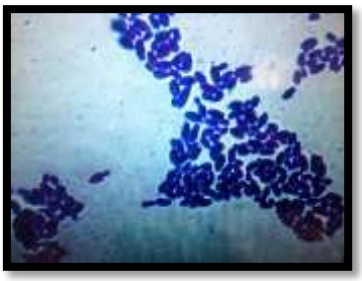


Microscopic picture of *Candida albicans* Using Gram stain

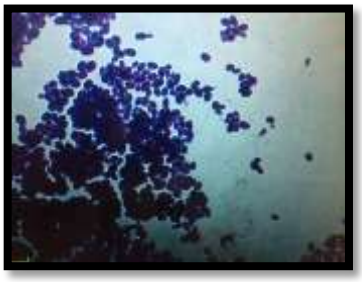


Microscopic picture of *Candida famata* Using Gram stain

Microscopic picture of *Candida glabrata* Using Gram stain



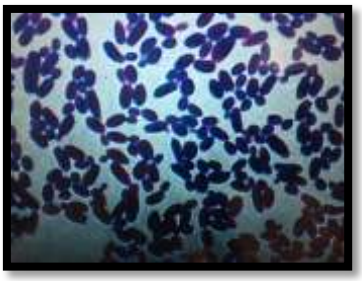
Microscopic picture of *Candida guilliermondii* Using Gram stain



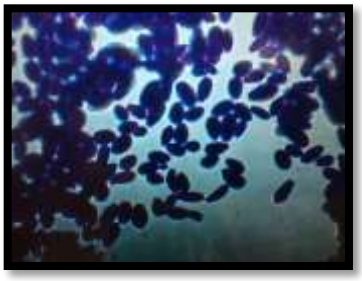
Microscopic picture of *Candida kefyr* Using Gram stain

Microscopic picture of *Candida krusei* Using Gram stain

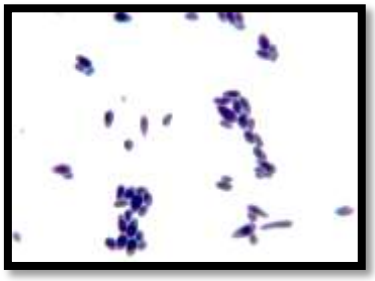
Microscopic picture of *Candida norvegenesis* Using Gram stain



Microscopic picture of *Candida parapsilosis* Using Gram stain



Microscopic picture of *Candida sphaerica* Using Gram stain



Microscopic picture of *Candida tropicalis* Using Gram stain

Figure (3): Gram staining of *Candida* species
Diagnosis by germ tube formation

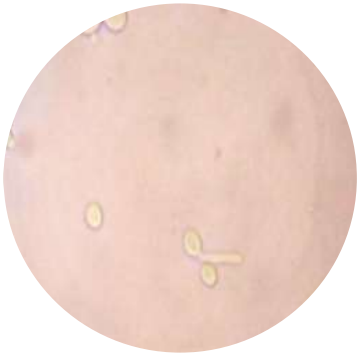


Figure (4): *Candida albicans* susceptibility to germ tube formation under microscope [40X]

Diagnosis by CHROMO agar

Figure (5): the growth of *C. albicans* on CHROMagar

Diagnosis of *candida spp* isolates using biochemical tests Diagnosis using the Vitek® apparatus

Yeast isolates were diagnosed as genus and species using biochemical tests. Positive results of the Vitek® system test showed that 115 species were diagnosed at the level of species and by (79%). The other isolates could not be read by the Vitek®. This may be due to these isolates are contaminated with other bacterial or fungal species or may be a mixture of more than one type of *candida* (Mixed infection), making it difficult to diagnose and not read by Vitek apparatus.

However, through the morphology examination, it was found that the rest of isolates (30) isolates (21%) belong to the genus *Candida albicans*, the most frequent yeast in Vitek examination is *C.albicans.* The number of isolates of this type (33) was isolated (29%) and the total number of isolates of white candidiasis was 63 (43%). This result is consistent with (3), they noted that the high frequency of *C.albicans* yeast in clinical samples compared with other species, Followed by *C. guiliermondii* (14) by (10%), *C. tropicalis* (13) (9%), C. *krusei* (12) (8%), C. *sphaeirica* (10) (7%), *C. parapsilosis & C. glabrata* (9) (6%), *kefyer* (7) (5%), *C. famata* (5) (3%) and C. norvegenesis (5) (3%). The results showed in Table (2).

Table (2): Distribution of *Candida* infections in clinical samples

Candida spp	No.
Candida albicans	63
Candida glabrata	9
Candida tropicalis	13
Candida krusei	12
Candida sphaeirica	10
Candida parapsilosis	9
Candida kefyer	7
Candida guiliermondii	14
Candida norvegenesis	3
Candida famata	5

Study effect of antibiotics on the *Candida*

A total of 145 clinical isolates of *Candida* species were tested for antibiotics susceptibility against three antifungal drugs namely, Fluconazole, Clotrimazole and Nystatin, The antibiotics susceptibility of *Candida* species was tested by disc diffusion (DD), as (Figure 5), *Candida spp* 26 (18%) percentage isolates were fluconazole, clotrimazole and nystatin resistant, and 48 (33%) were S-DD for Fluconazole, and 71 (49%) were susceptible to Floconazole, While the results show that 92 (63%) were S-DD to Clotrimazole and 27 (19%) were susceptible to Clotrimazole.

The test of Nystatin shows that 104 (72%) were S-DD for Nystatin, While 15 (10%) were susceptible to Nystatin (Table 3), these results agree with [25, 26, 27, 28, 29].

A **B**

Figure (5): Antifungal susceptibility test against *Candida* species by disk diffusion method. [A]: Resistance [B]: Sensitive

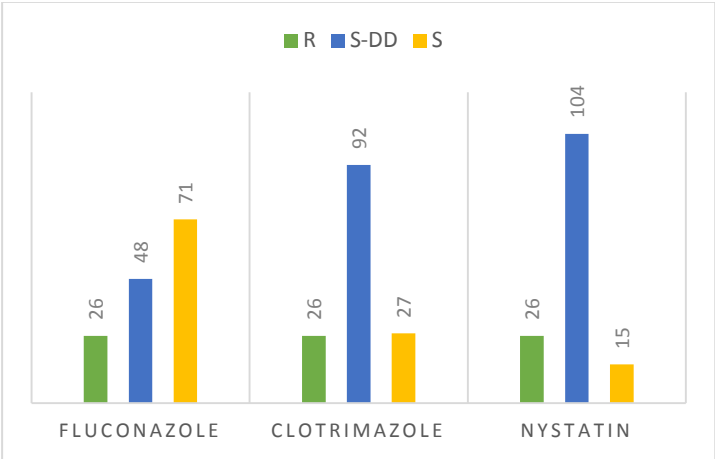


Figure (6): Antibiotics susceptibility test for *Candida spp*

Percentage of [Sensitive and resistance] *Candida* species against different antifungal agents

The results (Table 3) and (Figure 2) showed that (15) (54%) isolates of *Candida albicans* were resistant, [33] (41%) were isolated within normal growth and (13) (41%) isolates were sensitive to the three antifungal which used in this study, while was (2) (7%) resistance isolates from *Candida glabrata*, (7) (9%) were in the normal inhibition from total (9) and did not show a sensitive isolation of antibiotics.

Candida tropicalis showed (2) (7%) was resistant, (8) (10%) percentage was grown in S-DD inhibition, and no sensitive.

Candida krusei was (3) (11%) percentage are resistant, (5) (6%) percentage was grown in S-DD inhibition, and sensitive is (4) (13%) isolates.

Also the results showed *Candida parapsilosis, kefyer and guiliermondii* was (2) (7%) are resistant, while the isolates *Candida parapsilosis, kefyer* was by (4) (5%) and *Candida guiliermondii* was (9) (11%) are grown in S-DD inhibition, the isolates which was sensitive are *Candida kefyer* and *guiliermondii* in (3) by (9%).

For the isolates *Candida norvegenesis* was resistant in (1) by (4%), (2) (2%) are grown in S-DD, no sensitive. *Candida famata* showed (3) by (4%) grown in S-DD and (2) (6) was sensitive.

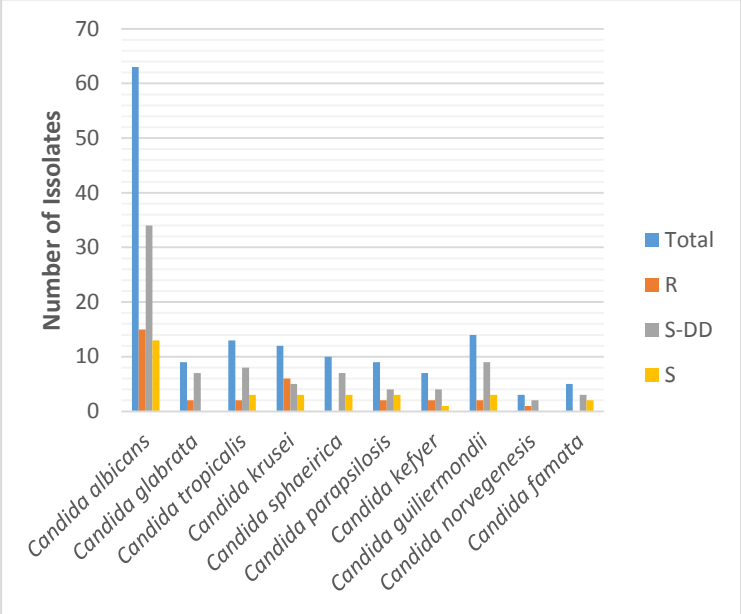


Figure (7): Types of *Candida spp* and antifungals susceptibility test for *Candida spp*

Comparison of isolates by source of collection

The samples were collected from different infectious areas of the body, taken from skin, oral thrux, lower respiratory tract, vaginal canal and urine. The inhibitory activity was tested on isolates under study. The results showed that most species isolated from the lower respiratory tract and vaginal tract were resistant to antibiotics, while the isolates from Skin, oral thrux and urine showed sensitivity to the antibiotics under study.

Comparison of isolates isolated from vagina

The results of the statistical analysis, as shown in (Table 3) showed that isolates isolated from the vaginal were effective resistance to the antifungals used comparison with criteria at (Table 3).

Table (3): Criteria of susceptibility for antifungals

Anti-F	Disk con.	[Zone diameter]mm		
		R	S-DD	S
FLU	25 µg	≤14	15-18	≥ 19
CLO	10 µg	≤11	12-19	≥ 20
NYS	100 UN	≤16	17-24	≥ 25

The presence of effective resistance for *candida spp* to fluconazole by 26 with 18% percentage against all Candida species (Table 3).

Candida albicans (8.8 ≤14) this is the rate of inhibition diameter of *Candida albicans*. While the rate of inhibition diameters of the same type against Clotrimazole (9.7 ≤11) and (12.7 ≤16) for Nystatin. Whereas the statistical analysis showed that *candida glabrata* were resistance by (13≤14), *candida guillermondii* (11≤14), and *candida parapsilosis* were (10≤14).

The following species *candida kefyer, candida krusei, candida famata* and *candida norvegensis* showed no resistance against fluconazole.

The total Nystatin group gave inhibitory effect to most of the isolates used in the study, while most showed significant physical resistance, such as *C. trpicalis, C. sphaerica, C. parapsilosis, C. guilliermonii, C. famata C.glabrata, C.parapsilosis, C.krusei, C.kefyr,* and some isolates of *C.albicans*, according to the source of the combination, also showed sensitivity when treated with the group of polyneses, but some isolates of candidiasis showed high resistance against the group of polynes, especially that candidiasis characterized by defensive mechanisms and high virulence factors in against of antifungals, and the cause of resistance to isolates taken from the Lower respiratory and vagina use to frequent use Anti-fungal or vital irregularly, leading to adapt to the pathogenic on these high doses of antifungal and consequently become able to overcome these antibiotics effect in activating the ferocity of fungus by acquiring resistance to this type of antibiotics through generations.

As for the isolates that showed their sensitivity to the antibody mentioned, the reason is that these fungi are present naturally in the areas mentioned, such as skin and oral mucus and genital canal vaginal, but it can turn into pathogen when the opportunity to seize the weakness of the immune system and others.

Comparison of isolates isolated from respiratory tract

The results showed that most of types as *Candida albicans, Candida tropicalis, krusei*, *Candida glabrata* and *Candida guillermondii* were resistance to all used antifungals in this study. While the other types as *Candida sphaerica, Candida kefyer* and *Candida parapsilosis* were sensitive and these results were similar for [30].

It was found that most of the isolates that showed resistance to the azole group (Fluoconazole and Clotrimazole) as well as the group of Polynes (Nystatin) were isolated from the lower respiratory tract. This supports the resistance of these species and their virulence. This is due to the widespread use of azoles at random without consulting a specialist, which increases the resistance of certain yeast species [31].

The randomly use of antibiotics used in the treatment of other infections in the body provides an opportunity for this disease and changes the microbiological environment, encouraging the colonization of Candida [32].

It may be caused by other chronic diseases that are responsible for reducing the immune system, creating an environment conducive to the stimulation and growth of these opportunistic organisms, and as a result they acquire actual resistance and increase their virulence [33,34].

These changes can be explained by pharmacological sensitivity with the time, the ability of yeast to tolerate the toxic effects of antimicrobial resistance and its ability to develop some resistance mechanisms. The differences in the results of the current study with the results of other studies in developed countries based on the environmental location and excessive intake of antimicrobial agents may explain the transmission of genetic resistance between resistant isolates to sensitive isolates Due to unhealthy and personal hygiene practices, including the introduction of sewage into drinking water, leading to the transmission of resistance genes [33,35].

The resistance of isolates from the lower respiratory tract is due to the frequent use of antifungal or non-systemic antibiotics, which has resulted in the adaptation of the pathogens to these high doses of antibiotics. As a result, they have been able to overcome the effect of these antibiotics in stimulating the virulence of fungi by acquiring resistance to this type of antigens throughout generations.

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