The Relation between PhynotypicFeatures andAntifungal Resistance among Some Candida species Isolates

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Abstract—The present study was aimed to determine some biological parameters among some clinical isolates of *Candida* species and their sensitivity or resistance towered some antifungal drugs.A total of 120 oral swabs were obtained from 120 patients suffering from oral thrush who attending Al-Sader Teaching Hospital in Al-Najaf (76 males and 44 females), 42 vaginal swabs were obtained from 42 women suffering from vaginal candidiasis who attending Al-Sader Teaching Hospital in Al-Najaf Provence, Iraq and 30 swabs from 30 healthy individualsof no obvious infections with oral thrush or vaginal candidiasis. The cultural and biochemical methods were used in *Candida* species isolation and identification besides API 20C Aux and CHROMagar*Candida* medium. Some virulence factors have been made including germ tube production, chlamydospore production, cyclohiximide resistance, adhesion, phospholipase production, and lipase production test.Drug sensitivity test in two methods disk division and minimal inhibition concentration were used to determinate the sensitive and resistance isolates towered some antifungal drugs. The results of swabs cultures revealed positive *Candida* species growth in 86/120 (71.67%) from the total oral swabs, 13/42 (30.9 %) from vaginal swabs samples, and only 8/30 (26.67%) from the healthy donors. The identification by the API 20C Aux showed five species, *C. albicans* 85/107 (79.4%), *C. tropicalis* 12 (11.3%), *C. parapsilosis* 5 (4.6%), *C. krusei* 3 (2.9%) and *C. rugosa* 2 (1.8%). The results of CHROMagar test were confirmed the results of the API 20C Aux yeast identification system. In this test, the colony color of *Candida* species on CHROMagar*Candida* medium weregreen for *C. albicans*,blue surrounded by a typical pink halo for *C. tropicalis*,pink for *C. krusei*,purple with halo for *C. parapsilosis* and pink with white border for *C. rugosa*. *C. albicans* isolates showed positive results for germ tube, chlamydospore production, growth in the presence of cycloheximide and adhesion test. Tobacco agar test was positive only to *C. krusei* and *C. rugosa* isolates, phospholipase assay was positive to *C. albicans, C. tropicalis* and *C. parapsilosis*. Lipase assay was positive to *C. albicans* and *C. krusei*.Disk diffusion method results revealed that the nystatin (NS 100 IU) was the most effective antifungal which inhibited 100% of *Candida* species isolates, followed by amphotericin-B (AP 100 IU) that inhibited 96.6% of the isolates. Clotrimazole (CC 10µg), ketoconazole (KT 10µg), and itraconazole (IT 10µg) inhibited 81%, 65.4% and 61.5% of the isolates respectively. Fluconazole (FLC 10µg) was the less effective antifungal which inhibited only 43.4% of all the isolates. The statistical analysis (p≤ 0.01) showed significant differences among the tested isolates after compared with the standard inhibition zone diameters. The MIC results demonstrated that nystatin was the most active antifungal drug that inhibit the growth of all *Candida* isolates (100%) by 0.07 µg/ml. (94%) of all isolates were determined susceptible to amphotericin B at 0.5µg/ml, (83.5%) to clotrimazole at 0.125 µg/ml, (69.7%) to ketoconazole at 0.125 µg/ml, (62%) to itraconazole at 0.125 µg/ml and (48.5%) to fluconazole at 64 µg/ml, the statistical analysis (p≤ 0.01) showed a significant differences among the tested isolates after compared with the standard values of MIC breakpoints of antifungal drugs.All the *Candida* isolates in this study showed a high level drug resistance and the differences in drug sensitivity among them were consistent with their phenotype features.

Index Terms—*C. albicans,C. tropicalis,C. parapsilosis*, *C. krusei, C. rugosa,*Antifungal drugs, Multidrug resestance,and Chrom agar.

1 INTRODUCTION

Candida species are part of the normal flora of the oral cavity in many individuals but are normally only present in small numbers. According to several studies, they colonized in 20% to 40% of healthy individuals, and they become predominant flora in more than 60% of immunocompromised subjects[1],especially in HIV patients, the elderly, neonates and patients undergoing chemotherapy, antibacterial therapy or invasive procedures, where they can cause opportunistic infections [2].*Candida* species are opportunistic fungal pathogen that cause severe blood and disseminated infections. The incidence of these infections has markedly increased over the past decade, due to the increase of immunocompromised and neutropenic patients after organ transplantation, cancer therapy or AIDS [3]. In these populations, *C. albicans* is associated with a high mortality rate and the obvious economic consequences [4]. There is a dramatic change in the incidence of different *Candida* species in candidiasis during the last five years [5].

The pathogenetic effects of only 22 of *Candida* species have been recognized in humans and include *C. albicans*, *C. tropicalis, C. krusei, C. parakrusei, C. parapsilosis, C. pseudotropicalis, C. zeylanoides, Candida glabrata*and *C. guilliermondii*[6].Factors such as transplant surgery and concomitant immunosuppressive therapies, anti-cancer therapies, medical devices that traverse the protective skin barrier (e.g., central venous lines, catheters, etc.), broad-spectrum antibacterial therapies, corticosteroid therapies, certain disease states (e.g., malignancy, human immunodeficiency virus infection, etc.), and others have contributed to increased numbers of immunocompromised individuals [7]. One of the major reasons for the increase in *Candida* infections is the development of its resistant strains to azole drugs, such as fluconazole used in the prophylaxis and treatment of candidiasis [8].Although some antifungal drugs are available, fluconazole is still considered the drug of choice to treat most *Candida* infections [9]. However, long-term exposure to fluconazole, as well as fluconazole underdosage during the empirical regimens leads to an increased resistance phenomena [10]. Thus, the understanding of the molecu-

lar mechanisms underlying azole resistance in *C. albicans* is necessary in order to discover new antifungal agents that circumvent drug resistance and could guide the choice of the appropriate antifungal treatment at the onset of infection [11].

In the present study aimed to determine some of biological parameters among some clinical isolates of *Candida* species based on their sensitivity or resistance to these parameters by using some phenotypic techniques, and some virulence factors determination.

2 METHODOLOGY

2.1 Patients Group

Oral Candidiasis: One-hundred twenty patients (76 males and 44 females), who attending Al-Sader Teaching Hospital in Al-Najaf their ages ranged from 1-10 years, they were suffering from oral thrush were included in this study.

Vaginal Candidiasis: Forty twowomen who attending Al-Sader Teaching Hospital in Al-Najaf their ages ranged from 25-50 years suffering from vaginal candidiasis were included in this study.All the patients were diagnosed clinically by specialist physician as oral thrush and vaginal candidiasis.

Control Group: Thirty subjects of no obvious infections with oral thrush or vaginal candidiasis were considered as control group.Their ages were same as patients group.

2.2 Specimens Collection

A total of 120 oral swabs were obtained from patients who suffering from oral thrush. And 30 swabs from healthy individuals. Specimens were taken using sterile swabs, and then transported to the laboratory for diagnosis. Forty two vaginal swabs were taken by the gynecologist from pregnant and non-pregnant women with excessive vaginal discharge, purities vulva dysuria, and irritation.

2.3 Isolation and Identification

Single colonies of each isolate were obtained by streaking each swab sample onto Sabouraud's Dextrose Agar (SDA) containing 0.05g/L chloramphenicol , and incubated at 37^{o}C for 48hrs. All the isolates were saved by culturing them for 24 h in vials containing slant of SDA, then 1ml of glycerol was added to each vial and kept at -20^{o}C.Identifications were made by reference to the API Analytical Profile Index using the API 20C AUX yeast identification system(BioMérieux, France). Results were considered correct when the profile was listed as excellent, very good or acceptable and if results were in agreement with the reference identification [12].

2.4 Tobacco Test

Tobacco agar plates were prepared according to Khan *et al* [13]. The plates werestreaked with a small amount of inoculums from the isolated colonies. The culture plates were incubated at 28^{o}C and observed daily up to 96 hours for colony characteristics, such as surface topography (rough or smooth), formation of hyphal fringes at the periphery and color.

2.5 CHROM Agar Test

This test was performed by inoculating CHROM Agar *Candida* Medium (Hi-Media, India)with isolated colony taken from *Candida* isolates culture grown on SDA for 24 hours, and then incubated at 30˚C for 24-48 hours. CHROM Agar test was used for the presumptive identification of *Candida* species by the color of the growth produced (*C.albicans*= green/blue green; *C. tropicalis* = blue; *C. krusei* = pink)[14].

2.6 Virulence Factors

2.6.1 Germ Tube Production

This used as a differential test for identification of *Candida albicans.* The procedure was carried out as follows; a small inoculum of the test yeast cells from a pure culture was suspended in 0.5 ml human serum. The suspension was incubated at 37^{o}C for exactly three hours after which a drop of the incubated serum was placed on a microscope slide and covered with a cover slip. The wet mounts were examined for presence of germ tubes using the 100× lens. The isolates were classified as either germ tube positive or germ tube negative [15].

2.6.2Chlamydospore Production

Chlamydospore production on corn meal agar was used as a presumptive confirmatory test for the identification of *Candida albicans***.** The test involved streaking and stabbing the media with a 48 hour old yeast colony and, incubated at 25 °C for 72 hours. A small portion of the colony was placed on a slide containing a drop of lactophenol cotton blue then covered with cover slip and examined under the microscope 40× lens. The isolates were categorized as chlamydospore positive or negative [16].

2.6.3 Resistance to Cycloheximide (Actidione) Test

SDA plates containingcycloheximide (0.5 g/L) were inoculated with a small portion of the yeast colony by a loop. The cultures were incubated at 30^{o}C for 7 days. Growth present indicates resistance of the isolate to cycloheximide[17].

2.6.4 Adherence Assay

The candidal adhesion assay was performed as follows: An overnight culture of *Candida* isolates on Sabouraud's dextrose agar was harvested by using a loop and suspended in PBS in a turbidity adjusted to match a 0.5 McFarland density standard resulting in a suspension containing 5×10^{6} yeast cells/ml. Then 1 ml from this suspension was mixed with 1 ml of a suspension containing buccal cavity cells, obtained by swabbing the buccal mucosa and suspended in PBS. The mixture was incubated in a shaking incubator operated at 80 rpm at 37^{o}C for 1 h. A drop of this mixture was mounted on a glass slide, air-dried, heat-fixed and stained with crystal violet for 1 min and adherence assayed microscopically at 40× lens[18].

2.6.5: Lipase Production Assay

This test was performed by preparing Rhan media which prepared according to Rodina[19].The plates were inoculatedwith isolated colony taken from *Candida* species culture

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grown on SDA for 24 hours, and then incubated at 30˚C for 1-5 days. The appearance of white sediment around the grown colonies refers to the positive result [19].

2.6.6 Phospholipase Production Assay

Candida species were screened for production of extracellular phospholipase activity by growing them on egg yolk agar and measuring the size of the zone of precipitation by the method of Price *et al*[20], as follows: A 10 µl suspension was placed on the surface of the egg yolk medium in a 90-mm-diameter Petri dish and left to dry at room temperature. The culture was then incubated at 37°C for 48h, after which the colony diameter and colony diameter plus precipitation zone were measured for each isolate. Calculation of of phospholipase activity was performed according to Price *et al.*[20]:

$$Colony\ diameter/Colony\ diameter\ +\ Zone\ of\ precipitation = Pz$$

Pz coefficient was classified as:Negative (Pz = 1.00), Positive(0.64 ≤ Pz< 1.00) and strongly positive (Pz<0.64).

2.7 Antifungal Susceptibility Test

2.7.1 Disk Diffusion Method: Six types of antifungal drug discs were used in current study: fluconazole (FLC 10µg), ketoconazole (KT 10µg), itraconazole (IT 10µg), clotrimazole (CC 10µg), nystatin (NS 100 IU) and amphotericin-B (AP 100 IU). The diameter of inhibition zone for individual antifungal drug was translated in terms of sensitive and resistant categories referring to an interpretation chart of CLSI document M44-A [21](Table 1).

Table 1
Standard values of growth inhibition zones diameter (mm) of antifungal drugs used in this study based on CLSI document M44-A [21]

Antifungal disk	Symbol	Potency	Zone diameter in mm		
			Sensitive (S)	Intermediate (I)	Resistance (R)
Amphotericin-B	AP	100 IU	≥ 15	14 – 10	< 10
Clotrimazole	CC	10µg	≥ 20	19 – 12	≤ 11
Fluconazole	FLC	10µg	≥ 19	18 – 15	≤ 14
Itraconazole	IT	10µg	≥ 23	22 – 14	< 13
Ketoconazole	KT	10µg	≥ 28	27 – 21	≤ 20
Nystatin	NS	100 IU	≥15	14 – 10	No zone

2.7.2 Determination of Minimal Inhibitory Concentrations (MICs): The minimal inhibitory concentrations (MICs) were established using agar dilution method.The following drugs were used in a suggested concentration according to CLSI M27-A2 [22]:

Amphotericin B 0.03 to16 µg/ml.
Ketoconazole 0.03 to16 µg/ml.
Clotrimazole 0.03 to16 µg/ml.
Itraconazole 0.03 to16 µg/ml.
Fluconazole 0.125 to 64 µg/ml.
Nystatin 0.7 to18.5 µg/ml.

The MIC was compared with Standards values of minimal inhibition concentration breakpoints of antifungal drugs (Table 2) based on CLSI M27-A2 [22].

Table 2
Standard values of MIC breakpoints of antifungal drugs used in this study based on CLSI M27-A2 [22]

Antifungal drug	Equivalent MIC Breakpoints(µg/ml)	
	Sensitive (S)	Resistance (R)
Amphotericin-B	≤ 1	≥ 2
Clotrimazole	≤ 0.125	≥ 1
Fluconazole	≤ 8	≥ 64
Itraconazole	≤ 0.125	≥ 1
Ketoconazole	≤ 0.125	≥ 0.5
Nystatin	≤ 0.125	≥ 0.5

2.7 Statistical Analysis

All the gain data were analyzed statistically (p ≤ 0.01) using ANOVA table to define the differences among tested treatments [23].

3: Results and Discussion

3.1Isolation and Identification

The results of swab cultures revealed that 86/120 (71.67%) gave positive *Candida* species growth on SDA plates while only 8/30 (26.67%) from the healthy donors were positive. These results were agree with Zarembaet *al*. (2006) in yeasts of *Candida* genus were isolated in 65/103 (63.1%) from adults oral cavities.While the results of vaginal swab cultures showed that 13/42 (30.9 %) out of the total vaginal samples gave positive growth culture for *Candida species* on SDA. This result was also conducted by Tanksale*et al*., [24] in *Candidaspecies* were detected in (29%) from study subjects, and with Darogha[25] who found that the highest infectious rate with virginities is belonging to *C.albicans* with 31.1%.

3.2 Tobacco Test

The result of this test revealed that all isolates were showed typical morphotype of *Candida* species, smooth whitish-cream color colonies with hyphal fringes for *C. krusei* and *C. rugosa*, and without hyphal fringes even after extended incubation for up to 10 days for *C. albicans*, *C. tropicalis* and *C. parapsilosis*. These results were agreed with Khan *et al*. [13] and Silveira-Gomes *et al*. [26].

3.3 API 20C AUX Yeast Identification System

The result of identification by the API 20C Aux yeast identification system revealed that the Candida species isolates from oral and vaginal samples belong to five different species, *C. albicans* 85/107 (79.4%), *C. tropicalis* 12 (11.3%), *C. parapsilosis* 5 (4.6%), *C. krusei* 3 (2.9%) and *C. rugosa* 2 (1.8%) (Figure 1).

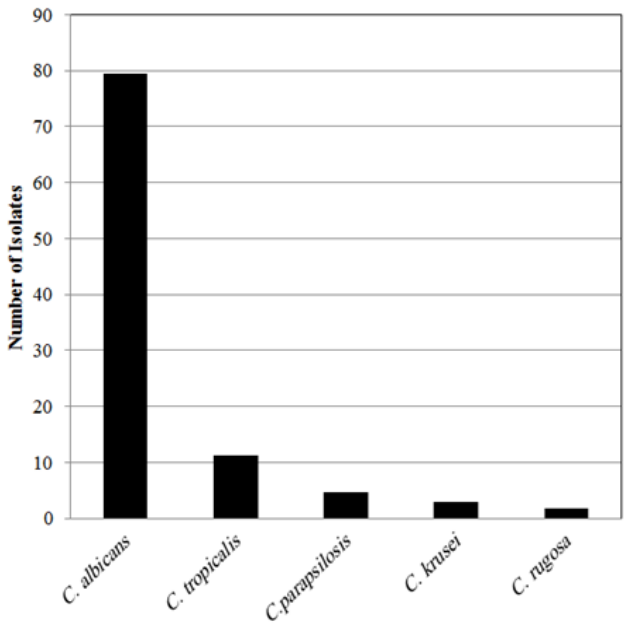

Figure 1: Percentages of Candida species isolated from oral and vaginal candidiasis

3.4 CHROMagar Test

The results of CHROMagar test were confirmed the results of the API 20C Aux yeast identification system. In this test, the colony color of CHROMagar Candida medium was green for *C. albicans*, blue surrounded by a typical pink halo for *C. tropicalis* and pink for C. krusei. These results were conducted with the instructions that described by the manufacturer of this medium, and agreed with the results of Raut and Varaiya[27].

Results of the current study showed that *C. parapsilosis* produced purple colonies with halo while *C. rugosa* produced pink colonies with white border. These results were agreed with Hospenthal*et al*. [28] who found that the colonies of *C. parapsilosis* on CHROMagar Candida were purple with variable shades of ivory, pink and lavender indistinguishable from each other and often inconsistent between isolates of the same species. And with Horvath *et al*. [29] who noted that *C. rugosa* strains produced a distinct appearance on CHROMagar Candida, they grew pink colonies that possessed a pale or white border.

In this study and many others like Momani [30] and Iyampillai*et al*. [31] indicated that CHROMagar Candida medium can be used for the presumptive identification of Candida species because it cost effective and quicker than conventional methods.

4.5 Virulence Factors

4.5.1 Germ Tube Production

C. albicans isolates were produced obvious germ tube after incubated them in human serum at 37ºC for three hours, while the non-albicans Candida species isolate showed a negative result (table 3). These results were agreed with Kumar and Shukla [32]. The importance of germ tube formation for the invasive capability of *C. albicans* has been stressed. It is commonly considered that the blastoconidia represent the morphological cell form associated with asymptomatic colonization of mucosal surfaces. Conversely, germ tube/hypha formations are assumed to represent a potentially tissue invasive form [33].

Table 3: Virulence factors of Candida species isolates

Candida species	virulence factors					
	Germ tube	Chlamydo-spore	Cyclohexi-mide	Adherence	Lipase	Phlosph o-lipase
C. albicans	+	+	+	+	+	+
C. tropicalis	–	–	–	–	+	–
C. parapsilosis	–	–	–	–	+	–
C. krusei	–	–	–	–	–	+
C. rugosa	–	–	–	–	–	–

4.5.2Chlamydospore Production

The chlamydospore results were showed that C .albicans was able to produce chlamydospores on corn meal agar (table 3), while the other Candida species were showed a negative result. These results were consistent with Bose *et al*. [34]. Chlamydospores are developing on pseudohyphal support cells, which in some forms lead to spontaneous phenotypic switching [35] which is the major virulence determinant of Candida species [36].

4.5.3 Resistance to Cycloheximide Test

All the isolates of *C. albicans* were resistance to cycloheximide and gave a positive growth on SDA plates supplemented with 0.5 g/L from this antibiotic (table 3), while all of the other species isolates were showed a negative results, these results were agreed with Takaku*et al*. [37]. Resistance to cycloheximide is a great indication for resistance to azole antifungal drugs, and the isolates that show resistance to cycloheximide are consedard as virulence multidrug isolates [38].

4.5.4 Adherence Assay

All the isolates of *C. albicans* showed a positive result to adhesion test after incubation with buccal cavity cells for 1h at 37ºC, while the non-albicans *Candida* species isolates showed a negative result (table 3). These results were agreed with Henriques*et al*. [39] and Jain *et al*.[40]. Adherence of *C. albicans* to host surfaces is thought to be a crucial step in the pathogenic process and a prerequisite for colonization of these surfaces, this adhesion occurs by the interaction between yeast and epithelial cell receptors, and a variety of mechanisms have been proposed [41].

4.5.5 Lipase Production Assay

The results of lipase production assayindicated that*Candida* species were differed in their ability to produce lipase.*C. albicans*, *C. tropicalis* and *C. parapsilosis* gave positive results by producing white sediments around the grown colonies after incubated their colonies for 72 h at 30ºC (table 3). These results were agreed with many studies, Paraje*et al*. [42] found that *C. albicans* was able to produce lipase, Neugnot*et al*. [43] study

had shown that *C. parapsilosis* gave a positive result for lipase test, and Gacser*et al*. [44]approvedthat *C. parapsilosis* isolates were able to produce lipase enzyme.The current study revealed that *C. rugosa* and *C. krusei* gave negative resultsfor lipase test; these results were agreed with results of Slifkin[45].Lipase has very important role in *Candida* species pathogenicity because it is able to digest lipids for nutrient acquisition, adhesion to host cells and host tissues [46].

4.5.6 Phospholipase Production Assay

The phospholipase production was verified by determination the Pz coefficient to the precipitation zone around the isolates colonies on egg yolk agar. The results were positive for *C. albicans* (Pz = 0.85) and *C. krusei* (Pz = 0.9) *isolates*, and negative (Pz = 1.00) among the other species for its production (table 3). These results are similar to that found by Ribeirol*et al*. [47]. The extracellular phospholipases of *Candida* species have a significant role in the pathogenesis of infections and invasion to mucosal epithelia [48].

4.6 Antifungal Susceptibility Test

4.6.1 Disk Diffusion Method

The results in the current study revealed that the nystatin (NS 100 IU) was the most effective antifungal which inhibited 100% of *Candida* species isolates, followed by amphotericin-B (AP 100 IU) that inhibited 96.6% of the isolates. Clotrimazole (CC 10µg) Ketoconazole (KT 10µg), and itraconazole (IT 10µg) inhibited 81%, 65.4% and 61.5% of the isolates respectively. Fluconazole (FLC 10µg) was the less effective antifungal which inhibited only 43.4% of all the isolates, the statistical analysis (p≤ 0.01) showed significant differences among the tested isolates after compared with the standard zone.

Most of the *C. albicans* isolates (98.5%) were resistant to fluconazole, while 0%, 12.5%, 57.5%, 65% and 87.5% were resistant to nystatin, amphotericin-B, clotrimazole, ketoconazole, and itraconazole respectively. All *C. rugosa* and *C. krusei* isolates (100%) were resistant to fluconazole, and they were sensitive to the other antifungal drugs. All of *C. tropicalis* and *C. parapsilosis* isolates were sensitive to all the antifungal drugs that included in the study. The diameter of growth inhibition zone of *Candida* species toward the antifungal drug disks were listed in tables 4.

The isolates that collected from *Candida* infected patients (infection isolates) were showed 100% resistant to fluconazole and different sensitivities to the other antifungal drugs, while all the isolates that collected from healthy donors (colonization isolates) were sensitive to all antifungal drugs that tested in current study. These results were agreed with Negri*et al*. [49] in that colonization yeasts were more susceptible to fluconazole, itraconazole, and amphotericin B than infection yeasts.

The levels of resistance among the isolates in our study were high comparing with most other studies, Madhavan*et al*. [50] results showed that 71% of *C. tropicalis*, *C. albicans*, *C. krusei*, *C. parapsilosis*, *C. rugosa*, *C. dubliniensis* and *C. glabrata* strains were susceptible to fluconazole. While a study done by Tulumoğlu*et al*. [51] revealed that all *C. albicans* strains were sensitive to itraconazole and amphotericine B while 3.63% of *C. albicans* strains were resistant to fluconazole, and 100% of *C. parapsilosis* strains were sensitive to amphotericine B, whereas two strains (5.88%) were resistant to itraconazole and one strain (2.94%) was less sensitive to fluconazole. *C. tropicalis* strains were found sensitive to all antifungal agents.

Table 4: The diameter of growth inhibition zone of Candida species toward different antifungal drug disks

Candida species	Growth inhibition zone diameter (mm)*					
	Antifungal disks					
	NS	KT	AP	FLC	CC	IT
C. albicans	27 (S)	17 (R)	14 (I)	0 (R)	19 (I)	13 (R)
C. tropicalis	32 (S)	37 (S)	19 (S)	25 (S)	25 (S)	23 (S)
C. parapsilosis	32 (S)	33 (S)	28 (S)	26 (S)	34 (S)	33 (S)
C. krusei	25 (S)	17 (R)	17 (S)	0 (R)	19 (I)	13 (R)
C. rugosa	26 (S)	16 (R)	16 (S)	0 (R)	19 (I)	12 (R)
C. albicans (control)	30 (S)	33 (S)	18 (S)	25 (S)	29 (S)	23 (S)

*All tested isolates were significant (p ≤ 0.01) after compared with the standard zone. NS: Nystatin, KT: Ketoconazole, AP: Amphotericin-B, FLC: Fluconazole, CC: Clotrimazole, IT: Itraconazole, S: sensitive, R: resistance, I: intermediate.

4.6.2 Minimal Inhibition Concentrations Method (MIC)

The MIC results demonstrated that nystatin was the most active antifungal drug that inhibit the growth of all *Candida* isolates (100%) by 0.07 µg/ml. (94%) of all isolates were determined susceptible to amphotericin B at 0.5µg/ml, (83.5%) to clotrimazole at 0.125 µg/ml, (69.7%) to ketoconazole at 0.125 µg/ml, (62%) to itraconazole at 0.125 µg/ml and (48.5%) to fluconazole at 64 µg/ml, the statistical analysis (p≤ 0.01) showed a significant differences among the tested isolates after compared with the standard values of MIC breakpoints of antifungal drugs (Table 5 and figure 2).

Table 5: The value of MICs of Candida isolates toward different antifungal drug

Antifungal drug	MIC µg/ml*
Nystatin	0.07
Amphotericin B	0.5
Clotrimazole	0.125
Ketoconazole	0.125
Itraconazole	0.125
Fluconazole	64

*All tested isolates were significant (p ≤ 0.01) after compared with the standard values of MIC breakpoints of antifungal drugs.

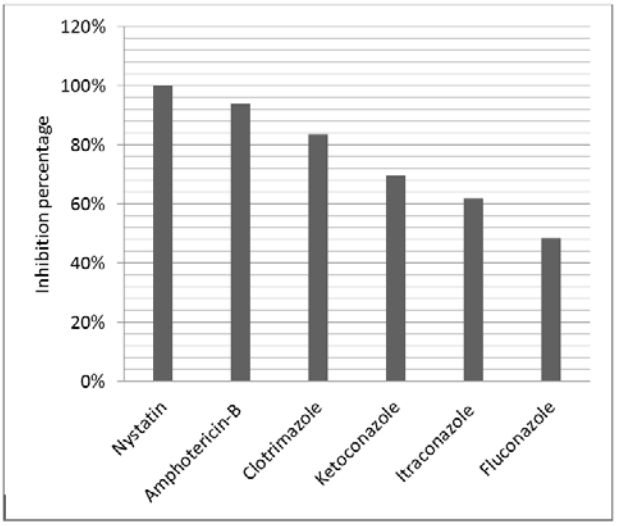

Figure 2: *Candida* isolates inhibition rates toward different antifungal drugs by MIC method

These results were agreed with the disk diffusion method results and partially with some other studies, as Negri*et al*. [49] who showed that 83% from *Candida* isolates were susceptible for amphotericin B, 63% for itraconazole, and 64% for fluconazole.The results ofPfaller*et al*. [52] revealed that 2 from 14 *Candida* isolates were susceptible for fluconazole, confirming their lack of susceptibility to azole antifungal agents.

Conclusions and Recommendations

1: Conclusions

1. Chrome agar media was a good tool in the diagnosis and differentiation among *Candida* species based on the colony color.
2. *Candida* species have some virulence factors that aid in the differentiation between C. *albicans* and non-albicans *Candida* species.
3. *C. albicans* show a high resistance to azole antifungal drugs.

2: Recommendations

1. Phenotypic characteristics on general media of fungi alone are not sufficient in the identification of *Candida* species and must support by using chrome agar media for differentiation among the species.
2. Virulence factors such as chlamydospore, germ tube production and cycloheximide resistance are an important diagnostic tool for confirming the differentiation of *C. albicans* from non-albicans *Candida* species.
3. Due to the resistance of *C. albicans* to the azole antifungal drugs, we recommend to finding substitute drugs or new generations of the commercial drugs to minimize of these resistances or side effects.

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