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Original Article

Detection of Virulence Factors from *Candida* spp. Isolated from Oral and Vaginal Candidiasis in Iraqi Patients

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Abstract

Yeast-like fungi (YLF) of the genus Candida are unicellular microorganisms of relatively large size and rounded shape, aerobes, and belong to conditionally pathogenic microorganisms. The genus Candida includes approximately 150 species, which are classified as Deuteromycetes due to the absence of a sexual stage of development. This study aimed to identify virulence factors from *Candida* spp. isolated from oral and vaginal candidiasis. Fifty-eight oral and vaginal swab specimens were collected from patients, including (28) oral swabs from children and (30) vaginal swabs from different infected women. All isolates were subjected to direct examination, Morphological tests, Germ tube formation, growth at 45ºC, CHROM agar Candida culture, and VITEK 2 Compact system to ensure this diagnosis. (31) isolates were identified as Candida spp., including (21) (*C. albicans* (14), *C. glabrata* (1), *C. guilliermondii* (2), *C. dubliniensis* (3), *C. parapsilosis* (1)) were isolated from oral swabs and (10) isolates included*C. parapsilosis* (4), *C. albicans* (6) were isolated from vaginal swabs. Moreover, these isolates had been detected to have some virulence factors, including phospholipase, esterase, proteinase, coagulase, hemolysin, and biofilm formation. Different species of Candida were isolated and identified from oral and vaginal. Phospholipase (Pz), Esterase (Ez), and Proteinase (Prz) were produced by 19 (61.29%), 16 (51.61%), and 26(83.87%), respectively, out of 31 isolates, whereas. All isolates produce coagulase enzyme except *C. dubliniensis*, which did not produce coagulase enzyme. All *Candida* spp. isolates produce hemolysin and biofilm formation in different percentages.

Keywords: Yeast-Like Fungi, Unicellular Microorganisms, Deuteromycetes

1. Introduction

Yeast-like fungi (YLF) of the genus Candida are unicellular microorganisms of relatively large size and rounded shape, aerobes, and belong to conditionally pathogenic microorganisms. The genus Candida includes approximately 150 species, which are classified as Deuteromycetes due to the absence of a sexual stage of development. From a medical point of view, seven of them are recognized as the most important disease-causing species: *C. albicans, C. tropicalis, C. glabrata, C. krusei, C. guilliermondii, C. parapsilosis,* and *C. Kefir* (1). Approximately 30 container species cause injury to humans. *C. albicans* is the most common species, then other pathological species containing *C. glabrata, C. tropicalis, C. krusei, C. parapsilosis, C. lusitaniae, C. guilliiermondii, C. kefyr, C. rugosa, C. inconspicua, C. dubliniensis, C. famata, C. lipolytica, C. sake, C. apicola, C. pulcherriima, C. zeylanoides, C. pelliculosa, C. valida, C. intermedia, C. haemulonii, C. humicola, C. utilis, C. ciferrii, C. lambica, C. holmii, C. marina, C. humicola, C. sphaerica* and *C. stellatoidea, C. colliculosa* ect. Candida colonies appear small, creamy, or whitish colonies with a characteristic yeast odor on SDA; on CHROM agar, Candida appears as glistening green color colonies for

C. alibicans and another color for each Candida species. As well, under the microscopic, all *Candida* spp. showed that all species produce blastoconidia. Blastoconidia may be round or elongate; most species produce pseudohyphae which may be extended, branched, or curved.

Candida spp. infections are caused by several virulence mechanisms, including adhesion to the host and abiotic medical surfaces, biofilm formation, and the production of hydrolytic enzymes. Furthermore, *Candida albicans* resistance to antimicrobials, particularly azoles, is widely documented, particularly when Candida cells are in biofilm form. The role of *Candida virulence* factors that mediate their success are as follows: pathogens, such as membrane and cell wall barriers, dimorphism, biofilm formation, signal transduction pathway, proteins related to stress tolerance, hydrolytic enzymes, e.g., proteases, lipases, hemolysins and toxin production (2). Phospholipases are the enzymes that can hydrolyze the ester bonds in glycerophospholipids. These enzymes cause cell membrane breakdown; conversely, proteases can break down various host cell proteins, including collagen, keratin, mucus, antibodies, complements, and cytokines are all broken down by these enzymes (3). Esterases are enzymes that help to break down an ester group. Esterase synthesis was detected in *Candida albicans, Candida tropicalis, Candida parapsilosis,* and *Candida lipolytica*, as well as their low activity in *C. parapsilosis* (4). Hemolysins are involved in Candida pathogenicity because they are responsible for red blood cell destruction and iron uptake. Iron and other inorganic elements are required to develop Candida cells and create the infection process (5). Biofilms are complex microbial populations that are very heterogenous and provide their residents with increased chances of survival (2). Therefore, this study aimed to detect virulence factors from *Candida* spp. isolated from oral and vaginal candidiasis.

2. Materials and Methods

2.1. Isolation of Candida Isolates

From May 2021 to the end of June 2021, fifty-eight specimens were collected from patients, including twenty-eight oral swabs from children in AL-elwia pediatric teaching hospital and thirty vaginal swabs from infected women in Imamein kadhimen medical city in Baghdad and transported under aseptic conditions to the laboratory for Candida spp. isolation. The samples were streaked directly onto Sabouraud Dextrose agar (SDA) enriched with chloramphenicol (250 mg/liter) and chromogenic candida agar and incubated aerobically at 37°C for 48 hours (6).

2.2. Identification of Candida Isolates

2.2.1. Cultural Examination

Candida spp. were identified based on morphological characteristics of sabouraud dextrose agar and chromogenic candida agar, which included colony shape, color, texture, size, opacity, and margin. The isolates were subcultured on chromogenic candida agar for 48 hours at 37°C after being obtained from sabouraud dextrose agar (SDA) at 37°C for 48 hours. Chromogenic candida agar can be used as a selective or differential media for Candida spp. After incubation, yeast identification was done based on colony color.

2.2.2. Microscopical Examination

The isolates were stained with gram stain after placing the specimen on a clean slide with a drop of 10% (KOH) and examining it under a microscope. Furthermore, a fragment of the colony was extracted with a sterile stick and placed on a clean slide, which was then combined with a drop of lactophenol cotton blue, covered with a cover-slip, and viewed under a light microscope to look for blue budding Candida cells (7).

2.3. Germ Tube Formation (GT)

Transferring a tiny piece of isolated colonies using a sterile stick, mixing them with 0.5 ml of human serum, and incubating them at 37°C for two and a half hours, the generation of germ tubes by Candida spp. isolates were investigated. Due to the propensity of other yeast

species to create germ tubes, the incubation period must not exceed 3 hours. Using a Pasteur pipette, a drop of the cultured serum was deposited on a slide, covered with a cover - slip, and examined under the microscope for the presence of germ tubes (8). In this test, serum was prepared according to Yin, Peter (9). According to the method previously described by Mariano, Gonçalves (10), the distinction and detection of *Candida* spp., performed by Growth at 45°C for 48 h.

2.4. Identification Using Vitek 2 Compact System

The Vitek 2 Compact device is committed to clinically confirming important Candida organisms' detection using the manufacturer's protocols (bioMerieux Inc., Durham, NC 27712, USA).

2.5. Detection of Some Virulence Factors of *Candida* spp. Isolated from Vagina and Oral Cavity

2.5.1. Extracellular Phospholipase Production

The egg yolk agar plate method was used to determine extracellular phospholipase production. The modified egg yolk agar was inoculated with ten microliters of newly generated yeast inoculum (consisting of a 24-hour-old *Candida* spp. colony suspended in 5 ml of sterile normal saline) equivalent to McFarland (1.5×10^8 CFU/ml) and incubated at 37°C for 48 hours. The result was achieved by measuring the colonies' diameter and the opacity zone's diameter and calculating the Pz value as follows.

$$Ez = \frac{\text{Thecolonydiameter (a)}}{\text{thediameterofcolony} + \text{precipitationzone (b)}}$$

Pz value = 1 indicates that the test isolate is negative for phospholipase, 0.80-0.89 indicates low phospholipase activity, 0.70 - 0.79 indicates moderate phospholipase activity, and 0.70 - 0.79 indicates high phospholipase activity (11).

2.5.2. Esterase Activity

This investigation assessed the esterase activity of Candida isolates using the Tween 80 opacity test. An aliquot of 10 ul of the yeast suspension was incubated at 37ºC for 5 days after being inoculated on the test medium. The precipitation zone around the colonies was used to determine the esterase activity. To evaluate esterase activity, the colony diameter (a) and the colony plus precipitation-zone diameter (b) were measured (12).

2.5.3. Proteinase activity

In terms of bovine serum albumin (BSA) degradation, An aliquot 10ul of the yeasts suspension was inoculated on the test medium and incubated at 37ºC for 7 days; the proteinase activity was measured as the diameter of clear zones around the colonies, proteinase activity was determined using the amido black staining solution. The ratio of the colony to the diameter of the proteolytic unstained zone was used to calculate the value of proteinase activity (Prz). A Prz value of 1 showed no proteinase was produced, whereas a Prz value of 1 indicated that proteinase activity was present (13).

$$\text{Przvalue} = \frac{\text{Colonydiameter}}{\text{proteolyticunstainedzonediameter}}$$

2.5.4. Coagulase Activity

Candida isolates were tested for coagulase production using the traditional test tube method (14). In a tube containing 500ul of EDTA rabbit plasma, 0.1 ml of an overnight Candida spp. the culture was aseptically introduced. For 4 hours, the tubes were incubated at 35 °C. A positive coagulase test revealed the existence of a clot that could not be resuspended by gentle shaking. The tube was reincubated and reexamined if no clot formed after 24 hours.

2.6. Haemolysin Production

2.6.1. PlateMethod(agarmedium)

A loop - full of pure *C. albicans* culture was inoculated into sabouraud dextrose agar and incubated at 37ºC for 24 h. This Growth was used to prepare a suspension compared to McFarland 0.5. Ten microliters of this suspension were placed on sabouraud dextrose blood agar medium (with 3% glucose), and then the plates were incubated at 37°C for 48 h. A translucent halo around the colony indicates positive hemolysin

production. Hemolytic index, which represented the intensity of hemolysin production, was estimated by dividing the colony diameter by the diameter of the colony plus the hemolysis zone; based on the Hi index, hemolytic activity recorded to the scores: Hi 1 means not a hemolytic activity, 0.64 - 0.99 indicate moderate hemolytic activity, 0.63 ≤ indicates vigorous hemolytic activity (15).

2.6.2. SpectrophotometricMethod(Liquidmedium)

The hemolysin production in a liquid medium was determined using a spectrophotometric method reported by Lee, Kim (16), in which (20) µl of Candida spp. suspension compared to (0.5) McFarland was added to (180) µl of sabouraud dextrose broth and incubated at 37º C for 24 hours. Human red blood cells were prepared by centrifugation at 3000 rpm for 5 minutes and washed three times with PBS buffer. The pellet was diluted in PBS buffer (330 µl red blood cells were added to 10 ml PBS buffer), and 100 µl candidal culture was added to 10 ml red blood cells previously prepared and incubated at 37º C for 1 hour. After centrifugation at 12,000 rpm for 10 minutes, the supernatants were collected, and the amount of hemoglobin released from lysed erythrocytes was measured using a spectrophotometer at 543 nm. Blood cells with only a medium served as the experiment's control.

2.6.3. Biofilm Formation

The biofilm formation was determined using polystyrene 96-well microplates, following the methods reported by Deepa, Jeevitha (17) with some modifications. Candida spp. were inoculated into a tube containing 2 ml of Yeast extract peptone dextrose (YPD) broth and incubated at 37°C for 24 hours. All tubes were diluted at a ratio of 1:20 with freshly prepared YPD broth, and a 96 flat bottom well microtiter plate was filled with 200 µlof this final solution, while the control wells were filled with 200 µlof YPD. Microtiter plates were sealed with lids and incubated at 37°C for 24 hours; after that, the media in the wells was removed and washed twice with sterile phosphate buffer solution (PBS) to remove any unattached Candida spp. cells then dried at room temperature for 15 minutes. After that, each well was filled with 200 liters of crystal violet and left for 20 minutes. The crystal violet was taken from the wells and washed three times with PBS (pH 7.2) to remove the unbound dye, then dried at room temperature. Finally, a 200 µl combination of acetone and ethanol (20:80 v/v) was added to each well, and O.D was read at 450 nm by an ELISA reader. These OD values were used to determine if *Candida* could form biofilms (18) (Table 1).

Table 1. OD values used to determine the ability of biofilms production by *Candida*

OD values	Biofilm formation
>0.320	Strong
0.120–0.320	Moderate
<0.120	Non/weak

3. Results and Discussion

3.1. Cultural Characteristics

The morphology of *C. albicans* colonieson sabouraud dextrose agar was white colored, smooth, andyeast-like, as indicated in figure 1,*C. parapsilosis* colonies were white to creamy, shiny, and smooth, *and C. glabrata* colonies were smooth and cream colored.

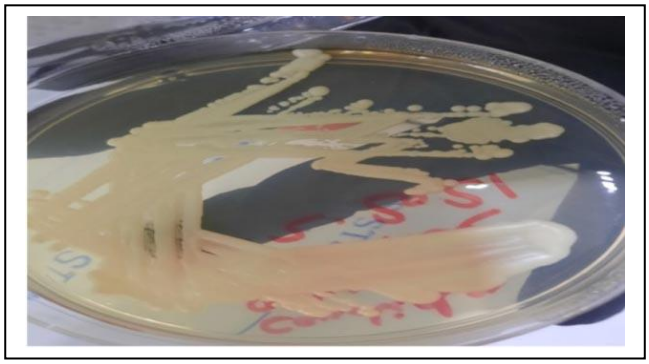

Figure 1.*Candida albicans* growth on sabouraud dextrose agar (SDA) (24 h/ 37º C)

Chromogenic Candida agar is a differential and selective media that promotes the rapid isolation of *Candida* from mixed culture sand can differentiate *Candida* species from other organisms based on colony morphology, as indicated in figure 2. Chromogenic

Candida agar has a unique reactive substrate with specific enzymic belonging to *Candida* spp. specifically *C. albicans* and *C. glabrata*. The current study's results agree with previously published work. The color of colonies showed various species of *Candid*a, which were as. *C. albicans* or *C. dubliniensis* orgreen color, *Candida dubliniensis* isolates grow well at 30°C and 37°C on commonly used culture media for *Candida* spp. It is impossible to differentiate between *C. albicans* and *C. dubliniensis* colonies on conventional solid media. Colonies of the two species can be distinguished following primary isolation from clinical specimens using the newly developed Chromogenic Candida agar. The results showed that the colonies of *C. dubliniensis* are pale green and easily distinguished from those of other *Candida* spp. The recorded data in the current study agreed with. *C. glabrata* for pink-purple, *C. parapsilosis* for white to pale pink, and the other species developing colonies from white to rose. These results agreed with a study by Hospenthal, Beckius (19).

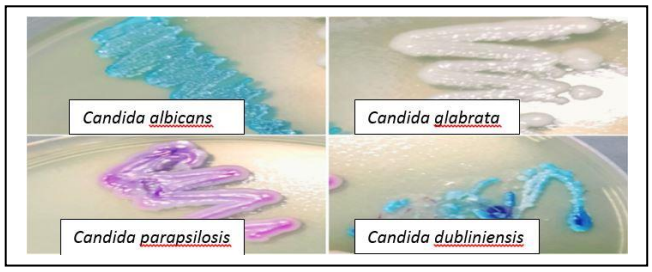

Figure 2. *Candida* spp. growth on CHROM agar Candida (CaC) (24 h / 37 ºC)

3.2. Microscopical Examination

After staining with Methylene blue dye, the *Candida* spp. cell shape and size were identified. *Candida* spp. isolated appeared oval, spherical cell shape with budding yeast-like cells.

3.3. Germ Tube Formation

The results indicated that isolates of *C. albicans* generate germ tubes in the form of long tube-like projections extending from the yeast cells. Within two hours of incubation with human serum, germ tubes formed. The findings of this investigation corroborated with those of Matare, Nziramasanga (8), who indicated that all *C. albicans* isolates could form germ tubes when examined directly from the colony. Developing germ tubes in human serum allows for the quick detection of *C. albicans*. Conversion of *C. albicans* from yeast to filamentous or mycelial Growth occurs due to the production of germ tubes in human serum. These morphological changes frequently reflect the fungus's response to changing environmental conditions and may enable the fungus to adapt to different biological factors (20). At the same time, the other species from non *C. albicans* showed a negative result except *C. dublinienesis*. These results are in agreement with. *C. albicans* and other candida spp. could differentiate between them by germ tube formation. *Candida albicans* formatted germ tube, but others have not formatted (8)

All *C. albicans* isolates grew well at 45ºC, while the others failed to grow. This result was in agreement with Kalaiarasan, Singh (21), who mentioned that the Growth at 45º C was deemed a viable method for differentiating *C. albicans* from *C. dubliniensis*. Moreover, indicated that fungi growth at 45º C was performed on all isolated fungi. The *C. albicans* have grown, but others have not.

3.4. Identification of *Candida* spp. by Vitek 2 Compact System

The Vitek 2 system is fully automated equipment for the susceptibility and identification of microorganisms. It appears to be a superior alternative detection method for clinical laboratories performing fungal diagnostics. The isolates were finally identified using the bioMerieux (USA) Vitek 2 compact technology. Identifications of isolates based on 64 biochemical tests for an unknown biopattern were compared to a database of reactions for each taxon, followed by a numerical likelihood estimate.

3.5. Detection of Virulence Factor for *Candida* Species

Extracellular phospholipase activity of pathogenic *Candida* spp. Isolated from the oral cavity and vaginal

infection were tested using the egg yolk agar plate method. In 21 isolates of Candida spp.isolated from the oral cavity, the results showed that 8 isolates were vigorous phospholipase activity with a Pz value of < 0.70, while 4isolates were moderate phospholipase activity with a Pz value of (0.70 - 0.79), and two isolates were weak phospholipase activity with Pz value of (0.80- 0.89) (Table 2).As for these 7 isolates, they do not produce a phospholipase enzyme. In ten isolates of *Candida* spp. isolated from vaginal infections, results showed that 2 isolates were strong phospholipase activity, while 2 isolates had moderate phospholipase activity, and only one isolate had weak phospholipase activity. As for these 5 isolates, they do not produce a phospholipase enzyme. Phospholipases are enzymes that hydrolyze the ester bonds in glycerophospholipids, causing cell membrane breakdown (3). Hence, phospholipases could bind to the cell surface or be released into the environment (22). Secreted phospholipases are extracellular hydrolytic enzymes (23). According to the study conducted by Lan, Hou (13), phospholipase synthesis is strain-dependent. de Paula Menezes, de Melo Riceto (24) found that phospholipase activity was identified in 99% of *C. albicans* isolates and another research the production of these enzymes was observed among 38% (21), 69% (25) or 81% (11) of *C. albicans* isolates. On the other hand, (24), Abi-Chacra, Souza (26) showed that Phospholipase activity was not detected within *C. parapsilosis* isolates.

3.6. Production of Proteinase Activity

Proteinase activity of pathogenic *Candida* spp. isolated from the oral cavity, and vaginal infections were tested using the Agar plate method. In 21 isolates of *Candida* spp. isolated from the oral cavity. The results shown in table 2 revealed that 12 isolates were strong proteinase activity with a Prz value of < 0.70, while 2 isolates were moderate proteinase activity with a Prz value of (0.70 - 0.79), and 3isolates were weak proteinase activity with Prz value of(0.80-0.89). These 4 isolates were negative Proteinase activity. In ten isolates of *Candida* spp. isolated from vaginal infections, results showed that 5 isolates had strong proteinase activity, while 2isolates were moderate proteinase activity, and 2 isolates had weak proteinase activity. As for these, only one isolate was negative proteinase activity; proteinase is a crucial factor contributing to *Candida* infection that facilitates microbial invasion by damaging mucosa (28). On the other hand, these enzymes down protease breakdown in various host cell proteins, including collagen, keratin, mucus, antibodies, complement, and cytokines (3).

Table 2. Phospholipase, Esterase, and Proteinase activity production by *Candida* spp. from the oral cavity and vaginal infection

Candida isolates	Pz value	Ez value	Prz value
C. albicans (O1)	0.633	0.38	0.436
C. glabrata(O2)	0.766	0.61	0.333
C. guilliermondii (O3)	0.806	0.71	0.523
C. albicans (O4)	0.576	0.42	0.413
C. dubliniensis (O5)	0.673	0.66	0.646
C. albicans (O6)	0.560	0.38	0.360
C. guilliermondii (O7)	0.703	0.42	-
C. dubliniensis (O8)	0.740	0.61	-
C. albicans (O9)	0.663	0.43	0.613
C. albicans(O10)	0.640	0.46	0.543
C. albicans(O11)	0.550	-	0.455
C. albicans(O12)	-	-	0.890
C. albicans(O13)	0.840	0.65	0.771
C. albicans(O14)	0.782	-	0.683
C. albicans(O15)	0.690	-	0.650
C. albicans(O16)	-	-	0.844
C. albicans(O17)	-	-	0.562
C. albicans(O18)	-	-	-
C. albicans(O19)	-	-	-
C. dubliniensis (O20)	-	-	0.770
C. parapsilosis (O21)	-	-	0.802
C. parapsilosis (V22)	0.800	0.62	0.346
C. parapsilosis (V23)	0.660	0.44	0.630
C. parapsilosis (V24)	0.713	-	0.296
C. albicans (V25)	0.570	0.37	0.506
C. albicans (V26)	0.736	0.44	0.423
C. albicans (V27)	-	-	0.810
C. albicans (V28)	-	-	0.782
C. albicans (V29)	-	-	-
C. albicans (V30)	-	-	0.830
C. parapsilosis (V31)	-	0.44	0.780

Pz, Ez, Prz < 0.70 Strong, Pz, Ez, Prz (0.70-0.79) Moderate, Pz, Ez, Prz (0.80-0.89) Weak

In general, the results of this study showed that the number of *Candida* spp. Isolates secreted proteinase was higher than the number that produced phospholipase. Proteinase activity is observed among 70-100% of clinical *C. albicans* isolates (24). While the variable numbers of clinical *C. parapsilosis* isolate producing proteinase were reported at 38%, 87% (29), and 100% (26). The obtained data are in accordance with previous reports demonstrating the high proteinase production among all tested isolates such as *C. albicans, C. parapsilosis*, and rare species.

3.7. Coagulase Activity

Coagulase activity of pathogenic *Candida* spp. isolated from the oral cavity, and vaginal infections were tested by using rabbit plasma. The highest coagulase activity was seen with (100%) in *C. albicans, C. parapsilosis*, and *C. guilliermondii* with (85%), (60%), and (50%) respectively. In contrast, *C. dubliniensis* does not produce coagulase enzyme (Table 3). In a study conducted by Yigit, Aktas (30), they found that the coagulase activities of the different species tested after 24 h incubation at 37° C were: (43%) *C. albicans* isolates and (37%) non- *albicans Candida* strains were positive for coagulase in rabbit plasma.

Table 3. Coagulase Activity of Pathogenic *Candida* spp. after 24 hincubation with Rabbit plasma at 37 ° C

Species	Total No. of isolates	Coagulase Activity
C. albicans	20	17(85%)
C. glabrata	1	1(100%)
C. guilliermondii	2	1(50%)
C. dubliniensis	3	0(0%)
C. parapsilosis	5	3(60%)

3.8. Haemolysin Production

Haemolysin activity of pathogenic *Candida* spp. isolated from the oral cavity, vaginal infections were tested using Sabouraud dextrose agar with blood. In 21 isolates of *Candida* spp. isolated from the oral cavity, results showed that 14 isolates were strong hemolys in production with Hi<0.63, while 7 isolates were moderate hemolysin production with Hi=0.64-0.99. In ten isolates of Candida spp. isolated from vaginal infections, the results revealed that 5 isolates were strong hemolysin production, while 5 isolates were moderate hemolysin production (Table 4).

Table 4. Hemolysin production by *Candida* spp. isolated from the oral cavity and vaginal infection by using the plate method

Candida spp. isolates	Hz value	Candida spp.isolates	Hz value
C. albicans (O1)	0.38	*C. albicans*(O17)	0.54
C. glabrata(O2)	0.66	*C. albicans*(O18)	0.38
C. guilliermondii (O3)	0.83	*C. albicans*(O19)	0.65
C. albicans (O4)	0.81	*C. dubliniensis* (O20)	0.61
C. dubliniensis (O5)	0.62	*C. parapsilosis* (O21)	0.65
C. albicans (O6)	0.42	*C. parapsilosis* (V22)	0.67
C. guilliermondii (O7)	0.82	*C. parapsilosis* (V23)	0.82
C. dubliniensis (O8)	0.61	*C. parapsilosis* (V24)	0.69
C. albicans (O9)	0.47	*C. albicans* (V25)	0.55
C. albicans (O10)	0.58	*C. albicans* (V26)	0.46
C. albicans (O11)	0.40	*C. albicans* (V27)	0.64
C. albicans(O12)	0.84	*C. albicans* (V28)	0.61
C. albicans(O13)	0.61	*C. albicans* (V29)	0.42
C. albicans(O14)	0.48	*C. albicans* (V30)	0.42
C. albicans(O15)	0.60	*C. parapsilosis* (V31)	0.73
C. albicans(O16)	0.39		

O: Isolated from the oral cavity, V: Isolated from vaginal infection
Hi=1 Not hemolysis production, Hi=0.64-0.99 Moderate production, Hi≥0.63 Strong production

Haemolysin activity of pathogenic *Candida* spp. isolated from the oralcavity, and vaginal infections were tested using the spectrophotometric method in 21 isolates of *Candida* spp. The results showed that 16 isolates had strong hemolysin production isolated from the oral cavity, while 5 isolates had low hemolysin production in 10 *Candida* spp. isolated from vaginal infections, the results showed that 5 isolates were strong hemolysin production, while 5 isolates were low hemolysin production (Table 5).

The biofilm formation of the pathogenic *Candida* spp. isolated from the oralcavity, and vaginal infections were tested using the microtiter plate method. Biofilm formation was foundinall *Candida* spp. isolates. In 21 isolates of *Candida* spp. isolated from oral cavity results showed in table 6 revealed that 4 isolates were strong biofilm formation with O.D values>0.320, while16isolates were

moderate biofilm formation with O.D values of 0.120–0.320. In 10 isolates of *Candida* spp. Isolated from vaginal infections, the results showed that 2isolates were strong biofilm formation, while 8 isolates were moderate biofilm formation. The results showed that biofilm formation was changed according to the strains. However, it was found that biofilm formation was generally high.

Candida biofilm growth is the predominant reason for *Candida* antifungal resistance (31, 32). Evidence suggests that *Candida* spp. the pathogenic potential is also influenced by their ability to form biofilms on the abiotic and biotic surfaces (33). Silva, Henriques (34) reported that *Candida albicans* isolates isolated from the vagina produced high levels of biofilms. Paiva, Vidigal (35) reported that *C. tropicalis* produced high levels of biofilm. They reported that *C. parapsilosis and C. glabrata* produced less biofilm than pathogenic *C. albicans*. Rudenko, Vatnikov (1) showed that biofilm production is considered one of the most potent pathogenic traits contributing to treatment failures and recurrent infections and described that *C. tropicalis* produces strongly adherent biofilm followed by *C. albicans*, while *C. parapsilosis* produce no biofilm, which is, in contrast, to a study by Tellapragada, Eshwara (36), where higher biofilm production was seen in *C. albicans*.

Table 5. Haemolysin production by *Candida* spp. Isolated from oral cavity and vaginal infection by using spectrophotometric

***Candida* spp.isolates**	**O.D (543)nm hemolytic**	***Candida* spp.isolates**	**O.D (543)nm hemolytic**
C. albicans (O1)	0.436	*C. albicans*(O17)	0.429
C. glabrata(O2)	0.383	*C. albicans*(O18)	0.437
C. guilliermondii (O3)	0.390	*C. albicans*(O19)	0.429
C. albicans (O4)	0.423	*C. dubliniensis* (O20)	0.410
C. dubliniensis (O5)	0.440	*C. parapsilosis* (O21)	0.429
C. albicans (O6)	0.406	*C. parapsilosis*(V22)	0.386
C. guilliermondii (O7)	0.380	*C. parapsilosis*(V23)	0.373
C. dubliniensis (O8)	0.370	*C. parapsilosis*(V24)	0.413
C. albicans (O9)	0.433	*C. albicans*(V25)	0.426
C. albicans(O10)	0.400	*C. albicans*(V26)	0.433
C. albicans(O11)	0.413	*C. albicans* (V27)	0.422
C. albicans(O12)	0.410	*C. albicans* (V28)	0.410
C. albicans(O13)	0.428	*C. albicans*(V29)	0.405
C. albicans(O14)	0.432	*C. albicans* (V30)	0.427
C. albicans(O15)	0.420	*C. parapsilosis* (V31)	0.390
C. albicans(O16)	0.412		

O: Isolated from the oral cavity, V: Isolated from vaginal infection

Table 6. Biofilm Formation of *Candida* spp. Isolated from oral cavity and vaginal infection after 24 h

***Candida* spp.isolates**	**Detection of biofilm (O.D 450 nm)**	**Candida spp.isolates**	**Detection of biofilm (O.D 450 nm)**
C. albicans (O1)	0.200	*C. albicans*(O17)	0.363
C. glabrata(O2)	0.140	*C. albicans*(O18)	0.300
C. guilliermondii (O3)	0.316	*C. albicans*(O19)	0.204
C. albicans (O4)	0.240	*C. dubliniensis* (O20)	0.300
C. dubliniensis (O5)	0.313	*C. parapsilosis* (O21)	0.353
C. albicans (O6)	0.330	*C. parapsilosis*(V22)	0.176
C. guilliermondii (O7)	0.300	*C. parapsilosis*(V23)	0.206
C. dubliniensis (O8)	0.273	*C. parapsilosis*(V24)	0.280
C. albicans (O9)	0.286	*C. albicans*(V25)	0.156
C. albicans(O10)	0.266	*C. albicans*(V26)	0.283
C. albicans(O11)	0.192	*C. albicans* (V27)	0.352
C. albicans(O12)	0.270	*C. albicans* (V28)	0.260
C. albicans(O13)	0.207	*C. albicans* (V29)	0.238
C. albicans(O14)	0.180	*C. albicans* (V30)	0.306
C. albicans(O15)	0.194	*C. parapsilosis*(V31)	0.360
C. albicans(O16)	0.370		

O: Isolated from the oral cavity, V: Isolated from vaginal infection
O.D values>0.320 Strong, O.D values 0.120–0.320 Moderate, O.D values<0.120 Non/weak

Authors' Contribution

Study concept and design: A. A. H. A.
Acquisition of data: J. A. S. S.
Analysis and interpretation of data: H. A. A.
Drafting of the manuscript: H. A. A.
Critical revision of the manuscript for important intellectual content: A. A. H. A.
Statistical analysis: J. A. S. S.
Administrative, technical, and material support: A. A. H. A.

Ethics

The study protocol was approved by the ethics committee of the Mustansiriyah University, Baghdad, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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