Clin. Lab. 2025;71:XXX-XXX

ORIGINAL ARTICLE

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

Sara S. Maryoush, Hamzia A. Ajah, Raghad A. Abdulrazaq

Department of Biology, College of Science, Al-Mustansiriyah University, Baghdad, Iraq

SUMMARY

Background: **Candidiasis can be present as a cutaneous, mucosal, or deep-seated organ infection, which is caused by more than 20 types of *Candida* spp., with *C. albicans* being the most common. Hence, this work aimed to estimate some virulence factors, including phospholipase and biofilm formation, in some *Candida* spp.**
Methods: **A total of eighty-six specimens were collected from patients with oral and vaginal candidiasis and subjected into different examinations, including cultural characteristic (on Sabouraud Dextrose Agar SDA and chromogenic *Candida* agar) and microscopic examination and germ tube formation (GT)to isolate *Candida* spp. In addition, the egg-yolk agar plate method was used to determine the extracellular phospholipase production, and the microtiter plate method was used to determine biofilm formation of *Candida* spp. Vitek Compact equipment was used to identify the highest phospholipase and biofilm-producers of *Candida* spp.**
Results: **As a result of all examinations, 58.1% (n = 50/86) of isolates of *Candida* spp. were obtained, including 26.7% (n = 23/86) isolates of *Candida* spp. from oral cavity and 31.3% (n = 27/86) isolates of *Candida* spp. from vaginal cavity. These isolates included 58% (n = 29/50) *C. albicans*, 10% (n = 5/50) *C. glabrata*, 6% (n = 3/50) *C. parasilosis*, 6% (n = 3/50) *C. krusei*, 6% (n = 3/50) *C. lusitaniae*, 6% (n = 3/50) *C. kefyr*, 6% (n = 3/50) *C. tropicalis*, and 2% (n = 1/50) *C. ciferrii*. To quantify extracellular phospholipase production, the egg-yolk agar plate method was utilized. The results indicated that the majority of isolates (n = 33; 66%) were phospholipase-strong producers, 18% (n = 9) of isolates were phospholipase-moderate producers, 5% (n = 10) were phospholipase-weak producers, and 6% (n = 3) were non-phospholipase producers. Microtiter plate method was utilized to estimate formation of biofilm by *Candida* spp. obtained from vaginal and oral cavities. The majority of *Candida* spp. isolates (n = 32; 64%) were biofilm-strong producers, followed by 30% (n=15) moderate-biofilm producers and 6% (n = 3) weak-biofilm producers. The results of VITEK 2 system indicated that the probability of *C. albicans*, *C. krusei*, *C. kefyr*, *C. tropicalis*, *C. lusitaniae*, *C. glabrata*, and *C. ciferrii* was 98, 95, 94, 91, 85, 93, and 85 %, respectively.**
Conclusions: ***Candida albicans*** **was the most frequent isolate among all isolates.**
(Clin. Lab. 2025;71:xx-xx. DOI: 10.7754/Clin.Lab.2024.240304)

Correspondence:
Sara Salim Maryoush
Department of Biology
College of Science
Al-Mustansiriyah University
Baghdad
Iraq
Email: sarahsalim670@gmail.com

Manuscript accepted June 4, 2024

KEYWORDS

Candida spp., phospholipase production, candidiasis, virulence factors

LIST OF ABBREVIATIONS

VVC - Vulvovaginal candidiasis
T2DM - Type 2 diabetes mellitus
SDA - Enriched Sabourauddextrose agar
KOH - Potassium hydroxide
GT - Germ tube

YPD - Yeast peptone dextrose
PBS - Phosphate-buffered saline

INTRODUCTION

There are more than 20 different forms of *Candida* spp., with *C. albicans* being the most prevalent, and they may all cause candidiasis, which can manifest as a cutaneous, mucosal, or deep-seated organ infection. This pathogenic yeast is often found in the wholesome microbiome [1]. Candidiasis includes different types, such as vulvovaginal and oral candidiasis. Congenital candidiasis may result from a mother's case of vulvovaginal candidiasis (VVC), which is frequent in the latter months of pregnancy. VVC infections can also lead to miscarriage or premature birth [2,3].

Candida spp. vaginal infections, the second most frequent kind of illness, produce severe vulvovaginal candidiasis in most women. An overgrowth of the fungus *Candida*, most often *Candida albicans*, causes oral candidiasis, a frequent opportunistic infection of the mouth. The prevalence changes with age and other risk variables [4]. *Candida albicans* is the most prevalent species in colonization of the oral mucosa in healthy and immunocompromised individuals. In addition, *Candida albicans* was the most frequent fungus isolated from type 2 diabetes mellitus(T2DM) in Iraqi patients [5]. Nevertheless, *C. dubliniensis, C. guilliermondii, C. krusei, C. glabrata, C. tropicalis,* and *C. parapsilosis* have also been reported [6]. In order to produce disease, *Candida* spp. need some virulence factors to invade the host and survive, including biofilm formation and phospholipases [7,8].

Biofilms are populations of microorganisms that are commonly connected to a surface and wrapped in an extracellular polysaccharide matrix created by the bacteria. The four secreted phospholipases A through D (PLA, PLB, PLC, and PLD) hydrolyze one or more ester bonds of glycerophospholipids on the host cell membrane and are hence crucial players in tissue invasion. These enzymes are responsible for the breakdown of cell membranes and may break down a broad range of host cell proteins such as cytokines, complements, collagen, keratin, antibodies, and mucus [9].

The objectives of the present study were to investigate biofilm formation and phospholipase production from some isolates of *Candida* spp. obtained from Iraqi patients with vaginal and oral candidiasis.

MATERIALS AND METHODS

Isolation of *Candida* isolates

Eighty-six specimens were collected from patients with oral and vaginal candidiasis who attended the AL-Baladi Teaching Hospital during the period from November 2022 to May 2023. These specimens included forty-eight vaginal and forty oral swabs. All specimens, including 40 oral cavity swabs and 46 vaginal cavity swabs, were collected and transported under aseptic conditions to the laboratory. Then, all specimens were cultured on chloramphenicol (250 mg/L) enriched-Sabouraud dextrose agar (SDA) (Himedia/India) and incubated for 48 hours at 37°C under aerobic conditions.

Identification of *Candida* isolates

Cultural examination

Using SDA, the cultural characteristics, including size, texture, color, shape, opacity, and margin of colonies, were investigated. Then, the *Candida* spp. isolates were recultured on selective and differential medium, called chromogenic Candida agar (Himedia/India), for 48 hours at 37°C. After incubation, the color of colonies was utilized as indicator for identification of yeast. CHROMagar Candida is a ready-to-use chromogenic medium that distinguishes *C. krusei*, *C. tropicalis*, and *C. albicans* based on colony color and shape. *C. albicans* colonies are light to medium green, *C. tropicalis* colonies appear dark blue to metallic blue, and *C. krusei* and *C. lusitaniae* colonies are pink with a white border. Other yeasts, such as *C. glabrata*, can generate either light to dark mauve or cream hues on isolation media. Colonies of *C. ciferrii* develop well, are regular spherical with a blue center and white edge, and become rough with gyrus-like grooves and uneven edges [6,10].

Microscopic examination

The isolates were also stained with Gram stain after being placed on a clean slide with a drop of physiological saline and were examined under microscope [10]. Furthermore, a section of the colony was checked with a sterile stick and deposited on a clean slide, which was then mixed with a drop of lactophenol cotton blue, coated with a cover slip, and tested under a light microscope [10].

Germ tube formation (GT)

Germ tube formation by *Candida* spp. isolates was studied by transferring a small portion of colonies using a sterile stick, mixing with 0.5 mL of human serum, and incubating the mixture at 37°C for 2.5 hours. Other yeast species tend to form germ tubes during incubation, therefore keeping the incubation time under 3 hours is essential. Drops of the cultured serum were placed on microscope slides using a pasteur pipette, and any germ tubes that formed were counted. The human serum was prepared based on the method described by Yin et al. [11]. The detection and distinction of *Candida* spp. was performed after 48 hours of incubation at 45°C.

Detection of some virulence factors of *Candida* spp.

Extracellular phospholipase production

To quantify extracellular phospholipase production, the egg yolk agar plate method was utilized. Ten microliters of fresh inoculum of *Candida* (containing 24-hour-old colonies re-suspended in five milliliters of sterile nor-

mal saline with 1.5×10^8 CFU/mL) was added into egg yolk agar medium. The plates were placed into an incubator for 48 hours at 37°C. The opacity zone's diameter and the colonies' diameters were estimated to calculate the value of phospholipase (Pz) with the following equation [12]:

$$Pz = \frac{The colony diameter\ (a)}{The diameter of colony + Precipitation zone\ (b)}$$

The results are illustrated in Table 1.

Table 1. The ranges used to evaluate phospholipase activity.

Value of Pz	Phospholipase activity
1	**Negative**
0.80 - 0.89	**Weak**
0.70 - 0.79	**Moderate**
< 0.70	**Strong**

Biofilm formation

The formation of biofilm was estimated using polystyrene 96-well microplates. Two milliliters of broth of yeast peptone dextrose (YPD) were utilized to culturing *Candida* spp. for 24 hours at 37°C. Serial dilution was performed to obtain 1:20 *Candida*-cultured broth of YPD as a final solution. Each well plate was filled with two hundred microliters of *Candida*-cultured YPD broth, except for the control wells, which filled with 200 µL of fresh YPD. A lid was used to seal the plate. The plate was placed into an incubator for 24 hours at 37°C. Then, the media in the wells was discarded and the plate was cleaned twice using sterile phosphate buffer saline (PBS) to eliminate any unbonded cells. The plate was left for fifteen minutes at room temperature. Then, 200 µL of crystal violet were filled into each well, and the plate was left to stand for twenty minutes. The content of the wells was discarded by using PBS three times in order to eliminate unattached stain, and the plate was left to dry at room temperature. Two microliters of solution (20:80 v/v; acetone:ethanol) were filled in each well. ELISA reader was utilized to read the OD at 450nm. The results were determined as described by Tartor et al., 2022 [13], as shown in Table 2.

Table 2. The values used to evaluate biofilm formation.

Value of OD	Formation of biofilm
> 0.320	**Strong**
0.120 - 0.320	**Moderate**
< 0.120	**Weak**

VITEK 2 system identification of *Candida* spp.

The Vitek Compact equipment, manufactured by bio-Merieux Inc. in Durham, NC 27712, USA, was used to identify *Candida* spp. in accordance with the manufacturer's protocol. To meet the standards set out in 21 CFR sections 11, which deal with electronic records and signatures, these protocols incorporate the Vitek Compact instrument. Each of the reagent cards has 64 wells, and those wells are specifically made to hold a specific kind of test substrate. Acidification, alkalinization, enzyme hydrolysis, and compound formation are just a few of the many metabolic activities that may be quantified using substrates in the presence of inhibitory substances. An optically clear covering coats both sides of the card, which together form a sealed vessel that prevents oxygen delivery from interacting with the substrate admixtures of the organism. Inserting the card into the device reveals information about the product's category, lot number, expiration date, and sample's unique identification through the bar codes on the outside.

RESULTS

Figure 1 shows the color of the colonies; each colored colony indicated presence of specific species of *Candida*. Colonies of *C. albicans* appeared as light green colonies, while the fuzzy, purple colonies indicated presence of *C. krusei*.

As a result of all examinations, 58.1% (n = 50/86) of isolates of *Candida* spp. were obtained, including 26.7% (n = 23/86) isolates of *Candida* spp. from oral cavity and 31.3% (n = 27/86) isolates of *Candida* spp. from vaginal cavity. These isolates include 58% (n = 29/50) *C. albicans*, 10% (n = 5/50) *C. glabrata*, 6% (n = 3/50) *C. parasilosis*, 6% (n = 3/50) *C. krusei*, 6% (n = 3/50) *C. lusitaniae*, 6% (n = 3/50) *C. kefyr*, 6% (n = 3/50) *C. tropicalis*, and 2% (n = 1/50) *C. ciferrii*, as shown in Figure 2.

Production of phospholipase enzyme was estimated among isolates of *Candida* spp. by using egg yolk agar plate method, whereas the results indicated that the majority of isolates (n = 33; 66%) were phospholipase-strong producers, 18% (n = 9) of isolates were phospholipase-moderate producers, 5% (n = 10) were phospholipase-weak producers, and 6% (n = 3) were non-phospholipase producers, as mentioned in Tables 3 and 4. Microtiter plate method was utilized to estimate formation of biofilm by *Candida* spp. obtained from vaginal and oral cavities. Based on the Tables 5 and 6, the majority of isolates (n = 32; 64%) were biofilm-strong producers, followed by 30% (n = 15) moderate-biofilm producers and 6% (n = 3) weak-biofilm producers.

VITEK 2 system identification of *Candida* spp.

According to the manufacturer's protocol, Vitek Compact equipment was used to identify *Candida* spp., as represented in Figures3 A, B, C, D, E, F, and G. The re-

Table 3. Phospholipase values of vaginal and oral *Candida* spp.

Candida spp. (CV)	Pz value	*Candida* spp. (CO)	Pz value
C. albicans CV1	0.52	*C. albicans*CO28	0.41
C. albicans CV2	0.57	*C. albicans*CO29	0.75
C. albicans CV3	0.77	*C. albicans*CO30	0.82
C. albicans CV4	0.48	*C. albicans*CO31	0.55
C. albicans CV5	0.46	*C. albicans*CO32	0.64
C. albicans CV6	0.35	*C. albicans*CO33	0.52
C. albicans CV7	0.49	*C. glabrata*CO34	0.32
C. albicans CV8	0.76	*C. glabrata*CO35	-
C. albicans CV9	0.55	*C. glabrata*CO36	0.81
C. albicans CV10	0.82	*C. glabrata*CO37	0.54
C. albicans CV11	0.56	*C. glabrata*CO38	0.36
C. albicans CV12	0.85	*C. krusei*CO39	0.32
C. albicans CV13	0.33	*C. krusei*CO40	0.71
C. albicans CV14	0.49	*C. krusei*CO41	0.46
C. albicans CV15	0.54	*C. lusitaniae*CO42	0.73
C. albicans CV16	0.50	*C. lusitaniae*CO43	0.72
C. albicans CV17	0.72	*C. kefyr*CO44	-
C. albicans CV18	0.62	*C. kefyr*CO45	0.35
C. albicans CV19	0.44	*C. kefyr*CO46	0.86
C. albicans CV21	0.54	*C. tropicalis*CO48	0.74
C. albicans CV20	0.63	*C.tropicalis*CO47	0.73
C. albicans CV22	0.35	*C. tropicalis*CO49	0.46
C. albicans CV23	0.42	*C. ciferrii*CO50	0.43
C. parasilosis CV24	-		
C. parasilosis CV25	0.43		
C. parasilosis CV26	0.49		
C. lusitaniae CV27	0.54		

*** CV - *Candida* species isolated from vagina, CO - *Candida* species isolated from oral cavity.**

Table 4. The degree of phospholipase production in vaginal and oral *Candida* spp.

Isolate source	Phospholipase production				Total
	negative	weak	moderate	strong	
Oral isolates	2 (4%)	3 (6%)	6 (12%)	12 (24%)	23 (46%)
Vaginal isolates	1 (2%)	2 (4%)	3 (6%)	21 (42%)	27 (54%)
Total	3 (6%)	5 (10%)	9 (18%)	33 (66%)	50 (100%)

sults indicated that the probability of *C. albicans*, *C. krusei*, *C. kefyr*, *C. tropicalis*, *C. lusitaniae*, *C. glabrata*, and *C. ciferrii* was 98, 95, 94, 91, 85, 93, and 85%, respectively.

DISCUSSION

The size, texture, color, shape, opacity, and margin colonies were estimated on SDA. The results revealed that some colonies appeared as yeast-like, smooth with

Table 5. The biofilm OD of vaginal and oral *Candida* spp.

Candida spp. (CV)	OD value	*Candida* spp. (CO)	OD value
C. albicans CV1	1.041	*C. albicans*CO28	0.904
C. albicans CV2	0.123	*C. albicans*CO29	0.524
C. albicans CV3	0.121	*C. albicans*CO30	0.233
C. albicans CV4	0.832	*C. albicans*CO31	0.420
C. albicans CV5	1.152	*C. albicans*CO32	1.395
C. albicans CV6	1.342	*C. albicans*CO33	0.780
C. albicans CV7	0.451	*C. glabrata* CO34	1.125
C. albicans CV8	0.823	*C. glabrata* CO35	0.90
C. albicans CV9	0.071	*C. glabrata* CO36	0.240
C. albicans CV10	0.301	*C. glabrata* CO37	0.493
C. albicans CV11	0.081	*C. glabrata*CO38	1.230
C. albicans CV21	0.093	*C. krusei*CO39	0.561
C. albicans CV13	0.395	*C. krusei*CO40	0.311
C. albicans CV14	0.672	*C. krusei*CO41	1.831
C. albicans CV15	0.525	*C. lusitaniae*CO42	0.192
C. albicans CV16	0.185	*C. lusitaniae*CO43	0.816
C. albicans CV17	0.875	*C. kefyr*CO44	0.190
C. albicans CV18	0.380	*C. kefyr*CO45	0.730
C. albicans CV19	0.141	*C. kefyr*CO46	0.267
C. albicans CV20	0.183	*C. tropicalis*CO47	0.584
C. albicans CV21	0.530	*C. tropicalis*CO48	0.344
C. albicans CV22	0.301	*C. tropicalis*CO49	1.141
C. albicans CV23	0.966	*C. ciferrii*CO50	1.240
C. parasilosis CV24	0.251		
C. parasilosis CV25	0.175		
C. parasilosis CV26	1.131		
C. lusitaniae CV27	0.820		

*** CV - *Candida* species isolated from vagina, CO - *Candida* species isolated from oral cavity, Pz - Phospholipase.**

Table 6. Distribution of *Candida* biofilm formation according to infection site.

Isolate source	Biofilm formation			Total
	weak	moderate	strong	
Oral isolates	0 (0%)	6 (12%)	17 (34%)	23 (46%)
Vaginal isolates	3 (6%)	9 (18%)	15 (30%)	27 (54%)
Total	3 (6%)	15 (30%)	32 (64%)	50 (100%)

white color, which expected to be colonies of *C. albicans*, while some colonies observed as smooth, shiny, and creamy with white color, which expected to be colonies of *C. parapsilosis*. Also, colonies of *C. glabrata*, *C. kefyr*, *C. ciferii*, and *C. tropicalis* appeared as smooth, glossy, and cream-colored, while *C. krusei* shows rough colonies on this medium; these results correspond with a previous study conducted by Al-Dabbagh et al., 2023 [10].

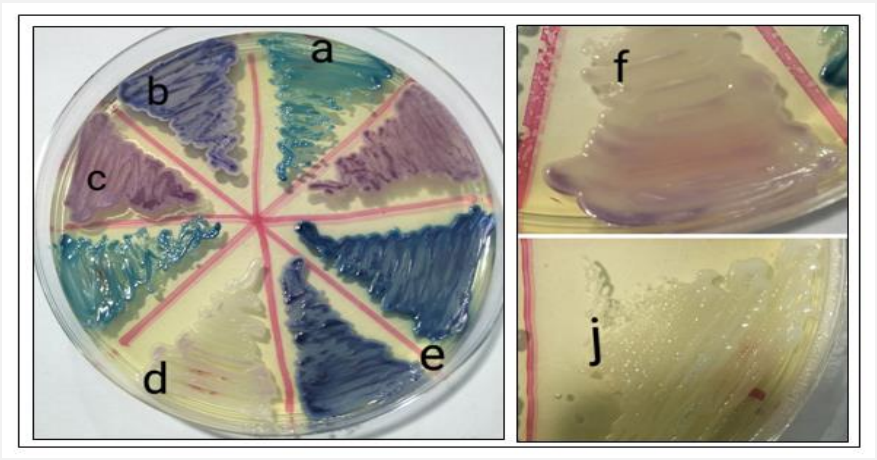

Figure 1. *Candida* spp. growth on CHROMagar Candida (CaC) (24 hours/37ºC).

a) *C. albicans*; b) *C. krusei*; c) *C. glabrata*; d) *C. kefyr*; e) *C. tropicalis*; f) *C. parasilosis,* and j) *C.ciferrii.*

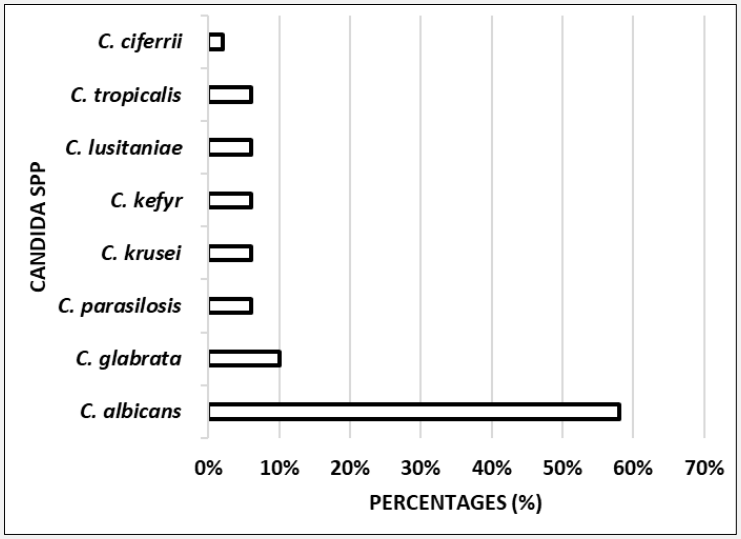

Figure 2. The distribution of *Candida* spp. among the samples.

The colony morphology of *Candida* species can be used to distinguish them from other organisms when using chromogenic Candida agar, which is a differential and selective medium that facilitates the quick separation of *Candida* from mixed culture. *Candida* spp., specifically *C. albicans* and *C. glabrata*, produce specific enzymes that react only with the chromogenic Candida agar's reactive substrate [14].

According to Figure 1, each colored colony indicated the presence of specific species of *Candida*. Colonies of *C. albicans* appeared as light green colonies, while the fuzzy and purple colonies indicated the presence of *C.*

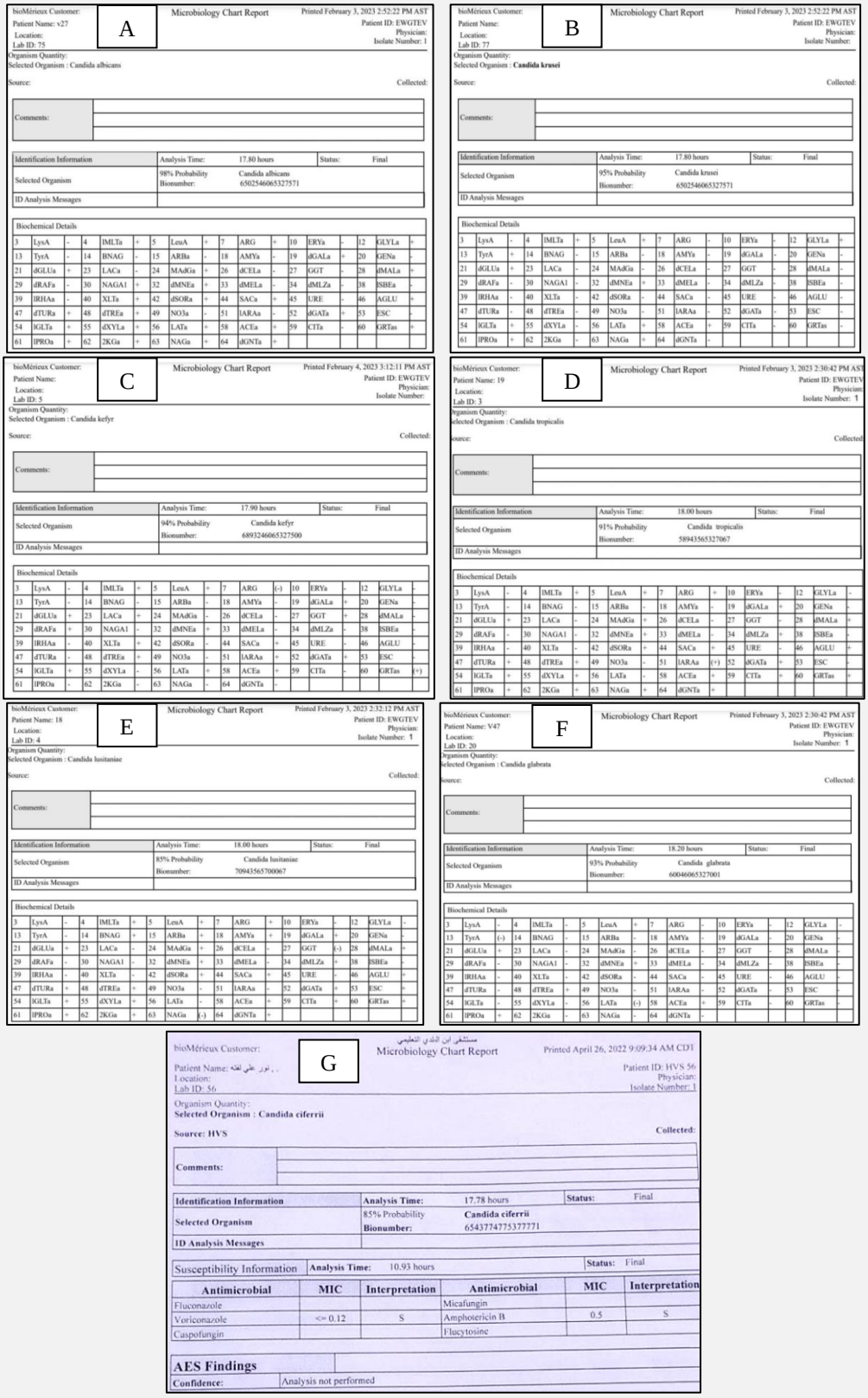

Figure 3. Results of *Candida* spp. identification using VITEK 2 system.

A) *C. albicans*, B) *C. krusei*, C) *C. kefyr*, D) *C. tropicalis*, E) *C. lusitaniae*, G) *C. glabrata*, and F) *C. ciferrii*.

krusei. Also, pink colonies indicated *C. glabrata*, cream to white colonies with slight pink center indicated *C. kefyr*, blue to purple colonies indicated *C. tropicalis*, creamy to slight purple colonies indicated *C. parasilosis,* and white to creamy colonies indicated *C. ciferii* and *C. lusitaniae*. A different study, conducted by Bayona et al., 2020 [15], is in agreement with these results. Under microscopy, the size and shape of cells of *Candida* spp. were identified. The results indicated that cells of *Candida* were spherical or of an oval shape with budding like a yeast, which is in agreement with Al-Dabbagh et al., 2023 [10] and Bayona et al., 2020 [15]. In a study conducted by Ajah et al., 2020 [16], 41.98% of the cases (n = 56 out of 111 cases) showed positive results for *Candida* spp., including 56 *Candida* isolates from vaginal swabs, whereas the majority of isolates was *C. albicans* (n = 41; 73%), which is in agreement with findings of this study, followed by *C. glabrata* 5 (9%), *krusei* 2 (3.5%), and other spp. Also, there were 55 different *Candida* spp. isolates from oral swabs, including *C. tropicalis* 1(1.8%), *C. krusei* 2 (3.6%), and *glabrata* 7 (13%), while 36 (65%) were *C. albicans*.

In addition, it has been reported that *C. famata*, *C. glabrata,* and *C. albicans* were the most common species of yeast obtained from vaginal infection, with percentages 14.8%, 44.5%, and 45%, respectively [17].

The findings showed that *C. albicans* isolates produce germ tubes, which are lengthy, tube-like extensions from yeast cells, and the results indicated that the other non-*C. albicans* species showed to be negative, which is in agreement with Al-Dabbagh et al., 2023 [10]. After being incubated with human serum, the germ tubes produced for just two hours. After being incubated with human serum, the germ tubes produce in just two hours. All *C. albicans* isolates in this study had the ability to generate germ tubes when analyzed in a colony setting, which is consistent with the findings conducted by Matare et al., 2017 [18]. *C. albicans* can be detected rapidly to the development of germ tubes in human serum. *C. albicans* produce germ tubes in human serum, which causes the fungus to transform from yeast to a filamentous or mycelial growth. Fungi often respond to changing environmental conditions by undergoing morphological changes, which may help the fungus to accommodate to new biological elements. *Candida albicans* and *C. dubliniensis* are the only organisms known to have successfully formatted a germ tube [18,19].

In a study conducted by Mohammedet al., 2017 [17], it has been reported that 7 isolates were non-phospholipase producers, 2 of the isolates were strong-phospholipase producers, and 2 of the isolates were moderate-phospholipase producers. Also, 87.3% (n = 48) out of 55 isolates revealed activity of phospholipase and 7 (12.7%) had no activity of phospholipase in the study conducted by Ajah et al., 2020 [16]. Released phosphorlipases are extracellular hydrolytic enzymes. According to the study conducted by Larkin et al., 2017 [20], synthesis phospholipase is dependent on the strain of producer. De Paula et al., 2016[21], reported that activity of phospholipase was detected in 99% of *C. albicans* isolates [21]. Phospholipases are enzymes that cause the breakdown of cell membranes by hydrolyzing the ester linkages in glycerophospholipids, this means phospholipases can either bind to the outside of cells or be discharged into the surrounding environment [22].

The outcomes demonstrated that biofilm development varied between strains. Biofilm production was shown to be prevalent among *Candida* spp. In agreement with this study, it has been reported that the majority (n = 10) of *Candida* species were moderate- and strong-biofilm producers [14]. Antifungal resistance in *Candida* is mainly caused by the development of biofilms. The capacity to form biofilms on abiotic and biotic surfaces has been linked to the pathogenic potential of *Candida* spp. [23].

CONCLUSION

The findings of this study suggested that the majority of *Candida* spp., especially *C. albicans*, poses a risk to human health, which may be attributed to their ability to secrete phospholipase and form biofilm.

Acknowledgment:
The authors thank Mustansiriyah University in Iraq (https://www.uomustansiriyah.edu.iq/) for their support of the present research.

Ethical Approval:
The study protocol was approved by the Ethics Committee of the Mustansiriyah University, Baghdad, Iraq.

Source of Funds:
Self-funded.

Declaration of Interest:
The authors declare that they have no conflicts of interest.

References:

1. Bhattacharya S, Sae-Tia S, Fries BC. *Candidiasis* and Mechanisms of Antifungal Resistance. Antibiotics (Basel) 2020;9(6): 312. (PMID: 32526921)

2. Samuel O, Ifeanyi O, Ugochukwu O. Prevalence of *Candida* species among vaginitis symptomatic pregnant women attending ante-natal clinic of Anambra State University Teaching Hospital, Awka, Nigeria. Bioengin Biosci 2015;3(2):23-7. https://www.hrpub.org/download/20150510/BB2-10103802.pdf

3. Al-Baqer TM, Al-Gharrawi SAR, Saeed NAA. Causative Microorganisms and Antibiotics Susceptibilities in Children with Urinary Tract Infection. Al-Mustansiriyah J Sci 2021;32(1):5. https://www.researchgate.net/publication/349484270_Causative_Microorganisms_and_Antibiotics_Susceptibilities_in_Children_with_Urinary_Tract_Infection

4. Akpan A, Morgan R. Oral candidiasis. Postgrad Med J 2002; 78(922):455-9. (PMID: 12185216)

5. Al-Badri AS, Ali EN, Ali Ajah HH, Ali Ajah HH. Effect of*IL-23 Receptor* Gene Polymorphism (Rs1884444) on the Prevalence of Oral Fungal Infection in Patients with Type 2 Diabetes Mellitus: A Case-Control Study in Iraqi Patients. Arch Razi Inst 2022; 77(5):1553-60. (PMID: 37123139)

6. Eghtedar Nejad E, Ghasemi Nejad Almani P, Mohammadi MA, Salari S. Molecular identification of *Candida* isolates by Real-time PCR-high-resolution melting analysis and investigation of the genetic diversity of *Candida* species. J Clin Lab Anal 2020; 34(10):e23444. (PMID: 32656934)

7. Amona FM, Wend-Lasida Ouedraogo R, Sangare I, Elola A. The Role of *Candida* Albicans in Routine Clinically Suspected Otomycosis. West Afr J Med 2021;38(8):743-8. (PMID: 34499919)

8. Abid SA, Aziz SN, Saeed NAAA, et al. Investigation of Virulence Factors in Microbial Organisms that Associated with Public Health Risk Isolates from Different Environmental Regions. Al-Mustansiriyah J Sci 2023;33(5):1-7. https://mjs.uomustansiriyah.edu.iq/index.php/MJS/article/view/1303

9. Cerminati S, Paoletti L, Aguirre A, Peirú S, Menzella HG, Castelli ME. Industrial uses of phospholipases: current state and future applications. Appl Microbiol Biotechnol 2019;103(6): 2571-82. (PMID: 30729255)

10. Ali Hameed Al-Dabbagh A, Ali Ajah H, Abdul Sattar Salman J. Detection of Virulence Factors from *Candida* spp. Isolated from Oral and Vaginal Candidiasis in Iraqi Patients. Arch Razi Inst 2023;78(1):465-74. (PMID: 37312700)

11. Yin P, Peter A, Franken H, et al. Preanalytical aspects and sample quality assessment in metabolomics studies of human blood. Clin Chem 2013;59(5):833-45. (PMID: 23386698)

12. Fule SR, Das D, Fule RP. Detection of phospholipase activity of *Candida* albicans and non albicans isolated from women of reproductive age with vulvovaginal candidiasis in rural area. Indian J Med Microbiol 2015;33(1):92-5. (PMID: 25560009)

13. Tartor YH, Elmowalid GA, Hassan MN, Shaker A, Ashour DF, Saber T. Promising Anti-Biofilm Agents and Phagocytes Enhancers for the Treatment of *Candida albicans* Biofilm-Associated Infections. Front Cell Infect Microbiol 2022;12:807218. (PMID: 35846767)

14. Vecchione A, Florio W, Celandroni F, Barnini S, Lupetti A, Ghelardi E. Comparative evaluation of six chromogenic media for presumptive yeast identification. J Clin Pathol 2017;70(12): 1074-8. (PMID: 28663328)

15. Mulet Bayona JV, Salvador García C, Tormo Palop N, Gimeno Cardona C. Evaluation of a novel chromogenic medium for *Candida* spp. identification and comparison with CHROMagar™ *Candida* for the detection of *Candida* auris in surveillance samples. Diagn Microbiol Infect Dis 2020;98(4):115168. (PMID: 32927410)

16. Ajah HA, Hassan AS, Rahi GK. Isolation and Identification of *Candida* Species from oral and vaginal and determination of virulence factor. Plant Arch 2020;20(1):2697-706. https://www.plantarchives.org/20-1/2697-2706%20(6014).pdf

17. Mohammed NA, Ajah HA, Abdulbaqi NJ. Detection the prevalence of adhesins and extracellular hydrolytic enzymes genes in *Candida* albicans biofilm formation. Iraqi J Sci 2017;58(2):988-1000. https://ijs.uobaghdad.edu.iq/index.php/eijs/article/view/5931/1870

18. Matare T, Nziramasanga P, Gwanzura L, Robertson V. Experimental Germ Tube Induction in *Candida albicans*: An Evaluation of the Effect of Sodium Bicarbonate on Morphogenesis and Comparison with Pooled Human Serum. Biomed Res Int 2017;2017: 1976273. (PMID: 28656137)

19. Deorukhkar SC, Santosh S, Jadhav P. Evaluation of different media for germ tube production of *Candida albicans* and *Candida dubliniensis*. Int J Biomed Adv Research 2012;3(9):704-7. https://www.researchgate.net/publication/272761632_EVALUATION_OF_DIFFERENT_MEDIA_FOR_GERM_TUBE_PRODUCTION_OF_CANDIDA_ALBICANS_AND_CANDIDA_DUBLINIENSIS

20. Larkin E, Hager C, Chandra J, et al. The Emerging Pathogen *Candida* auris: Growth Phenotype, Virulence Factors, Activity of Antifungals, and Effect of SCY-078, a Novel Glucan Synthesis Inhibitor, on Growth Morphology and Biofilm Formation. Antimicrob Agents Chemother 2017;61(5):e02396-16. (PMID: 28223375)

21. de Paula Menezes R, de Melo Riceto ÉB, Borges AS, de Brito Röder DVD, dos Santos Pedroso R. Evaluation of virulence factors of *Candida* albicans isolated from HIV-positive individuals using HAART. Arch Oral Biol 2016;66:61-5. (PMID: 26913969)

22. Mba IE, Nweze EI. Mechanism of *Candida* pathogenesis: revisiting the vital drivers. Eur J Clin Microbiol Infect Dis 2020;39(10): 1797-819. (PMID: 32372128)

23. Jawdat S. Determination of some virulence factors of *Candida* spp. isolated from locally produced cheese in Diyala Governorate-Iraq. Al-Mustansiriyah J Sci 2017;27(4):1-6. https://www.researchgate.net/publication/314129240_Determination_of_some_virulence_factors_of_Candida_spp_isolated_from_locally_produced_cheese_in_Diyala_Governorate-Iraq