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Molecular Detection of *Candida spp.* Isolated from Female Patients Infected with COVID-19 in Baghdad City

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Abstract: Coronavirus disease 2019 (COVID-19) is an infectious disease with severe acute respiratory syndrome and first recognized in Wuhan, China, and it has since spread to the world, resulting in the coronavirus pandemic to 2020. The present study aimed to evaluate Molecular study of some types of vaginal fungi isolated from recovered women from Covid-19 in Baghdad governorate. The study was conducted on 213 samples collected between December 2021 and March 2022, where the number of positive samples reached 188 with percentage 88.26%, while the number of negative samples reached 25 with percentage 11.73% by taking vaginal swabs from various female patients in Al- Kadhimiya Teaching Hospital. Three of *Candida* spp. were isolated: *Candida albicans*, which appeared in 60 samples with a percentage 41.37%, 50 isolates from *Candida glabrata* with a percentage 34.48% , 58 isolates from *Candida pichia* with a percentage 24.13% , all *Candida spp*. It was conclude that molecular diagnosis results of fungal isolates using the polymerase chain reaction technique

Keywords: Vaginitis, vaginal fungi, molecular diagnoses, PCR, Covid-19 virus.

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Introduction

As a medical term, vaginitis refers to any ailment characterized by atypical vaginal symptoms such as discharge, odor, and irritation, itching, or burning. Vaginitis is a common condition that affects almost every woman at some point. Both patients and clinicians tend to Many pathogenic components of these microorganisms are responsible for the capacity of fungus of the genus *Candida*, notably *C. albicans*, to colonize or infect different places (tissues) in the human body (1). Understate the severity of vulvovaginal candidiasis (VVC), despite the fact that it is a leading cause of vaginal illness in women across the globe. More recent research has focused on the complexities of the interaction between yeast-like fungi and host cells, the host's reaction to infection, and how these factors influence the progression and therapy of VVC (2). The 2019 coronavirus illness (COVID-19) is a global emergency due to its fast spread and high fatality rate. Infections with the severe acute respiratory syndrome coronavirus (SARS-CoV) (3).The virus responsible for COVID-19 are rising quickly across the globe. There is an elevated risk of pneumonia, acute respiratory distress syndrome (ARDS), and multiple organ failure in COVID-19-infected patients (3). *Candida* is the genus responsible for the opportunistic

fungal infection known as candidiasis. COVID-19-associated candidiasis (CAC) is bringing new challenges to the already difficult task of preventing Candida infections (2). By using Polymerase Chain Reaction (PCR), to subjecting a sample of starting material to a series of temperature cycles in order to produce multiple copies of a specific nucleic acid sequence. This is a huge benefit, as it means that any sample, regardless of whether it can be cultured or not, can have a greater yield (4).

Materials and methods fungal isolation

This study was done between December 2022 and March 2022. A female 213 patients were recruited in this study in Al-Kadhimiya teaching hospital. According to morphological features, microscopic examination and PCR techniques of three species of *Candida* was detected in women patients. These species were *Candida albicans*, The ages of this patients between 21 – 60 years .The specimens were taken by sterilized cotton swabs and then put them in containers that contain a gel to keep the fungi available for long times until they are examined and cultured in laboratory and PCR technique. the specimen was cultured on SDA medium for 2-5 days in 37 ºC , and smear examined immediately under microscope for direct examination.

Analysis through both microscopic and macroscopic means:

This investigation included a fungal identification step. This distinction was based on the following:

1- The Marks of a Colony (color, consistency and topography).
2- Negative colonial trend (color, significant pigment).
3- Morphology at the microscopic level.

Quantitation of DNA

Extracted DNA concentration was measured using a Quantus fluorometer to ensure the quality of samples for further analysis. An amount of 1 µl of DNA was mixed with 200 µl of diluted.

Primer preparation

The primer used in this study listed in (Table 1):

Table (1): The primer used in this study.

Primer Name	Vol. of nuclease free water (µl)	Concentration (pmol/µl)
ITS1	300	100
ITS4	300	100

Lyophilized primer produced by the Macrogen Company. After being lyophilized, the primers were reconstituted in nuclease-free water at a concentration of 100 pmol/µl so that they could be used as a stock solution. In order to get a working solution with a concentration of 10 p mol/µl, we began by diluting 10 µl of the primer stock solution, which had been stored in the freezer at a temperature of -20 °C, with 90 µl of nuclease-free water as shown in (Table 2).

Table (2): Components of mixture with their size.

Interaction components	Size (ml)
Green master Mix	**12.5**
Primer forward	**1**
Primer Revers	**1**
Deionized D.W	**7.5**
DNA	**2**
Total	**25**

PCR Program: The optimum condition of detection used in this study listed in (Table 3).

Table (3): DNA amplification program.

Steps	Temperture(°C)	Time	Cycle
Initial Denaturation	95	5 min	1
Denaturation	95	30 sec	30
Annealing	55	30sec	
Extension	72	30sec	
Final extension	72	30sec	1
Hold	10	10 min	

Agarose gel electrophoresis

After carrying out of the genomic DNA extraction from *Candida* spp. isolates, the agarose gel electrophoresis was adopted to confirm the presence and integrity of this extracted genomic DNA according to the gel electrophoresis protocol which included the following steps:

Solutions

The DNA ladder marker, 1 X TAE buffer, and Ethidium bromide (10 mg/ml).

Preparation of agarose

1. A flask was filled with 100 cc of 1X TAE.
2. The buffer was supplemented with 1 g of agarose (1 percent).
3. To ensure that the gel particles were completely dissolved, the solution was brought to a boil (in a water bath).
4. Agarose was mixed with ethidium bromide (10 mg/ml) at a concentration of 1 Ethidium bromide (10 mg/ml) was added to agarose at a concentration of 1 µl.
5. For proper mixing and to prevent bubble formation, the agarose was swirled.
6. We let the solution cool down to around 50-60 degrees Celsius**.**

Casting of the horizontal agarose gel

The agarose solution was poured into the gel tray and allowed to set for 30 minutes at room temperature after being taped shut on both sides. We took off the comb and carefully poured the gel into the tray. The tray was filled with 1X TBE-electrophoresis buffer to a depth of about 3-5 mm above the gel's surface.

DNA loading

The PCR products were directly loaded. In this case, 5 l of PCR product was added to the wells straight away. For 60 minutes, 100v/mAmp of electricity was on. Positive cathode to anode gene transfer. Using gel imaging

equipment, bands in gel that were stained with ethidium bromide could be detected as shown in (Table 4).

Standard sequencing

Sanger sequencing was performed on PCR products using an ABI3730XL from Macrogen Corporation in Korea. The data was sent through email and processed using cutting-edge programs.

Table (4): DNA concentration (ng/µl)

Sample	Concentration
1	20
2	25
3	21
4	17
5	22
6	19

Results and Discussion

In the present study ,the most common isolated was *C. albicans* (41.37%), *C. glabrata* (34.42%), *C. pichia* (24.13%). Colony characteristic and morphology, color, texture and cell shape as show in figure (1).

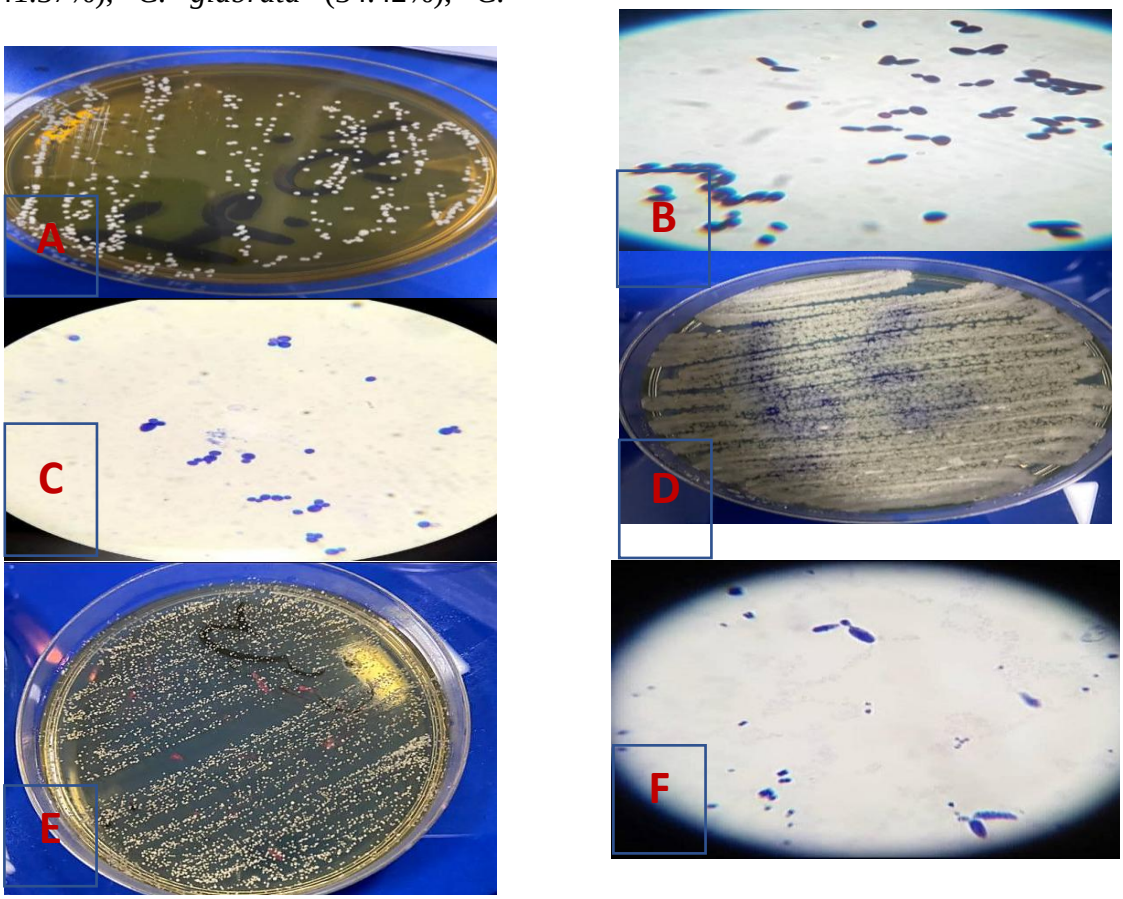

Figure (1): *Candida* spp. after 2-5 days of incubation in SDA medium at 37 ºC :(A) Top view: a colony of *Candida albicans* (B) microscopic features of *Candida albicans* (40X) (C) Top view: *a* colony of *Candida glabrata* (D) microscopic features of *Candida glabrata* (40X) (E) Top view: a colony of *Candida Pichia* (F) microscopic features of *Candida Pichia* (40X).

To confirm the identification of some *Candida* spp., three isolates were identified by PCR and sent for gene sequence. In line with the traits previously mentioned for this fungus, these isolates demonstrated the production of concentric rings typical of *Candida* spp.It is required to validate the species by molecular approaches, one of which is a sequencing test, since although colony morphology is useful for identifying fungus of this genus, it is inadequate to differentiate the species. As show in table (5).

Table (5): The concentration of DNA in each *Candida* spp.

Sample No.	*Candida* spp.	DNA Concentration (ng/µl)
1	*C.albicans*	20
2	*C.albicans*	25
3	*C.albicans*	21
4	*C.glabrata*	17
5	*C.pichia*	22
6	*C.pichia*	19

Next, the agarose gel electrophoresis using the Ethidium Bromide. M: 100bp ladder marker and a concentration between 37·1 to 72·5 µg ml^{-1} validated the DNA's purity (1.4-1.8). The results in lanes 1–6 seem like PCR samples. To conduct molecular characterisation, many vaginal fungal isolates were tested by amplification of a segment of nuclear rDNA including the ITS1, ITS4, and 5.8S rRNA gene. All the isolates had their intergenic spacer region effectively amplified, and all of the isolates from a particular species consistently gave a product size of roughly ~ 100 bp in figure (2).

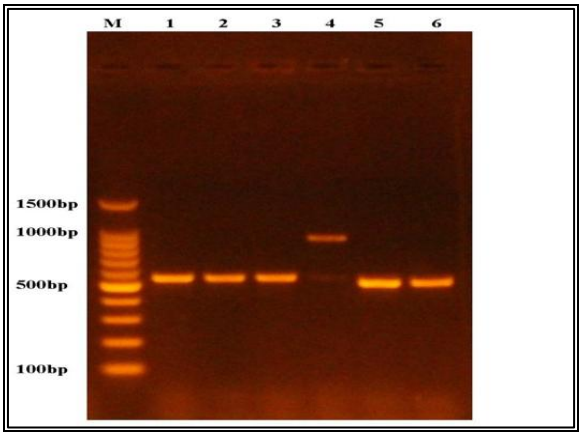

Figure (2): On an electrophoretic 1 percent agarose gel stained with Eth.Br. M: 100bp ladder marker, we separated the amplification products of the ITS1,ITS4 gene from fungi of unknown species. PCR-type products may be seen in lanes 1–6.

The polymerase chain reaction (PCR) has shown to be a reliable method for identifying a wide range of bacteria, suggesting that it might be a valuable diagnostic tool. This finding is consistent with results from other research showing that ITS sequencing analysis provides a more rapid, accurate, and reliable diagnosis at the species and subspecies levels than traditional laboratory approaches (9). The ITS region is the starting point in the tower spices typing approach for identifying certain species of *Candida*;

the ITS1 and ITS4 PCR primers are a highly specific and efficient method for identifying and characterizing some species of *Candida*.

Of several *Candida* spp. and 50 µl (10 pmol) of forward primer PCR product, three samples were submitted for sequencing analysis. For this gene were submitted to the Macrogene business to evaluate the DNA sequencing in these genes. The sequence analysis was analyzed by using a blast search on the NCBI website (http://www.ncbi.nlm.nih.gov) using the BioEdit application to look for polymorphism. After sequencing analysis on blast website, acquired results allowed to determine some *Candida* at species level. Result indicated the presence of three different species which is *C. albicans, C. glabrata, C. pichia* .

Based on their findings, authors Schoch *et. al*., (10) and Bengtsson-Palme (11) the authors conclude that clinical isolates may be identified to the species level using PCR-automated DNA sequencing, and the internal transcribed spacer (ITS) region has the best likelihood of successful identification for the widest variety of fungi. As a very easy and quick technique, this method offers a useful molecular tool for diagnosing mycological infections. In addition, identified *Candida* spp. using the same primer and exhibited almost identical PCR results (12).

These sequences came from the *C. albicans, C. glabrata, and C. pichia Candida* strain 600313/19. They allow partial sequences of the genes for small subunit ribosomal RNA, internal transcribed spacer 1, and 5.8S ribosomal RNA as well as the whole sequences of internal transcribed spacer 4 and large subunit ribosomal RNA (13), as shown in the Table (6).

Table (6): 18S ribosomal RNA gene sequencing of the fungi

No.	Type of substitution	Location	Nucleotide	Sequence ID with compare	Sequence ID with Submission	Source	Identities
1	------	-----	----	**ID: ON908683.1**	-----	*C.albicans*	100%
2	------	-----	----	**ID: MK026347.1**	----	*C.glabrata*	100%
3	------	-----	----	**ID: ON853557.1**	-----	*C.pichia*	100%

Conclusion

In this study, the patients infected with COVID 19 are more infected with the fungus than others patients by isolating vaginal fungi samples. By isolating the fungi it appeared the *Candida albicans, Candida glabrata,* and *Candida pichia* were highly effective and resistant, while *Candida albicans* was more effective and resistant than others fungus .*Candida albicans* , *Candida glabrata* and *Candida pichia* were molecular diagnosed by PCR technology. The research presented here provides scientific support for molecular systems use of PCR technology.

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