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Research Article

Molecular diagnosis of Candida species isolates from skin diseases in Karbala Governorate

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Abstract:

The present work was designed to identify *Candida* species associated with skin infections in patients from Karbala Governorate, Iraq, through the application of molecular approaches. Out of one hundred clinical specimens examined, seventy-seven fungal isolates were confirmed as belonging to the genus *Candida*. For molecular identification, the Internal Transcribed Spacer (ITS) region was targeted using three species-specific primers: *CALB1* for *Candida albicans*, *CGL1* for *C. glabrata*, and *CTR1* for *C. tropicalis*. Polymerase chain reaction (PCR) analysis generated reproducible bands of 273 bp for *C. albicans*, 423 bp for *C. glabrata*, and 357 bp for *C. tropicalis*, confirming the efficiency of the primers in species recognition. Among the isolates, *C. albicans* was the most frequent species, followed by *C. glabrata* and *C. tropicalis*. The predominance of *C. albicans* emphasizes its epidemiological significance as a leading cause of skin mycoses in the studied population. The findings of this investigation provide useful baseline data on the distribution of *Candida* species in Iraq and demonstrate the value of PCR-based methods for achieving accurate and rapid diagnosis. Such information may assist in guiding appropriate antifungal therapy and improving patient management in clinical practice.

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Introduction

The skin is the largest organ of the body, and its primary function is to protect against temperature changes, injury, and inflammation. It also contains a large number of bacteria and fungi that live as natural flora on the skin [1]. Under the influence of a number of external and internal factors, these organisms transform into pathogens and cause skin diseases [2]. Fungal infections of the skin are a common health problem throughout the world, and their prevalence is expected to reach 20-25% of the world's population, and their incidence continues to rise [3]. There are many types of fungi that cause skin infections in humans, including *dermatophytes (Trichophyton, Microsporum, Epidermophyton), Malassezia* forfur, *Candida*, and, less commonly, *Aspergillus*, *Cladosporium*, and *Fusarium* species [4].

The genus *Candida* is a type of fungus that causes an infection called candidiasis. It typically lives on the skin and inside the body, in the mouth, digestive tract, and vagina, without causing any disease [5]. Under conditions including AIDS, cancer, treatment with broad-spectrum antibiotics and corticosteroids, immune suppression, diabetes, and malnutrition, these fungi transform from a yeast state to a pathogenic state, causing various infections ranging from infections of the superficial mucous membranes and skin to systemic infections affecting any organ within the body [6]. The genus *Candida* includes approximately 30 species, 20 of which cause disease in humans. The most important of these species medically are *C. albicans, C. tropicalis, C. glabrata, C. parapsilosis*, and *C. krusei* [7]. Many studies have indicated that morphological characteristics cannot be relied upon in the diagnosis of fungi, including *Candida*, because they require sufficient experience in the field of fungal classification, especially when working with closely similar fungal groups, in addition to requiring a lot of time and effort. Also, the sizes, shapes, and colors of fungal spores and colonies can be affected by different environmental factors, and therefore many researchers do not rely on them, although they sometimes give accurate results. The accuracy and sensitivity of many classification techniques based on molecular biology have contributed to the detection and study of genetic differences among many organisms, including *Candida*, in addition to eliminating the disadvantages of traditional methods. Polymerase chain reaction (PCR) is one of the molecular techniques based on targeting and amplifying a specific region of the organism's genome to reveal the different genetic relationships between fungal species, through which the results of the phenotypic diagnosis of fungi can be supported. Given the importance of accurate diagnosis of *Candida*, this study aimed to diagnose different fungal isolates using polymerase chain reaction (PCR) [8].

Materials and Methods

Pathogenic Specimens Collection

One hundred skin samples were collected from patients with various skin diseases following clinical diagnosis by a specialist physician. The patients were patients of both sexes and varying ages, attending the Children's Teaching Hospital, the Dermatology Consultation Clinic, and the Burns Center at Imam Hussein Hospital and Imam Hassan Hospital in the holy city of Karbala. The samples were collected over a period of five months, from September 8, 2024, to February 2, 2025. Cotton swabs containing the carrier medium were used to collect the samples after sterilizing the affected area with 70% ethanol to eliminate contaminating fungi and bacteria.

Culture and Isolation

The study samples were cultured directly using the stromal method on saproidium dextrose agar medium supplemented with amoxicillin, with two replicates for each sample. The plates were then incubated at 27°C for 24–48 hours, after which fungal growth was observed and diagnosed [9].

Identification

Morphology examination

After fungal growth appeared on the surface of Sabouraud dextrose agar medium supplemented with amoxicillin, the external appearance of the growing colonies was

examined, including their shape, color, diameter, and height. [11], [10]

Microscopic examination

A sample of the fungal growth was taken and transferred to a glass slide containing a drop of lactophenol blue stain. The slide was covered with a coverslip and examined under a microscope at 40x magnification to observe the shape, size, and buds. [12], [11], [10]

Molecular Identification of *Candida* Isolates

PCR testing was performed to confirm the morphological, microscopic, biochemical, and physiological identification of *Candida* species isolated from various skin lesions, as follows:

1 - Cultivation of *Candida* Isolates

Candida isolates were activated by culturing them on Saproid agar medium supplemented with amoxicillin. The plates were incubated at 27°C for 24-48 hours.

2 - Extraction of Deoxyribonucleic Acid (DNA)

DNA was extracted using the GENOMIC DNA mini kit provided by the Korean company Macrogen, following the steps described by the manufacturer.

3- PCR reaction

The PCR test was performed using a master mix prepared by the Korean company Interon to amplify the ITS1 and ITS2 regions, with a total volume of 25 microliters, including 2 microliters of DNA, 1 microliter of forward primer, and 1 microliter of reverse primer, as shown in Tables (1) and (2).

Table (1):PCR Master Mix

Component	Amount (µl)
DNA sample	2
Primer (F)	1
Primer (R)	1
Nuclease free water	16
Master Mix tube	5
Total volume	25

Table (2): Primers used to diagnose *Candida* isolates [13].

Reference	Candida	Primer name	Primer sequence 5 → 3	PCR reaction product (bp)
[14] [15] [16] [17] [18] [13]	*C.albicans*	*CALB1* *CALB2*	TTT ATC AAC TTG TCA CAC CAG A ATC CCG CCT TAC CAC TAC CG	273
	C. glabrata	*CGL1* *CGL2*	TTA TCA CAC GAC TCG ACA CT CCC ACA TAC TGA TAT GGC CTA CAA	423
	C.tropicalis	*CTR1* *CTR2*	CAA TCC TAC CGC CAG AGG TTA TTGG CCA CTA GCA AAA TAA GCGT	357

4- PCR Conditions

The isolated DNA was amplified using the PCR reaction conditions shown in Table (3).

Table (3): PCR reaction conditions for identifying isolated Candida species.

Primer Name	Temperature (c) / Time				
	Initial Denaturation / Time	Cycling Condition			Final extension / Time
		Denaturation	Annealing	Extension	
CALB1	94c/5min 1 cycle	94c/1min 35 cycle	56c/ 1min 35 cycle	72c/2min 35 cycle	72c/7min 1cycle
CGL1	94c/5min 1 cycle	94c/1min 35 cycle	56c/ 1min 35 cycle	72c/2min 35 cycle	72c/7min 1cycle
CTR1	94c/5min 1 cycle	94c/1min 35 cycle	56c/ 1min 35 cycle	72c/2min 35 cycle	72c/7min 1cycle

5- Analysis of PCR Results

DNA content was assessed using 1.5% agarose gel electrophoresis. Ultraviolet light was used to detect DNA content. PCR products were then analyzed according to the agarose gel electrophoresis protocol [14].

Results and Discussion

Isolation and Diagnosis

One hundred samples were collected from various clinical cases of individuals suffering from various skin diseases. Laboratory examination results showed growth in 77 samples (77%), while 23 samples (23%) showed no significant growth. This indicates that clinical diagnosis does not provide significant accuracy in identifying the pathogen, as some diseases have similar clinical manifestations to other skin diseases. Therefore, laboratory confirmation using direct microscopic examination, culture, or molecular techniques is necessary to obtain an accurate diagnosis and appropriate treatment [19].

Diagnosis of isolated Candida.

The results of morphological examination of yeast colonies growing on SDA medium showed the appearance of white, discoid or oval colonies with a smooth surface, elevated, and creamy at the base (Figure (1)). These characteristics were consistent with those reported by [20], [21], and [22]. SDA medium is an ideal medium for isolating *Candida* because the low pH of this medium promotes its growth and inhibits the growth of many types of bacteria, and the addition of an antibacterial increases its selectivity [23].

With regard to microscopic diagnosis, all isolates showed single, spherical to oval, or elongated cells with budded cells, and the results were consistent with those reported by [24].

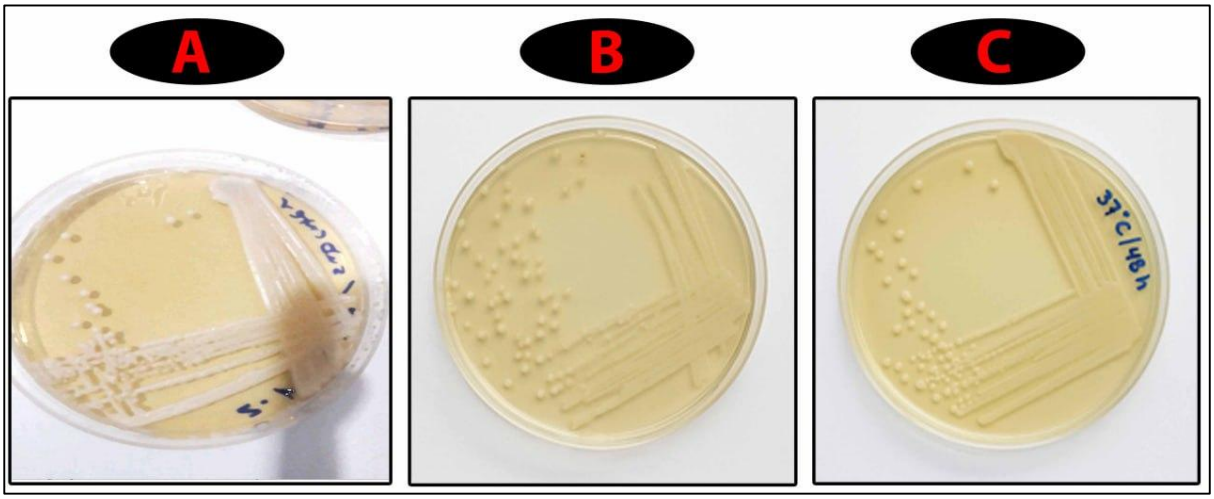

Figure (1): A- *C.albicans* colonies on SDA medium after incubation at 37°C for 48 hours. B- *C.tropicalis* colonies on SDA medium after incubation at 37°C for 48 hours. C- *C.glabrata* colonies on SDA medium after incubation at 37°C for 48 hours.

Molecular Identification of Isolated Candida Species

Molecular identification of isolated *Candida* species, identified using conventional methods, was performed within three species: *C. tropicalis, C. glabrata, and C. albicans*. This was accomplished using three ITS region-specific primers: *CALB1*, *CTR1*, and *CGL1*. [25] indicated that ITS regions are important for studying the relative origins of various fungal species and understanding the evolutionary relationship between these species. The results of using these primers were characterized by success and high accuracy in molecular identification of the isolated species, as PCR results were obtained with a size of 273bp for 58 isolates. This confirms that all isolates belong to the *C. albicans* species (Figure 2: a, b, c). This result confirms what was identified using conventional methods, and the current result is consistent with the findings of numerous researchers, including [27], [26], [17], [15], and [14]. The polymerase chain reaction (Figure (3)) also showed that 11 isolates had a molecular weight of 423 bp, confirming that the isolates belong to the *C. glabrata* species. This result is consistent with the findings of [26], [17], and [16]. Eight isolates isolated during the current study were confirmed as belonging to the *C. tropicalis* species, as molecular diagnosis results showed an amplification product of 357 bp (Figure (4)). This result was confirmed by some researchers, including [27], [26], and [17]. [25] indicated that there are significant differences and variations in the ITS regions of fungal DNA, and these differences help in identifying fungi at the level of species, strains, and other taxonomic groups. They also help in understanding and studying evolutionary relationships and how new species and strains arise.

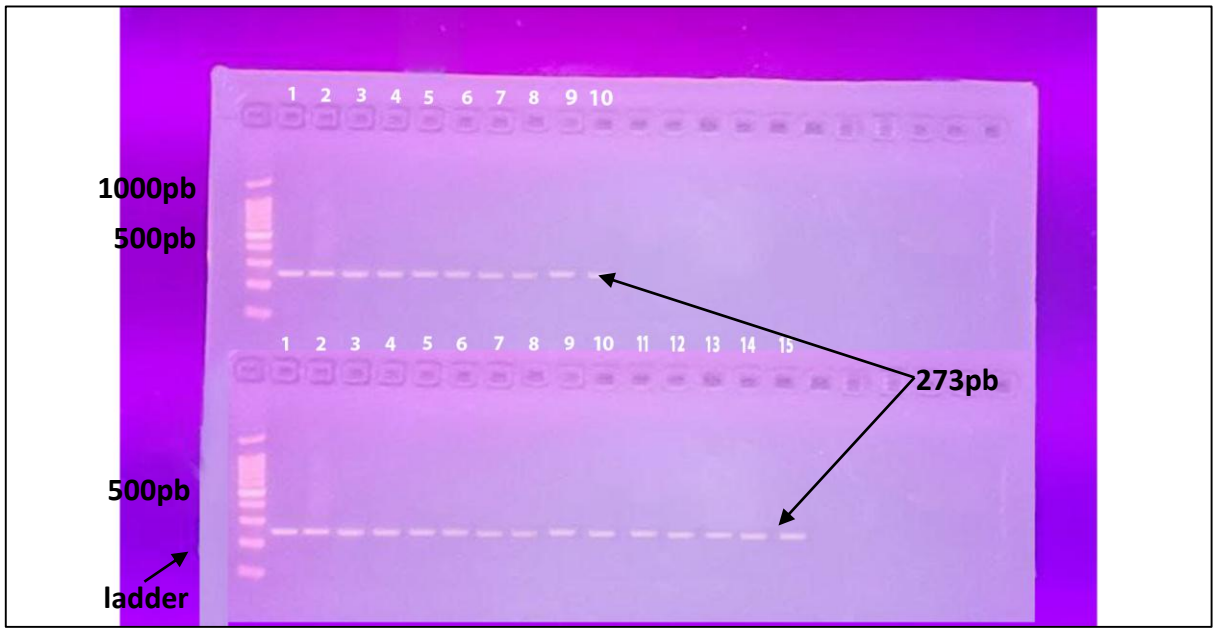

Figure (2): A- Electrophoresis of the PCR reaction product of *C. albicans* isolates using the specific primer *CALB1* (273bp) at a gel concentration of 1.5%.

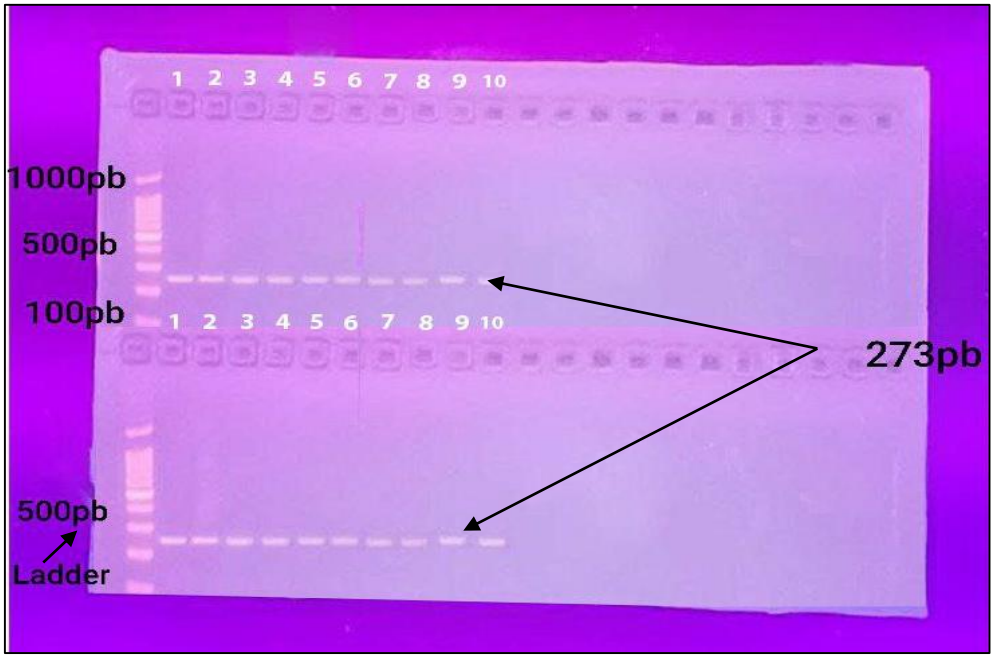

Figure (2): B- Electrophoresis of the PCR reaction product of *C. albicans* isolates using the specific primer *CALB1* (273bp) at a gel concentration of 1.5%.

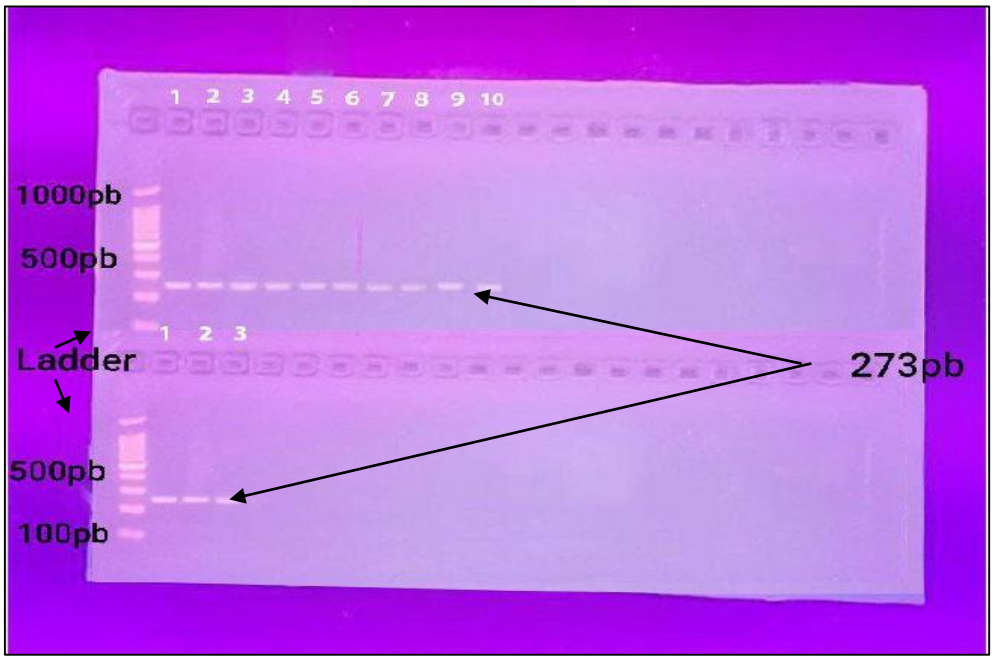

Figure (2): C- Electrophoresis of the PCR reaction product of *C. albicans* isolates using the specific primer *CALB1* (273bp) at a gel concentration of 1.5%.

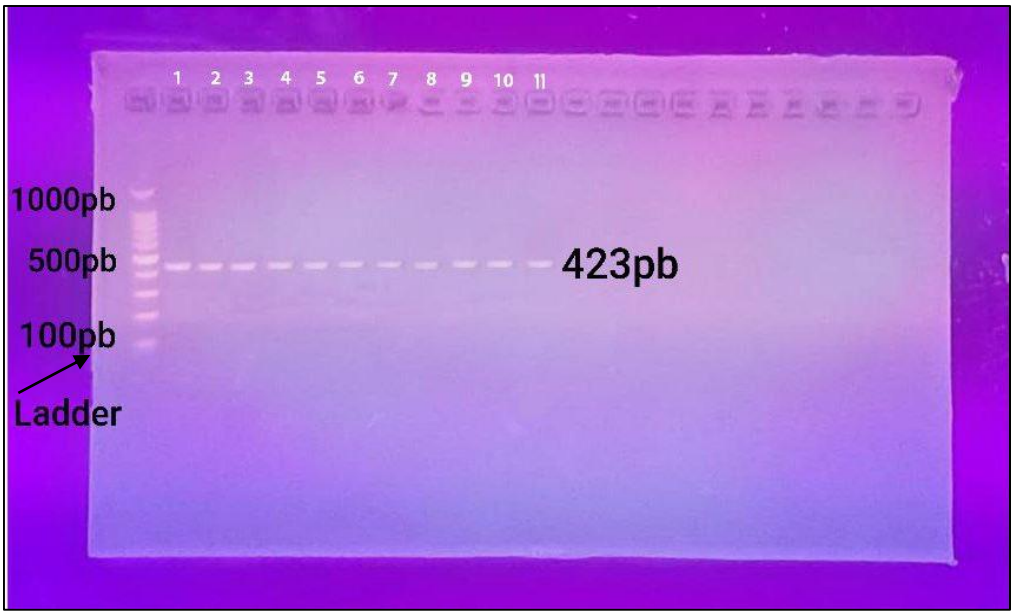

Figure (3): Electrophoresis of the PCR reaction product of *C. glabrata* isolates using the specific primer *CGL1*(423bp) at a gel concentration of 1.5%.

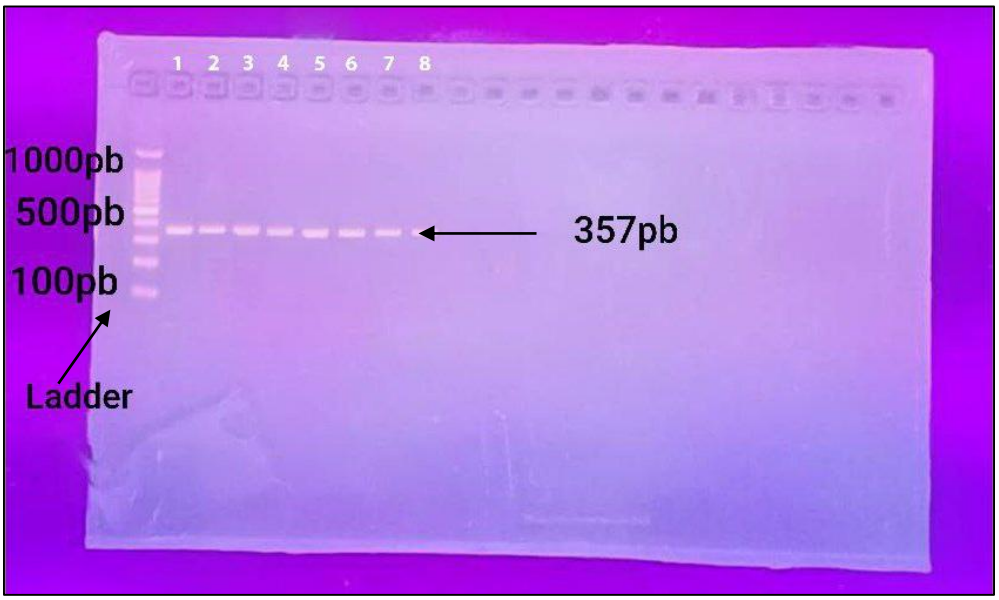

Figure (4) : Electrophoresis of the PCR reaction product of *C.tropicalis* isolates using the specific primer *CTR1*(357bp) at a gel concentration of 1.5%.

Conclusion

The results of the study demonstrated that the use of the ITS region as a molecular marker is an effective and sensitive tool for diagnosing *Candida* species, given the high level of variability between different species. Furthermore, the use of three specific primers (*CALB1, CGL1, and CTR1*) enabled accurate discrimination between the studied strains, helping to account for the potential for cross-contamination with other morphologically similar fungi. This contributes to improving therapeutic strategies and selecting the most appropriate antifungal.

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