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Evaluation of Candida albicans Diagnosis by Conventional Methods via Employing a PCR-Based Test

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ORIGINAL ARTICLE

Evaluation of *Candida albicans* Diagnosis by Conventional Methods via Employing a PCR-based Test

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Abstract

Background: **The current study uses a single pair of primers, SC1F and SC1R, in a polymerase chain reaction (PCR)-based test to amplify a "670-bp segment of the KER1 gene," of *C. albicans*.**

Objective: **To evaluate the conventional methods for diagnosing *C. albicans*, as accurate identification of Candida species has significant prognostic and therapeutic implications.**

Methods: **In the current in vitro study, 100 oral swab samples were collected from patients aged between 3 and 80 years who exhibited clinical presentations of oral thrush and had malignant diseases at the Hematology Center/Baghdad Teaching Hospital and the Children Welfare Teaching Hospital in the Medical City of Baghdad. The diagnosis of *C. albicans* was performed using traditional diagnostic techniques. Favorable outcomes were then confirmed with those of molecular approach.**

Results: **In the present investigation, 49% of the studied swab samples were from females, while 51% were from males. The average age of patients with malignant diseases who experienced oral thrush was 37.72 years. Upon direct microscopic examination of the samples, only 85% revealed positive results. However, upon culturing these samples, 74% showed the growth of *C. albicans* colonies on SDA. Additionally, in the microscopic examination of these colonies, 74% revealed positive results. The germ tube test revealed positive results in 35 out of 74 isolates. In the PCR test, 30 out of 35 isolates produced positive findings.**

Conclusion: **Conventional diagnostic methods are prone to inaccuracies due to the phenotypic similarities between *C. albicans* and other Candida species.**

Keywords: *C. albicans*, KER1 gene, PCR

Introduction

Candida species are responsible for most fungal infections in humans. These species include *C. albicans*, the most frequent pathogen of opportunistic infections; the drug-resistant *C. glabrata*; *C. auris*, an emerging global public health danger; and other developing species such as *C. parapsilosis*, *C. krusei*, and *C. tropicalis* (Lopes & Lionakis, 2022).

C. albicans, the human's primary fungal pathogen, is accountable for approximately 15% of nosocomial infections (Chow et al., 2021). It possesses multiple virulence factors, including hyphal development, adhesion, phenotypic switching, proteinase production, and biofilm formation. It manifests as a commensal in most individuals' oral cavities, genitourinary tract, gastrointestinal tract, and skin, restricted by epithelial barriers, the local microbiota, and immune defenses (Mohammed et al., 2021). Disruption of any one of these barriers can lead to infections that range from simple skin infections to potentially lethal candidemia (D'Enfert et al., 2021; Pappas et al., 2018). The rate of candida infection rises annually due to several contributing factors, including the increasing number of immunocompromised individuals, the widespread use of antibiotics and immunosuppressants in cancer chemotherapy, and the substantial advancement of organ transplantation. Of all the fungi responsible for candidiasis, *C. albicans* was found in 70%−90% of

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cases (Chen et al., 2020). The mortality rate in people with systemic fungal infections is unacceptably high, in several circumstance approaching 50%. This can be attributed to the fact that fungal infections are often challenging to diagnose and treat effectively (D'Enfert et al., 2021). Accurately identifying *Candida* species is highly significant, as it has prognostic and therapeutic implications that allow early and effective antifungal therapy (Taher, 2019). The molecular identification methods are increasingly gaining popularity as these techniques are precise and extremely specific (Arafa et al., 2023; Ibrahim et al., 2022). In the current investigation, we aimed to assess the precision of conventional diagnostic techniques by using a PCR-based test.

Methods

Ethical approval

The project was approved by the research ethics committee at the College of Medicine, University of Baghdad, according to the code number (UVB 23.12) on (21/8/2023).

Sample collection

In the current in vitro study, a total of 100 oral swab samples were obtained from patients of both genders, who are between 3 and 80 years old, who exhibited clinical presentations of oral thrush and were afflicted with malignant diseases at the Hematology Center/Baghdad Teaching Hospital and the Children Welfare Teaching Hospital in the Medical City of Baghdad/Iraq from November 2022 to March 2023.

Samples processing

Direct microscopic examination

All the collected samples were subjected to a direct microscopic examination with a drop of 10% KOH (potassium hydroxide) solution to identify the presence of pseudohyphae and budding yeast cells (Mardani et al., 2020).

Samples culturing

The oral swab samples were streaked on SDA (Sabouraud dextrose agar) (Liofilchem, Italy) and incubated at 37 °C for 24–48 h (Abidullah et al., 2021; Saber Ali et al., 2015).

Identification of *Candida albicans*

Macroscopic identification

The smell, texture, shape, and color of the colonies were screened for *C. albicans* colony features on SDA (Chongtham et al., 2022; Mohsin & Ali, 2021).

Microscopic identification

To examine the distinctive microscopic characteristics of *C. albicans* following growth on SDA, such as pseudohyphae and budding yeast cells, a staining method using lactophenol cotton blue (HiMedia, India) was employed. Smears were made from a well-isolated colony on SDA (Sriramajayam et al., 2023).

Germ tube formation test

The test was conducted by inserting only a tiny portion of a pure cultivated colony of candida isolates into 0.5 mL of human serum. Following that, the suspension was incubated for two to 3 h at 37 °C. After the incubation period, a drop from this suspension was inspected under the microscope at 40x magnification for the formation of the germ tube (Qasim, 2020; Waikhom et al., 2020).

Molecular identification

DNA extraction

By using the Presto Mini gDNA Yeast Kit (Geneaid, Taiwan), fungal DNA was extracted following the manufacturer's instructions from candida colonies that showed a positive germ tube test.

The purity of extracted DNA

The purity of the extracted DNA was measured by utilizing the UV-Vis spectrophotometer Q5000 (Quawell, USA) to detect the quality of extracted samples for downstream applications.

PCR amplification

The PCR amplification was performed in a 25 µl solution made up of the following:

12.5 µL of GoTaq master mix (Promega, USA).

1.25 µL of forward primer (F) 10 pmol (Scientific Researcher, Iraq), Table 1.

Table 1. The list of oligonucleotide primers used in the study.

Primer	Primer sequences	Amplicon size (base pairs) of SCI	Reference
SC1-KER1gene-F	5′ CGGAGATTTTCTCAATAAGGACCAC 3′	670 bp	GalÃ¡n et al (2006)
SC1-KER1gene-R	5′ AGTCAATCTCTGTCTCCCCTTGC 3′		

1.25 µL of reverse primer (R) 10 pmol (Scientific Researcher, Iraq), Table 1.

5 µL of sample DNA, then with nuclease-free water (Promega, USA), the volume was completed to 25 µl.

The PCR was performed using the thermal cycler GeneAmp PCR System 9700 (Applied Biosystem, Singapore), the cycling conditions including time and temperature were displayed in Table 2.

Agarose gel electrophoresis

After the PCR amplification process, agarose gel electrophoresis was performed, in which 5 µL from each of the PCR samples and the standard molecular weight marker (DNA ladder) 100 bp (Promega, USA) were electrophoresed on 1.5% agarose gel (Promega, USA), supplemented with 5 µL of ethidium bromide solution, molecular grade (Promega, USA), at 90 V for approximately 60 min in a gel electrophoresis system (Major Science, Taiwan). Thereafter, by using the Molecular Imager Gel Doc XR with Image Lab Software (Bio-Rad, USA), the agarose gel was inspected under UV light for documentation and determination of anticipated DNA bands.

Statistical analysis

The Statistical Package for Social Sciences, version 20.0 for Windows, was used to analyze the data. The mean and standard deviation were used to illustrate quantitative data. Counts and percentages were utilized to show the qualitative data.

Results

The current study included 51% male patients and 49% female patients. The average age of patients with malignant illnesses who developed oral thrush was 37.72 years, with a standard deviation (SD) of ±21.95 years. Upon direct microscopic examination of the collected oral swab samples with a 10% KOH solution, only 85 out of 100 samples revealed positive results (pseudohyphae and budding yeast), as displayed in Fig. 1.

Whereas only 74 out of 100 of the collected oral swab samples revealed the growth characteristics of *C. albicans* colonies on SDA (pasty, smooth, creamy, and convex colonies), as displayed in Fig. 2.

Upon microscopic examination of these clinical isolates after staining with lactophenol cotton blue stain, 74 isolates revealed positive results (pseudohyphae and budding yeast cells), as displayed in Fig. 3.

From these 74 clinical isolates, only 35 exhibited positive test results in the germ tube formation test

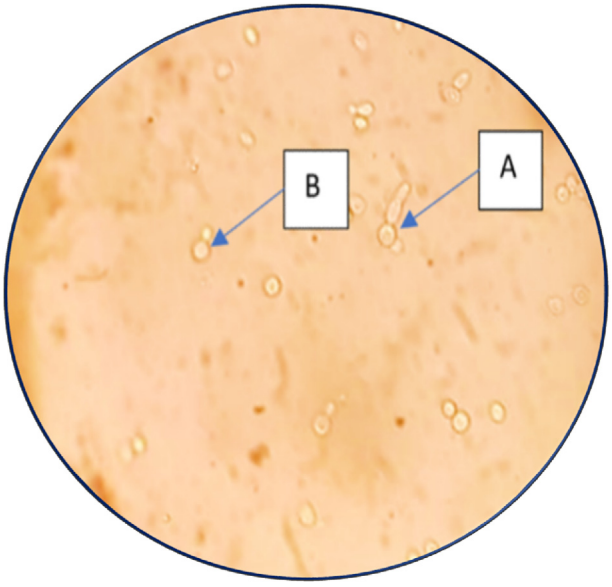

Fig. 1. Direct microscopic examination of clinical samples: A-Pseudohypha, B-Budding yeast.

Table 2. PCR Cycling conditions for amplification of the "670-bp DNA fragment" of C. albicans.

PCR steps	Temperature	Time	Cycle	Reference
Initial denaturation	94	3 min	1	GalÃ¡n et al. (2006)
Denaturation	94	40 s	30	
Annealing	60	30 s		
Extension	72	2 min		
Final extension	72	12 min	1	

Fig. 2. C. albicans colonies on SDA.

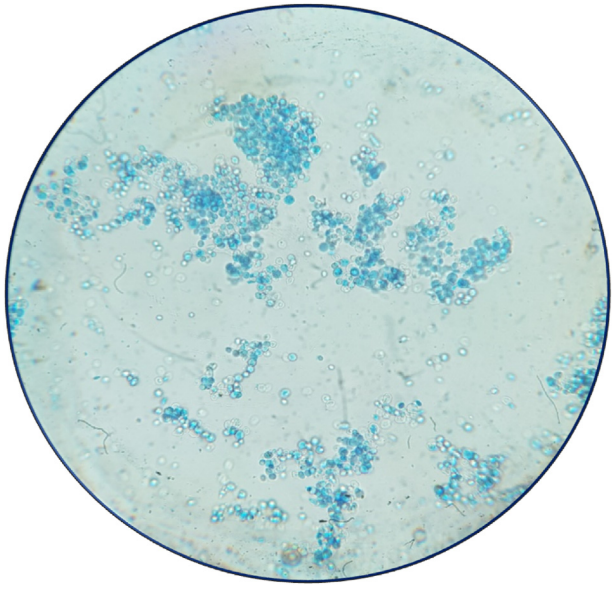

Fig. 3. Lactophenol cotton blue staining of Candida isolates.

(they formed elongated structures that have parallel-sided walls and do not display constriction at the mother cell's point of origin), as displayed in Fig. 4, and Table 3.

Regarding the PCR-based approach for the identification and differentiation of *C. albicans*, the extracted DNA from all suspected isolates (which had positive germ tube formation tests) was evaluated for downstream applications. The results of this evaluation indicated that the purity of the extracted DNA was within the range of 1.75–1.95. Subsequently, the extracted DNA was subjected to PCR

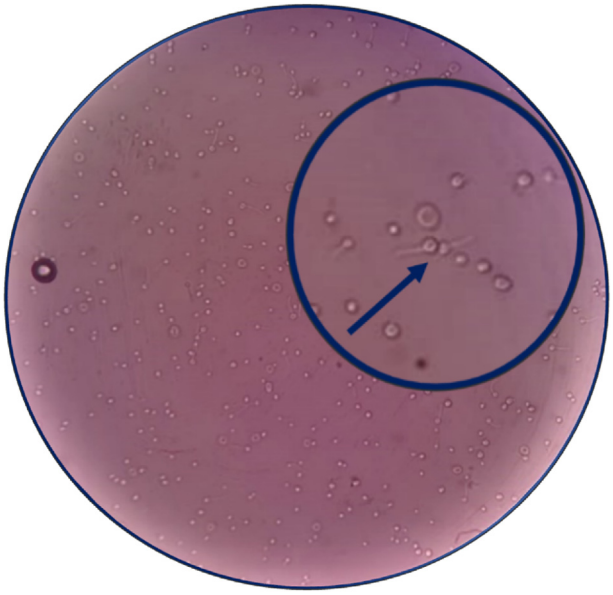

Fig. 4. Germ tube test positive.

Table 3. Germ tube test results (n = 74).

Germ tube test	Count	Percentage
Positive	35	47.3%
Negative	39	52.7%

amplification and agarose gel electrophoresis, which confirmed that 30 out of 35 isolates had amplified "670-bp DNA fragment of the KER1 gene," as displayed in Table 4 and Fig. 5.

Discussion

About 20 of the more than 150 Candida species are known to cause infections in humans. In both adult and pediatric patients, *C. albicans* is the primary cause of fungal infections and the main culprit behind candidiasis. The development of yeast infections is becoming more frequent among the increasing number of people with predisposing conditions such as HIV infections, cancer chemotherapy, and organ transplants. The management of these infections could be enhanced with the provision of more accurate and expedient diagnostic and treatment options (Macias-Paz et al., 2023; Ojaimi Mahdi Al-Dahlaki et al., 2023; Pappas et al., 2018). Molecular identification methods are increasingly

Table 4. PCR amplification results (n = 35).

PCR	Count	Percentage
Positive	30	85.7%
Negative	5	14.3%

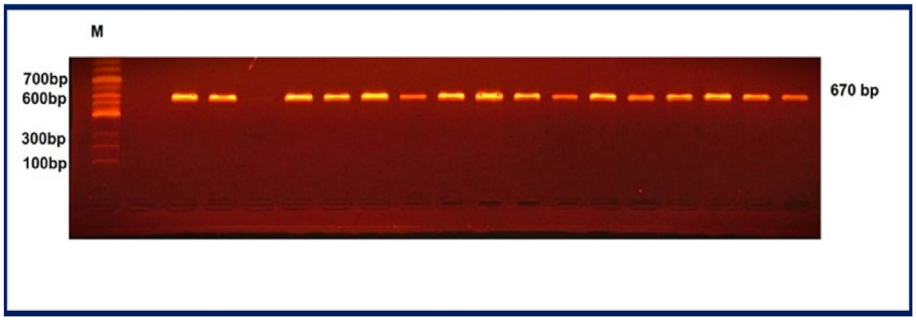

Fig. 5. The image of electrophoresed PCR products. M: 100 bp ladder marker.

gaining popularity since they are accurate and highly specific (Arafa et al., 2023). In the present study, conventional methods were employed for the primitive diagnosis of *C. albicans*, and the positive results were verified with those of the molecular approach. In the direct microscopic examination of oral swab samples, 85% of the taken samples showed positive results (pseudohyphae and budding yeast cells), while upon culturing these samples on SDA (the most frequently used primary isolation media for *Candida* spp.), because of its low pH, this medium prevented the growth of many species of oral bacteria while allowing Candida spp. to proliferate (Mastammanavar et al., 2014), only 74% of the collected oral swab samples showed the growth features of *C. albicans* colonies on SDA, so 11% of the collected samples had positive results in the direct microscopic examination but negative results with macroscopic identification (not detected on SDA). This result was consistent with those of (Mardani et al., 2020). This discrepancy between culturing on SDA and direct microscopic examination is being accepted even though the direct microscopic examination is useful in distinguishing between bacterial and yeast infections but it is less sensitive than the culture approach (Swain et al., 2021). Afterward, for the differentiation of *C. albicans* from other Candida species, the clinical isolates that showed positive findings on macroscopic and microscopic identification were subjected to a germ tube test. The results of this test showed that 47.3% of clinical isolates developed elongated structures that have parallel-sided walls and do not exhibit constriction at the mother cell's point of origin (germ tube). The outcomes of this test agree with the findings of an Iraqi study conducted by (Abdulla & Mustafa, 2020). Then all clinical isolates that showed positive results during germ tube testing were subjected to a PCR-based technique that involved the use of "primers derived from the pH-regulated KER1 gene of *C. albicans*, which encodes a novel lysine/glutamic acid-rich protein" with no clear similarity to other known sequences (Galán et al., 2004). This technique facilitates the detection, identification, and discrimination of *C. albicans* from other Candida species, such as the azole-resistant *C. glabrata* and *C. krusei*, as well as the phenotypically correlated *C. dubliniensis*. The results of this test showed that the ability to amplify the "670-bp fragment of the KER1 gene" was present in only 85.7% of clinical isolates that had a positive germ tube formation test. The findings of this test are somewhat lower than those mentioned by (GalÃ¡n et al., 2006), who found that 94.6% of clinical isolates that had been identified by traditional microbiological techniques including germ tube formation test, agglutination test, and sugar assimilation test succeeded in amplifying a 670-bp DNA fragment. Regarding the discrepancy between the results of the PCR-based test and the germ tube formation test in the current study (five samples positive by the germ tube test appeared negative by the PCR-based test as *C. albicans*), the possible explanation is that in the germ tube test, the misidentification of the phenotypically correlated *C. dubliniensis* as *C. albicans* has occurred since *C. dubliniensis* is also capable of producing a germ tube (Asadzadeh et al., 2019).

Conclusion

In conclusion, the traditional diagnostic techniques may be inappropriate for the accurate identification of *C. albicans* due to the possibility of misidentification with other Candida species. While PCR provides high specificity, its widespread application in resource-limited settings may be constrained by cost and technical demands. Future initiatives should focus on developing cost-effective molecular diagnostic platforms.

Ethics information

The project was approved by the research ethics committee at the College of Medicine, University of

Baghdad, according to the code number (UVB 23.12) on (21/8/2023).

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Author's contributions

Rawaa Yousif Sadkhan: MSC student. Samara Mowafaq Ali: supervisor.

Conflicts of interest

None.

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