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Detection and Characterization of *Candida albicans* Isolated from Clinical Specimens

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Abstract

Candida albicans are reportedly the most common human fungal pathogens. The objective was to analyse the distribution of *C. albicans* among isolates from various clinical specimens and to ascertain how their virulence factors were expressed. Methods: *C. albicans* isolates were collected from clinical specimens at Karbala hospitals between March 2022 and November 2022, a descriptive study on the makeup of *Candida* specimens' identification by phenotype and genotype test. One hundred clinical specimens, 35 oral swabs (patient Diabetes), 35 oral swabs (patient non-diabetes) and 30 throat swabs, were obtained from various locations and grown on chromogenic media. The total (n=60) isolates were identified as *C. albicans* using biochemical and partial sequence of 18S rDNA sequences analysis. All *C. albicans* isolates named CA1 to CA 60 were characterised by drug resistance (CDR1) gene and multidrug resistance (MDR1) gene. Results: *C. albicans* was the most prevalent (60). Our findings support the quick and precise

identification of *C. Albicans*

infections in clinical specimens using the 18S rDNA PCR diagnostic workflow. *C. albicans* isolates showed 55 % and 40 % (CDR1 and MDR1) genes, respectively. The PCR results reported here suggest that amplification with CDR1 and MDR1 genes may be helpful for specifically identifying *C. albicans* strains.

Introduction

Candida is a common human commensal fungus that resembles yeast. They transform into pathogens and cause infections locally or broadly when the host's resistance to infection is compromised [1]. *Candida* species, which range in severity from non-life-threatening mucocutaneous candidiasis to life-threatening invasive conditions such as bloodstream candidiasis, genitourinary [2] candidiasis, vulvovaginal candidiasis, and oropharyngeal candidiasis, are responsible for the majority of fungal infections. *Candida*, a common commensal organism of the gastrointestinal tract, can grow on the skin [3]. Invasive infections by *Candida* species are the most common cause of death in patients undergoing invasive procedures and immunocompromised people [4]. *Aspergillus*, *Candida*, and *Cryptococcus* are opportunistic microorganisms that can cause fungal infections in people with weaker immune systems,

which can have detrimental effects [5]. About 60–80% of infections resulting in candidiasis are caused by *Candida albicans*. Nonalbicans species have become more prevalent during the past few decades [6]. According to the literature, the oral cavity has been home to *C. albicans*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, *C. guilliermondii*, *C. dubliniensis*, and *C. glabrata*, are some examples of *Candida* species. Compared to other species of *Candida*, *C. albicans* possesses a pathogenic site factor that makes it more likely to cause disease [7].

Candida-caused invasive infections have a high risk of death. An expanding population of individuals is vulnerable to and impacted by invasive fungal diseases due to better treatment options and survival rates for some critical illnesses, such as hematologic malignancies [8]. Patients who are immunosuppressed or neutropenic and those in intensive care units are more likely to develop invasive candidiasis [9]. Previously, it was believed that *C. albicans* was the species most usually in charge of candidiasis. However, some studies over the past few decades asserted that there has been a steady shift from *C. albicans* to non-albicans. NAC candida species include *C. glabrata*, *C. tropicalis*, and *C. krusei* [10]. Due to the stability of genomic markers and better level of typing resolution, genotypic methods are preferred to phenotypic methods for identifying and characterising fungal isolates [11]. Symptomatic and asymptomatic forms of candiduria exist. Most patients with *Candida* excretion in their urine don't have any symptoms. On the contrary, Patients with prostatitis, pyelonephritis, cystitis, epididymorchitis, and renal candidiasis, on the other hand, exhibit symptomatic candiduria [12], pregnancy, diabetes, and age [13].

Compared to other *Candida* species of medical importance, *C. tropicalis* shares a closer genetic relationship with *C. albicans* [14]. In this study, we wanted to determine if the rDNA ITS region could be used as a molecular marker for assessing genetic diversity within and among therapeutically important and developing *Candida* species from a sizable Iraq yeast collection characterised by conventional plus molecular approaches.

Materials and Methods

processing of samples

The protocol for identifying *C. albican* was followed during the isolation. Of one hundred (n=100) clinical specimens, 35 oral swabs of Supernatants were eliminated after being spun at 3000 rpm for 5 minutes, and precipitated pellets. They were absorbed immediately in Sabouraud agar and then kept at 30 °C for 24-48 hours [15].

The germ tube test

The suspension of Candida cells was dipped into 0.5 ml of human serum and incubated for 3 hours at 37 °C for 24 hours. After incubation, a period microscopy examination reveals that the germ tube has a lateral tube without a septum and no constrictions at the commencement point [16].

Molecular Identification Isolation of Genomic DNA

Using a DNA Extraction Kit (Promega, USA), the bacterial DNA was isolated from 2 ml of the overnight culture maintained at 30°C on an orbital shaker (200 rpm). The Maestro Nano Spectrophotometer (Maestro Gen, USA) was used to measure the absorbance at 260 nm and 280 nm to evaluate the concentration and purity of the extracted DNA. Before usage, the extracted DNA was kept at -20°C [17]. To identify virulence genes Utiliz PCR amplification Primers for detecting the Drug resistance genes were considered during this study, whereas the primers for detecting the Drug resistance CDR1 gene and multi-drug resistance MDR1 gene are shown in Table

1. The PCR reaction is shown in Table 2.

Electrophoresis on agar

Following PCR amplification, 1 µL of each DNA extract was electrophoresed at 95 V for minutes on extracted genomic DNA Agarose gels (1% w/v) in 1xTAE buffer. GeneRuler™ 1 kb DNA ladder containing 0.1% was dyed in 1xTAE buffer and then post-stained in the same buffer. As a DNA size marker, Maestrosafe™ Nucleic Acid was utilized to pre-stain the gel [16]. Finally, UV trans- illuminator Gel Documentation System (Syngene, UK) was used to evaluate and record all associated gel [18].

Table 1: Oligonucleotide primers for detecting the toxin genes of *Candida albicans*

Species	Primer Sequence	Cyclic program	Product sizes (bp)	Reference
<i>Candida albicans</i> 18SrRNA gene	5'- GCCGCCAGAGGTCTAAACTT-3' 5'- AGTTCAGCGGGTAGTCCTAC -3'	94 °C- 5 min	415	19
		94 °C -60 sec		
		55°C- 2 min 72°C-1 min 72°C -10 min		
<i>Drug resistance</i> (<i>CDR1</i>) gene	F: 5'- TGGTGTTCCTCGGTGGTGAAA-3' R: 5'-CCCTGGTGTGATCGTTCA -3'	95 °C- 5 min	503	18
		95 °C -60 sec		
		50°C- 1 min 72°C-2 min 72°C -10 min		
<i>Multi drug</i> <i>resistance MDRI</i> gene	F: 5'- TTGGTTATGGTGTGGCCCA-3' R: 5'- ACCGGTGATGGCTCTCAATC-3'	95 °C- 5 min	458	20
		95 °C -60 sec		
		55°C- 1 min 72°C-2 min 72°C -10 min		

Table 2: Reaction mixture of PCR products

Component	Volume (μL)
GoTaq Green Master (25)	25.0
Upstream primer (10 μM)	2.5
Downstream primer (10 μM)	2.5
DNA Template	2.5
Nuclease-free water	17.5
Total	50.0

Results and discussion

According to reports, *Candida albicans* is humans' most prevalent fungal infection. The goal was to determine how the virulence factors of *C. albicans* were expressed and to analyse the dispersion of the organism across isolates from various clinical specimens. Between March and November 2022, medical specimens from Karbala hospitals were used to isolate *C. albicans*. The present study aims to explore the prevalence of *C. albicans*. The protocol for *C. albicans* detection was followed during the isolation. Out of 100 clinical specimens, oral swabs from patients with diabetes accounted for 71.42%, oral swabs from patients without diabetes for 51.42%, and throat swabs from patients with diabetes accounted for 56.66%. Of the 100 clinical specimens, 60% tested positive for *C. albicans*, indicating bright green colonies when grown onto Sabouraud dextrose agar (SDA) or broth medium containing 6.5% NaCl. The

18s rDNA sequences were used to select and identify the sixty (60) isolates as *C. albicans*. When 18S rDNA blasts in the “NCBI database” (Figure 1) showed the amplicons of *B. cereus* isolates utilizing 18s rDNA universal primers, which produced 250 bp in size, all *C. albicans* isolates, known explicitly as CA1 to CA60, were additionally identified. The PCR results were consistent with and matched those of [21,22].

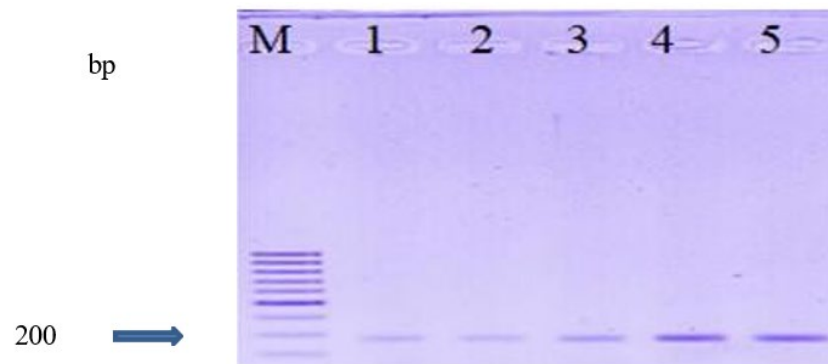


Figure 1: Amplicons of *C. albicans* using *18s rDNA* universal primers on 1.5 % (w/v) agarose gel. Lane “M: 1 kb DNA ladder”; Lane 1-4: *C. albicans* isolates (CA1-CA5); Lane 5: Positive control.

Table 3: *Candida albicans* isolation rate from different clinical samples

Specimens	No of specimens	<i>C. albicans</i> No (%)	Percentage %
oral swabs (patient Diabetes)	35	25	71 .42%
oral swabs (patient non-Diabetes)	35	18	51.42%
throat swabs	30	17	56.66%
Total	100	60	

Table 4: Detection of *Drug resistance (CDR1) gene* and *Multi drug resistance MDR1 gene* *Candida albicans* isolation rate from different clinical samples

Specimens	No (%)	<i>Drug resistance (CDR1) gene</i>	<i>Multi drug resistance MDR1 gene</i>
oral swabs (patient Diabetes)	25	10	8
oral swabs (patient non-Diabetes)	18	6	4
throat swabs	17	4	2
Total	60	20	14

The finding was consistent with polymerase chain reaction (PCR) analysis. The *C. albicans* 60 isolates manifested favourable toward *C. albicans* Drug resistance by targeting two genes of (*CDR1*) gene and *Multidrug resistance MDR1*. The amplicon generated from PCR analysis showed a particular band of 503 bp (*CDR1*) and 458 bp *Drug resistance (CDR1) gene*, respectively, on agarose gel (Figure 1). The results indicate that (*CDR1*) (10/35) oral swabs (patient Diabetes, (6/35) oral swabs (patient non- Diabetes and (4/30) throat swabs. *Multidrug resistance MDR1* 8 /35 oral swabs (patient Diabetes, oral swabs (patient non-diabetes and (4/30) and 2/35 oral swabs (figure 1) and table 4 of *C. albicans* isolates comprise drug resistance genes complex which encode genes. On the other hand, the development of biofilms by azole-resistant isolates of *C.albicans* causes a variety of challenges in the management of invasive candidiasis, including alterations in the expression of the CDR1 and CDR2 genes in the early stages of the disease [23].

Conclusion

Comparative investigation of the virulence factors in this study showed no appreciable variations in the virulence factors and pathogenesis of the 60 strains between the two strains. Patients with candidiasis and the nature of these strains. However, additional samples must be analysed to validate our findings.

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