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(RESEARCH ARTICLE)



## RT-PCR diagnosis of invasive *Candida albicans* strain predominant among a private school in Kirkuk City

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### Abstract

This work was carried out to investigate the incidence of the invasive *Candida albicans* fungus in oral swabs collected from a private primary school and to confirm its invasiveness by RT-PCR. Accordingly, the total of (368) students in the age range of (7-12) years from both sexes was orally examined for the incidence of *Candida albicans* from October 2019 to March 2020. Wet oral swabs were directly cultured on Sabouraud agar medium, and positive cultures were examined microbiologically. Results of Gram's staining revealed the isolation of the invasive *Candida albicans* confirmed by the germ tube test. This fungus was significantly ( $P < 0.05$ ) prevalent in most of the school students, where males were more affected (64.53%) than female students (35.47%) across all age groups. Moreover, the molecular analysis of the isolated fungal strains confirmed the invasiveness of the isolates.

**Keywords:** RT-PCR; Molecular diagnosis; *Candida albicans*; Private school

### 1. Introduction

The discomfort of thrush has been known to occur in people for over 2000 years, where *Candida* species presented as both commensals and opportunistic pathogens in the oral cavity [1-3]. For decades, it has captivated the clinicians to investigate its pathogenicity and to improvise newer therapeutic regimens based on the updated molecular research [2-4]. On the other hand, *Candida albicans* fungal strains were found as a commensal in the oral cavity of about (46%) of one-year-old infants and (39%) of 1–6-year-old children [5]. The turning of *Candida albicans* into a pathogen is usually determined by the host immune system where candida infections might range from non-life threatening superficial mucocutaneous disorders to an invasive disseminated disease involving multiple organs [6-8]. In recent years, a remarkable increase in Candidiasis has been observed, as reflected by the increased incidence of *Candida albicans* infections among children frequently [7&8]. The probable explanations included changes in the practice of medicine like the introduction of broad-spectrum antibiotics, immunosuppressive agents, and morbid conditions such as diabetes and severe malnutrition in children [9-11]. On the other hand, oral Candidiasis became a significant source of morbidity, as it can cause chronic pain or discomfort upon eating, limiting nutrition intake in children of different ages [10 &11].

Therefore, this study was carried out to estimate the prevalence of invasive *Candida albicans* fungus in oral swabs collected from a private primary school and to confirm the invasiveness of the pathogen by RT-PCR.

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## 2. Material and methods

### 2.1. Children included in the study

The total of (368) children of Kirkuk private primary school were the subject of this study. Students were living in different districts of Kirkuk city/Iraq and they were recruited for the study according to the criteria cited in [12].

### 2.2. Oral sample collection

Oral samples obtained by swabbing the oral mucosa (palatal mucosa and tongue dorsal) of all patients with sterile swabs. Then, samples were directly cultured on Sabouraud's dextrose agar medium (Himedia/India) supplemented according to the procedure mentioned in [13]. Plates were incubated at 37 °C for 24-72 hours.

### 2.3. Microbiological tests

#### 2.3.1. Gram's Staining

Single colonies picked with a loop and were stained according to the manufacturing company Kit protocol (Himedia/India). Slides were examined the magnification of (100 X) objective of a light microscope and were photographed.

#### 2.3.2. Germ tube test:

This procedure is followed according to [14] for identifying *Candida albicans*. The isolated, purified strains were incubated in human serum at 37 °C for 2 hours, and the ability to produce short, slender, tube-like structures (germ tubes) were recorded as a pathogenic strain.

### 2.4. Molecular analysis of invasiveness

#### 2.4.1. Extraction of DNA

All isolates were subcultured twice on Sabouraud's dextrose agar and were incubated at 37 °C for 48–72 h before molecular analysis. Crude DNA extracts were prepared by a simple boiling method according to [15]. A loopful colony was suspended in (2 ml) of Sabouraud's dextrose broth medium and was incubated overnight at 37 °C. One milliliter of this suspension was transferred to a sterile Eppendorf tube, centrifuged at 13,000 rpm for 10 min. A volume of (200 µl) of sterile distilled water was then added to the pellet and boiled at 100 °C for 15 min. Then, extracted DNA was transferred to a clean tube and stored at -20°C before RT-PCR.

#### 2.4.2. Identification of the 310 bp genotype by RT-PCR

Two primer pairs used to span the site of the transposable intron in the 25S rRNA were CA-INT-L (50-ATAAGG GAA GTC GGC AAA ATA CCG TAA-30) and CA-INT-R (50-CCT TGG CTG TGG TTT CGC TAG ATA GTA GAT-30) as described by [15]. Amplification reactions were performed in 50 µl final volume containing 3.5 mM MgCl<sub>2</sub>, 200 µM dNTP mix, 1 U Taq DNA polymerase (Sigma, USA), 100 pmol (each) of the primers and 4 µl DNA template. Then, the Genotype of the isolates were investigated by restriction endonuclease analysis by HaeIII and MspI (Thermo Scientific/ France), according to Karahan *et al.* [16]. DNA extracts of *Candida albicans* isolates were digested with HaeIII and MspI restriction endonucleases along with the extracted DNA of the standard invasive *Candida albicans* ATCC 10231 (WDCM 00054 Vitroids™ /Sigma-Aldrich/Germany) and run on RT-PCR (BioRad/USA). The products were characterized by electrophoresis on 2% agarose gels and visualized in U.V. transilluminator (BioRad/USA) after ethidium bromide staining.

### 2.5. Statistical analysis

Data from the study were analyzed using a T-test of the SPSS program Ver.10 for Windows, where a *P* value of <0.05 was considered indicative of a statistically significant difference.

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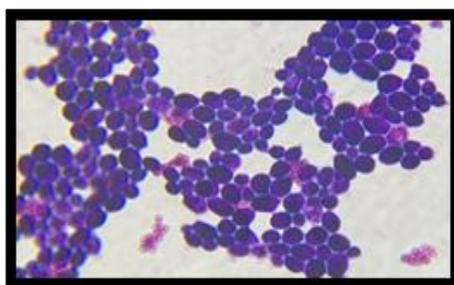
## 3. Results and discussion

The fungus *Candida albicans* is a dimorphic, opportunistic pathogen that causes infections in immuno-compromised individuals where the virulence of this organism is a function of a multiplicity of factors working jointly to overwhelm the host defenses [1,2,3]. Recent findings showed an increase in the global burden of fungal infection, especially for

*Candida albicans*, where the proportion represented by children is mainly unknown [6, 7, 11]. Besides, most of the local studies had neglected the incidence of the pathogenic *Candida albicans* in the oral cavity of small children [17, 18, 19].

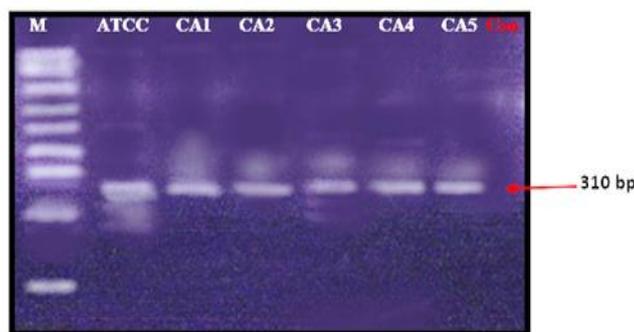
Accordingly, the total of (368) of school children were included in this study, and the results of the oral examination showed a high prevalence of Candidial infections among many students who volunteered for the inspection and results indicated that about (198) students out of (368) students examined from the particular primary schools had displayed suspected Candidiasis with an incidence rate of (58.37%) among all ages and in both sexes. The same results were obtained by [12, 18], where they declared a percentage of (52.33% and 47.3%) of Candidiasis among preschool and school children in different parts of the world, respectively.

Subsequently, the suspected Candidiasis oral samples were microbiologically analyzed after the cultivation on selective medium for the identification of the fungal strain and the outcomes of the Gram staining of the wet oral samples and germ tube test had revealed the isolation of Gram's positive budding yeast cells that was almost bearing a resemblance to *Candida albicans* as shown in (Figure 1).



**Figure 1** A photograph of light microscope under (100X) magnification showing Gram's positive budding yeast cells of suspected *Candida albicans* strain isolated from a ten years old male student during the study.

As a consequence, molecular analysis was conducted for the identification of invasive *Candida albicans* isolates against the genotype sequence of the standard virulent strain of *Candida albicans* ATCC 10231 as described by [19].



**Figure 2** Electrophoretic pattern of RT-PCR amplified DNA products. Lane M, 100-bp marker; Lane 1, *C. albicans* ATCC 10231 (310 bp); Lanes (CA1-5), the tested *C. albicans* isolates of the study; Lane Con, negative control, no DNA template (distilled water).

Other Studies [17, 18, 20] had identified virulent strains of *Candida albicans* strains at different genotypic base pairs probably because this fungus has significant phenotypic and genetic diversity [20, 21]. It contains a diploid genome with 14.4 megabases that are arranged as eight chromosomes [21]. The heterozygosity and heterozygous of the genome are thought to be related to *C. albicans* virulence [24]. Also, it is now well documented that the genomic instability of *C. albicans* plays a significant role in its pathogenesis.

Since the genetic variability of *C. albicans* can modulate the candida behavior at the host defense mechanism [21]. Thus, the predominance of the invasive strains was analyzed according to the study levels of the infected school children and results were declared in (Table 1)

**Table 1** Distribution of invasive *Candida albicans* among student in a private primary school.

Patient's Gender	Infected Students Numbers per grades					
	1st	2nd	3rd	4th	5th	6th
Males	7	10	21	22	26	38
Females	5	7	11	12	16	20
Total	12	17	32	34	42	58
Percentages	6.15%	10.30%	16.41%	17.44%	21.54%	29.74%

From the results of the table above, it could be concluded that mouth thrush predominated all ages of students recording a percentage of (64.53 %) in male students and (35.47 %) in female students respectively. These results agree with the results obtained previously by [25, 26, 27], where they exposed that, mouth thrush was very prevalent among preschool and School children due to increased oral cavities due to teeth loss at these ages. However, in this study, it was noticed that the percentage of Candidiasis was significantly higher in males than female students, probably due to poor oral hygiene practices by boys. Moreover, it could be noticed from the table above that the predominance in *Candida albicans* incidence increased with the age of the students where students of 11-12 years old were more affected by Candidiasis compared to the younger ones probably due to a relationship between personal hygiene with nutrition. The same results obtained by [26, 27], where they concluded that girls had better hygiene practices and eating routines than the boys. Also, the majority of the health problems affecting school children are preventable by the promotion of hygienic practices and healthy eating habits.

#### 4. Conclusion

The Maintenance of a healthy mouth environment, a balanced microbial ecology is very crucial to children's health. Besides, invasive *Candida albicans* was significantly high ( $P < 0.05$ ) among male students than females of a primary school in 2019. Still, the predominance of invasive *Candida albicans* increased with student's age.

#### Compliance with ethical standards

##### Acknowledgments

The present work was carried out at the Medical Laboratory Techniques Department/ Kirkuk / Iraq.

##### Disclosure of conflict of interest

The author declares no conflict of interest.

##### Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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