Archives of Razi Institute, Vol. 78, No. 1 (2023) 475-483

DOI: 10.22092/ARI.2022.359466.2422

Original Article

Diagnosis of Oral *candidiasis* in Patients under 12 Years: 18*S rRNA* as a Marker of Molecular Characterization of *Candida tropicalis*

Alwaily, E. R[1], Abood, M. S[2], Al Uobody, R. M[1]

1. College of Pharmacy, Al-Ayen University, Thi-Qar, 64001, Iraq
2. Department of Biology, College of Education for Pure Science, Thi-Qar University, Thi-Qar 64001, Iraq

Received 18 July 2022; Accepted 6 August 2022
Corresponding Author: enas.kazem@alayen.edu.iq

Abstract

Candida tropical has been found as the most abundant pathogenic yeast species under the group Candida-non-albicans. Despite this, it is taxonomically related to *C. albicans* and has many of its pathogenic characteristics. Infection with *Candida tropicalis* is closely associated with many virulence factors encoded by multiple virulence genes. This study aims to diagnose *C. tropicalis* based on the presence of *18SrRNA* and to detect many virulence genes. *C. tropicalis* isolates were collected from oral candidiasis patients. Children infected with oral thrush ranging in age from infants to 12 years old provided 150 samples. *C. albicans* (66.68 %), *C. tropicalis* (13.21 %), *C. krusie* (9.43 %), *C. parapsilosis* (7.55 %), and *C. glarata* were isolated as *C. tropicalis* types, according to the findings of the present study (2.83%). The presence of the *18SrRNA* gene was confirmed in the isolates. All isolates were positive for *cph1* and *hwp1*, while some were positive for *sap1* (78.5%) and *plb1* genes (71.4%). Using sequences and phylogenetic trees, it was determined that there was negligible genetic variation between local isolates and global strains. These virulence factor genes play a crucial role in developing infections.

Keywords: Oral candidiasis, *sap1, hwp1, cph1, plb1*

1. Introduction

Candida tropical has been identified as the most prevalent pathogenic yeast species from the group Candida-non-albicans, but despite this, it is taxonomically close to *C. albicans* and shares many pathogenic traits and itis one of the most widespread pathogens and colonizers, causing many human diseases in tropical countries (1), this disease vary depending on the location where the species colonizes such as oropharyngeal candidiasis, balanoposthitisoral thrush or oral candidiasis and vulvovaginal candidiasis (2).

The most prevalent mucocutaneous mycosis of the oral cavity is oral candidiasis, brought on by the species *Candida spp*. As a common commensal organism, *Candida* is present in the oral cavity of the general population in about 53% of cases. Around 150 species in this genus have been isolated from the oral cavity, and *Candida albicans* is the most prevalent species, accounting for 80% of the isolates. When combined with *Candidaglabrata* or *Candida tropicalis*, *Candida albicans* can colonize the oral cavity. This combination is seen in 7% of healthy individuals and 80% of candidiasis cases (3).

The pathogenicity or virulence factors of *C. tropicalis* due to its ability to form a biofilm, produce lytic enzymes like protease and phospholipase enzymes, adhere to epithelial cells, and switch from bud to

hyphae (phenotypic switching) and the filamentous growth of *C. tropicalis* only have the ability to invade and colonize oral epithelial cells (4, 5).

C. tropicalis needs some cofactors to enter and cause infection in the host; once attached to the host′s cells, excretion of extracellular enzymes known as protease enzymes facilitate the penetration of the *C. tropicalis* and permit it to interfere with the host defense system. Produced aspartic proteases (*sap*) support the ability of *C. tropicalis* to penetrate deep into the host tissue and attach to affect the organs because proteases hydrolyze peptide bonds. Phospholipases hydrolyze phospholipids and help to destroy the structure of the epithelial cell membrane allowing the tip of a hypha to enter the cytoplasm (6). Moreover, *hwp1* is another virulence factor that was shown to have a considerable role in hyphae production, host tissue injury, and biofilm formation, respectively (7).

C. tropicalis is essential for many diseases like oral candidiasis, so molecular methods to determine some. *C. tropicalis* virulence factors necessary to control disease, wherefore this study aims to investigate some virulence genes (*hwp1, sap1, cph1, plb1*) in *C. tropicalis* that may increase pathogenicity for it.

2. Materials and Methods

2.1. Samples Collection from Oral Swabs

Randomly, 150 Oral swabs samples were collected from children infected with oral thrush. Their ages ranged from newborns to 12 years who attended the consulting clinic at Al-Rifai General Hospital and Mohammad Al- Mousawi Children′s Hospital in AL-Nasiriyah City, Thi-Qar province, south of Iraq. The current study was conducted from the beginning of January to the end of December 2020. All Oral swabs were cultivated on sabouraud dextrose agar to diagnose *Candida* spp. that may grow.

2.2. *C. tropicalis* Isolates

The phenotype of fungal colonies was checked and identified after the cultivation and incubation on SDA depending on, microscopic and traditional tests (Germ tube formation, Sugar fermentation, Chlamydospore formation, and Differential medium CHROM agar *Candida*) were used to identify *Ctropicalis* (8).

2.3. Genomic DNA Extraction

Using EZ-10 Spin Column Fungal Genomic DNA Mini-Preps Kit, genomic DNA of *Candida* spp. was extracted according to the produced company protocol, and then DNA was checked with Nanodrop-spectrophotometer. Finally, the extracted DNA was stored at -20˚C until use.

2.4. PCR Amplification

In the study, the 18S *rRNA* gene was used to identify *C. tropicalis* isolates, according to Fatima, Bashir (9). The conventional PCR technique was also used to determine virulence genes (*hwp1, plb1, sap1, cph1*) in all *C. tropicalis* isolates. The primers were designed online with the NCBI database and Primer 3. They were provided by Bioneer Company (Korea) (Table 1). According to the AccuPower®PCR PreMix kit, the PCR master mix (Bioneer, Korea). The PCR master mix reaction components were inserted in conventional PCR tubes and included 5 µl of DNA template, 10 pmol of each F and R primer, and 12µl of PCR water. The PCR thermocycler's settings were 95 °C for five minutes of initial denaturation, followed by 30 cycles of 95 °C for 20 seconds, 60 °C for 20 seconds, and 72 °C for one minute. The final extension was at 72 °C for 5 min. PCR products of each gene were electrophoresed using 1% agarose gel with 3µL of ethidium bromide. In each comb well, 10 µl of PCR product was added, and 5 µl of the ladder (100bp) was added in one well. Next, the gel tray was fixed and filled with a (1X) TBE buffer inside the chamber. After that, the current was set at 100 volts and 80 mA for 1 hr. Finally, PCR amplification products were imaged with a UV transilluminator.

Table 1. Oligonucleotide primers designed and used in PCR technique

Primer		Sequence (5'-3')	Product Size bp
Candida tropicalis	F	ATGCCCTTAGACGTTCTGGG	415
18S rRNA gene	R	ATCGATGCGAGAACCAAGAGA	
Adhesion	F	CCTCAACCAGATCAGCCATGT	598
hwp1 gene	R	AGGTTTCACTGGCAGGCATG	
Phospholipase	F	GGTGGGTCATGGTTAGTGGG	489
PLB1 gene	R	CGGCCATCATTTTAGCAGCC	
proteinase	F	CCGTACCAAGTTCTGAGTTTGC	460
SAP1 gene	R	TGTCGCTGCTGGAGGAAATT	
hyphal formation	F	ACTGCTCCAGCTAATTGGCA	224
CPH1 gene	R	ACCAAGACCAACAGCAGCAT	

2.5. Phylogenetic Analysis

The gene samples were purified using a purification kit, and one sample was sent to Macrogene Company (Korea) for sequencing and deposited into GenBank for accession numbers. The recorded nucleotide sequences of *C .tropicalis* isolates were compared with *C .tropicalis* strains in NCBI GenBank to detect mismatching between gene sequences using the NCBI-Blast, (http://blast.ncbi.nlm.nih. gov/Blast.cgi). Phylogenetic tree analysis has based on molecular evolutionary genetics analysis using Mega V. 6.

2.6. Statistical Analysis.

Analysis of findings was done by excel 2010 and statistical packages for social science-version 23 (SPSS). Chi-squared was used to assess these significant differences at $P \leq 0.05$ (10).

3. Results

3.1. Diagnosis of *Candida* spp.

The study found that 106 (70.66 %) of 150 isolates were positive for *Candida spp.* Five different species of this genus were identified, as follows: 71 (66.98 %) *C. albicans*, 14 (13.21%) *C. tropicalis*, *C. krusie*10 (9.43%), 8 (7.55%) *C. parapsilosis*, and 3 (2.83%) *C. glabrata* are all included in table 2. The study also confirmed the existence of significant differences between the isolated fungal species.

3.2. Molecular Study of *Candida tropicalis*

The isolates of *Candida tropicalis*, which were identified by biochemical tests and CHROM gen agar *Candida* have supported their diagnosis by a detective the presence of *18Sr*RNA gene at 415bp (Figure 1A). All isolates of this genus were positive for this gene. All *Candida tropicalis* isolate positive to *hwp*1 and *cph*1gene (100%) at 598 bp and 224 bp, respectively (Figure 1B and 1C). In 11 (78.5%) *Candida tropicalis* isolates, the sap1 gene was found at 460 bp, and in 10 (71.4%) of these isolates, the plb1 gene was found at 489 bp (Figure 1D and 1E).

3.3. DNA Sequence results for *Candida tropicalis*

Among all *C. tropicalis* isolates containing virulence genes in their genome, one isolate contained virulence genes; it was sent for sequencing for multiple alignment analysis. Nevertheless, the nucleotide sequence of the 18*S rRNA* gene showed slight genetic variation between the local *C. tropicalis 18Sr RNA* gene and NCBI BLAST *C.tropicalis* isolates. Phylogenetic tree analysis was intended to reveal the extent of genetic similarity between this isolate and the registered strains globally, where local *C. tropicalis* isolate (MZ025916) has shown a genetic similarity to NCBI-Blast strains (MF925001.1/China/2018) with Identity: 99.00 %at total genetic change (0.006-0.001) (Figure 2A).

Table 2. Numbers and percentages different yeast species

Species	Number	Percentage
Candida albicans	71	66.98
Candida tropicals	14	13.21
Candida krusie	10	9.43
Candida glabrata	3	2.83
Candida parapsilosis	8	7.5
Total	106	100

$x^2 = 14.60$ df= 4 P-valu= 0.00

Depending on the multiple alignment analysis, the sequencing of partial virulence gene *cph1*gene for local *C. tropicalis* isolate (MZ032148) showed (99.15 %)homology identity to NCBI- BLAST isolate (XM_002549816.1/USA /2018) (Table 3) and by using the phylogenetic tree analysis between *C.tropicalis* isolates the genetic variation was (1.5 -0.5%) (Figure 2B). AS for *hwp1* gene of local *C. tropicalis* isolate (MZ032147) recorded simple genetic variation. It was near NCBI- BLAST isolate (EU616624.1/Iran/2018), with identity: 99.38 % at genetic change (0.6 - 0.1 %) (Figure 2C). Phylogenetic tree of *sap1* gene of local *C. tropicalis* isolate (MZ032149) showed genetically similarity related to NCBI-Blast *C. tropicalis* isolate (MF925001.1/China/2018) (Identity: 99.09 %) at total genetic change (0.004 -0.001 %), (Figure 2D). While local *C. tropicalis* isolate of *plb1*gene (MZ032146) showed homology identity to NCBI-BLAST isolate (AY394565.1/Malaysia/2005) (Identity: 98.58 %) at total genetic change was (0.007 -0.001 %) (Figure 2E).

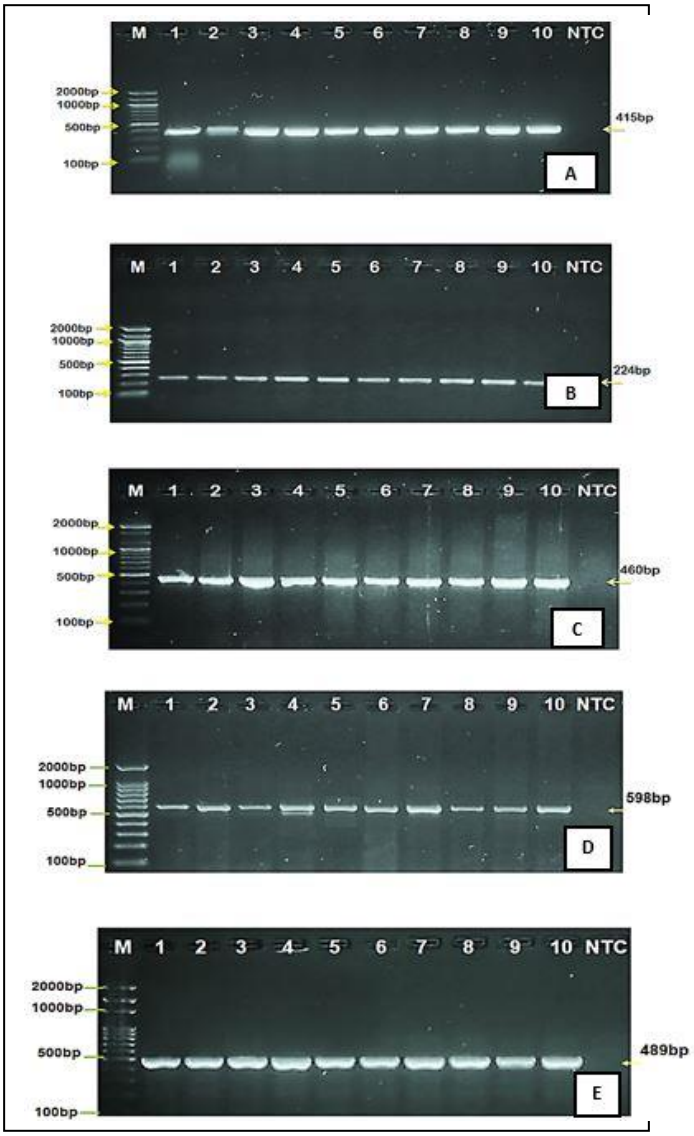

Figure 1. Agarose gel electrophoresis of *C.tropicalis*. Where Marker ladder (100-2000bp), lane (NTC): Non template negative control. **A:** Lanes (1-10) positive for 18*S rRNA* gene (A) at 415bp, **B:** *cph1* gene at 224bp, **C:** *hwp1* adhesion gene at 589bp, **D:** *sap1* proteinase gene at 460bp and **E:** *plb1* gene at 489bp, at 100 volts and 80 mA for 1 hr. Finally

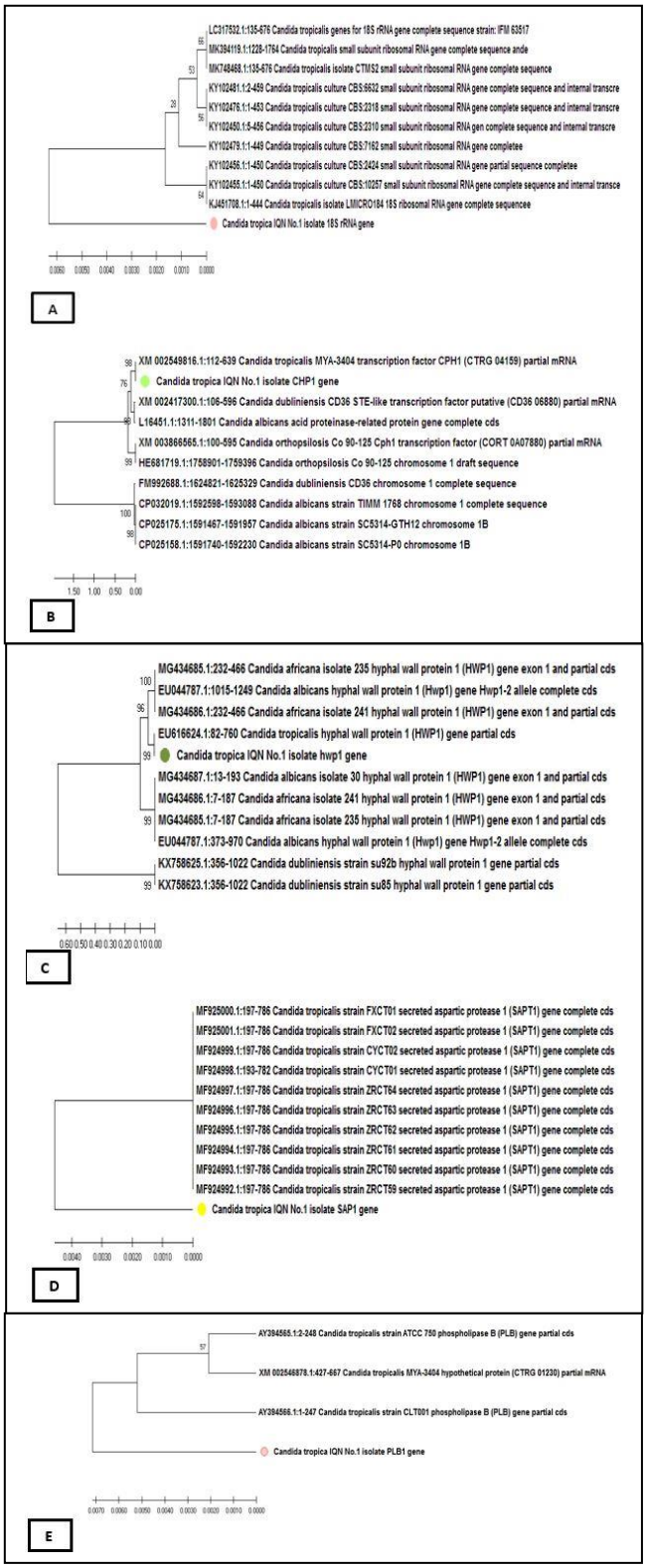

Figure 2. Phylogenetic analysis using Maximum Likelihood method (MEGA 6.0 version). **A:** 18*S rRNA* gene sequencing of local *C. tropicalis* (MZ025916) isolate at total genetic changes (0.006-0.001). , **B:** *cph1* gene partial sequence in local *C. tropicalis* (MZ032148) isolate at total genetic changes (1.5 -0.5), **C:** *hwp1* gene partial sequence in local *C. tropicalis* (MZ032147) isolate at total genetic changes (0.6 - 0.1 %), **D:** *sap1* gene partial sequence in local *C. tropicalis*(MZ032149) isolate at total genetic changes (0.004 -0.001%), **E:** *plb1* gene partial sequence in local *C. tropicalis(MZ032146)*isolate at total genetic changes (0.007 -0.001%)

Table 3. NCBI-BLAST Homology Sequence identity (%) between local *C.tropicalis* IQN isolates and NCBI-BLAST isolates

Local isolate No.1	Genbank Accession number	NCBI-BLAST Homology Sequence identity (%)		
		Identical NCBI BLAST gene-related isolate	Genbank Accession number	Identity (%)
18SrRNA	MZ025916	Candida tropicalis strain FXCT02	MF925001.1	99.00%
CPH1	MZ032148	Candida tropicalis MYA-3404	XM_002549816.1	99.15%
HWP1	MZ032147	Candida tropicalis	EU616624.1	99.38%
PLB1	MZ032146	Candida tropicalis strain ATCC 750	AY394565.1	98.58%
SAP1	MZ032149	Candida tropicalis strain FXCT02	MF925001.1	99.09%

4. Discussion

The colonization of the NAC species in the oral cavity was progressively increasing. The determination of non-*C. albicans* species in oral cavity candidiasis is essential where some of these species resist the azole compounds used to treat this infection. Many species of non-*C .albicans* have been registered in this study.

According to the study of Candida tropical (13.21 %), the results were comparable to those of Ambe, Longdoh (11), who found a prevalence of 9.4% for *C. tropicalis* in patients with oral candidiasis. Other studies report that 6.06% of *C. tropicalis* that isolated from the oral cavity of a patient with oral candidiasis (1), the study by Muadcheingka and Tantivitayakul (12) found that *C. tropicalis* recorded (10.4%) when have been isolated from the oral cavity. According to Costa, Passos (13), oral isolates were created when *Candida tropicalis* and *Candida albicans*. They observed that compared to *C. albicans*, *C. tropicalis* showed a more remarkable ability to adhere to laminin and fibronectin.

In the current study, *C. tropicalis* was identified using *the 18SrRNA* gene, and the results revealed that all isolates have this gene at 415pb. Traditional diagnostic techniques that depend on biochemical studies are not very reliable; in contrast, molecular techniques like conventional PCR are highly accurate (14). The most popular approach to differentiating different fungus species is based on 18S rRNA sequences (14-16). In the current study, some virulence genes have been found in the genome of *C. tropicalis*. Several therapeutic bacteria use lytic enzymes, including proteinases, phospholipases, and hemolysins, to alter or destroy the host membranes, causing the host cells to malfunction or lacerate to encourage invasions of the host tissues (17).

sap1 gene was detected In this study with 11 isolates (78.5%) at 460pb. Several secreted aspartyl protease superfamily members have been considered virulence factors in the genus *Candida*. Among them *Candida tropicalis* (18). According to Dabiri, Shams-Ghahfarokhi (19), the *sap* expression is dependent on strain and source, and that plays a significant role in infections with *Candida* spp; high proteinase activity belongs to oral candidiasis .*sap1-4* genes have been identified in species *C. tropicalis* and *sap1* is the prevalent enzyme that produced in vitro. At the same time, the expression level of the residual 3 *sap* genes is low and can only be measured by RT-PCR (20). Das, Mangayarkarasi (21) found that all isolates positive to *sap1* genes 100% (3-3) that isolated from clinical vulvovaginal candidiasis symbiotic and (4-4) 100% asymptotic. On the contrary, Costa, Passos (13) estimated proteinase enzyme activity of 15 isolates of *Candida albicans* and 15 isolates of *Candida tropicalis* isolated from the saliva of dental inflammation patients. Also, Compared to *C. albicans* isolates, all *C. tropicalis* isolates had increased enzymatic secretion. These findings go opposite to the majority of other research findings that *C. albicans* has stronger (20-22)

Many studies refer to fungal phospholipases that may promote virulence by damaging the host cell membranes (23, 24). This study also *found that the plb1* gene was detected in 10 isolates (71.4%) at 489pb.

Phospholipids are the primary components of cell membranes. Therefore, the production of phospholipases is considered an essential attribute for host epithelial invasion; in addition, the fracture of these molecules encourages instability in host cells, resulting in the lysis of cells (23, 24). The study by Bassyouni, Wegdan (25) revealed that 100% of *Candida* isolates caused VVC in diabetic women secretion phospholipase, and the gene *plb1* was identified in (87.5 %) of 35 isolates. Another study with 29 *C. tropicalis* isolates isolated from various anatomic sites obtained from hospitalized patients revealed low or no activity for phospholipase in *C. tropicalis* (26). While Jiang, Li (27) utilized 52 strains of *C. tropicalis* that were isolated from various clinical sources and found phospholipase enzyme activity in 31 isolates.

The *hwp1* gene controls another significant adhesin found on the hyphal cell wall, which was present in all isolates (100%) of *C. tropicalis* in this study. Several studies have shown that *C. tropicalis* is the second species after *Candida albicans* in terms of its ability to adhere (28, 29). According to specific investigations, hwp1p is found in high concentrations at hyphal cell walls while in low concentrations in blastoconidia and pseudohyphae (30). Modrzewska and Kurnatowski (31) have mentioned that *als*, *epa*, *and hwp1* are considered the most important adhesins found on the cell wall of *Candida* spp. It is also considered the responsible gene for fungal hyphae formation. Naglik, Fostira (30) have mentioned that expression of the *hwp1* gene is high among strains isolated from patients with candidiasis. Moreover, the research conducted by Wan Harun, Jamil (32) in Malaysia revealed that adhesin was reported for *C. tropicalis* and that utilizing mRNA expression, it was possible to prove the existence of the *hwp1*gene in NCAC species. The constant regulation of *hwp1* mRNA transcription for *C. tropicalis* suggests that this species can express the gene for this adhesin. This contradicts ten Cate, Klis (33) claim that the *hwp1* gene is exclusively detected in *C. albicans.*

Additionally, this study demonstrates that all isolates can produce the *cph1* gene, which is necessary for morphogenesis and the transcription of the relevant downstream proteins at 224pb. de Barros, Rossoni (34) demonstrated that cph1 has a minor role in the transcriptional control of cell wall genes throughout hyphal and yeast growth. It is well documented that to create a biofilm, pathogenicity, as well as hyphae, are required (35).

In conclusion, this study confirmed that the species *Candida tropicalis* is no less important than the species *Candida albicans* in terms of pathogenicity and its ability to cause diseases including oral candidiasis and that by having virulence factors that are closely related to the events of infection also use the molecular strategies in diagnosis this species and detecting the genes encoding some virulence factors are necessary due to its high accuracy and the oral candida infection rate increases with age, also are prone to develop in certain populations as a warning sign and present in potentially malignant oral disorders of significance.

Authors' Contribution

E. A. conceived the presented idea, designed the study, and collected the samples. M. F. and M. A. investigated from the practical aspect and verified the analytical methods. M. A. wrote the original draft. K. H. and M. F. wrote, reviewed, and edited the final manuscript. K. H. supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

Ethics

The current study protocol was obtained from the college of pharmacy, Al-Ayen University, and agreed upon by the management of the training and human development department/ Thi-Qar Health Office. Also, participants filled out written consent on this study, which was voluntary. All authors have seen and approved the study protocol.

Conflict of Interest

There are no conflicts of interest among authors. We hereby confirm that all the Figures and Tables in the manuscript are mine.

Acknowledgment

The authors would thank the staff of Al-Rifai General Hospital and Mohammad Al-Mousawi Children's Hospital in Thi-Qar province for their help collecting the samples. The study is not supported financially.

References

1. Hu L, He C, Zhao C, Chen X, Hua H, Yan Z. Characterization of oral candidiasis and the Candida species profile in patients with oral mucosal diseases. Microb Pathog. 2019;134:103575.
2. Chamorro-Petronacci C, Garcia-Garcia A, Lorenzo-Pouso AI, Gomez-Garcia FJ, Padin-Iruegas ME, Gandara-Vila P, et al. Management options for low-dose methotrexate-induced oral ulcers: A systematic review. Med Oral Patol Oral Cir Bucal. 2019;24(2):e181-e9.
3. Bensadoun RJ, Patton LL, Lalla RV, Epstein JB. Oropharyngeal candidiasis in head and neck cancer patients treated with radiation: update 2011. Support Care Cancer. 2011;19(6):737-44.
4. Sardi JCO, Scorzoni L, Bernardi T, Fusco-Almeida AM, Mendes Giannini MJS. Candida species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. J Med Microbiol. 2013;62(Pt 1):10-24.
5. Zuza-Alves DL, Silva-Rocha WP, Chaves GM. An Update on Candida tropicalis Based on Basic and Clinical Approaches. Front Microbiol. 2017;8:1927.
6. Chai LY, Denning DW, Warn P. Candida tropicalis in human disease. Crit Rev Microbiol. 2010;36(4):282-98.
7. Kothavade RJ, Kura MM, Valand AG, Panthaki MH. Candida tropicalis: its prevalence, pathogenicity and increasing resistance to fluconazole. J Med Microbiol. 2010;59(8):873-80.
8. Gupta V, Abhisheik K, Balasundari S, Devendra NK, Shadab K, Anupama M. Identification of Candida albicans using different culture media and its association in leukoplakia and oral squamous cell carcinoma. J Oral Maxillofac Pathol. 2019;23(1):28.
9. Fatima A, Bashir G, Wani T, Jan A, Kohli A, Khan MS. Molecular identification of Candida species isolated from cases of neonatal candidemia using polymerase chain reaction-restriction fragment length polymorphism in a tertiary care hospital. Indian J Pathol Microbiol. 2017;60(1):61.
10. Bryman A, Cramer D. Quantitative data analysis with IBM SPSS (21): A guide for social scientists Rutledge. 2012.
11. Ambe NF, Longdoh NA, Tebid P, Bobga TP, Nkfusai CN, Ngwa SB, et al. The prevalence, risk factors and antifungal sensitivity pattern of oral candidiasis in HIV/AIDS patients in Kumba District Hospital, South West Region, Cameroon. Pan Afr Med J. 2020;36:23.
12. Muadcheingka T, Tantivitayakul P. Distribution of Candida albicans and non-albicans Candida species in oral candidiasis patients: Correlation between cell surface hydrophobicity and biofilm forming activities. Arch Oral Biol. 2015;60(6):894-901.
13. Costa CR, Passos XS, e Souza LK, Lucena Pde A, Fernandes Ode F, Silva Mdo R. Differences in exoenzyme production and adherence ability of Candida spp. isolates from catheter, blood and oral cavity. Rev Inst Med Trop Sao Paulo. 2010;52(3):139-43.
14. Fricke S, Fricke C, Schimmelpfennig C, Oelkrug C, Schonfelder U, Blatz R, et al. A real-time PCR assay for the differentiation of Candida species. J Appl Microbiol. 2010;109(4):1150-8.
15. Schabereiter-Gurtner C, Selitsch B, Rotter ML, Hirschl AM, Willinger B. Development of novel real-time PCR assays for detection and differentiation of eleven medically important Aspergillus and Candida species in clinical specimens. J Clin Microbiol. 2007;45(3):906-14.
16. Zhang J, Hung GC, Nagamine K, Li B, Tsai S, Lo SC. Development of Candida-Specific Real-Time PCR Assays for the Detection and Identification of Eight Medically Important Candida Species. Microbiol Insights. 2016;9:21-8.
17. Sanita PV, Zago CE, Mima EG, Pavarina AC, Jorge JH, Machado AL, et al. In vitro evaluation of the enzymatic activity profile of non-albicans Candida species isolated from patients with oral candidiasis with or without diabetes. Oral Surg Oral Med Oral Pathol Oral Radiol. 2014;118(1):84-91.
18. Parra-Ortega B, Cruz-Torres H, Villa-Tanaca L, Hernandez-Rodriguez C. Phylogeny and evolution of the aspartyl protease family from clinically relevant Candida species. Mem Inst Oswaldo Cruz. 2009;104(3):505-12.

19. Dabiri S, Shams-Ghahfarokhi M, Razzaghi-Abyaneh M. SAP (1-3) gene expression in high proteinase producer Candida species strains isolated from Iranian patients with different candidosis. J Pure Appl Microbiol. 2016;10(3):1891-6.
20. Zaugg C, Borg-Von Zepelin M, Reichard U, Sanglard D, Monod M. Secreted aspartic proteinase family of Candida tropicalis. Infect Immun. 2001;69(1):405-12.
21. Das KH, Mangayarkarasi V, Sen M. Genotypic Patterns of Secreted Aspartyl Proteinase Gene in Various Candida Species Isolated from Antenatal Women with Vulvovaginal Candidiasis. J Pure Appl Microbiol. 2019.
22. Bianchi CM ,Bianchi HA, Tadano T, Paula CR, Hoffmann-Santos HD, Leite DP, Jr., et al. Factors Related to Oral Candidiasis in Elderly Users and Non-Users of Removable Dental Prostheses. Rev Inst Med Trop Sao Paulo. 2016;58:17.
23. Schaller M, Borelli C, Korting HC, Hube B. Hydrolytic enzymes as virulence factors of Candida albicans. Mycoses. 2005;48(6):365-77.
24. Staniszewska M, Bondaryk M, Piłat J, Siennicka K, Magda U, Kurzątkowski W. Czynniki zjadliwości Candida albicans. Przegl Epidemiol. 2012;66:629-33.
25. Bassyouni RH, Wegdan AA, Abdelmoneim A, Said W, AboElnaga F. Phospholipase and Aspartyl Proteinase Activities of Candida Species Causing Vulvovaginal Candidiasis in Patients with Type 2 Diabetes Mellitus. J Microbiol Biotechnol. 2015;25(10):1734-41.
26. Galan-Ladero MA, Blanco MT, Sacristan B, Fernandez-Calderon MC, Perez-Giraldo C, Gomez-Garcia AC. Enzymatic activities of Candida tropicalis isolated from hospitalized patients. Med Mycol. 2010;48(1):207-10.
27. Jiang C, Li Z, Zhang L, Tian Y, Dong D, Peng Y . Significance of hyphae formation in virulence of Candida tropicalis and transcriptomic analysis of hyphal cells. Microbiol Res. 2016;192:65-72.
28. Biasoli MS, Tosello ME, Luque AG, Magaro HM. Adherence, colonization and dissemination of Candida dubliniensis and other Candida species. Med Mycol. 2010;48(2):291-7.
29. Lyon JP, de Resende MA. Correlation between adhesion, enzyme production, and susceptibility to fluconazole in Candida albicans obtained from denture wearers. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;102(5):632-8.
30. Naglik JR, Fostira F, Ruprai J, Staab JF, Challacombe SJ, Sundstrom P. Candida albicans HWP1 gene expression and host antibody responses in colonization and disease. J Med Microbiol. 2006;55(Pt 10):1323-7.
31. Modrzewska B, Kurnatowski P. Adherence of Candida sp. to host tissues and cells as one of its pathogenicity features. Annals of parasitology. 2015;61(1).
32. Wan Harun WH, Jamil NA, Jamaludin NH, Nordin MA. Effect of Piper betle and Brucea javanica on the Differential Expression of Hyphal Wall Protein (HWP1) in Non-Candida albicans Candida (NCAC) Species. Evid Based Complement Alternat Med. 2013;2013:397268.
33. ten Cate JM, Klis FM, Pereira-Cenci T, Crielaard W, de Groot PW. Molecular and cellular mechanisms that lead to Candida biofilm formation. J Dent Res. 2009;88(2):105-15.
34. de Barros PP, Rossoni RD, Freire F, Ribeiro FC, Lopes L, Junqueira JC, et al. Candida tropicalis affects the virulence profile of Candida albicans: an in vitro and in vivo study. Pathog Dis. 2018;76(2).
35. Senanayake I, Rathnayaka A, Marasinghe D, Calabon M, Gentekaki E, Lee H, et al. Morphological approaches in studying fungi: Collection, examination, isolation, sporulation and preservation. Mycosphere. 2020;11(1):2678-754