Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org
Original Research

Molecular detection of *ALS1*, *ALS3*, *HWP1* and *SAP4* genes in Candida Genus isolated from hospitalized patients in Intensive Care Unit, Tehran, Iran

Seyed Hossein Ardehali[1], Taher Azimi[2], Fatemeh Fallah[3], Nicka Aghamohammadi[4], Shadi Alimehr[5], Ahmad Moein Karimi[6], Leila Azimi[3*]

[1] Department of Anesthesiology and Critical Care, Shohadaye Tajrish Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[3] Pediatric Infection Research Center, Research Institute for Children Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[4] Department of Dental Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[5] Department of Mycology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[6] Tehran Medical Science Branch, Islamic Azad University, Tehran, Iran

Correspondence to: leilaazimi1982@gmail.com

Received October 16, 2018; **Accepted** April 9, 2019; **Published** April 30, 2019

Doi: http://dx.doi.org/10.14715/cmb/2019.65.4.3

Copyright: © 2019 by the C.M.B. Association. All rights reserved.

Abstract: Candida species are considered as one of the important cause of nosocomial and community infections. Candidacies are fourth caused by septicemia in some countries and possess extra cost to the health care system. The aim of this study was survey the presence of virulence factors associated with various candida geniuses in samples which have been collected from the intensive care unit. In this cross-sectional study, various clinical specimens have been collected from patients which hospitalized in the Intensive Care Unit (ICU) of Milad hospital, Tehran, Iran. The species of candida has been determined by CHROM agar. Finally, adherence factors genes and proteinase gene have been detected by PCR. In this research, 100 samples have been collected from patients that colonized with candida. *C. albicans* (63%) and *C.glabrata* (19.4%) are the most identified species, respectively. The species of four specimens have been not detected according to the color of CHROM agar candida medium and two different genus of candida has been isolated from 7 patients. The frequency of *Als1*, Als3, *HWP1 and SAP1* genes among *C. albicans* was (92%), (94%), (95%) and (88%), respectively. The most detected virulence factor was HWP1 and SAP4 was the lowest one. At least two virulence factors have been detected in 95% of different Candida species that can cause invasive fungal properties. These results are important for infection control committee in the hospital because invasive fungal diseases can make a serious problem for patients that hospitalized in ICU.

Key words: *Candida albicans*; Polymerase chain reaction; Intensive Care Unit.

Introduction

Candida albicans (*C. albicans*) can cause several types of infections in patients and healthy humans (1, 2). Candidiasis that caused with *C. albicans*, is an opportunistic infection (3). Nowadays *C. albicans* emerged as the fourth most common nosocomial infection in a different part of the world (4, 5). Under normal conditions and in healthy humans, *C. albicans* inhabit in a gastrointestinal tract (GI) and urogenital. In GI tract of healthy individuals, the proliferation and growth of *C. albicans* is inhibited by the existence of microbiome, as well as by actions of the immune system (2, 4, 6) In several condition such as HIV infection, cardiac or abdominal surgery, organ transplantation, consumption of immunosuppressive drugs, extensive use of broad-spectrum antibiotics and in malignant diseases includes neoplasia and cancer, candidiasis has been increased (7-10). Finding a study in one cancer hospital has revealed the occurrence of candidiasis was 2.9% with an attributed mortality of nearly 50% (11). Moreover, based on published studies, 10.2% of all cases of septicemia and 25% of all urinary tract infections (UTI) in hospital wards, especially intensive care unit (ICU) caused by *C. albicans* (12, 13). On the other hand, *C. albicans* accounted for 80% percent of all cases of vulvovaginal candidiasis (VVC) (14). Candida species have a several putative virulence factor such as phospholipases, secreted aspartyl proteinases, agglutinin-like sequence (*ALS*) gene, hyphal wall protein *(HWP)* and cell wall glycoproteins (adhesions), which contributed to adherence of *C. albicans* cells to various targets included other microorganism's cells, abiotic surfaces and different host cells (3, 14-16). *ALS* family of *C. albicans* consists of eight genes (*Als1–7* and *Als9*) that encode large glycosylphosphatidylinositol (GPI)-linked cell surface glycoproteins (17, 18). Among these essential proteins, *ALS3* is particularly serious for adhesion (2). Moreover, it revealed that in various condition, within VVC and infection of oral epithelial cells, the expression level of the *ALS1* gene is up regulated (19). *C. albicans ALS1* gene product as a cell surface protein could facilitate adherence to endothelial and epithelial cells and the results of studies on biofilm formation mechanism revealed that the expression level of the *ALS1* gene is enhanced. The finding suggested that the *ALS1* gene have an important role in biofilm formation process(20, 21). Furthermore, secreted aspartyl proteinases (*SAPs*)

are another virulence factor of *C. albicans* that has a significant role in pathogenicity process and encoded by SAP family genes. Genes belonging to the *SAP* family (*SAP1-10*) may contribute to colonization and infection by degrading components of host cell membranes. Moreover, these genes are expressed at several phases of the infection procedure and might play a critical role in tissue invasion. On the other hand, indicated that the hyphae production rate depends on the expression level of *SAP* genes(14, 15). *HWP1,* as a glycosylphosphatidylinositol linked mannoprotein like the *ALS* proteins, up-regulated throughout the biofilm formation process and contributed to covalent attachment of *C. albicans* to several surfaces and host cells(14, 22). There are several studies that survey the pathogenic mechanism of fungi, virulence factors of fungi and antifungal resistance; however, more studies are still needed on these subjects. In the current study, we aimed to investigate the existence of *ALS1*, *ALS3*, *HWP1* and *SAP4* genes by polymerase chain reaction (PCR) in different identified Candida species isolated from hospitalized patients in ICU in Tehran, Iran. Another objective of the current study was to evaluate which of the Candida species have the highest and lowest pathogenicity and prevalence among patients.

Materials and Methods

Ethics Statement

The study was approved by the Ethics committee of the Pediatric Infections Research Center, Shahid Beheshti University of Medical Sciences (SBMU), Tehran, Iran. (no.IR.SBMU.MSP.REC.1396.234). Informed written consent was obtained from subjects enrolled. All data collected were analyzed anonymously.

Sample collection and identification of Candida strains

In the current study, from March 2016 until the end of February 2017, various clinical specimens have been collected from patients hospitalized in ICU from Milad hospital, Tehran, Iran. Specimens were collected from patients with bloodstream infection (BSI) and Urinary candidiasis. If a patient hospitalized at ICU ward for more than 48 h and had a positive blood culture for Candida species with significant clinical symptoms, this patient considered that have a BSI. Moreover, if patients had a positive urine culture with ≥103 yeast colonies/ml, these patients considered that have urinary candidiasis. The process of sample collection was performed with hospital personals and all specimens were taken from separate patients. Specimens transferred to pediatric infections research center laboratory in Mofid hospital after collection. Candida species identification was performed according to conventional microbiological tests such as culture on Sabaouraud Dextrose agar containing chloramphenicol, direct microscopic observation, germ tube formation in fetal calf serum at 37°C and culture on CHROM agar candida (Merck, Germany) medium. Furthermore, we used cornmeal-Tween 80 agar to evaluate the morphological characteristics.

DNA extraction and Polymerase Chain Reaction (PCR)

Genomic DNA was extracted from candida species colonies grown on CHROM agar candida medium using the specific kit according to the Manufacturer's instructions and preserve in -80°C. Polymerase chain reaction (PCR) was conducted for *ALS1, ALS3*, *HWP1* and *SAP4* genes using specific primer pairs. The primers sequences used for PCR reaction are revealed in Table 1. The PCR was performed in a 25 µl reaction mixture containing 3 µl of 10x PCR buffer without MgCl2, 2.5 mM MgCl2, 0.5 µl of 10 mM of each deoxynucleoside triphosphate (dNTPs), 0.5 µM of each primers (10mM), 1 units of Taq polymerase (Cinnagene, Iran), 3 µl of template DNA and sterile distilled water up to 25 µl. The PCR reactions were carried out with the following condition: One cycle of 95 °C for 5min, followed by 32 cycles of 95 °C for 1 minute, 55 °C for 1 minute, and 72 °C for 1minute with a final extension at 72°C for 10 min following the last cycle. Finally, the PCR product was screened on an agarose gel, stained with DNA safe stain (SinaClon Co., Iran) and visualized by a UV transilluminator.

PCR products were purified with the Silica Bead DNA Gel Extraction Kit (K0513) and the sequencing of PCR amplified products was carried out by the Sanger sequencing method. The sequencing was performed to confirm the presence of the examined genes. For these purpose, PCR was performed in a reaction mixture with the total volume of 50 µl containing 10 µl template DNA (40 ng),5 µl 10X Taq polymerase buffer [100 mM Tris/HCl (pH 8.3), 500 mM KCl, and 15 mM MgCl2], 0.5 µl, (100 pmol/ µl) each of primers, 0. 5 µl dNTPs (10 mM), 0.4 µl (5U/ µl) Taq DNA polymerase and 33 µl sterilized distilled water.

Statistical analysis

Information of patients such as gender, age and hospitalized ward were collected from the hospital database. The frequency and prevalence of Candida species among male and female were analyzed using the Chie square test and Fisher's exact test. A P-value of ≤ 0.05

Table 1. *Als1, ALS3 HWP1* and *SAP4* primers sequence for PCR amplification

Primer name		*Sequence (5'→3')*	*PCR Product size (bP)*
Als1	F	ACCAGAAGAAACAGCAGGTG	319
	R	GACTAGTGAACCAACAAATACCAG	
Als3	F	CCAAGTGTTCCAACAACTGAA	185
	R	GAACCGGTTGTTGCTATGGT	
HWP1	F	ATGACTCCAGCTGGTTC	503
	R	TAGATCAAGAATGCAGC	
SAP4	F	GAGTGTTCTTGCTTTCGCTTTA	201
	R	TTGCCCACATCATTTCTACC	

was considered statistically significant.

Results

Identification of Candida species

In this cross-sectional study, one hundred specimens have been collected from 100 non duplicated samples from patients (52 male and 48 female) that colonized with Candida species in Tehran, Iran. Specimens included blood, cerebrospinal fluid (CSF), peritoneal fluid, urine, Foley catheter, tracheal catheter and nasogastric tube. The mean age of the patients was 51.5 years old (1-95 years old). Twenty three and seventeen percent of patients had cancer and diabetes, respectively and other diseases such as seizure were less reported. The species of four specimens have been not detected according to the color of CHROM agar candida medium and two different genera of candida have been isolated from 7 patients. Totally, according to conventional microbiological tests, the frequency of various candida genus was as follows: *C.albicans* (n = 65; 63%), *C. glabrata* (n = 20; 19.4%), *C. tropicalis* (n = 8; 7.76%), *C. krusei* (n = 4; 3.88%), *C. lusitani* (n = 3; 2.9%), *C. dubliniensis* (n = 2; 1.9%), *C. kefyr* (n = 1; 0.9%). The frequency of Candida species isolated with CHROM agar medium is shown in Table 2. Moreover, Candida species distribution among sterile body sites and foreign bodies are shown in Table 3. Totally, Candida species were isolated from urine sample (n = 46; 44.6%), blood (n = 30; 29.1%), Foley catheter (n = 9; 8.7%), CSF (n = 7; 6.8%), peritoneal fluid (n = 5; 4.8%), tracheal catheter (n = 3; 3%) and nasogastric tube (n = 3; 3%). Amongst 46 Candida species isolated from urine specimen, the most frequently found species were *C. albicans* (n = 29), *C. glabrata* (n = 9), *C. tropicalis* (n = 3), *C. krusei* (n = 2) and C. lusitani, *C. dubliniensis* and *C. kefyr* each with one isolate. Thirty candida species were isolated from blood specimen of which 21 isolates (32.4%) revealed *C. albicans*, three (15%) showed *C. glabrata*, and C. lusitani, *C. dubliniensis* and, *C. krusei* each with one isolate. Of the nine *Candida* species isolated from a Foley catheter, most isolates (n = 5) were *C. glabrata* and four isolates were *C. albicans*. *C.albicans* (n = 4) and *C. glabrata (n = 1)* isolate were obtained from peritoneal fluid and C. albicans (n = 5) and C.tropicalis (n = 2) were isolated from CSF specimens. *Candida albicans (n = 2) and C. lusitani (n = 1)* were attained from nasogastric tube, while *C. glabrata (n = 2)* and *C. krusei (n = 1)* were attained from tracheal catheter. Evaluating the dissemination of candida genus among male and female gender has shown that *C. glabrata* isolates have the highest prevalence among females (P-value: 0.019). *C. dubliniensis* and C. *kefyr* have not been detected in specimens that were taken from females. The frequency of Candida species among male and female patients is shown in Table 4.

Table 2. The frequency of Candida species in clinical specimens.

Candida species	*Frequency*	*Percentage (%)*
C.albicans	65	63
C. glabrata	20	19.4
C. tropicalis	8	7.76
C. krusei	4	3.88
C. lusitani	3	2.9
C. dubliniensis	2	1.9
C. kefyr	1	0.9
Total	103	100

The frequency of virulence genes among Candida species

PCR product was screened on an agarose gel, stained with DNA safe stain (SinaClon Co., Iran) and visualized by a UV transilluminator. The results of gel electrophoresis for examined genes in Candida albicans isolates are shown in Figure1 and 2. In the next step, to confirmation of the presence of examined genes, purified PCR products were sequenced and results of sequencing were analyzed using the CLUSTALW, CHROMAS, and NCBI BLAST Software. The results of sequencing confirm the presence of examined gene and the finding

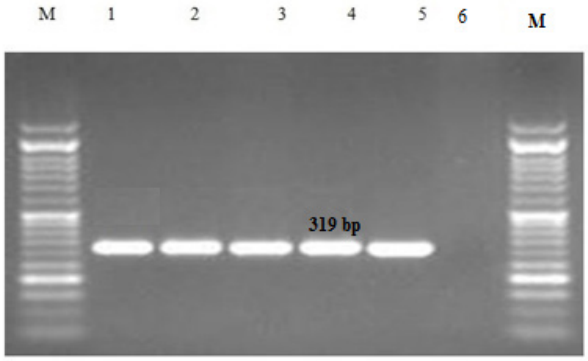

Figure 1. Polymerase Chain Reaction-based identification of the *Als1* gene, using species primer pairs in Candida albicans. M: DNA Ladder 50bp, 1: Internal positive control, 2-5: Positive sample; 6: Negative control.

Table 3. *Candida* species distribution among sterile body sites and foreign bodies.

Candida species	Total	Blood	CSF	Peritoneal fluid	Urine	Foley catheter	Tracheal catheter	Nasogastric tube
C.albicans	65	21(32.4%)	5(7.7%)	4(6.1%)	29(44.6%)	4(6.1%)	-	2(3.1%)
C. glabrata	20	3(15%)	-	1(5%)	9(45%)	5(25%)	2(10%)	-
C. tropicalis	8	3(37.5%)	2(25%)	-	3(37.5%)	-	-	-
C. krusei	4	1(25%)	-	-	2(50%)	-	1(25%)	-
C. lusitani	3	1(33.3%)	-	-	1(33.3%)	-	-	1(33.3%)
C. dubliniensis	2	1(50%)	-	-	1(50%)	-	-	-
C. kefyr	1	-	-	-	1(100%)	-	-	-
Total	103	30(29.1%)	7(6.8%)	5(4.8%)	46(44.6%)	9(8.7%)	3(3%)	3(3%)

CSF: Cerebrospinal fluid.

Table 4. The frequency of candida species isolated from clinical specimens on CHROM Agar medium among male and female patients.

Gender	Candida species																					
	Candida albicans			*Candida glabrata*			*Candida tropicalis*			*Candida krusei*			*Candida lusitani*			*Candida dubliniensis*			*Candida kefyr*		
	Yes (65)	No (38)	p	Yes (20)	No (83)	p	Yes (8)	No (95)	P*	Yes (4)	No (99)	P*	Yes(3)	No (100)	P*	Yes (2)	No (101)	P*	Yes (1)	No (102)	P*
Male	36 (55.4%)	19 (50%)	0.597	6 (30%)	34 (41%)	0.019	6 (75%)	49 (51.6%)	0.279	2 (50%)	53 (53.5%)	0.099	1 (33.3%)	54 (54%)	0.597	2 (100%)	53 (52.5%)	0.497	1 (100%)	54 (52.9%)	0.99
Female	29 (44.6%)	19 (50%)		14 (70%)	49 (59%)		2 (25%)	46 (48.4%0		2 (50%)	46 (46.5%)		2 (66.7%)	46 (46%)		0 (0%)	48 (47.5%)		0 (0%)	48 (47.1%)	

*Fishers exact test.

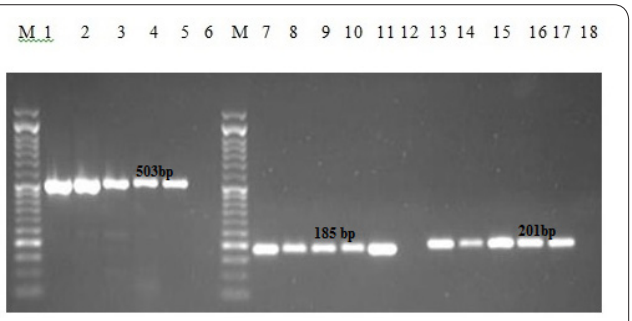

Figure 2. Polymerase Chain Reaction-based identification of the *HWP1*, *Als3*, and *SAP4* genes, using specific primer pairs in candida albicans. M: DNA Ladder 50bp,1: Internal positive control *HWP1*,2-5: Positive samples *HWP1*,6: Negative control *HWP1*,7: Internal positive control *Als3*,8-11: Positive sample *Als3*,12: Negative control *Als3*,13: Internal positive control *SAP4*,14-17: Positive sample *SAP4*,18: Negative control *SAP4*.

of our study revealed that among Candida species, the frequency of *Als1* and *Als3* genes was as follows: *C. albicans* (n = 60/65; 92% and 61/65; 94%), *C. glabrata* (n = 8/20; 40% and 11/20; 55%), *C. dubliniensis* (n = 2/2;100% and n = 2/2;100%), *C. lusitani* (n = 2/3;67% and n = 3/3; 100%), respectively. The *Als1* gene was not detected in *C. tropicalis*, *C. kefyr, and C. krusei.* The prevalence of *Als3* gene in *C. tropicalis* was 12.5% (n = 1/8). Also, gel electrophoresis results in Candida species have revealed the frequency of *HWP1*and *SAP4* genes was as follows: *C. albicans* (n = 62/65; 95% and n = 57/65;88 %), *C. dubliniensis* (n = 2/2;100% and n = 2/2;100%), C. *glabrata* (n = 11/20;55% and n = 12/20; 60%) and *C. lusitani* (n = 3/3;100% and n = 3/3;100%), respectively. PCR analysis detected the presence of *HWP1* in 25% (n = 2/8) of *C. tropicalis* isolates. In *C. kefyr* and *C. krusei* the PCR results for all investigated genes were negative. The frequency of existences of *ALS1, AlS3, HWP1* and *SAP4 genes* of Candida species are shown in Table 5.

Discussion

Generally, Candida species, particularly *C. albicans* are the main cause of candidiasis, worldwide (20). Based on published studies, Candida species have a several virulence factor includes adhesions, catabolic proteins, hyphae production, protease and phenotypic switching which are critical and necessary for survival and pathogenicity of the microorganism (14). Adherence is the first and most important step in candida infection especially in biofilm formation (23). *C. albicans* could attach on microorganism's cells, abiotic surfaces such as medical devices and different host cells (3). The *ALS* proteins that were coded by *Als1-Als9* genes are essential extracellular components to adhesion and colonization (17, 24). Moreover, *SAP4* and *HWP1* are other virulence factors that were shown has a significant role in hyphae Production, host tissue damage and biofilm development, respectively (15, 22). The result of our study revealed that *C. albicans* and *C. glabrata* have the highest prevalence at 63% and 19.4% with *C. kefyr* lowest at 0.9%. These results were similar to published studies that were conducted by L. Klingspor and J. ZHU(4, 25). In these studies stated that 64% of their 86 candida species and 56.6% of their 105 candida strains were *C.albicans*, respectively. Moreover, Roudbary et al. have been shown the *C. albicans* is the most Candida species isolated from clinical samples (3). *C.albicans* was the most important species isolated from male and female specimens. However, in the comparison between the two groups, the frequency of *C. glabrata* was higher among the female group (70%; p-value: 0.019). Moreover, the result of our study revealed that C. kefyr and C. dubliniensis had not been detected between female specimens. Among infected patients, cancer was the main underlying disease. Several factors including consumption of corticosteroids and broad-spectrum antibiotics, the use of a central venous catheter, mucosal damage due to chemotherapy may contribute to cancer in candidiasis(26).It has been well established that contaminated environmental surfaces play an important role in the transmission of infectious diseases in the healthcare setting. Moreover, despite routine cleaning, persistent contamination can occur because of some microorganisms such as C.albicans ability to form biofilms. As a result, the proper management of healthcare environmental surfaces is an integral part of the infection control of transmissible diseases(27).According to that candida infections arise from patient's endogenous microflora or healthcare environment, healthcare institutes should devise control programs against these infections. Administration, workers, and individuals admitted or visiting the hospital must take into account such programs to play their role in the prevention of infections(28).In this study *C. albicans* isolated frequently in the urine specimen, which is by other studies that described *C. albicans* as the most prevalent isolate in patients with urinary candidiasis(29-32). However, these results were dissimilar to other studies that revealed Candida non-*albicans* group such as C.glabrata as the most predominant isolate in patients with urinary candidiasis (33, 34). In this study, thirty candida species were isolated from blood specimen of which 21 isolates (32.4%) revealed *C. albicans* which is in accordance with other studies that were conducted by Pfaller et al. (35), Rodero et al. (36), Sandven et al. (37), Alvarado et al. (38) and Rahbar et al. (33). C.tropicalis and C.gla-

Table 5. The frequency of existences of *ALS1, AlS3, HWP1* and *SAP4 genes* of Candida species.

Candida species	Als1	Als3	HWP1	SAP1
C.albicans	60 (92%)	61 (94%)	62 (95%)	57 (88%)
C. glabrata	8(40%)	11 (55%)	11 (55%)	12 (60%)
C. tropicalis	0 (0%)	1 (13%)	2 (25%)	0 (0%)
C. krusei	0 (0%)	0 (0%)	0 (0%)	0 (0%)
C. lusitani	2 (67%)	3(100%)	3 (100%)	3 (100%)
C. dubliniensis	2 (100%)	2 (100%)	2 (100%)	2 (100%)
C. kefyr	0 (0%)	0 (0%)	0 (0%)	0 (0%)

brata were the most prevalent *Candida* non-*albicans* species in a blood specimen. This result is by other studies that were conducted by Rahbar et al. (33), St-Germain et al. (39) and Sandven et al. (37). In our study, the presence of *ALS1*, *ALS3*, *HWP1,* and *SAP4* Genes among Candida species were analyzed with PCR assays. The results of our study indicated that 94%, 55%, 100%, 13% and 100 % of *C. albicans, C. glabrata, C. Lusitania, C. tropicalis,* and *C. dubliniensis* strains were positive for *Als3,* respectively. Roudbary et al. investigated the existence of *Als3* gene in the vaginal swab, by using the PCR method. They found that the *Als3* gene was present in 90.5% of isolated *C. albicans* from these samples (3). The presence of the *ALS3* gene was seen in 90% > strains to contain the *HWP1* gene. Finding of several studies revealed that the *ALS3* gene of the *ALS* family and the *HWP1* gene have a significant role in candida species attachment and biofilm formation(40, 41). Thus, detecting the existence of the *HWP1* and *ALS3* genes in *Candida species* isolated from various clinical samples will help to discover the roles of these genes in colonization and disease. On the other hand, a previously published study revealed that *Als3* gene had detected in 35.8% of *C.albicans* strains (20). This difference may come from the differences in the type of samples and the techniques applied for diagnosis. The finding showed that the *ALS1* gene was present in 92%, 40%, 67% and 100 % of the *C. albicans, C. glabrata*, *C. lusitani,* and *C. dubliniensis* strains, respectively. These findings were similar to a published study that was conducted by Roudbary et al. and reported that 83% of their 44 *C. albicans* isolates carried the *Als1* gene(3). *HWP1* protein is revealed that contributed in *C. albicans* covalently binding to epithelial cells and data of published studies have shown that *HWP1* surface protein and *ALS1/3* association is necessary to initiation and development of biofilm formation. A complementary role was proposed for *ALS1/3* and *HWP1* genes in biofilm formation(40, 42). Also, the finding of many studies suggested that several genes included *HWP1* and *HWP2* have the main role in biofilm formation, but between them, *HWP1* was the essential factor(22, 43). Furthermore, the results of this study have been shown that *Als1* gene was not detected in *C.tropicalis*, *C. kefyr, and C.Krusei*. In the current study, the polymerase chain reaction finding has revealed the frequency of *HWP1* and *SAP4* genes is highest in *C.albicans, C. dubliniensis* and *C. lusitani* than other Candida species. These results were similar to a published study that was conducted by Nas et al. and reported that *HWP1* gene was expressed in 60% and 73% of the strains isolated from vaginal swab specimens taken from prenatal, and reproductive-aged women with VVC, respectively (14). *HWP1* is proposed as an essential substrate that could contribute to covalent attachment of *C. albicans* to host cells (40). This result supports the hypothesis that *HWP1* and *SAP4* genes have a significant role in the initiation and development of candida infections at the various tissue site. Several studies have described the co-isolation of several pathogenic bacteria such as Staphylococcus aureus*, Streptococcus spp, Pseudomonas aeruginosa* and *C. albicans* from various biofilm-associated diseases(44, 45). Generally, the co-isolation of these pathogenic microorganisms from a collection of acute and chronic infection such as cystic fibrosis, periodontitis, burn wounds, keratitis, denture stomatitis, ventilator-associated pneumonia, urinary tract, and bloodstream infections is well documented(46, 47). In candida species, especially *C.albicans*, the hyphae formation is the main step in adherence and penetrating to host tissue and are therefore responsible for mucosal infections, particularly oral candidiasis(48). Results of published studies revealed that in hospital wards particularly ICU which patients usually have immune deficiencies opportunistic pathogens such as Staphylococcus aureus or *Pseudomonas aeruginosa* bind to candida Als3 adhesion and hyphae and through biofilm formation, can cause various drug-resistant infections(44, 45). Thus, it is likely that the association of opportunistic pathogens with *C. albicans* hyphae, as they penetrate host tissue, may allow these pathogens to gain entry into deeper tissues and initiate infection, with dire consequences for the host, particularly in critically ill patients(44). Furthermore, it is possible that biofilm formation with pathogenic bacteria may contribute to antibiotic resistance in the Candida species, especially *C. albicans*. In conclusion, it can be assumed that co-colonization *C.albicans* and pathogenic bacteria such as Staphylococcus aureus could complicate treatment process in immunocompromised patients (44, 45, 49).

There are some limitations of the present study; I) this study is oriented toward demonstrating the *ALS1*, *ALS3*, *HWP1,* and *SAP4* Genes in DNA with PCR method and this is not a gene expression study. II) Only two of several genes implicated in ALS family (ALS1-7, 9) in clinical strains of Candida species are focused on. Other ALS family gene and HWP or SAP genes should be evaluated in further widespread studies. III) The strains included in the study were obtained from the various sample and we do not have demographic information about samples and patients.

This finding suggested that *C. albicans* and *C. glabrata* are the most identified species, respectively. On the other hand, among the Candida species, *C. albicans* have the highest frequency of *Als1 Als3, HWP1,* and *SAP4* genes and is more virulent. This high rate of virulence factors detection *C. albicans* as a most identified Candida species is very important data and need to serious health action to control that. According to the various problems in treatment and eradication of infection that is caused by co-colonization of *C.albicans* and opportunistic pathogens such as Staphylococcus aureus, we suggest that in patients with candida infection, to prevent of potential problems in the treatment of these patients, the possibility of infection by other pathogens is also evaluated. Moreover, we suggest that control and duration treatment of candidiasis and other infection is based on source control and clinical improvement.

Conflict of interest

None.

Acknowledgements

This study was conducted with the financial support of Pediatric Infections Research Center (Grant No. 7294), Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Authors' contributions

Study design: Leila Azimi, Seyed Hossein Ardehali
Data collection and molecular analysis: Taher Azimi, Nicka Aghamohammadi
Manuscript writing and editing: Taher Azimi, Fatemeh Fallah, Leila Azimi.

References

1. Shibasaki S, Karasaki M, Aoki W, Ueda M. Molecular and Physiological Study of Candida albicans by Quantitative Proteome Analysis. Proteomes. 2018;6(3):34.
2. Al-Ahmar SD, Al-Attraqhchi AA, Ali AS. Molecular detection of ALS1 virulence gene of Candida albicans isolated from groups of Iraqi patients. Journal of the Faculty of Medicine. 2015;57(1):79-83.
3. Roudbary M, Roudbarmohammadi S, Bakhshi B, Farhadi Z. Relation of ALS 1 and ALS3 genes and fluconazole resistance in Candida albicans isolated from vaginal candidiasis. Inter J Mol Clin Microbiol. 2012;2:170-4.
4. Klingspor L, Jalal S. Molecular detection and identification of Candida and Aspergillus spp. from clinical samples using real-time PCR. Clinical microbiology and infection. 2006;12(8):745-53.
5. Mancuso R, Chinnici J, Tsou C, Busarajan S, Munnangi R, Maddi A. Functions of Candida albicans cell wall glycosidases Dfg5p and Dcw1p in biofilm formation and HOG MAPK pathway. PeerJ. 2018;6:e5685.
6. Poulain D. Candida albicans, plasticity and pathogenesis. Critical reviews in microbiology. 2015;41(2):208-17.
7. Berman J, Sudbery PE. Candida albicans: a molecular revolution built on lessons from budding yeast. Nature Reviews Genetics. 2002;3(12):918.
8. Kim J, Sudbery P. Candida albicans, a major human fungal pathogen. The Journal of Microbiology. 2011;49(2):171.
9. Berzaghi R, Colombo AL, de Oliveira Machado AM, de Camargo ZP. New approach for diagnosis of candidemia based on detection of a 65-kilodalton antigen. Clinical and Vaccine Immunology. 2009;16(11):1538-45.
10. Bineshian F, Yadegari MH, Sharifi Z, Eidgahi MA, Nasr R. Identification of Candida species using MP65 gene and evaluation of the Candida albicans MP65 gene expression in BALB/C Mice. Jundishapur journal of microbiology. 2015;8(5).
11. Mirhendi S, Makimura K. PCR-detection of Candida albicans in blood using a new primer pair to diagnosis of systemic candidiasis. Iranian Journal of Public Health. 2003;32(1):1-5.
12. Reiss E, Tanaka K, Bruker G, Chazalet V, Coleman D, Debeaupuis J, et al. Molecular diagnosis and epidemiology of fungal infections. Medical mycology. 1998;36:249-57.
13. Gudlaugsson O, Gillespie S, Lee K, Berg JV, Hu J, Messer S, et al. Attributable mortality of nosocomial candidemia, revisited. Clinical Infectious Diseases. 2003;37(9):1172-7.
14. Nas T, Kalkanci A, Fidan I, Hizel K, Bolat S, Yolbakan S, et al. Expression of ALS1, HWP1 and SAP4 genes in Candida albicans strains isolated from women with vaginitis. Folia Microbiol (Praha). 2008;53(2):179-83.
15. Khodavandi A, Alizadeh F, Harmal NS, Sidik SM, Othman F, Sekawi Z, et al. Expression analysis of SIR2 and SAPs1-4 gene expression in Candida albicans treated with allicin compared to fluconazole. Tropical biomedicine. 2011;28(3):589-98.
16. Garcia MC, Lee JT, Ramsook CB, Alsteens D, Dufrêne YF, Lipke PN. A role for amyloid in cell aggregation and biofilm formation. PloS one. 2011;6(3):e17632.
17. Murciano C, Moyes DL, Runglall M, Tobouti P, Islam A, Hoyer LL, et al. Evaluation of the role of Candida albicans agglutinin-like sequence (Als) proteins in human oral epithelial cell interactions. PLoS One. 2012;7(3):e33362.
18. Zordan R, Cormack B. Adhesins in opportunistic fungal pathogens. Candida and Candidiasis, Second Edition: American Society of Microbiology; 2012. p. 243-59.
19. Wächtler B, Wilson D, Haedicke K, Dalle F, Hube B. From attachment to damage: defined genes of Candida albicans mediate adhesion, invasion and damage during interaction with oral epithelial cells. PloS one. 2011;6(2):e17046.
20. Monroy-Pérez E, Paniagua-Contreras GL, Rodríguez-Purata P, Vaca-Paniagua F, Vázquez-Villaseñor M, Díaz-Velásquez C, et al. High virulence and antifungal resistance in clinical strains of Candida albicans. Canadian Journal of Infectious Diseases and Medical Microbiology. 2016;2016.
21. Wenwen S, Liuping Z, Xiaoyan L, Lei F, Sun S. The synergistic antifungal effects of sodium phenylbutyrate combined with azoles against Candida albicans via regulating Ras/cAMP/PKA signalling pathway and virulence. Canadian journal of microbiology. 2018(ja).
22. Inci M, Atalay MA, ÖZER B, EVİRGEN Ö, Duran N, KOÇ AN, et al. Investigations of ALS1 and HWP1 genes in clinical isolates of Candida albicans. Turkish Journal of Medical Sciences. 2013;43(1):125-30.
23. Cavalcanti YW, Morse DJ, da Silva WJ, Del-Bel-Cury AA, Wei X, Wilson M, et al. Virulence and pathogenicity of Candida albicans is enhanced in biofilms containing oral bacteria. Biofouling. 2015;31(1):27-38.
24. MOECKLI B. Influence of CgPDR1 hyperactivity on the expression of EPA-family adhesins in Candida glabrata: Université de Lausanne, Faculté de biologie et médecine; 2014.
25. Zhu J, Cao J, Zhang T, Li T, Li X, Shen W. Multilocus sequence typing for Candida albicans strains from the intestinal tract of patients with cirrhosis. European review for medical and pharmacological sciences. 2018;22(18):6063-71.
26. Dimopoulos G, Velegraki A, Falagas ME. A 10-year survey of antifungal susceptibility of candidemia isolates from intensive care unit patients in Greece. Antimicrobial agents and chemotherapy. 2009;53(3):1242-4.
27. Ku TS, Walraven CJ, Lee SA. Candida auris: Disinfectants and Implications for Infection Control. Frontiers in microbiology. 2018;9:726.
28. Khan HA, Baig FK, Mehboob R. Nosocomial infections: Epidemiology, prevention, control and surveillance. Asian Pacific Journal of Tropical Biomedicine. 2017;7(5):478-82.
29. Levy I, Rubin LG, Vasishtha S, Tucci V, Sood SK. Emergence of Candida parapsilosis as the predominant species causing candidemia in children. Reviews of Infectious Diseases. 1998;26(5):1086-8.
30. Iman K, Shorouk K, Muhmoud M. Candida infection associated with urinary catheter in critically ill patients. Identification, antifungal susceptibility and risk factors. Res. J Med Sci. 2010;5(1):79-86.
31. Passos XS, Sales WS, Maciel PJ, Costa CR, Miranda KC, Lemos JdA, et al. Candida colonization in intensive care unit patients' urine. Memórias do Instituto Oswaldo Cruz. 2005;100(8):925-8.
32. Colombo A, Nakagawa Z, Valdetaro F, Branchini M, Kussano E, Nucci M. Susceptibility profile of 200 bloodstream isolates of Candida spp. collected from Brazilian tertiary care hospitals. Medical mycology. 2003;41(3):235-9.
33. Rahbar M, Vossoghian S, Alimehr S, Abad HSE, Mohammadzadeh M, Fallah F, et al. Prevalence of Candida Infection at the Intensive Care Unit with Nested Polymerase Chain Reaction (PCR) Using Primer Mixes Specific to Candida DNA Topoisomerase II Genes. Archives of Clinical Infectious Diseases. 2016;11(4).
34. Occhipinti D, Gubbins P, Schreckenberger P, Danziger L. Frequency, pathogenicity and microbiologic outcome of non-Candida albicans candiduria. European Journal of Clinical Microbiology and Infectious Diseases. 1994;13(6):459-67.

35. Pfaller M, Jones R, Doern G, Sader HS, Hollis R, Messer S. International surveillance of bloodstream infections due to Candida species: frequency of occurrence and antifungal susceptibilities of isolates collected in 1997 in the United States, Canada, and South America for the SENTRY program. Journal of clinical microbiology. 1998;36(7):1886-9.
36. Rodero L, Davel G, Cordoba S, Soria M, Canteros C, Hochenfellner F. Multicenter study on nosocomial candidiasis in the Republic of Argentina. Revista Argentina de microbiologia. 1999;31(3):114-9.
37. Sandven P, Bevanger L, Digranes A, Gaustad P, Haukland HH, Steinbakk M. Constant low rate of fungemia in Norway, 1991 to 1996. Journal of clinical microbiology. 1998;36(12):3455-9.
38. Alvarado D, Díaz MC, Silva V. Identificación y susceptibilidad antifúngica de Candida spp aisladas de micosis invasora. Influencia del porcentaje de inhibición del crecimiento para la determinación de CIM. Revista médica de Chile. 2002;130(4):416-23.
39. St-Germain G, Laverdiere M, Pelletier R, Bourgault A-M, Libman M, Lemieux C, et al. Prevalence and Antifungal Susceptibility of 442Candida Isolates from Blood and Other Normally Sterile Sites: Results of a 2-Year (1996 to 1998) Multicenter Surveillance Study in Quebec, Canada. Journal of Clinical Microbiology. 2001;39(3):949-53.
40. Sundstrom P. Adhesion in Candida spp. Cellular microbiology. 2002;4(8):461-9.
41. Nobile CJ, Nett JE, Andes DR, Mitchell AP. Function of Candida albicans adhesin Hwp1 in biofilm formation. Eukaryotic cell. 2006;5(10):1604-10.
42. Staab JF, Bradway SD, Fidel PL, Sundstrom P. Adhesive and mammalian transglutaminase substrate properties of Candida albicans Hwp1. Science. 1999;283(5407):1535-8.
43. Nobile CJ, Schneider HA, Nett JE, Sheppard DC, Filler SG, Andes DR, et al. Complementary adhesin function in C. albicans biofilm formation. Current Biology. 2008;18(14):1017-24.
44. Peters BM, Ovchinnikova ES, Krom BP, Schlecht LM, Zhou H, Hoyer LL, et al. Staphylococcus aureus adherence to Candida albicans hyphae is mediated by the hyphal adhesin Als3p. Microbiology. 2012;158(Pt 12):2975.
45. Schlecht LM, Peters BM, Krom BP, Freiberg JA, Hänsch GM, Filler SG, et al. Systemic Staphylococcus aureus infection mediated by Candida albicans hyphal invasion of mucosal tissue. Microbiology. 2015;161(Pt 1):168.
46. Cuesta AI, Jewtuchowicz V, Brusca MI, Nastri ML, Rosa AC. Prevalence of Staphylococcus spp and Candida spp in the oral cavity and periodontal pockets of periodontal disease patients. Acta Odontológica Latinoamericana. 2010;23(1):20-6.
47. Sudbery P, Gow N, Berman J. The distinct morphogenic states of Candida albicans. Trends in microbiology. 2004;12(7):317-24.
48. Nobbs AH, Vickerman MM, Jenkinson HF. Heterologous expression of Candida albicans cell wall-associated adhesins in Saccharomyces cerevisiae reveals differential specificities in adherence and biofilm formation and in binding oral Streptococcus gordonii. Eukaryotic cell. 2010;9(10):1622-34.
49. Harriott MM, Noverr MC. Candida albicans and Staphylococcus aureus form polymicrobial biofilms: effects on antimicrobial resistance. Antimicrobial agents and chemotherapy. 2009;53(9):3914-22.