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

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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 adherence (2). Moreover, it revealed that in various condition, within VVC and infection of oral epithelial cells, the expression level of the ALS3 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 Sabauraud 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 (10nm), 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 1 minute 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 Chi 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**

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence (5'→3')</th>
<th>PCR Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Als1</td>
<td>F ACCAGAAAGAAACGCAGGGT</td>
<td>319</td>
</tr>
<tr>
<td></td>
<td>R GACTAGTGAAACCAAAATACCG</td>
<td></td>
</tr>
<tr>
<td>Als3</td>
<td>F CCAAGTGTTCACACGTTA</td>
<td>185</td>
</tr>
<tr>
<td></td>
<td>R GAAACCGGTGTGGCTGATGT</td>
<td></td>
</tr>
<tr>
<td>HWP1</td>
<td>F ATGACTCAGCTGGTTT</td>
<td>503</td>
</tr>
<tr>
<td></td>
<td>R TAGATCAAGAATGCCGAC</td>
<td></td>
</tr>
<tr>
<td>SAP4</td>
<td>F GAGTGTCTTCTGCTTCTTA</td>
<td>201</td>
</tr>
<tr>
<td></td>
<td>R TTGCCACATCATTTCTACC</td>
<td></td>
</tr>
</tbody>
</table>
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 = 20; 19.4%), 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.

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.](image)

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

<table>
<thead>
<tr>
<th>Candida species</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>65</td>
<td>63</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>20</td>
<td>19.4</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>8</td>
<td>7.76</td>
</tr>
<tr>
<td>C. krusei</td>
<td>4</td>
<td>3.88</td>
</tr>
<tr>
<td>C. lusitani</td>
<td>3</td>
<td>2.9</td>
</tr>
<tr>
<td>C. dubliniensis</td>
<td>2</td>
<td>1.9</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>Total</td>
<td>103</td>
<td>100</td>
</tr>
</tbody>
</table>

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

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

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

<table>
<thead>
<tr>
<th>Gender</th>
<th>Candida albicans</th>
<th>Candida glabrata</th>
<th>Candida tropicalis</th>
<th>Candida krusei</th>
<th>Candida lusitani</th>
<th>Candida dubliniensis</th>
<th>Candida kefyr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>p</td>
<td>Yes</td>
<td>No</td>
<td>p</td>
<td>Yes</td>
</tr>
<tr>
<td>Male</td>
<td>36 (55.4%)</td>
<td>19 (50%)</td>
<td>0.597</td>
<td>6 (30%)</td>
<td>34 (41%)</td>
<td>0.019</td>
<td>6 (75%)</td>
</tr>
<tr>
<td>Female</td>
<td>29 (44.6%)</td>
<td>19 (50%)</td>
<td>(70%)</td>
<td>14 (59%)</td>
<td>49 (25%)</td>
<td>(48.4%)</td>
<td>2 (50%)</td>
</tr>
</tbody>
</table>

*pFishers exact test.
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-
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 ALSI, ALS3, HWPI, and SAP4 Genes among Candida species were analyzed with PCR asays. 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. Roudbari 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 HWPI1 gene. Finding of several studies revealed that the ALS3 gene of the ALS family and the HWPI1 gene have a significant role in candida species attachment and biofilm formation(40, 41). Thus, detecting the existence of the HWPI1 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 Roudbari et al. and reported that 83% of their 44 C. albicans isolates carried the Als1 gene(3). HWPI1 protein is revealed that contributed in C. albicans covalently binding to epithelial cells and data of published studies have shown that HWPI1 surface protein and ALSI/3 association is necessary to initiation and development of biofilm formation. A complementary role was proposed for ALSI/3 and HWPI1 genes in biofilm formation(40, 42). Also, the finding of many studies suggested that several genes included HWPI1 and HWPI2 have the main role in biofilm formation, but between them, HWPI1 was the essential factor(22, 43). Furthermore, the results of this study have been shown that AlsI1 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 HWPI1 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 HWPI1 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). HWPI1 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 HWPI1 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-associataed 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 ALSI, ALS3, HWPI, 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 HWPI 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 AlsI1 Als3, HWPI1, 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.

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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
Molecular detection virulence factors genes in Candida Genus.


