

## DETECTION OF VIRULENCE GENES AND ABC GENOTYPES IN CANDIDA ALBICANS ISOLATES FROM CANDIDEMIA PATIENTS

Burak Pural<sup>1</sup> , Sevda Ozel Yildiz<sup>2</sup> , Gonca Erkoş Genc<sup>1\*</sup> 

<sup>1</sup>Department of Medical Microbiology, Istanbul University Istanbul Faculty of Medicine, Istanbul, Türkiye

<sup>2</sup>Department of Biostatistics, Istanbul University Istanbul Faculty of Medicine, Istanbul, Türkiye

Submitted on 06 January 2026, accepted on 24 March 2026

**Abstract:** Candidemia is the most common form of invasive fungal infection, and is associated with high mortality rates particularly in intensive care patients. In this study, 50 *C. albicans* strains isolated from blood samples were genotyped by 25S intron analysis, and the presence of virulence genes was investigated by PCR. Genotyping revealed that 28 isolates (56%) were genotype A, 14 (28%) genotype B, and eight (16%) genotype C. The *SAP1* gene was detected in 96% of isolates, *SAP2* and *SAP4* in 98%, *ALS1* and *HWPI* in 92%, and *PLB1* in 94%. A comprehensive understanding of *Candida* virulence factors, particularly in *C. albicans* which is the most prevalent *Candida* species acting as both a commensal organism and an opportunistic pathogen, is essential for improving diagnostic methods, and guiding the design of novel antifungal agents targeting virulence mechanisms.

1. Introduction. 2. Materials and Methods. 2.1. Isolates. 2.2. ABC genotyping. 2.3. Detection of the virulence genes. 2.4. Statistical analysis. 3. Results. 4. Discussion.

**Keywords:** *Candida albicans*, Invasive candidiasis, Candidemia, ABC genotyping, Virulence genes.

### 1. Introduction

Invasive fungal infections pose a significant global health threat due to their high mortality rates and the recent increase in antifungal resistance. Candidemia is the most common form of invasive fungal infection, particularly affecting immunocompromised individuals and patients in intensive care units, where it is associated with high mortality rates (Shrivastava and Whiteway 2025). The high incidence in these patient groups is associated with risk factors such as the use of broad-spectrum antibiotics, chemotherapy, neutropenia, and invasive procedures (Dawoud et al. 2024). The epidemiology of candidemia varies considerably across geographic regions, even among healthcare facilities within the same country. In recent years, there has been a notable rise in cases caused by non-*albicans* species, including *Nakaseomyces glabratus* (formerly *Candida glabrata*), *Candida tropicalis*, *Candida*

*parapsilosis*, and *Pichia kudriavzevii* (formerly *Candida krusei*). Furthermore, *Candidozyma* (formerly *Candida*) *auris* has emerged as an important cause of candidemia, gaining attention due to outbreaks in healthcare settings (Chavez et al. 2025). Nevertheless, *Candida albicans* remains the most prevalent species, both as a member of the microbiota and as an infectious agent (Shrivastava and Whiteway 2025).

In the development of candidemia, both host immune factors and the virulence attributes of the causative species play a crucial role (Dawoud et al. 2024). *C. albicans* possesses several major virulence factors, including adhesion, phenotypic switching, hyphal formation, biofilm production, and the secretion of extracellular hydrolytic enzymes such as hemolysins, proteases, and phospholipases. These factors contribute to the pathogenicity of *C. albicans* by enabling adhesion and proliferation on host cell surfaces and abiotic materials such as catheters, promoting biofilm formation,

\* Corresponding author: Gonca ERKOŞ GENC, Istanbul University, Istanbul Faculty of Medicine, Department of Medical Microbiology, 34093 Fatih Istanbul Türkiye; e-mail: gonca.genc@istanbul.edu.tr

© 2026 Burak Pural

This is an open access article licensed under the Creative Commons Attribution-NonCommercial-NoDerivs License (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

#### Cite as:

Detection of Virulence Genes and ABC Genotypes in *Candida albicans* isolates from Candidemia Patients. Pural B. et al., ADV MICROBIOL-NY, 2026, 65, 2, 103-109, <https://doi.org/10.2478/am-2026-0008>

facilitating the yeast-to-hypha transition, producing tissue-damaging enzymes, and allowing evasion from immune defenses. Through these mechanisms, *C. albicans* can multiply, colonize, invade tissues, and cause extensive damage (Shrivastava and Whiteway 2025; Saiprom et al. 2023).

The first step in the infection process is the attachment of *C. albicans* cells to host cell surfaces, a process in which specific surface proteins called adhesins play a crucial role. Among these adhesins are glycosylphosphatidylinositol (GPI)-anchored cell surface proteins encoded by the eight-member agglutinin-like sequence (ALS) gene family (*ALS1*–*ALS7* and *ALS9*). Another major adhesin of *C. albicans* is hyphal wall protein 1 (*HWP1*), a hypha-associated GPI-anchored mannoprotein located on the outer surface and expressed during germ tube and hyphal growth. The *HWP1* and *ALS* gene families are essential for *C. albicans* adhesion to host tissues, invasion, and biofilm formation. Another important virulence factor is secreted aspartyl proteinases (SAP), encoded by ten different genes (*SAP1*–*SAP10*). Among these, *SAP1*–*SAP3* are primarily associated with mucosal infections, while *SAP4*–*SAP6* are linked to systemic infections (Talapko et al. 2021). Seven genes, *PLA*, *PLB1*, *PLB2*, *PLC1*, *PLC2*, *PLC3*, and *PLD1*, are involved in the synthesis of phospholipase enzymes, with phospholipase B1 (*PLB1*), *PLB2*, *PLC1*, and *PLD1* being particularly associated with virulence. *PLB1* is a glycoprotein that exhibits both hydrolase and lysophospholipase transacylase activities, disrupting the structural integrity of cell membranes by hydrolyzing the ester bonds of phospholipids. Enzyme activity is especially concentrated at the tips of hyphae, where it contributes to tissue invasion (Shrivastava and Whiteway 2025).

*C. albicans* is divided into genotypes based on the presence or absence of a transposable group I intron in the ITS1 region of the 25S rDNA. This method, also referred to as 25S intron analysis or ABC genotyping, relies on ribosomal sequencing and classifies *C. albicans* isolates as genotype A (450 bp), genotype B (840 bp), or genotype C (450 and 840 bp) according to the size of the PCR amplification products. Genotyping is a very useful tool for epidemiologic analysis. Studies have shown that genotype A is the most prevalent genotype worldwide in all clinical forms of candidiasis, while the frequencies of genotypes B and C vary according to geographical region and the type of infection involved. Genotypes B and C are predominantly isolated from oral samples (Moron and Cabrera 2019; Kumar and Tejashree 2022; Ali et al. 2024).

The aim of this study was to determine the genotypes of *C. albicans* strains, isolated from blood samples of patients with candidemia, by 25S intron analy-

sis and to investigate the presence of virulence genes by PCR, including *ALS1* and *HWP1*, which are involved in biofilm formation and adhesion; *PLB1*, which is associated with phospholipase activity; *SAP1*, *SAP2*, and *SAP4*, which are related to secreted aspartyl proteinase activity.

## 2. Material and methods

### 2.1. Isolates

This study included a total of 50 strains isolated from the blood cultures of patients with candidemia and identified as *C. albicans* using either the API ID 32 C (bioMérieux, France) or the VITEK 2 YST (bioMérieux, France) systems in the Mycology Laboratory of the Department of Medical Microbiology, Istanbul University, Istanbul Faculty of Medicine, between 2021 and 2025. These strains were stored at -80 °C in brain heart infusion broth (BD Difco, USA) supplemented with 3% glycerol. *C. albicans* ATCC 10231 was used as the control strain.

The isolates were inoculated onto Sabouraud dextrose agar (BD Difco, USA) and incubated at 37 °C for 24–48 hours.

DNA isolation was performed with the DNA Extraction Kit (MicroLine, Türkiye) following the manufacturer's instructions.

### 2.2. ABC genotyping

ABC genotyping was based on the presence or absence of a transposable group I intron in the ITS1 region of rDNA. Amplification of the 25S rDNA region was performed using the primers CABC Forward (5'-ATAAGGGAAGTCGGCAAATAGATCCGTAA-3') and CABC Reverse (5'-CCTTGGCTGTG-GTTTCGCTAGATAGTAGAT-3'). The PCR reaction mixture was prepared according to the manufacturer's instructions and consisted of 12.5 µl 2x qPCRBIO Taq Mix (PCR Biosystems, USA), 1 µl forward primer, 1 µl reverse primer, 5.5 µl sterile ultrapure water, and 5 µl DNA, for a total volume of 25 µl. PCR amplification was carried out using the Labcycler Basic (SensQuest, Germany), with an initial denaturation at 95 °C for one minute, followed by 40 cycles of denaturation at 95 °C for 15 seconds, annealing at 60 °C for 15 seconds, extension at 72 °C for 2 minutes, and a final extension at 72 °C for 5 minutes (Mashaly and Zeid 2022; Fornari et al. 2016).

The amplified products were separated by size using agarose gel electrophoresis. The genotypes of *C. albicans* isolates were determined based on their band sizes by comparison with a DNA ladder as genotype A

(450 bp), genotype B (840 bp), and genotype C (two bands of 450 and 840 bp) (Mashaly and Zeid 2022).

2.3. Detection of the virulence genes

The presence of *SAP1*, *SAP2*, *SAP4*, *ALS1*, *HWPI*, and *PLB1* genes in *C. albicans* isolates was investigated by PCR. The primers used are listed in Table 1. The PCR reaction mixture was prepared according to the manufacturer’s instructions and had a total volume of 25 µl, consisting of 12.5 µl 2x qPCRBIO Taq Mix (PCR Biosystems, USA), 1 µl forward primer, 1 µl reverse

primer, 5.5 µl sterile ultrapure water, and 5 µl DNA. The same PCR conditions were applied for all genes and amplification was carried out using the Labcycler Basic (SensQuest, Germany), with an initial denaturation at 95 °C for one minute, followed by 40 cycles of denaturation at 95 °C for 15 seconds, annealing at 60 °C for 15 seconds, extension at 72 °C for 2 minutes, and a final extension at 72 °C for 5 minutes. The amplified products were visualized by agarose gel electrophoresis with a UV trans-illuminator using a DNA ladder (Costa et al. 2010; Dikmen et al. 2021).

Table 1. Primers used for the detection of virulence genes

| Gene   | Primer sequence (5'-3')        | Amplicon size (bp) | Reference   |
|--------|--------------------------------|--------------------|-------------|
| SAP1-F | TCAATCAATTTACTCTTCCATTTCTAACA  | 161                | Costa 2010  |
| SAP1-R | CCAGTAGCATTAACAGGAGTTTAATGACA  |                    |             |
| SAP2-F | AACAACAACCCACTAGACATCACC       | 178                |             |
| SAP2-R | TGACCATTAGTAACTGGGAATGCTTTAGGA |                    |             |
| SAP4-F | CATTTCATTCCCTTTAATACCGACTATC   | 156                |             |
| SAP4-R | GGTAACAAACCCTGTAGATCTTTTAA     |                    |             |
| ALS1-F | GACTAGTGAACCAACAAATAC          | 318                | Dikmen 2021 |
| ALS1-R | CCAGAAGAAACAGCAGGTGA           |                    |             |
| HWP1-F | ATGACTCCAGCTGGTTC              | 572                |             |
| HWP1-R | TAGATCAAGAATGCAGC              |                    |             |
| PLB1-F | ATGATTTTGCATCATTTG             | 751                |             |
| PLB1-R | AGTATCTGGAGCTCTAC              |                    |             |

2.4. Statistical analysis

Statistical analyses were performed using SPSS version 30.0 (IBM Corp., Armonk, NY, USA). The presence of virulence genes was compared among different *C. albicans* genotypes using the Freeman-Halton extension of Fisher’s exact test. This test was applied because the number of cells with expected values below 3 exceeded 20% of the total number of cells in the n×r contingency tables. A p-value of <0.05 was considered statistically significant.

3. Results

A total of 50 *C. albicans* isolates included in the study were obtained from eight pediatric (0-18 years, 16%) and 42 adult (>18 years, 84%) patients. Of these patients, 28 (56%) were female and 22 (44%) were male. The mean age of the patients was 54.08 years. Among the adult patients, six (12%) were aged 18-44 years, 14

(28%) were aged 45-65 years, and 22 (44%) were over 65 years. Overall, 31 patients (62%) were hospitalized in surgical units, 10 (20%) in intensive care units, and nine (18%) in internal medicine units.

Based on the amplicon sizes observed after gel electrophoresis, 28 (56%) of the 50 *C. albicans* isolates were identified as genotype A, 14 (28%) as genotype B, and eight (16%) as genotype C.

The *SAP1* gene was positive in 48 (96%) and negative in two (4%) isolates; *SAP2* and *SAP4* were positive in 49 (98%) and negative in one (2%) isolate; *ALS1* and *HWPI* were positive in 46 (92%) and negative in four (8%) isolates; and *PLB1* was positive in 47 (94%) and negative in three (6%) isolates (Table 2).

No significant differences were observed in the distribution of virulence genes among the different genotypes of *C. albicans*. As all p-values were greater than 0.05, each gene showed a similar distribution across the groups (Table 2).

**Table 2.** Distribution and relationship between genotypes and the presence of virulence genes (n, %).

| Gene | Total     | Genotype A<br>(n=28) | Genotype B<br>(n=14) | Genotype C<br>(n=8) | p value |
|------|-----------|----------------------|----------------------|---------------------|---------|
| SAP1 | 48 (96.0) | 26 (92.8)            | 14 (100)             | 8 (100)             | 0.680   |
| SAP2 | 49 (98.0) | 28 (100)             | 13 (92.8)            | 8 (100)             | 0.439   |
| SAP4 | 49 (98.0) | 28 (100)             | 13 (92.8)            | 8 (100)             | 0.439   |
| ALS1 | 46 (92.0) | 26 (92.8)            | 13 (92.8)            | 7 (87.5)            | 0.801   |
| HWP1 | 46 (92.0) | 25 (89.3)            | 13 (92.8)            | 8 (100)             | 0.999   |
| PLB1 | 47 (94.0) | 26 (92.8)            | 13 (92.8)            | 8 (100)             | 0.999   |

4. Discussion

*Candida* species cause a variety of clinical conditions, usually of endogenous origin, ranging from mild skin and mucosal infections to severe invasive infections, particularly in individuals with weakened immune systems. In addition to host-related risk factors, strain-specific virulence factors also play an important role in the development of infection. *C. albicans* is the most common *Candida* species, both as a member of the normal microbiota and as a pathogenic agent. It possesses several major virulence factors, including adhesion, phenotypic switching, hyphal formation, biofilm production, and the secretion of hydrolytic enzymes such as hemolysins, proteases, and phospholipases. Through these virulence mechanisms, *C. albicans* multiplies, colonizes, invades host tissues, and causes cellular damage (Shrivastava and White-way 2025). Among *Candida* species, *C. albicans* has been reported to exhibit the highest virulence due to its strong biofilm-forming capacity and high levels of proteinase and melanin production. Moreover, virulence genes have been found to be more prevalent in *C. albicans* than in other *Candida* species (Makled et al. 2024). Makled et al. (2024) also reported that the antifungal resistance profiles of *Candida* isolates were associated with the presence of virulence genes, particularly noting a correlation between flucytosine resistance and the presence of *HLP* and *HWP* genes.

Some researchers have reported a correlation between the body sites from which *Candida* spp. are isolated and the expression of virulence factors. *Candida* species isolated from blood have been found to be more virulent and to possess a greater number of virulence factors, likely due to their ability to adhere to catheters and secrete extracellular enzymes that facili-

tate tissue invasion (Talapko et al. 2021; Makled et al. 2024). Samaranayake et al. (2013) demonstrated that, in vitro, the presence of serum enhanced *C. albicans* virulence gene expression and promoted the formation of a thicker biofilm layer. However, other researchers reported no significant differences in virulence factor profiles among strains isolated from different clinical samples (Chen et al. 2021; Jabeen et al. 2023).

SAPs, which are hydrolytic enzymes of *C. albicans*, comprise ten members encoded by the *SAP* gene family and are expressed at different stages of infection. These enzymes play crucial roles in host tissue invasion, immune evasion, adhesion, hyphal growth, and biofilm formation, thereby contributing to tissue damage and dissemination (Gerges et al. 2023; Pawar et al. 2022). In vitro studies have shown that *SAP1*, *SAP2*, and *SAP3* are expressed exclusively by yeast cells, whereas *SAP4*, *SAP5*, and *SAP6* are expressed during the hyphal phase. *SAP1*-*SAP3* are primarily associated with mucosal infections, while *SAP4*-*SAP6* are linked to systemic infections (Mohammed et al. 2017). Among these, *SAP1* plays a particularly important role in promoting tissue damage and facilitating host invasion (Dikmen et al. 2021).

Makled et al. (2024) reported the presence of the *SAP1* gene in 26 (54.2%) of 48 *C. albicans* strains isolated from various clinical specimens, including 28 from blood. They also found that all isolates showing phenotypic proteinase activity were *SAP1*-positive (100%). Shrief et al. (2019) detected the *SAP1* gene in 65 (65%) of 100 *C. albicans* isolates, 56 of which were isolated from blood samples. Dawoud et al. (2024) identified the *SAP2* gene in nine (52.9%) of 17 *C. albicans* isolates obtained from pediatric and adult patients with candidemia. Bilgin et al. (2020) investigated the presence of the *SAP4* gene, known to play a key role in systemic

infections, in 50 *C. albicans* strains isolated as causative agents of candidemia and found it to be positive in 49 isolates (98%). Similarly, Ardehali et al. (2019) detected the *SAP4* gene in 57 (88%) of 65 *C. albicans* strains isolated from various clinical specimens, including 21 blood samples, obtained from patients in intensive care units. In the present study, among the 50 *C. albicans* isolates examined, the *SAP1* gene was detected in 48 (96%) isolates, while *SAP2* and *SAP4* were detected in 49 (98%) isolates each.

ALS, a family of glycoproteins, constitutes the largest group of adhesins in *C. albicans*. *ALS1*, one of the members of this family, is a cell surface protein that enhances adhesion to endothelial and epithelial cells and plays a crucial role in adhesion and hyphal development (Dikmen et al. 2021; Obais et al. 2023). Together with *HWP1*, *ALS1* is also important for mature biofilm formation, and the expression of these genes has been shown to increase during biofilm development (Makled et al. 2024; Gerges et al. 2023). Obais et al. (2023) reported that *ALS1* and *ALS3* play a significant role in the development of systemic candidiasis.

Makled et al. (2024) detected the *ALS1* gene in 28 of 48 *C. albicans* strains (58.3%) isolated from various clinical specimens, including 28 from blood. Dawoud et al. (2024) reported that 13 of 17 *C. albicans* isolates (76.5%) from blood cultures were *ALS1*-positive. Shrief et al. (2019) found the *ALS1* gene in 65 of 100 isolates (65%), 56 of which were from blood samples. Ardehali et al. (2019) reported the presence of *ALS1* in 60 of 65 isolates (92%), including 21 from blood. Similarly, İnci et al. (2013) detected this gene in 11 of 13 blood isolates (84.6%). In the present study, 46 of the 50 isolates examined (92%) were *ALS1*-positive.

The  $\beta$ -glucan-bound mannoprotein encoded by the *HWP1* gene, located in the hyphal cell wall, is an important protein that enables *C. albicans* to adhere to various surfaces and host cells, including epithelial cells (Makled et al. 2024; Gerges et al. 2023; Obais et al. 2023). Expression of *HWP1* has been shown to be particularly high during the early stages of biofilm formation (Mohammadi et al. 2023). *HWP1* plays a critical role in hyphal development and the induction of tissue damage in the host. In addition, *HWP1*, together with *ALS1* and *ALS3*, has been reported to contribute significantly to the development of systemic candidiasis (Obais et al. 2023).

Makled et al. (2024) reported that 44 of 48 *C. albicans* isolates (91.7%), including 28 from blood, were *HWP1*-positive. Dawoud et al. (2024) detected *HWP1* in 14 of 17 blood isolates (82.4%), and found that all 12 biofilm-forming isolates were positive (100%). Shrief et al. (2019) examined 100 *C. albicans* isolates, 56 from blood, and found *HWP1* in 77 isolates (77%). Ardeh-

ali et al. (2019) reported *HWP1* positivity in 62 of 65 strains (95%), including 21 blood isolates. İnci et al. (2013) detected this gene in only one of 13 blood isolates (7.7%). In the present study, *HWP1* was detected in 46 of 50 isolates (92%) and absent in four (8%).

Phospholipases are enzymes that hydrolyze phospholipids, leading to disruption of host cell membranes. Among them, *PLB1* and *PLD1* play key roles in the pathogenicity of *C. albicans* (Mohammadi et al. 2023). By cleaving the ester bonds of glycerophospholipids in host cell membranes, phospholipases lyse cells and facilitate tissue penetration and invasion. In addition, they promote adhesion by altering the surface properties of host cells (Mashaly and Zeid 2022). *C. albicans* exhibits stronger phospholipase activity than other *Candida* species (Saiprom et al. 2023).

Tunç et al. (2021) detected *PLB1* in all 30 *C. albicans* strains (100%) isolated from blood, with 26 strains (87.7%) exhibiting phenotypic phospholipase activity. In the same study, 30 *C. albicans* strains isolated from the oral cavity were also 100% *PLB1*-positive, with 80% showing phospholipase activity, and no statistically significant difference was observed between the two groups. Shrief et al. (2019) reported the presence of *PLB1* in 52 of 100 isolates (52%), 56 of which were from blood samples. In the present study, *PLB1* was detected in 47 of 50 isolates (94%) and absent in three (6%).

*C. albicans* is genotyped by 25S intron analysis based on the presence or absence of a transposable group I intron in the ITS1 region of rDNA. Previous studies have shown that genotype A is the most prevalent genotype worldwide (Moron and Cabrera 2019; Kumar and Tejashree 2022; Ali et al. 2024). Mahmoodi et al. (2023) reported the genotyping results of 20 *C. albicans* strains isolated from blood cultures as genotype A (12/20; 60%), genotype C (6/20; 30%) and genotype B (2/20; 10%). Similarly, Ali et al. (2024) investigated 55 *C. albicans* isolates from blood cultures and found that 28 strains (50.9%) were genotype A, 17 (30.9%) were genotype B, and 10 (18.2%) were genotype C. Karahan et al. (2012) determined the distribution of 121 blood culture isolates as genotype A (77/121; 63.7%), genotype B (24/121; 19.8%), and genotype C (20/121; 16.5%). In the present study, among the 50 *C. albicans* isolates analyzed, genotype A was the most common (28 isolates, 56%), followed by genotype B (14 isolates, 28%) and genotype C (eight isolates, 16%).

There are few studies comparing ABC genotypes of *C. albicans* in terms of virulence factors, and no comparative study has specifically addressed the presence of virulence genes. Ali et al. (2024) determined the genotypes of 55 *C. albicans* isolates from blood cultures and additionally assessed their phospholipase,

protease, esterase, hemolysin, coagulase, and biofilm formation properties using phenotypic methods. They found no statistically significant differences in virulence factors among the genotypes. Mashaly et al. (2022) genotyped 88 *C. albicans* strains isolated from various clinical samples of pediatric patients, including 19 from blood, and evaluated proteinase, phospholipase, and biofilm formation using phenotypic methods. The study reported no significant differences in virulence factors among genotypes or clinical sample types. Similarly, Gharaghani et al. (2021) analyzed *C. albicans* isolates from urine samples of pediatric patients, grouping them only as genotype A (72%) and C (28%) with no genotype B detected. A statistically significant difference was found only in esterase activity, with genotype A strains showing higher activity than genotype C, while no differences were observed for other virulence factors. Moron et al. (2019) examined 26 *C. albicans* isolates from various clinical samples and investigated phospholipase, esterase, hemolysin production, biofilm formation, and phenotypic transformation using phenotypic methods. No significant differences in virulence factors among genotypes were detected. Da Silva-Rocha et al. (2014) grouped 76 *C. albicans* isolates from the oral cavities of kidney transplant patients as colonizers or causative agents according to genotype and evaluated phospholipase activity, phenotypic change, and resistance to phagocytosis. Since only three isolates were identified as genotype B, statistical comparisons were made between genotype A (n=58) and genotype C (n=15). While no differences were found in phospholipase activity or phenotypic change, genotype C strains exhibited greater resistance to phagocytosis. In the present study, no statistically significant differences were observed among genotypes A, B, and C regarding the presence of *SAP1*, *SAP2*, *SAP4*, *ALS1*, *HWP1*, and *PLB1* virulence genes.

As a result, in the present study, a total of 50 *C. albicans* strains isolated from blood samples were genotyped by 25S intron analysis and examined for the presence of virulence genes *SAP1*, *SAP2*, *SAP4*, *ALS1*, *HWP1* and *PLB1*. Genotype A was found to be the most common (56%), consistent with reports from other regions worldwide. The *SAP1* gene was detected in 96% of the isolates, *SAP2* and *SAP4* in 98%, *ALS1* and *HWP1* in 92%, and *PLB1* in 94%. A better understanding of the virulence factors and the pathogenesis of infections caused by *Candida* species, particularly *C. albicans*, is essential for improving diagnostic approaches, developing infection control strategies, guiding antifungal therapy, and discovering novel agents targeting virulence factors that could be used in combination with antifungal drugs. Considering the increasing incidence of antifungal resistance, efforts to

design new therapeutic strategies targeting virulence factors have become particularly important. Studies in this field will contribute to a deeper understanding of fungal pathogenesis and help identify potential targets for more effective therapeutic approaches.

#### Funding

This study was supported by the Scientific Research Projects Coordination Unit of Istanbul University. Project No. 40642.

#### Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

#### ORCID

Burak Pural <https://orcid.org/0009-0005-5413-9176>

Sevda Ozel Yildiz <https://orcid.org/0000-0003-1357-6933>

Gonca Erkoş Genc <https://orcid.org/0000-0002-8983-9675>

## References

- Ali SAI, Ghamry AA, Ibrahim FA. ABC genotyping, virulence factors production and antifungal resistant pattern of *Candida albicans* isolated from immunocompromised patients with candidemia. *Microbes Infect Dis.* 2024 Oct; 5:1621–1630. <https://doi.org/10.21608/MID.2024.297534.1998>
- Ardehali SH, Azimi T, Fallah F, Aghamohammadi N, Alimehr S, Karimi AM, Azimi, L. Molecular detection of *ALS1*, *ALS3*, *HWP1* and *SAP4* genes in *Candida* genus isolated from hospitalized patients in intensive care unit, Tehran, Iran. *Cell Mol Biol.* 2019 May; 65:15–22. <http://dx.doi.org/10.14715/cmb/2019.65.4.3>
- Bilgin K, Birinci A, Yıldırım T. Typing of candidemia agents of *Candida albicans* isolates by molecular methods and investigation of *SAP4* gene presence. *Flora.* 2020 Sept; 25:316–324. <https://doi.org/10.5578/flora.69022>
- Chávez JF, Ortiz B, López R, Muñoz C, Aguilar K, Láinez-Arteaga I, Galindo C, Rivera L, Ballesteros-Monreal MG, Montes K, et al. Virulence factors and molecular identification of *Candida* species causing candidemia in Honduras. *J Fungi.* 2025 Jun; 11:470. <https://doi.org/10.3390/jof11070470>
- Chen J, Hu N, Xu H, Liu Q, Yu X, Zhang Y, Huang Y, Tan J, Huang X, Zeng L. Molecular epidemiology, antifungal susceptibility, and virulence evaluation of *Candida* isolates causing invasive infection in a tertiary care teaching hospital. *Front Cell Infect Microbiol.* 2021 Sept; 11:721439. <https://doi.org/10.3389/fcimb.2021.721439>
- Costa CR, Jesuino RS, de Aquino Lemos J, de Fátima Lisboa Fernandes O, Hasimoto e Souza LK, Passos XS, do Rosário Rodrigues Silva M. Effects of antifungal agents on Sap activity of *Candida albicans* isolates. *Mycopathologia.* 2010 Feb; 169(2):91–98. <https://doi.org/10.1007/s11046-009-9232-6>
- da Silva-Rocha WP, de BritoLemos VL, Svidizisnki TIE, Milan EP, Chaves GM. *Candida* species distribution, genotyping and virulence factors of *Candida albicans* isolated from the oral cavity of kidney transplant recipients of two geographic regions of Brazil. *BMC Oral Health.* 2014 Mar; 14:20. <https://doi.org/10.1186/1472-6831-14-20>
- Dawoud AM, Saied SA, Torayah MM, Ramadan A, Elaskary SA. Antifungal susceptibility and virulence determinants profile of

- Candida* species isolated from patients with candidemia. Scientific Reports. 2024 May; 14:11597. <https://doi.org/10.1038/s41598-024-61813-w>
- Dikmen N, Duran N, Ay E, Cimen F, Tek E. Genotyping, drug resistance and virulence factors of *Candida* species isolated from patients using long-term inhaled steroids. Int J Clin Pract. 2021 Sept; 75:e14820. <https://doi.org/10.1111/ijcp.14820>
- Fornari G, Vicente VA, Gomes RR, Muro MD, Pinheiro RL, Ferrari C, Herkert PF, Takimura M, Carvalho NS, Queiroz-Telles F. Susceptibility and molecular characterization of *Candida* species from patients with vulvovaginitis. Brazilian J Microbiol. 2016 Apr; 47(2):373–380. <https://doi.org/10.1016/j.bjm.2016.01.005>
- Gerges MA, Fahmy YA, Hosny T, Gandor NH, Mohammed SY, Mohamed TMA, Abdelmoteleb NEM, Esmael NE. Biofilm formation and aspartyl proteinase activity and their association with azole resistance among *Candida albicans* causing vulvovaginal candidiasis, Egypt. Infect Drug Res. 2023 May; 16:5283–5293. <https://doi.org/10.2147/IDR.S420580>
- Gharaghani M, Rezaei-Matehkolaei A, Hardani AK, Mahmoud-abadi AZ. Pediatric candiduria, epidemiology, genotype distribution and virulence factors of *Candida albicans*. Microb Pathog. 2021 Nov; 160:105173. <https://doi.org/10.1016/j.micpath.2021.105173>
- İnci M, Atalay MA, Özer B, Evirgen Ö, Duran N, Motor VK, Koç AN, Önlen Y, Kilinç Ç, Durmaz S. Investigations of *ALSI* and *HWPI* genes in clinical isolates of *Candida albicans*. Turkish J Med Sci. 2013; 43:125–130. <https://doi.org/10.3906/sag-1205-90>
- Jabeen G, Naz SA, Rangel DEN, Jabeen N, Shafique M, Yasmeen K. In vitro evaluation of virulence markers and antifungal resistance of clinical *Candida albicans* strains isolated from Karachi, Pakistan. Fungal Biology. 2023 Jul; 127(7–8):1241–1249. <https://doi.org/10.1016/j.funbio.2023.04.003>
- Karahan ZC, Saran B, Yenice S, Ağırbaşı H, Arıkan Akan Ö, Tekeli A. 25S intron analysis followed by restriction enzyme digestion performed for genotyping *Candida albicans* isolates. Mikrobiyol Bul. 2012 Apr; 46:257–265
- Kumar KPP, Tejashree A. Genotyping of *Candida albicans* and comparison of its antifungal resistance pattern in the South Indian region. J Pure Appl Microbiol. 2022 Sept; 16:2123–2130. <https://doi.org/10.22207/JPAM.16.3.69>
- Mahmoodi M, Nouraei H, Nasr R, Zomorodian K, Khodadadi H, Pakshir K. Phenotypes characterization and ABC genotypes distribution of clinical *Candida albicans* isolates. J Clin Lab Anal. 2023 Apr; 37:e24888. <https://doi.org/10.1002/jcla.24888>
- Makled AF, Ali SAM, Labeed AZ, Salman SS, Shebl DZM, Hegazy SG, Sabal MS. Characterization of *Candida* species isolated from clinical specimens: Insights into virulence traits, antifungal resistance and molecular profiles. BMC Microbiol. 2024 Oct; 24:388. <https://doi.org/10.1186/s12866-024-03515-x>
- Mashaly GE, Zeid MS. *Candida albicans* genotyping and relationship of virulence factors with fluconazole tolerance in infected pediatric patients. Infect Drug Res. 2022 Apr; 15:2035–2043. <https://doi.org/10.2147/IDR.S344998>
- Mohammadi F, Charkhchian M, Mirzadeh M. Phenotypic and genotypic characterization of virulence markers and antifungal susceptibility of oral *Candida* species from diabetic and non diabetic hemodialysis patients. BMC Oral Health. 2023 May; 23:261. <https://doi.org/10.1186/s12903-023-02970-8>
- Mohammed NA, Ajah HA, Abdulbaqi NJ. Detection the prevalence of adhesins and extracellular hydrolytic enzymes genes in *Candida albicans* biofilm formation. Iraqi J Sci. 2017;58:988–1000. <https://doi.org/10.24996/ijcs.2017.58.2C.3>
- Moron LS, Cabrera EC. ABC genotyping and putative virulence factors of *Candida albicans* clinical isolates. Malaysian J Microbiol. 2019 May; 15:400–407. <http://dx.doi.org/10.21161/mjm.180307>
- Obais AA, Al-shukri MSM, Al-Rubaye AFM. Molecular detection of *Candida albicans* virulence genes that isolated from periodontitis patients in Al-Hillah City. Med J Babylon. 2023 Apr; 20:347–351. [http://dx.doi.org/10.4103/MJBL.MJBL\\_55\\_23](http://dx.doi.org/10.4103/MJBL.MJBL_55_23)
- Pawar MY, Hatolkar SM, Misra RN. Phenotypic and molecular detection of virulence factor genes *SAP4* and *PLB* in *Candida albicans* isolates from the Western part of India. Med J Armed Forces India. 2022 Jul; 78:271–276. <https://doi.org/10.1016/j.mjafi.2020.03.023>
- Saiprom N, Wongsuk T, Oonant W, Sukphopetch P, Chantrata N, Boonsilp S. Characterization of virulence factors in *Candida* species causing candidemia in a tertiary care hospital in Bangkok, Thailand. J Fungi. 2023 Mar; 9:353. <https://doi.org/10.3390/jof9030353>
- Samaranayake YH, Cheung BPK, Yau JYY, Yeung SKW, Samaranayake LP. Human serum promotes *Candida albicans* biofilm growth and virulence gene expression on silicone biomaterial. PLoS ONE. 2013 May; 8:e62902. <https://doi.org/10.1371/journal.pone.0062902>
- Shrief R, Zaki ME, El-Sehsah EM, Ghaleb S, Mofreh M. Study of antifungal susceptibility, virulence genes and biofilm formation in *Candida albicans*. Open Microbiol J. 2019 Sept; 13:241–248. <https://doi.org/10.2174/1874285801913010241>
- Shrivastava M, Whiteway M. *Candida albicans*: A historical overview of investigations into an important human pathogen. Canadian J Microbiol. 2025 Jun; 71:1–21. <https://cdnsiencepub.com/doi/epdf/10.1139/cjm-2025-0036>
- Talapko J, Juzbašić M, Matijević T, Pustijanac E, Bekić S, Kotris I, Škrlec I. *Candida albicans*-The virulence factors and clinical manifestations of infection. J Fungi. 2021 Jan; 7(2):79. <https://doi.org/10.3390/jof7020079>
- Tunç B, Gürbüz ED, Dereli MD. Investigation of phospholipase activity in *Candida albicans* strains isolated from blood and oral cavity specimens. Turk Mikrobiyol Cemiy Derg. 2021; 51:50–60. <https://doi.org/10.5222/TMCD.2021.84429>