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Prevalence of *HWP1* and *SAP* Genes Among Biofilm-Forming *Candida*: Impacts on Antifungal Resistance Patterns

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Abstract
The study aimed to correlate the existence of biofilm and resistance to antifungal agents. 125 clinical samples were collected from cancer patients: 81 (64.8%) oral swabs and 44 (35.2%) vaginal swabs (21 (16.8%) from diabetic patients and 23 (18.4%) from pregnant women). The age range was 1-61 years, with 29 (23.2%) males and 96 (76.8%) females. Biofilm assay was performed using Congo red agar method and microtiter plate assay, in addition to determination of *HWP1*, *SAP1*, and *SAP4* genes. The results showed that the majority of isolates were Candida albicans 26 (81.25%) followed by C. glabrata 2 (6.25%). The study demonstrated that *SAP4* is more prevalent than other genes 24 (75%) and is responsible for resistance. Additionally, the current research showed that the presence of the three genes together *HWP1, SAP1*, and *SAP4*, provides more resistance. In conclusion, the percentage of *SAP4* gene in yeast isolates is higher than other genes.
Key words: Antifungal resistance, Candidiasis, Slim layer, *HWP1, SAP1, SAP4* gene.

Introduction

Candidiasis, caused by the fungus *Candida* species, is a major global health issue that manifests as oral thrush, cutaneous candidiasis, and deep infections. Individuals with impaired immune systems, such diabetic patients, pregnant women, and children with malignant are particularly susceptible since the fungus can infiltrate the body and spread through the circulation (Al-Garawi et al., 2022)[1]. Furthermore, *Candida* species can cause a variety of diseases, from minor to severe, including hospital-acquired bloodstream infections and infections of the mouth, skin, and vagina in immunocompromised patients(Khan et al., 2023)[2]. The discovery of antifungal medications is obstructed by similarities between fungus and host cells, which makes therapy difficult. Consequently, nearly 90% of invasive candidiasis (IC) is caused by *Candida albicans*, *C. glabrata*, *C. tropicalis*, and *C. krusei* (Bhosale et al., 2025)[3].Up to 75% of women will suffer vulvovaginal candidiasis (VVC), caused by factors like local defense mechanism dysfunctions, gene polymorphisms, and hormonal influences (Satora et al., 2023)[4], Felix *et al*., 2019[5]. The fungus can be activated by conditions like damaged mucosal integrity, altered microbiota, and impaired immune function, leading to superficial or mucocutaneous thrush and systemic candidiasis (d'Enfert et al., 2021)[6], ranging from traditional white plaques to mild erythematous lesions(Barayan *et al*., 2024)[7]. IC poses significant treatment challenges due to its wide range of symptoms, high morbidity and mortality rates

(Diez et al., 2025)[8]. *Candida albicans* virulence factors, such as *SAP* and *HWP1*, aid in host tissue penetration, adhesion, and immune evasion (Gerges et al., 2023)[9].

SAP contributed to colonization and infection by destroying host cell membrane components. Furthermore, these genes and *HWP1*are expressed at multiple stages of the infection process and play an important role in tissue invasion, *HWP1* was up-regulated during the biofilm formation process and contributed to the covalent attachment of *C. albicans* to various surfaces and antifungal resistance (Azim, 2019)[10]. The current study focused on disease, yeast isolates, biofilm effect and the genes that responsible for biofilm formation.

MATERIALs AND METHODS

Collection and culturing samples

A total of 125 clinical sample were collected from immunocompromised patients who were melignant 81 (64.8%) oral swab, and vaginal swab were collected from diabetic 21(16.8%) and pregnant 23 (18.4%). Patients ages ranged from 2 year to 61 years, from both sex (29 male and 96 female), from Al-Faiha Teaching Hospital, Faiha Specialized Diabetes Endocrine Metabolism Center (FDEMC), and Specialized Pediatric Hospital, Basrah Governorate, Iraq. For data collection, a questionnaire including name, gender, age, underlying illnesses, previous application of antifungal drugs, and the date of hospital admission was used. The specimens were collected during the period of the study from September 2024 to March 2025. Oral and vaginal swabs examination was used potassium hydroxide (KOH) 10%, and Gram stain. Thereafter, all samples were inoculated onto Sabouraud dextrose agar medium (SDA; Himedia, India) supplemented with chloramphenicol (250 mg/L) . The inoculated plates were incubated at 37°C for 1-7 days under aerobic circumstances with duplicates for each specimen. After the incubation period, the fungal isolates were investigated macroscopically to determine the shape, color, and texture of the developing colonies and microscopically using lactophenol cotton blue to examine true hyphae, pseudo hyphae, and clusters of conidia (Srivastava, 2023)[11].

Phenotypic investigation

1-In order to differentiate *Candida albicans* from other species, the capacity of each pure fungal isolate to generate germ tubes was assessed.
(Matare *et al*. 2017) [12].

2- A chromogenic culture medium designed specifically for the qualitative direct detection, differentiation of specific species of *Candida* based on color. (Karakoyun *et al*.,2024) [13].

Genotypic identification

The intergenic (ITS) ITS1 F-5'-TCC GTA GGT GAA CCT GCG G-3' and ITS4 R-5'-TCCTCC GCT TAT TGA TAT GC-3' was used to amplified genomic DNA of yeast isolates the extracted by DNA Extraction kit (Gained, Korea) (Fujita *et al* ., 2002)[14].

Total of PCR react 25 µl that 1 µl of each upstream and downstream primer, 5.5 µl of free nuclease water, 5 µl of DNA extraction, and 12.5 µl of master mix. Furthermore, a thermal cycler utilized beginning by denaturation at 95°C for 5 minutes and 1 cycle, while final denaturation for 30 seconds at 94°C, followed by annealing step for 30 seconds and 35 cycles at 58°C. As well as, the extension step was began for 60 seconds at 72°C, and followed by final extension for 7 minutes and 1 cycle at 72°C, according to(Fujita *et al* ., 2002)[14] with some modification.

The examination of PCR product was determine by use 1.5% agarose gels for 45 minutes at 70 v for

electrophoresis was used to show the amplicons' sizes.

Biofilm formation assay

Determined the ability of 32 yeast isolates to biofilm production phenotypically by two methods; **Congo red method** Saxena et al. (2014) [15] (Alrubayae et al., 2020) [16], and **microtiter plate method** Marak and Dhanashree 2018 [17] and Naser et al., 2023 [18].

Moreover, three genes including *HWP1, SAP1*, and *SAP4* used genotypically to detect the capacity to biofilm formation of yeast isolates. This process performed by using three primer, the sequence of primers for *HWP1* F--5'-ATG ACT CCA GCT GGT TC-3', *HWP1* R-5'-TAG ATC AAG AAT GCA GC-3', *SAP1* F-5'-GCT CTT GCT ATT GCT TTA TTA-3', *SAP1* R-5'-CAT CAG GAA CCC ATA AAT CAG-3', *SAP4* F-5'-GAGTGTTCTTGCTTTCGCTTTA-3' and *SAP4* R-5'-GAGTGTTCTTGCTTTCGCTTTA-3'

Hawever, the amplification of *HWP*1 and *SAP1* genes were done as fallow, 2.5 µl of each primer, 13 µl of Master Mix, 5 µl of DNA, and 2 µl of free water. The amplification conditions of *HWP1* were adjusted as follows: Initial denaturation for four minutes and one cycle at 94°C; final denaturation for thirty seconds at 94°C, followed by an annealing step for one minute and thirty-five cycles at 52°C; initial extension for two minutes at 72°C and final extension step for five minutes and one cycle at 72°C (Inci et al., 2013)[19].

The amplification conditions for *SAP1* were follows: initial denaturation at 95°C for 5 minutes and 1 cycle; final denaturation for 4 minutes and 95°C in accordance with an annealing step for 1 minute and 30 cycles at 49°C; initial extension for 1 minute at 72°C and final extention step for 5 minutes and 1 cycle at 72°C (Kalkanci et al., 2005)[20].

Afte that was amplified using 1 µl of both primer, 15 µl of Master Mix , along with 3 µl of DNA and 5 µl of free water. Through the amplification conditions were as follows: initial denaturation at 95°C for 5 minutes and 1 cycle, final denaturation for 1 minute and 95°C, annealing step for 1 minute and 32 cycles at 55°C, beginning extension for 1 minute at 72°C, and final extension step for 10 minutes and 1 cycle at 72°C (Nas et al., 2008[21]. Next, (1.5%) agarose gel electrophoresis was used to visualize the amplified products. Abdul-Lateef et al., 2015 [22] used a UV transilluminator to observe the PCR products in the gel and displayed the product size of the genes.

Antifungal susceptibility test

Four antifungals were utilized in this investigation against 32 yeast isolates include; amphotericin B (AMB) 50 mg (Abbott France, S.A.), ketoconazole tablets(KET) 200 mg (Pharmaline, Lebanon), clotrimazole droplets (CLO) 10 mg/ml (The Arab Drug Company, Cairo), and 2 mg of fluconazole capsule (FLU) (Pfizer, Amboise, France). To detect antifungal susceptibility test by microtiter plate method Niitani et al, 2025) [23]. According to clinical and laboratory standards institute (CLSI, 2008).

The assay was started by adding 100 µl of each drug solution to individual wells of 96-well microtiter plates. The suspension for each yeast isolate's prepared by adding activated colonies to saline solution then concentration was adjusted to $1*10^6$ CFU/ml

The minimum inhibitory concentration (MIC) was determined as the lowest dose of antifungal that inhibited growth by more than 90% as compared to the antifungal-free control(blank).

RESULTS AND DISCUSSION

In the current study a total of 125 clinical sample (oral and vaginal swabs) were collected from immunocompromised patients with malignant, diabetic and pregnant during the period of study from September 2024 to March 2025. The sample were collected from Al-Faiha Teaching Hospital, Faiha Specialized Diabetes Endocrine and Metabolism Center (FDEMC), and Specialized Pediatric Teaching Hospital. Depended on age and gender of the patient, the specimen were distributed into different age groups (table 1). The ages ranged from 2-61 years old. The majority of patients in (2-11). In contrast, the minority of patients was in (42-51) years old. The statistical analysis of age groups showed a significant differences ($p \geq 0.05$) and in sex found a significant differences ($p \leq 0.05$) (2-11), (22-31), (32-41), (42-51), and (52-61).

Table 1. Distribution of patients groups according to age and sex.

Age Groups	Female	Male
2-11	27(52%)	25(48%)
12-21	33(89.1%)	4(10.9%)
22-31	15(100%)	-
32-41	12(100%)	-
42-51	3(100%)	-
52-61	6(100%)	-
Total	96(76.8%)	29(23.2%)

Age groups significance difference($p \geq 0.05$).

Sex Insignificance difference($p \leq 0.05$).

Table 2. The source of clinical sample according to age groups

Age Groups	Oral swab cancer	Vaginal swab	
		DM	Pregnant with UTI
2-11	52(100%)	-	-
12-21	29(78.4%)	-	8(21.6%)
22-31	-	3(20%)	12(80%)
32-41	-	9(75%)	3(25%)
42-51	-	3(100%)	-
52-61	-	6(100%)	-
Total	81(64.8%)	21(16.8%)	23(18.4%)

Table 2 showed that 81 (64.4%) of the oral swabs collected from malignant patients in Specialized Pediatric Teaching Hospital suffer from oral thrush, while 21 (16.8%) of the vaginal swabs collected from diabetic patients in Faiha Specialized Diabetes Endocrine and Metabolism Center (FDEMC) struggle with UTI, vulvovaginal, and 23(18.4%) from pregnant women patients in Al-Faiha Teaching Hospital that suffer from itching, discharge, and

recurrent UTI.

Oral thrush presents a variety of clinical forms, including pseudomembranous, erythematous, angular cheilitis (fig 1), mucocutaneous, and oropharyngeal . (Lu, 2021)[24].

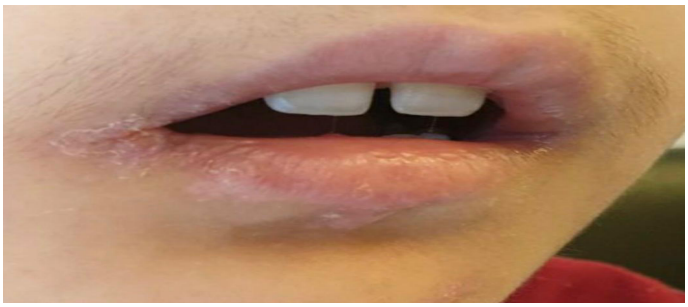

Figure 1. Clinical forms of pseudomembranous oral thrush.

Identification of fungal infection

Microscopic examination

125 of clinical sample were direct examination by 10% KOH and Gram stain only 32 (25.6%) of these were positive, 20 (62.5%) oral swab, 7 (21.9%) vaginal swab with DM, and 5 (15.6%) pregnant vaginal swab. Microscopic examination is required to identify and confirm fungal infection as pseudohyphae, true hyphae, and clusters of conidia arise (figs 2 and 3). This step is critical since it helps determine the best course of action. (Because of the nature of the infectious process, leukocytes (granulocytes, particularly neutrophils) are easily apparent in urine sediment with fungal formations. It is worth noting that yeast cells discovered in urine sediment can have structural similarities to erythrocytes, lipid droplets, and calcium oxalate monohydrate crystals (Poloni and Rotta, 2020)[25].

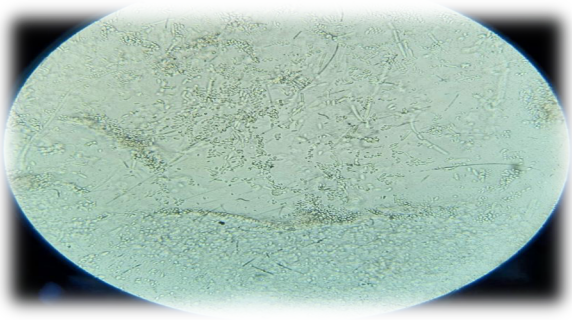

Fig 2. Microscopic direct examination of the vaginal swab using10 % KOH under the light microscope showed the yeast budding and true hyphae under 40x.

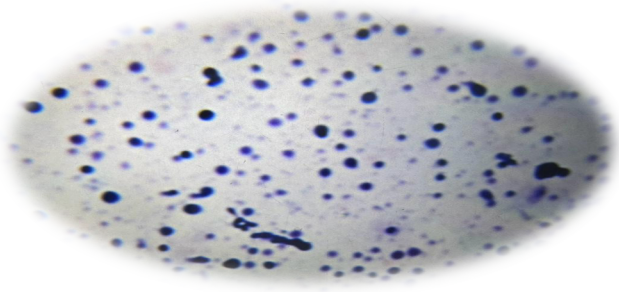

Fig 3. Gram stain in direct examination showed the yeast cells (100x).

Prevalence of fungal infections

The positive results appeared in 32 (25.6%) according to direct examination and cultured, oral swab 20(62.5%), and vaginal swab 12(37.5%). The percentage of females showed a higher prevalence, 24 (75%), while males were seen in 8 (25%). (2-11) which revealed a high presence of fungal infection with a significant differences ($p \geq 0.05$) in both males and female malignant patients, due to risk factors like underlying immunosuppression, extensive antibiotic therapy, steroid use, and intravenous catheters. Fungal is becoming more common in hematological malignancy patients as an opportunistic nosocomial infection agent (Sanlı *et al*., 2024)[26].Malignant patients are susceptible to a wide range of infections, with elevated susceptibility to fungal pathogens due to the adverse effect of cytotoxic chemotherapy on host defense mechanisms (Teoh and Pavelka, 2016; Akbar *et al*.,2025)[27,28]. Systemic fungal infections continue to pose a significant threat, resulting in elevated rates of mortality and morbidity in cancer patients (Hosseini *et al*., 2021)[29]. Prior research has identified oral candidiasis as a mark of systemic diseases, such as hematinic deficiency, diabetes mellitus, leukopenia, HIV/AIDS, malignancies, and carbohydrate-rich diets, drugs, or immunosuppressive conditions (Lu, 2021)[24]. Followed by (32-41) the potential risk factors for fungal infection during pregnancy include a number of clinical and behavioral disorders, as well as pregnancy-related variables, reduced immunity, increased sex, hormone levels, glycogen deposition, low vaginal pH, and diminished cell-mediated immunity (Disha and Haque, 2022)[30].While the minority of infections (52-61), World Health Organization found that despite the low fungal percentage, treatment for these illnesses can be challenging or even impossible (table 3).

Table 3. Prevalence of fungal infections in pregnant, DM and malignant patients according to the age groups.

Age Groups	Oral swab cancer	Vaginal swab	
		DM	Pregnant
2-11	14(43.7%)	-	-
12-21	6(18.7%)	-	-
22-31	-		3(9.3%)
32-41	-	5(15.6%)	2(6.3%)
42-51	-	-	-
52-61	-	2(6.3%)	-
Total	20(62.5%)	7(21.9%)	5(15.6%)

Insignificance difference($p \leq 0.05$).

Phenotypic identification

According to the current study the germ tube test results, *Candida albicans* or *C. dubliniesis* appeared with 24 (75%) (fig. 4), while other *Candida* spp. 8 (25%) unable to germ tube production, agreed with Obeed et al (2022)[31] who found thirty one (50%) yeast isolates shown the ability to form germ tubes. The test was used to distinguished *Candida albicans* from other species based on the development of unconstructed filaments in response to serum under adverse conditions. This results was consistent with numerous investigations:

Modrzewska and Kurnatowski (2015), Carradori *et al.* (2016), Matare *et al* .(2017), Abass *et al*. (2018), Abbas *et al*. (2021),Naser *et al.* (2023)[32,33,12,34,35,18], and CHROM agar's growth appearance is 26 (81.3%) bright green *Candida albicans or C. dubliniesis* (fig. 5, 6). These findings are agreed with AL-Darraji and Ameen (2023) [36], who found that light green *Candida albicans* (25) was more common than the other *Candida* species, and Naser et al. (2023) [18], who found that *C. albicans* or *C. dubliniensis* accounted for 50 (83.33%) of the isolates, with *C. tropicalis* coming in second with 5 (8.33%). There are significant differences ($p \leq 0.05$) between *C. albicans* and other species. These findings support the conclusion that the molecular technique is 32 (25.6%). These methods have been applied to enhance phenotypic analysis and produce more precise results in a shorter period of time (1.5–3 hours). Considering the rapidity and high accuracy of molecular typing methods and the rapid advancement of technology (Sudhan et al., 2016) [37].

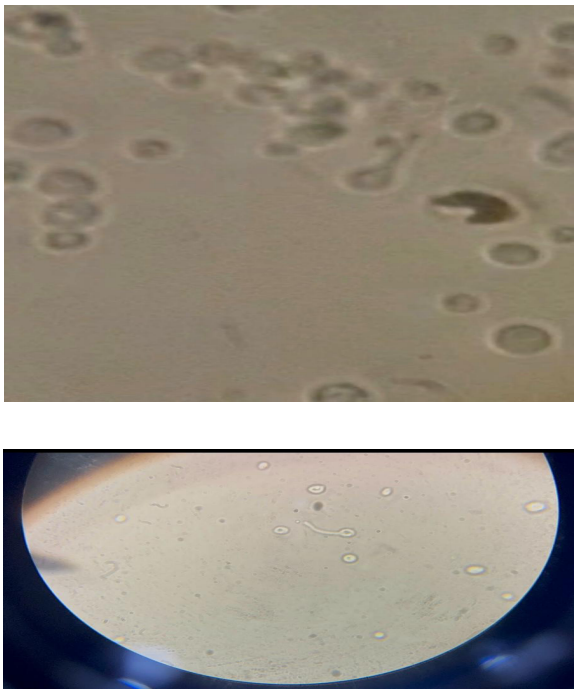

Fig 4. Germ tube formation after 3h incubation at 37º C in human serum (10x) (40x).

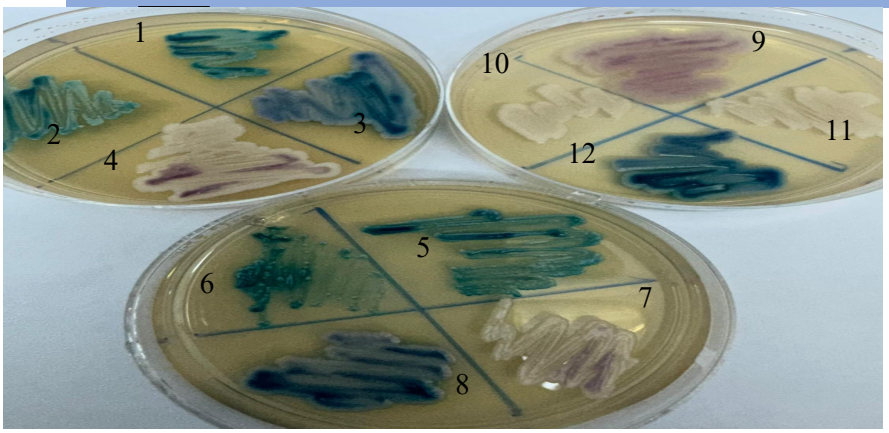

Figure 5. Differentiation of *Candida albicans* from other isolates according to colony color on Chrom *Candida* agar.1,2,5, light green *C. albicans*, *3,8,12 blue .C.tropicalis*, 9 purple *Pichia kudriavzevii*(*C.krusei*), *4,7, pink Kluyveromyces marxianus(C. kefyr), 10,11,C.white glabrata, 6, dark green C. dublineisis* .

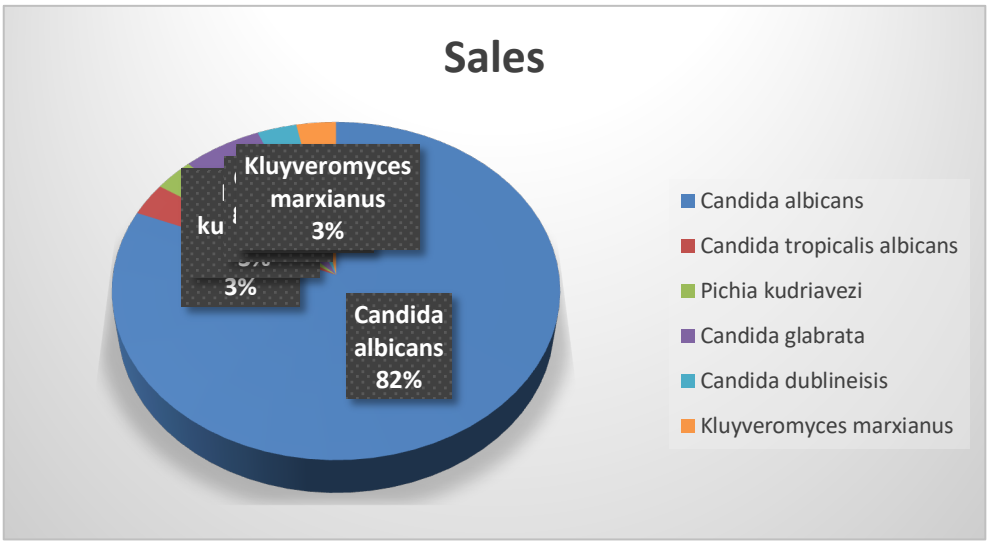

Fig 6. Percentage of pre-identified *Candida* spp. isolates according to colony color on chrome agar *Candida*.

Molecular identification of the isolates

As observed in figure (7), the PCR amplification findings showed that the yeast isolates had 500 bp.

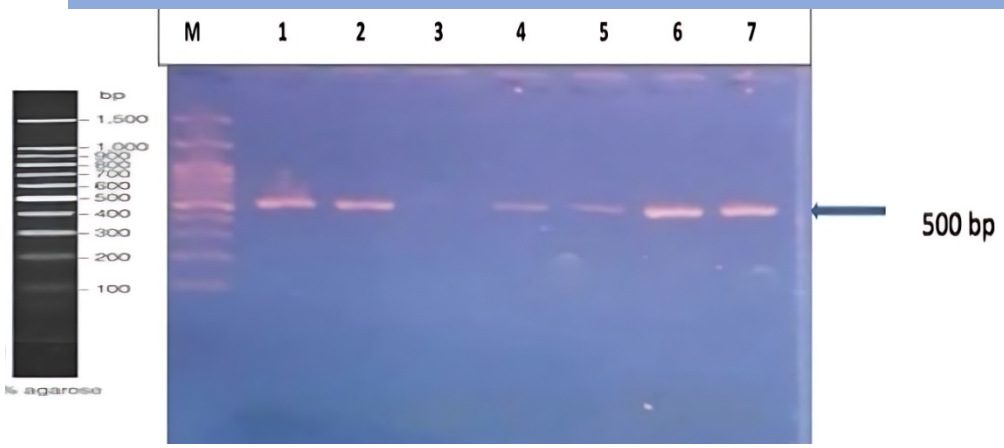

Fig 7. 1.5% agarose gel electrophoresis analysis for PCR products using FITS1 and RITS4 primers. 60 V. for 75 minutes 1: DNA marker 1500 bp; lane 2,3,5,6,7,8: PCR product 500 bp.

Sequencing

32 yeast isolates' PCR results were sent for processing by the Macrogen firm and identified using the NCBI's "BLAST" tool.

Causative agents

Table (4) and figure (8) showed that *Candida albicans* was the majority 26 (81.3%) than NAC 6 (18.7%), *Candida albicans* ability to transition from yeast to hyphae becomes a critical component of pathogenesis. Furthermore, *C. albicans* can create biofilms and hydrolytic extracellular enzymes (Cauchie *et al*., 2017)[38]. The results of current study disagree with Elkholy et al. (2025)[39]who found that the isolation rate of non-Albicans *candida* (NAC) was higher (64%) than that of *candida albicans* (36%), the most prevalent non-albicans species was *C. tropicalis*, and agreed with Hamzehee et al (2019)[40] Who found that the most common species was *C. albicans* (34.42%), followed by *C. glabrata* (24.59%), *C. krusei* (14.75%), C. kefyr (3.27%), and C. dubliniensis (1.63%), and disagreed with Abdel-Hamid et al.,(2023)[41] who concluded that Non-*albicans Candida* species caused the majority of infections, accounting for 70.5% of the 105 isolates. The results agreed with Khatun et al.,(2025)[42] found that *Candida albicans* (63.64%) was the most common species, followed by *Candida tropicalis* (12.73%) and *Candida glabrata* (10.90%).

The results in table (4) showed that malignant patients more susceptible to *Candida* infections 20 (62.5%) than DM 7 (21.8%) and pregnant 5 (15.7%) out of 32 (25.6%) cases, agreed with Hosseini et al 2021[29], who discovered that Cancer patients exhibit higher populations of harmful fungi, with *Candida* infections associated with esophageal, gastric, lung, cervical, cutaneous, and ovarian cancers. The oral cavity of cancer patients is particularly vulnerable to *Candida albicans* infections compared to diabetic and pregnant individuals, and agreed with(Pispero *et al*.,2022)[43] who found that neutropenia caused by cancer, as well as chemotherapy and/or radiotherapy therapies, enhance the transition of Candida species from carrier to pathogen.

Table 4. The source of *Candida* spp. (oral and vaginal swab), the number and percentage.

Yeast isolates (No)	Distribution of yeast isolates		
	Oral swab	Vaginal swab DM	Vaginal swab pregnant
C. albicans (26)	16(61.6%)	5(19.2%)	5(19.2%)
C. tropicalis (1)		1(100%)	0
C. glabrata (2)	1(50%)	1(50%)	0
Pichia kudriavzevii(1)	1(100%)	0	0
C. dubliniensis (1)	1(100%)	0	0
Kluyveromyces marxianus(C.kefyr)(1)	1(100%)	0	0
Total(32)	20(62.5%)	7(21.8%)	5(15.7%)

No significance difference($p \leq 0.05$).

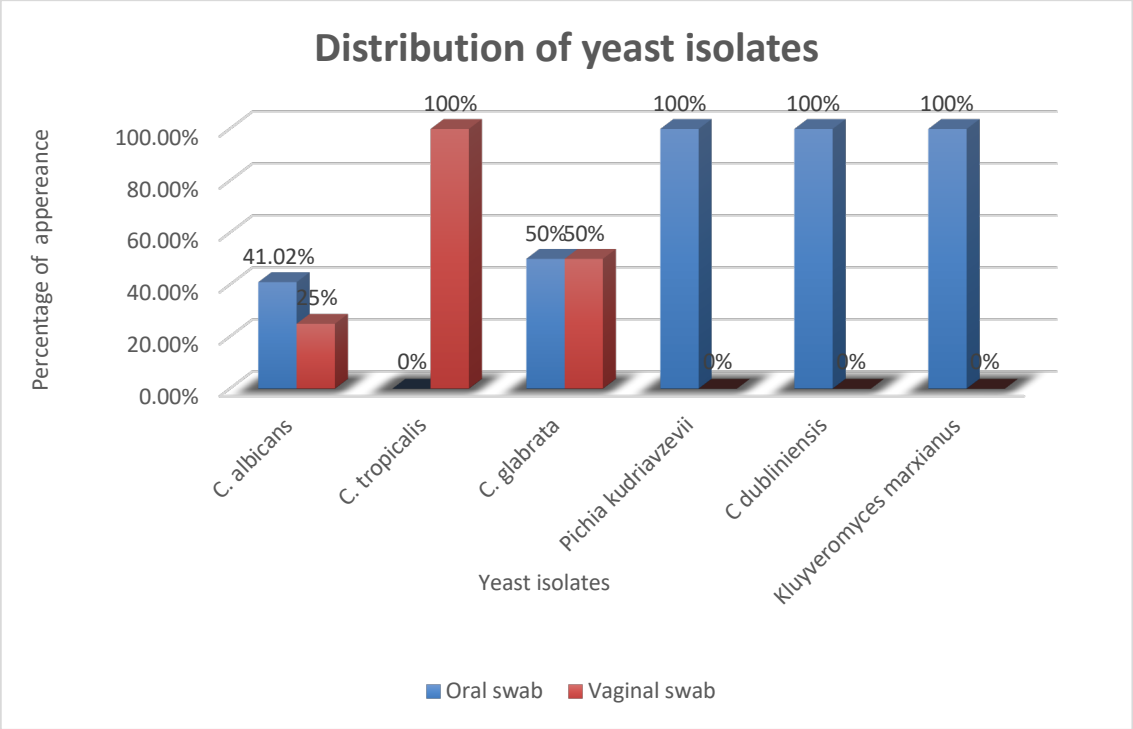

Fig 8. Based on the source of specimens (oral and vaginal swabs), the number and percentage.

Biofilm formation assay

Phenotypic method

The microtiter plate method revealed that 23 isolates (71.9%) had positive results; 6 isolates (18.7%) had weak activity, 3 isolates (9.4%) had moderate activity, and 14 isolates (43.8%) had strong activity. Only nine (28.1%) unable to produced biofilm (table 5 and Fig 9). Table (5) showed that the percentage of strong biofilm production was greater than the other 14 (43.8%), and *Candida albicans* 19(59.4%) predominate than NAC 4(12.5%), agreed with Malinový et al., 2023)[44] the following estimated occurrences of Candida species were reported in Europe

and the United States: are linked to around 50% of *Candida albicans*, 30% of *Candida glabrata*, 7% of *Candida tropicalis*, and 1% of *Candida krusei*, and (Jacobsen, 2023)[45] who found *C. albicans*, which is commonly cause of mucosal and disseminated infections (candidiasis), adapted to live closely with warm-blooded hosts, while disagreed with Shrief et al. (2019) [46] who reported that 58% of *Candida albicans* were assessed for biofilm formation, there were 20 isolates with strong, 21 with moderate, and 17 week biofilm formation, among biofilm-forming *C. albicans* isolates were compared to non-biofilm-forming isolates, was shown to be significantly more prevalent (P=0.0001).

The second method to detect the ability of biofilm formation was Congo red agar method, showed that 20 (62.5%) were able to produce a strong biofilm, 10 (31.3%) were week to form a biofilm, and 2 (6.3%) were unable to biofilm formation (table 6 and Fig 10). This result is in line with other studies Allaaeiby et al., 2020 [47]; Obeed et al., 2022 [31] that discovered a significant percentage of *C. albicans* can form a biofilm and agreed with Alrubayae et al 2020[16] who found the potential for biofilm formation was divided into three categories: strong, moderate, and weak. According to the findings, 79% of the isolates produced biofilm strongly, with 21% forming biofilm weakly. While Rabha *et al*.,2021)[48] was found Congo red medium to identified biofilm based on the degree of color of the colonies, the biofilm formation was classified into dark red colonies are labeled as biofilm-producers, and white colonies are evaluated as non-biofilm formation.

Our results are consistent with Tascini et al. (2018) [49], who demonstrated that biofilm formation by *Candida* species was associated with increased mortality rates in hospitalized patients in internal medicine wards, vulnerable patients, and patients taking medications (like azoles) that have no effect on biofilm.

Table (5): The rate of Biofilm formation assay of yeast isolates in Microtiter plate method.

Yeast isolates (No)	Negative	Positive		
		Weak	Moderate	Strong
***C. albicans* (26)**	**7(26.9%)**	**4(15.4%)**	**3(11.5%)**	**12(46.2%)**
***C. tropicalis* (1)**	**1(100%)**	**0**	**0**	**0**
***C. glabrata* (2)**	**0**	**1(50%)**	**0**	**1(50%)**
***Pichia kudriavzevii*(1)**	**0**	**1(100%)**	**0**	
***C. dubliniensis* (1)**	**0**	**0**		**1(100%)**
***Kluyveromyces marxianus*(1)**	**1(100%)**	**0**	**0**	**0**
Total (32)	**9(28.1%)**	**6(18.7%)**	**3(9.4%)**	**14(43.8%)**

No significance difference($p \leq 0.05$).

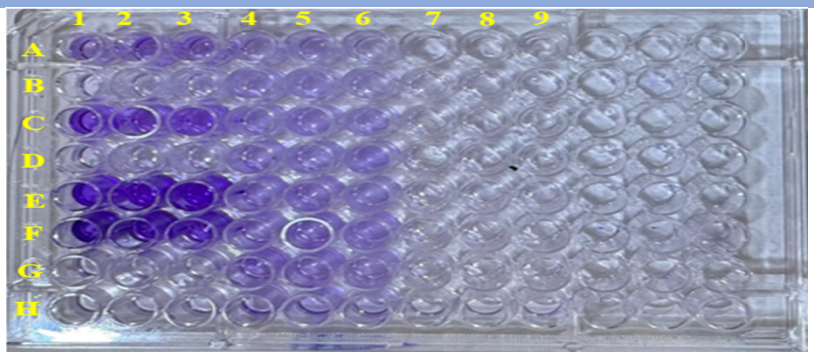

Figure 9: Biofilm formation assay according to microtiter plate technique: (1-3) tested isolates; (4-6) control (only medium); (7-9) blank (empty wells); (E,F) represented strong biofilm formation isolates (*Candida albicans* and *C. glabrata* ; (A,C) moderate biofilm formation isolates (2 of *Candida albicans*); (B, D, G, H) regarded nonproduction of biofilm (*Candida tropicalis and Kluyveromyces marxianus* (*C. kefyr) and one of Candida albicans*)

Figure (10): Congo red medium

Table(6) The color of yeast isolates on Congo red agar.

Yeast isolates (No)	Strong biofilm formation dark red color	Weak biofilm formation pink	Unable to formation biofilm white color
C. albicans(26)	16(61.5%)	9(34.6%)	1(3.8%)
C. tropicalis (1)	1(100%)	0	0
C.glabrata(2)	1(50%)	1(50%)	0
C. dubliniensis (1)	1(100%)	0	0
Pichia kudriavzevii (1)	1(100%)		
Kluyveromyces marxianus(1)	0		1(100%)
Total(32)	20(62.5%)	10(31.3%)	2(6.2%)

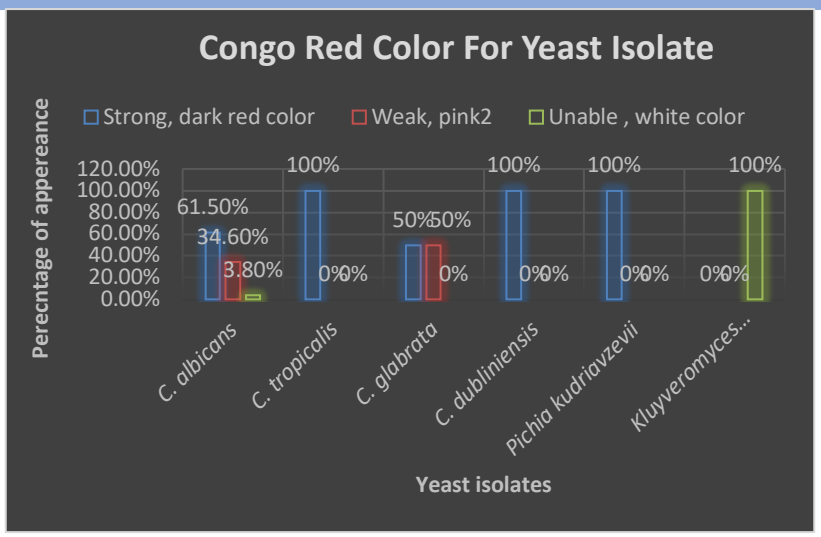

Figure (11): The color of yeast isolates and percentage of biofilm formation on Congo red agar.

Genotypic identification

In current study showed that *hwp1*gene of 32 isolates only 16 (50%), *sap1* gene was 8 (25%) that found in *Candida albicans* only and *sap4* was 24 (75%), had the ability to produce *hwp1,sap1* and *sap4* gene that play a roles in biofilms formation of *Candida* spp (fig. 12,13,14)(table7, 8).

The genotyping approach for *C. albicans* strain typing is useful in primary clinical and epidemiological research because it gives information on the relationships between strain types and features like as virulence and antifungal resistance. According to Saghrouni et al. (2013) [50], found that this approach divides *Candida albicans* into many genotypes based on the electrophoresis banding pattern.

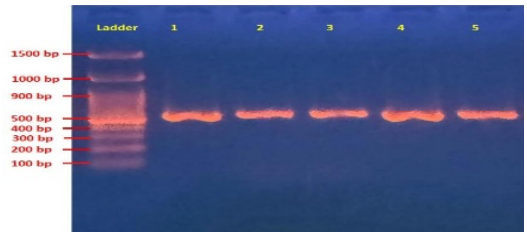

Figure 12. Molecular detection of Hyphal wall protein(*Hwp1)* was observed in(1-5) isolates of *Candida spp.* with longlength in (572bp).

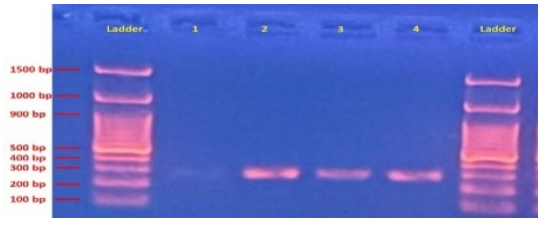

Fig 13. Gel electrophoresis of PCR of (*Sap1*) amplicon ; L (ladder) molecular weight marker of ladder (1500 bp), 2-4 samples obtained from malignant patients.

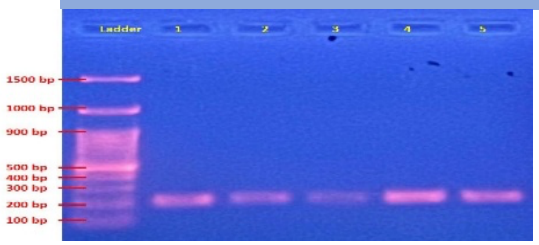

Fig 14. Gel electrophoresis of PCR of (*Sap4*) amplicon ; L (ladder) molecular weight marker of ladder (1500 bp), 1-5 samples obtained from diabetic patients.

Table (7). Distribution of *HWP1*, *SAP1* and *SAP4* in yeast isolates.

Yeast isolates NO.	*HWP1*	*Sap1*	*Sap4*
C. albicans (26)	14(53.8%)	8(30.7%)	20(76.9%)
C. tropicalis (1)	0	0	0
C. glabrata (2)	1(50%)	0	2(100%)
Pichia kudriavzevii (1)	0	0	1(100%)
C. dubliniensis (1)	1(100%)	0	1(100%)
Kluyveromyces marxianus(1)	0	0	0
Total 32	16(50%)	8(25%)	24(75%)

Table (8). Prevalence of the studied virulence genes in the 100 *C. albicans* isolates using PCR.

Gene	Samples Namber	Positive samples	%	P-value
HWP1	48	16	50%	0.032
SAP1	48	8	25%	0.142
SAP4	48	24	75%	<0.01

These results in line with Gerges et al. (2023) [9] who found that the frequency of *SAP4* was 88.7%, whereas *HWP1* was 74.5% and with Azimi (2019) [51], who discovered that the *HWP1* and SAP1 genes were found in 95% and 88% of *Candida albicans*, respectively. *HWP1* was the virulence factor most frequently found, whereas *SAP4* was the least. At least two virulence factors are present in 95% of the numerous species of *Candida*, which can lead to candidiasis, and disagree with Dawoud et al. (2024) [52] who found that *HWP1* is 82.4% of *C. albicans* and 85.7% of NAC, while Paniagua-Contreras et al. (2025) [53] found that *SAP1* was the most frequently expressed *SAP* gene (76/80). Whereas Makled et al. (2024) [54] concluded that the *HWP1* gene was present in 80.0% of isolates, but the *SAP* gene was present in 56.0%. *Candida albicans* possessed more virulence genes than *Candida* non-albicans (NAC). There was a considerable correlation between the presence of the *HWP1* gene and antifungal resistance, as well as between resistance patterns and virulence profiles. While Silva et al. (2014) [55],

found that aspartic proteinases (*Sap*) are linked to adhesion, invasion, and tissue injury, they are one of the major elements influencing pathogenicity. the findings of Monroy-Pérez et al. (2016)[56] showed that 37 (94.8%) isolates had *SAP1* and 35 (89.7%) had *HWP1*. In contrast, Shrief et al. (2019) [46] discovered that the frequency of the virulence genes under investigation was 65% for *SAP1* and 77% for *HWP1*. according to Fathy et al. (2023) [57].13 isolates (43.3%) have the *HWP1* gene.

Antifungal susceptibility testing (AFST) for yeast isolates

In vitro susceptibility of isolates to amphotericin B (AMB), fluconazole (FLC), ketoconazole (KTZ), and clotrimazole (CTZ) is summarized in table 9. Resistance was more common among *C. albicans* isolates was13(40.6%), 4 of these also resistance to other antifungals, than the non-*albicans Candida* isolates 3(9.3%). Overall, 84.6% of *Candida albicans* were susceptible to AMB, 92 % to both FLC and KTZ whereas to CTZ 65.4%. Resistance to CTZ was noted in *C. albicans* 34.6% , 2 originated from pregnant , 2 from diabetic women and were 5 isolated from malignant oral swabs, while *C. glabrata* 1(50%) isolated from diabetic (60 years old) vaginal swab, *Pichia kudriavezi* 1(100%) isolates from malignant oral swab, and *C. dubliniensis* was 1(100%) also from malignant oral swab were resistance to clotrimazole. Some exhibited severe sepsis, Central Venous Catheter (CVC), had received mechanical ventilation and other had been hospitalized for an extended period of time. Different MIC values were recorded for fluconazole, clotrimazole, ketoconazole and amphotericinB against the tested yeast isolates (Table 9). The minimum inhibitory concentrations were determined by the broth microdilution method CLSI, 2008 as described by the Clinical and Laboratory Standards Institute (Arikan, 2007;Cordeiro et al., 2013)**[58, 59]**.

Table (9): Antifungal activity against yeast isolates

Antifungals / No. yeast isolates	Minimum inhibitory concentration MIC (µg/ml) of antifungals							
	AMB	R	FLC	R	CTZ	R	KTZ	R
Candida albicans 26	32-0.1	4(15.4 %)	500-16	2(7.7 %)	500-32	9(34.6%)	500-32	2(7.7%)
Pichia kudriavezi 1	32-16	0	500-250	0	0	1(100%)	250-125	0
Candida tropicalis 1	16	0	500-125	0	250	0	250	0
C. glabrata (2)	8-16	0	250	0	500-250	1(50%)	250-62	0
C. dubliniensis	8-4	0	32-16	0	0	1(100%)	62-32	0

(1)								
Kluyveromyces marxianus(1)	16	0	62	0	125	0	125	0
Total 32		4(12.5 %)		2(6.25 %)		12(37.5 %)		2(6.25 %)

Insignificance difference($p \leq 0.05$). R= resistance, AMB=amphotericin B, CTZ=clotrimazole, FLC=fluconazole, KTZ= ketoconazole

The results of current study was disagreed with Dawoud *et al.* (2024)52, who found that *C.* albicans showed reduced biofilm development and higher susceptibility to antifungal drugs compared to NAC. However, there was no statistically significant difference (p-value > 0.05) between the two groups. *Candida albicans* had a susceptibility rate to FLC and AMB was 64.7% and 100% respectively, whereas NAC had a susceptibility rate 53.6% and 94.3%. It was more common for *C. glabrata* to be resistant to the azole antifungals, and disagree with Awad (2017)[60] who concluded antifungal drug resistance rates, particularly to fluconazole, are increasing as a result of in appropriate antifungal medication use. While Shrief *et al*. (2019 [46] demonstrated that the resistance to antifungal drugs, amphotericin B, it was 9% and for fluconazole was 8%. While Fathy et al. (2023) 57 found that, 93.3% of isolates were sensitive to amphotericin-B, whereas all isolates were susceptible to fluconazole.

Relationship between genes and antifungals resistance

Candida albicans is a prevalent possible fatal fungal infection in humans. Determining gene function can help to find virulence factors: key genes, or drug resistance regulators, that providing new targets for the development of antifungal medicines.(Lee *et al*.,2020)[61]. According to Elkholy et al. (2025)[39] discovered that in the critical care unit, both NAC and *Candida albicans* contribute to device-associated infections that are more likely to be fluconazole and amphotericin B resistant. Isolates that may form biofilms indicate greater antibiotic resistance. In the current investigation, it was discovered that the association between biofilm growth and virulence genes (*HWP1, SAP1*, and *SAP4*) is significant for antifungal resistance.

Table (10)

The current investigation came to the same conclusion as Shrief et al., 2019 [46] who showed that the connection between biofilm formation and gene expression in *Candida* spp. leading to resistance to antifungal drugs. *HWP1* and *SAP1* were the virulence genes that were shown to be 77% and 65% prevalent, respectively. The microplate approach revealed that 58% of *Candida albicans* had the ability to form biofilms, and 8% , 9% of them were resistant to the antifungal medication fluconazole and amphotericin B respectively.

conclusion

The current study focused on the ability of clinical yeast isolates to produce biofilms so that factor contribute to the establishment and enhancement of the pathogenicity of yeast, as well as the increase of fungal resistance to antifungals by various mechanisms, including preventing drugs from reaching fungal cells through biofilms, this ability serves to create and improve the pathogenicity of yeast and increases fungal tolerance to antifungals via a multitude of mechanisms, including blocking drugs from affecting fungal cells via biofilms.

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