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ORIGINAL ARTICLE

A Comparative Study between Invasive and Superficial *Candida albicans* Infections Regarding Biofilm Formation, ALS3 and SAP1-6 Genes Expression and Anti-Fungal Drug Susceptibility

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ABSTRACT

Background: *Candida species are usually found as a component of individual's normal microflora. Candida albicans capable of causing opportunistic infections varying from superficial infections in immunocompetent people, to life-threatening systemic infections in immunocompromised persons. A variety of pathogenicity factors contribute to colonization and pathogenesis of C. albicans during superficial and systemic infections.* ***Objective:*** *The aim of this work is to compare between the expression of ALS3 and SAP1-6 genes among Candida albicans isolated from superficial versus invasive candidiasis. To study the antifungal susceptibility pattern of the collected isolates.* ***Methodology:*** *This study was conducted on 25 isolates of C. albicans isolated from superficial candida infection and 25 isolates from invasive infections, The isolates were assessed for their capability to form biofilm, measurement of quantitative gene expression of ALS3, SAP1-6 genes by real time reverse transcription -polymerase chain reaction (RT-PCR), and antifungal susceptibility using the disk diffusion method.* ***Results:*** *There had been a high statistically significant difference between the two groups regarding biofilm formation p value < 0.001. Also, there was a high statistically significant difference in the expression of SAP 1-6 and ALS3 genes between the two studied groups (P < 0.001) with higher expression levels in the systemic candidiasis group. Resistance to antifungals was more common in systemic isolates.* ***Conclusion:*** *This study has found a relationship between the biofilm development and fluconazole and itraconazole resistance in C. albicans isolates. Moreover, the present study detected a correlation between the expressions of ALS3 gene and biofilm development.*

INTRODUCTION

Candida albicans, is one of the normal flora of our skin, oropharynx, and lower respiratory, gastrointestinal, and genitourinary tracts. It is an opportunistic fungus that settles in many human niches. *Candida* infections can be fatal, especially in patients with compromised immune systems. The different clinical symptoms of *Candida* species range from limited, superficial mucocutaneous illnesses to aggressive, potentially fatal diseases that affect numerous organ systems[1].

The term "candidiasis" unites three separate conditions: colonize asymptomatically a non-sterile location, such as the skin, digestive system; superficial mucocutaneous infections; and aggressive tissue infections[2].

Oral and vulvovaginal *Candida* infections are among the most prevalent human infections, yet they are not usually serious. 50% to 90% of human candidiasis is caused by *Candida albicans*, the most prevalent cause of oral and vulvovaginal candidiasis[3].

Invasive candidiasis has become a serious public health issue along with the increase in the number of critical patients getting wide spectrum antibiotics, immunosuppressant, and using invasive equipment. Both children and adults who have invasive candidiasis experience high mortality rates. The most typical type of invasive candidiasis is candidemia[4].

Candida species possess unique and specialized virulence features that are intrinsically linked to the factors that govern infection, including adhesion, invasion of host tissues, biofilm development, and immune system evasion[5].

Several virulence factors such as host tissue adhesion, hydrolase secretion and biofilm formation are associated with a change from symbiotic to pathogenic amongst candida spp. at different sites of the body.[6] Secreted hydrolases secreted as partyl proteases, phospholipases and haemolysesin are the uppermost common enzymes linked to the pathogenicity of

Candida which perform a crucial part in host damage, invasion, adhesion, and tissue penetration**.** Saps facilitate the attachment of the host tissues, and their subsequent host damage is associated with alterations to the host's immune system escape [8].

The emergence of *Candida* cells to host epithelial or medical device has been considered as an early stage for the formation of biofilms. The biofilm is a very organized microbial community, which irreversibly attaches itself to the surface and constitutes an extracellular matrix by its own creation. Living in a biofilm provides protection against fungal infections and contributes to survival under hostile environmental conditions [9].

Antifungal therapy available for treating Candida infections is limited to four major classes of antifungal drugs, including the most prescribed azoles, polyenes, fluoropyrimidines and the recently generated echinocandins. There has been an evolution of drug resistance in response to an increase in opportunistic fungal infections and widespread use of antifungals[9].

The aim of this work is to compare between the expression of *ALS3*, *SAP1-6* genes and antifungal susceptibility pattern among *Candida albicans* isolated from superficial versus invasive candidiasis

METHODOLOGY

Clinical Isolates:

The current study was carried out at the Department of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University during the period from February 2019 till September 2021. The research protocol acquired approval from the Ethical committee of the Faculty of Medicine, Cairo University (16/10/2018). The study included 150 samples obtained from clinically suspected cases of candidiasis. Samples were collected under strict aseptic precautions from (vaginal swabs, blood samples, pus, endotracheal tubes, and catheter tips) from neonatal intensive care unit (NICU), intensive care unit (ICU), Inpatient (Internal Medicine, Chest, Gynecology) and Outpatient Departments of Kasr El Ainy Hospital. Samples were subjected to direct microscopic examination and culture on Sabouraud Dextrose Agar (SDA) (Oxoid) with chloramphenicol followed by incubation at 25°C for 24 – 48hrs. Identification of *Candida* to Genus level by Gram stain and to species level by Germ Tube Test (GTT). Subculture on chromogenic medium (Oxoid, England, REF: CM1002B). *C. albicans* colonies were smooth and the color is pale green.. *C. tropicalis* appears as raised colonies that range in color from blue to metallic blue. *C. glabrata* colonies were smooth and cream to white, but *C. krusei* colonies were purple and hairy. Other *Candida* species can produce light mauve colonies, which were likewise made by yeast cells. Isolates were stored in glycerol broth at –30ºC.[10,11].

Antifungal Susceptibility of the yeast isolates:

All yeast isolates were evaluated using the CLSI disc diffusion method. The antifungal agents used for disc diffusion method were obtained by liofilchem s.r.l, Italy: Amphotericin B -100 units, Nystatin-100 units, Fluconazole – 10µg, Voriconazole -1 µ g, Ketaconazole-30 µg and Itraconazole- 30 µg and results were interpreted according to (CLSI M44-A 2018) guidelines[12].

Biofilm Development:

The ability of isolates of *Candida* to produce biofilm was evaluated using sterile 96-well micro plates. The optical density (OD) for every well was measured using an enzyme linked immunosorbent assay (ELISA) reader Stat Fax-2100 (GMI, Germany) at 450 nm. [13].

Measurement of virulence gene expression:

For each *Candida albicans* isolate, seven RT-PCRs were done. These reactions were to identify *SAP1-6* (*SAP1, SAP2, SAP3, SAP4*, *SAP5* and *SAP6*) and *ALS3* genes. Candida Actin (*ACT1*) gene was used as an internal mRNA control for evaluation of the sensitivity and efficacy of the RT-PCR analysis. Our study's real-time quantitative polymerase chain reaction (qPCR) differed from traditional PCR in that it included fluorescent reporter molecules in the reaction. These molecules increase in thermocyclers in direct proportion to the level of DNA amplification. RNeasy mini kit was used to extract total RNA from 50 *Candida albicans* isolates. (Qiagen GmbH, Hilden, Germany) according to the guidelines of the manufacturer [14]. RNA yield was determined by Nanodrop Spectrophotometer (ScanDrop, Delta, Germany) which was used to measure the absorbance of the isolated RNA at 260 nm and 280 nm and RNA purity was assessed by calculating the ratio of A260/A280. A ratio of 1.8–2.1 was considered acceptable. cDNA was synthesized by RT-PCR where the total RNA (1µg) was used for cDNA conversion using high-capacity cDNA reverse transcription kit (#K4374966, Thermo Fisher Scientific, USA). Relative Quantitation of *SAP1-6* genes expression by Real –Time PCR by using an Applied Biosystem with software version 3.1 (StepOne™, USA). The qPCR assay with the primer sets were adjusted at the annealing temperature. Every cDNA, even those from previously produced samples (for *SAP1-6* genes expression), internal control (for *ACT1* gene expression as housekeeping gene), and non-template control (water to verify that the reaction mixture is free of any DNA contamination), were duplicated. Relative Quantitation of *ALS3* gene expression by Real-Time PCR by were carried out with an Applied Biosystem with software version 3.1 (StepOne™, USA). The primer sets for the qPCR test were adjusted at the annealing temperature. Every cDNA, even those from previously produced samples (for *ALS3* gene expression), internal control (for the expression of *ACT1* gene as housekeeping gene), and

non-template control (water to verify that the reaction mixture is free of any DNA contamination), were duplicated. Relative Quantification (RQ) (relative expression) was calculated once the RT-PCR process was completed and the data were expressed in Cycle threshold (Ct). The PCR data sheet includes Ct values of assessed genes (for ***SAP 1-6 & ALS3*** genes expression) and the house keeping (reference) gene, the gene that the cell consistently and regularly expresses (***ACT1*** gene). To assess the gene expression of certain genes, a negative control (reference) sample was used. Therefore, target gene expression was assessed and related to reference (internal control) gene as follows:
Finally, RQ was calculated according to the following equation:

1. $\Delta \text{Ct} = \text{Ct}_{\text{assessed gene}} - \text{Ct}_{\text{reference gene}}$
2. $\Delta \Delta \text{Ct} = \Delta \text{Ct}_{\text{sample}} - \text{Ct}_{\text{internal control gene}}$
3. $\text{RQ} = 2^{-(\Delta \Delta \text{Ct})}$

Statistical analysis:

Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Data was summarized using mean and standard deviation for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Comparisons between groups were done using unpaired t test when comparing 2 groups and analysis of variance (ANOVA) with multiple comparisons post hoc test when comparing more than 2 groups [15] For comparing g categorical data, Chi square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5[16]. Correlations between quantitative variables were done using Pearson correlation coefficient[17]. P-values less than 0.05 were considered statistically significant.

RESULTS

Analysis of clinical isolates:

A total of 100 *Candida* isolates were obtained from 150 suspected cases of fungal infection. 70 isolates of *Candida albicans* (70%) were identified using germ tube test (GTT) and chromogenic medium (Oxoid, England, REF: CM1002B). (Figure 1)

Fig. 1: *C. albicans* on chrome agar showing greenish colonies

Out of these isolates 50 strains of *C. albicans* were chosen for studying the expression of *ALS3* and *SAP1-6* genes, biofilm development and the antifungal susceptibility pattern. The isolates were divided into two groups; Group (1): systemic candidiasis**:** 25 isolates from systemic candidiasis recovered from patients admitted to ICU departments, in Kasr El-Ainy University Hospitals and Group (2): 25 isolates obtained from superficial candidiasis were recovered from patients in Outpatient (Gynecology and E.N.T) Departments of Kasr El-Ainy Hospitals (Figure 2).

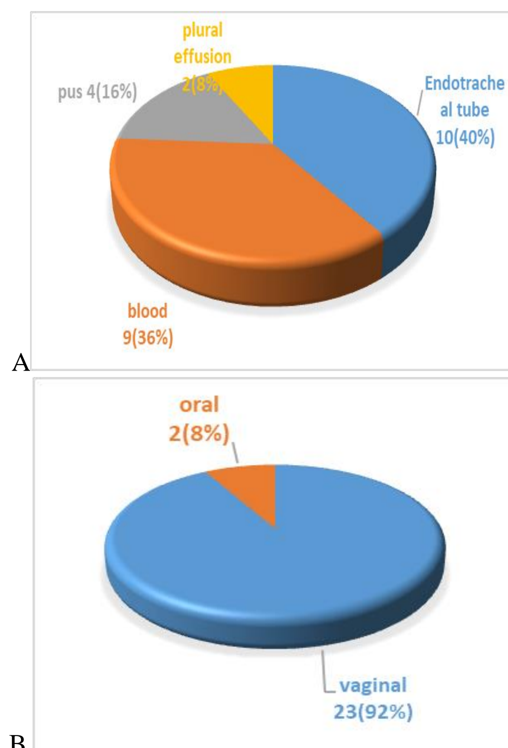

Fig. 2: Distribution of *C .albicans* isolates among different clinical samples. A: showing samples in systemic candidiasis (group 1) B: showing samples in superficial candidiasis (group 2).

Antifungal Susceptibility test results:

The disc diffusion method

Group 1 has shown that 72% of C. albicans isolates have been exposed to Amphotericin B, 60% are susceptible to Fluconazole and Itraconazole, 71% voriconazole, 76% ketoconazole and 88% nystatin. While in group 2; it shows that 92% of *C. albicans* isolates were susceptible to Amphotericin B, 88% were susceptible to fluconazole and voriconazole, 84% susceptible to Itraconazole, 96% susceptible to ketoconazole and nystatin. There was no statistically significant difference between the two groups in antifungal susceptibility test results except for fluconazole p value (0.024). (Figure 3a,b)

Fig. 3a: Antifungal susceptibility test of *C. albicans* isolates using the disc diffusion method. Amphotrecin B 100 (AP 100), Fluconazole 10 (FLC 10), Ketoconazole 30 (KT 30), Itraconazole 30 (IT 30), Voriconazole 1(VOC 1), Nystatin 100 (NYS 100). This isolate was sensitive to all except fluconazole.

Fig. 3b: Antifungal susceptibility test of *C. albicans* isolates using the disc diffusion method. Amphotrecin B 100 (AP 100), Fluconazole 10 (FLC 10), Ketoconazole 30 (KT 30), Itraconazole 30 (IT 30), Voriconazole 1(VOC 1), Nystatin 100 (NYS 100). This isolate was sensitive to amphotricine B, nystatin and resistant to the rest.

Biofilm formation:

Candida albicans isolates were screened for biofilm formation by the microplate method using sterile 96-well microplates. There were more biofilm forming isolates in systemic candidiasis [22 (88%)] than superficial candidiasis [12 (48%)]. (Figure 4)

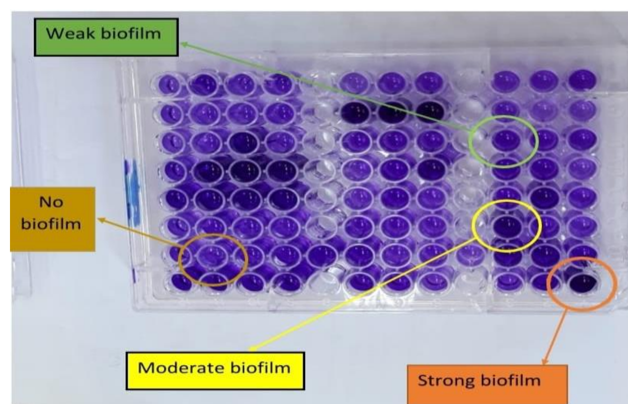

Fig. 4: Biofilm formation in microplate

A high statistically significant variation found between the two groups regarding biofilm formation p value < 0.001. (Figure 5)

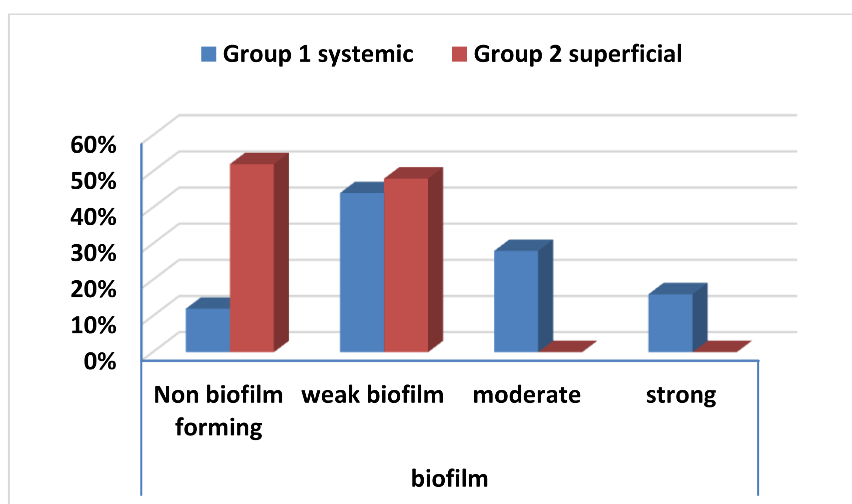

Fig 5: Comparison of biofilm formation in the two groups

Expression levels of Secreted aspartic proteinases (*SAP) 1-6* genes in group1and group 2

The *SAP1-6* expression levels were significantly lower in group 2 isolates in comparison to group 1 isolates (table 1)

Table 1: Comparison between expression levels of *SAP1-6* genes in the two groups.

	Group 1 systemic		Group 2 superficial		*P* value*
	Mean	Standard Deviation	Mean	Standard Deviation	
SAP 1	9.06	1.75	2.86	0.48	**< 0.001**
SAP 2	4.93	0.96	1.52	0.44	**< 0.001**
SAP 3	9.39	2.29	3.84	0.45	**< 0.001**
SAP 4	5.87	2.20	1.89	0.55	**< 0.001**
SAP 5	5.03	1.60	1.84	0.56	**< 0.001**
SAP 6	6.43	2.07	2.74	0.88	**< 0.001**

Expression levels of Agglutinin-like protein (*ALS3)* gene in group 1 and group 2.

There was a high statistical difference between the two groups ($P < 0.001$) with high expression levels in the systemic candidiasis (table 2)

Table 2: Comparison between expression levels of *ALS3* gene in group 1 and group 2

	Group 1 systemic		Group 2 superficial		*P* value*
	Mean	Standard Deviation	Mean	Standard Deviation	
ALS 3	3.42	1.16	1.66	0.49	**< 0.001**

*(Significant p value<0.05)

Correlation between the expression of *ALS3* and biofilm formation in the whole study population.

A high statistically significant variation revealed between biofilm producing isolates and non-biofilm producing in *ALS3* expression *p* value (< 0.001), *ALS3* were highly expressed among biofilm forming strains. (Table 3)

Table 3: *ALS3* expression in biofilm forming and non-biofilm forming isolates.

	Biofilm				
	Biofilm forming		**Non biofilm forming**		***P* value**
	Mean	**Standard Deviation**	**Mean**	**Standard Deviation**	
ALS 3	**2.97**	**1.23**	**1.64**	**0.74**	**< 0.001**

Correlation between the expression of *ALS3* and biofilm formation in each group.

There was statistically significant variation in expression level of secreted aspartic proteinases *(SAP) 1-2* genes between the resistant isolates to fluconazole, ketoconazole and itraconazole and the sensitive isolates, *p* value (0.008, 0.020, and 0.033) for *SAP 1* and (0.047, 0.036, and 0.049) for *SAP2* respectively with higher expression in the resistant isolates. (Table 4).

Table 4: comparison between the biofilm forming isolates and the non-biofilm forming isolates regarding the expression level of the *ALS3 gene.*

Group 1 systemic	**Biofilm**								
	Non biofilm forming		**weak biofilm**		**moderate**		**strong**		***P* value***
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	
ALS 3	**2.41**	**1.43**	**3.31**	**1.04**	**3.56**	**1.20**	**4.25**	**0.99**	**0.214**

Group 2 superficial	**Biofilm**				
	Non biofilm forming		**weak biofilm**		***P* value***
	Mean	**SD**	**Mean**	**SD**	
ALS 3	**1.46**	**0.39**	**1.88**	**0.51**	**0.027**

No significant variation was found between the non-biofilm producing isolates and the biofilm producing isolates regarding the expression level of *ALS3* in the first group, although there were significant differences in expression level of *ALS3* gene between both the non-biofilm forming isolates and the weak biofilm forming isolates in the second group.

Correlation between the antifungal susceptibility test results and the expression levels of secreted aspartic proteinases (*SAP) 1-6* genes and the *ALS3* gene.

There was statistically significant difference in expression level of secreted aspartic proteinases (*SAP) 1-2* genes between the resistant isolates to fluconazole, ketoconazole and itraconazole and the sensitive isolates, *p* value (0.008, 0.020, and 0.033) for *SAP 1* and (0.047, 0.036, and 0.049) for *SAP2* respectively with higher expression in the resistant isolates. (Table 5).

Table 5: Correlation between the antifungal susceptibility test results and the expression levels of secreted aspartic proteinases *(SAP) 1-6* genes and the *ALS3* gene.

	Fluconazole				
	resistant		sensitive		P value
	Mean	Standard Deviation	Mean	Standard Deviation	
SAP 1	8.05	2.90	5.22	3.25	**0.008**
SAP 2	4.11	1.64	2.92	1.87	**0.047**
SAP 3	7.56	2.95	6.28	3.32	0.225
SAP 4	4.14	2.31	3.79	2.67	0.669
SAP 5	4.04	1.95	3.22	2.00	0.206
SAP 6	5.72	2.90	4.19	2.16	0.051
ALS 3	2.96	1.34	2.40	1.21	0.168
	Ketoconazole				
	resistant		**sensitive**		**P value**
	Mean	**Standard Deviation**	**Mean**	**Standard Deviation**	
SAP 1	8.67	2.66	5.52	3.30	**0.020**
SAP 2	4.43	1.34	3.03	1.88	**0.036**
SAP 3	7.51	2.87	6.47	3.31	0.438
SAP 4	4.22	2.30	3.82	2.62	0.709
SAP 5	3.84	1.88	3.37	2.04	0.568
SAP 6	5.87	2.89	4.38	2.33	0.135
ALS 3	3.32	1.20	2.42	1.23	0.077
	Itraconazole				
	resistant		**sensitive**		**P value**
	Mean	**Standard Deviation**	**Mean**	**Standard Deviation**	
SAP 1	7.58	3.30	5.33	3.23	**0.033**
SAP 2	5.89	2.96	4.08	2.03	**0.049**
SAP 3	7.31	3.00	6.34	3.33	0.348
SAP 4	4.40	2.29	3.68	2.66	0.379
SAP 5	3.71	2.00	3.33	2.02	0.545
SAP 6	3.85	1.79	2.98	1.87	0.143
ALS 3	3.02	1.50	2.36	1.11	0.095

DISCUSSION

Candidiasis is a global fungal infection brought on by *Candida* fungus, the most prevalent of which is *Candida albicans*.[18] Infections can be either mucocutaneous or invasive candidiasis.[4] The ability of *Candida* to cause infections depends in part on its innate virulence factors. *C. albicans* are causing serious problems because it carries more virulence factors than non albicans *Candid*a isolates[18].

Our study has been designed to investigate the expression of virulence factor genes that play a major role in *C. albicans'* pathogenicity, as secreted aspartyl proteases genes 1-6 ***(SAP1-6)*** and agglutinin-like protein ***(ALS3)*** gene among *C. albicans* isolated from superficial *versus* systemic candidiasis and to assess

their antifungal susceptibility pattern. Standard mycological techniques, germ tube tests and morphological characteristics of chrome agar have identified 70 isolates, *C. albicans* was the highest prevalent isolated species (70%), whereas the non-albicans species were (30%). Similar results were recorded by Nagajothi et al.[19] and ElFeky et al.[20], who reported that prevalence of *C. albicans* was (70.59%) and 60.3% respectively. However, ElFeky and Gohar[21] reported (55.6%) C. *albicans* identification, while (44.4%) were identified as *non-albicans* among *Candida species* isolated from superficial and systemic candidiasis.

In our study, in-*vitro* antifungal susceptibility done by the disk diffusion technique showed that antifungals with the highest susceptibility in superficial candidiasis were ketoconazole and nystatin (96%), followed by amphotericin B (92%) and 88% for fluconazole. Similar results were detected by *Goulart et al.*[22] where 96.5% of the strains were susceptible to ketoconazole, Furthermore, Shi et al.[23] found that 100% sensitivity to amphotericin B and nystatin and 92.3% sensitivity to fluconazole. However, Waikhom et al.[24] reported lower rates of susceptibility, of *C. albicans* isolates were susceptible to fluconazole (21%).

It is widely accepted that Candida develops rapidly resistance to azoles and echinocandins. Nevertheless, resistance to amphotericin B remains extremely rare mostly because the fitness cost of developing modifications for survival is high[25].In our study, the *in vitro* sensitivity testing of *C. albicans* isolates, isolated from systemic candidiasis revealed that the antifungal with the greatest sensitivity was nystatin (88%) followed by ketoconazole and amphotericin B (76%, 72%) respectively, fluconazole and itraconazole susceptibility rates were (60%). Similarly, Khairat et al.[26] showed 94.4% sensitivity to nystatin. However, lower sensitivity rate was reported by Paul & Kannan[27] with only 29.4% sensitivity to fluconazole. Similarly, El-Houssaini et al.[9] detected a 100% fluconazole and voriconazole resistance. Different sources and locations of clinical samples can be attributed to variations in antifungal resistance profiles[9]. In our study, we detected statistically significant difference between systemic and superficial infections isolates in antifungal susceptibility testing results for fluconazole *p* value (0.024) with higher prevalence of fluconazole resistance in systemic than superficial isolates. This conclusion is consistent with the study performed before by El-Houssaini et al.[9]

Candida species pathogenicity is associated with its ability to biofilm formation[27]. The present study showed (88%) and (48%) of isolates in systemic candidiasis and superficial candidiasis were biofilm forming. High statistically significant variation between the two categories regarding biofilm formation p value < 0.001. In agreement with our study, Alikhani et al.[28] revealed (48.7%) positivity for biofilm production. However, Sriphannam et al.[27]reported 51.2% and 46% positivity of *C. albicans* isolates from systemic candidiasis.

Our study demonstrated a relation between the biofilm development and fluconazole and itraconazole resistance in *C. albicans* isolates, with statistically significant variations between those who develop biofilm and those who don't with more resistance to fluconazole and itraconazole. Similarly, Deng et al.[29] reported that the resistance to fluconazole, vioriconazole and itraconazole was higher for C. *albicans* with biofilm formation. However, Marak & Dhanashree[30] found no statistical correlation between the biofilm development and antifungal sensitivity ($p > 0.05$).

Through induction by surface contact, biofilm development raises the rates at which resistance genes are expressed[31]. The pathogenicity and adherence of *Candida albicans* to mucosa and epithelial cells are regulated by several genes, the most significant are the agglutinin-like sequence (ALS) genes[32].

Regarding our results, Agglutinin-like protein 3 (*ALS3)* quantitative gene was expressed in all isolates, which was matching with, Mohammed et al.[33] who showed *ALS3* gene detection in all tested *C. albicans* isolates from vaginal and oral infections. Also, Roudbarmohammadi et al.[32], reported the expressions in 77.7% of the isolates. In disagreement, Monroy-Pérez et al.[34] revealed that *ALS3* gene was detected in only 35.8% of *C. albicans* strains. Our research revealed a relationship between the expression of *ALS3* and the ability to biofilm formation with high statistically significant difference between *ALS3* expression in biofilm forming and non-biofilm forming isolates. Similar results were documented by Deng et al. [29] and Mohammed et al.[33]

The primary virulence determinants in *C. albicans* are secreted aspartic proteases, which are encoded by a family of 10 SAP genes[35]. Our result detected the *SAP1-6* expression in all isolates. Also, Ali et al.[36] reported 100% expression in *SAP4- SAP6* and 91% -92.3% in *SAP1-SAP3*.

Our result reported the quantitative expression levels of *SAP1-6* were statistically significantly lower in superficial isolates compared to systemic isolates ($P < 0.001$). Up to date studies comparing the quantitative *SAP 1-6* genes expression in superficial and systemic candidiasis has not been published yet**.** Additionally, we found a significant correlation between *SAP1, SAP 2* genes and resistant to fluconazole, ketoconazole and itraconazole, *p* value (0.008, 0.020, and 0.033) respectively with higher expression in the resistant isolates.

CONCLUSION & RECOMMENDATIONS

Prevalence of *Candida albicans* was more than *candida non- albicans* in isolated species. Biofilm formation is more prominent among the systemic

isolates. There is a correlation between biofilm production, and resistance of *C. albicans* to antifungal treatment. The expression of *SAP1-6* and *ALS3* were more evident in systemic isolates with a correlation between *ALS3* expression and biofilm production.

Therefore, our study recommends that prior to initiating therapy, a preliminary antifungal sensitivity test and identification of *Candida* species at the species level should be performed. In addition, In order to deal with the multiple factorial similarities of virulence factors in general, biofilm formation and antifungal resistance, it is necessary to carry out additional studies at Genetic and Molecular levels.

Declarations:

Consent for publication: Not applicable

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Author Contributions:

Marwa Ali: Carried out the experiments, performed the analysis, discussed the results, and wrote the final manuscript with input from all authors.

Hamida Goher: Supervision, Project Administration.

Reham Ali Dwedar: Supervision, Validation, Review & Editing.

Yasser Nassar: Resources, Supervision, Review & Editing.

Mona Gamal Eldin Nada: Manuscript writing, reviewing and final editing.

Maha Mahmoud Kotb: Planned and supervised the experiments, discussed the results, revised the work, and approved the final version to be published.

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