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Molecular Detection, Sequencing, and Expression Analysis of *ALS* Genes in Clinical *Candida* spp. Isolates with Antifungal Resistance

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ABSTRACT

The agglutinin-like sequence (*ALS*) family is crucial for *Candida* spp., particularly *C. albicans*, as it aids in adhesion to biotic and abiotic membranes and formation of virulence factors. This study was conducted to identify and measure the expression levels of *ALS* genes in *Candida* species isolates from clinical specimens. A total of 276 clinical samples (vaginal, oral, and blood) were obtained. The isolates were characterized, genetic material extracted, *ALS* genes detected, nucleotide sequence determined, and gene expression measured after antifungal treatment of oral and vaginal isolates. The study found a significant difference ($p \leq 0.05$) among the number of isolates; 135 (48.91%) *Candida* positive isolates, with *C. albicans* being the most common species. Also, 15% of isolates were resistant to nystatin. Most *Candida* species had *ALS* genes, except for *C. kefyr* and *C. lusitaniae*, which were not detected. The gene expression analysis before and after treatment with fluconazole and nystatin revealed that *C. albicans* isolates from the mouth had higher gene expression than vaginal isolates, with gene expression reaching 92.82 and 17.52, respectively, for the two antifungal agents, making them resistant to antifungals. The *ALS1* gene expression was highly elevated in antifungal-resistant isolates, while it was reduced in susceptible isolates. The study's findings highlight the prevalence of *ALS* genes in *Candida* species and could be used as a marker of drug resistance.

1. INTRODUCTION

Fungal infections frequently impact mucous membranes, including those of the respiratory, urinary, and genital tracts [1, 2]. *Candida* is a primary etiological agent of fungal infections, responsible for candidiasis, and ranks as the third leading cause of sepsis in Europe, with an estimated mortality rate of 37% within a 30-day period [3-5]. Several *Candida* species, including *C. tropicalis, C. albicans, C. krusei, C. parapsilosis*, and *C. glabrata*, are responsible for more than 90% of infections. In recent years, other *Candida* species have been identified and diagnosed as causative agents of infections, including *C. kefyr*, *C. lusitaniae*, and *C. guilliermondii* [6, 7]. Currently, invasive candidiasis is a common and widespread disease and is associated with high mortality and morbidity. Individuals most susceptible to candidiasis possess a compromised immune system, which may arise from various factors such as chemotherapy, use of broad-spectrum antibiotics, surgical interventions, kidney failure, and organ transplant surgery [8, 9].

The bacteria that colonize the mucous membranes, including those of the mouth and vagina, constitute a varied population that influences human health, particularly in women, notably during pregnancy. Many studies have demonstrated that an increased prevalence of infectious disorders is caused by any alteration of the microbial diversity in the mouth or vagina. It is estimated that 75% of women will suffer from vaginal infections at least once in their lifetime [10, 11]. Approximately 90% of these infections are caused by *Candida vaginitis* (bacterial vaginosis). The initial diagnosis of vaginosis is mainly based on clinical signs and symptoms and examination of vaginal discharge under a microscope [12]. Given the widespread prevalence and clinical impact of *Candida* infections, particularly among immunocompromised individuals, there is a growing interest in understanding the molecular mechanisms that contribute to the pathogenicity of these fungi. One such focus is the agglutinin-like sequence (*ALS*) gene family, which plays a critical role in *Candida* adhesion, biofilm formation, and virulence.

The *ALS* gene family of *Candida* spp. represents an important gene group for the genus *Candida*, especially *C. albicans*. This is due to the significant role of these genes in the process of adhesion of fungi (yeasts) to moist or wet membranes such as the surface of epithelial cells in the vagina, mouth, respiratory, urinary, or genital tract. Furthermore, the *ALS* gene family also plays an important and significant role in biofilm formation and other virulence factors, such as colonization, invasion, and pathogenicity [13]. *Candida dubliniensis*, *Candida parapsilosis*, *Candida orthopsilosis*, *Candida metapsilosis*, and *Candida tropicalis* have been shown to include genetic material and nucleotide sequences from *ALS* genes and the proteins that arise from their gene

expression. Although the presence of nitrogenous base sequences has made it possible for researchers to identify *ALS* genes, these sequences have limited research on the processes underlying the gene expression of these genes, as well as the reasons behind both elevated and decreased gene expression. According to recent research, *ALS* genes, in particular, the *ALS1* gene, play a significant role in modulating biofilm formation and, consequently, the connection between these genes and antifungal resistance [14, 15]. Research on the *ALS1* gene and its relationship to antibiotic resistance is scarce.

The present study aimed to detect the *ALS* genes and evaluate the effect of antifungal agents on the gene expression of isolates.

2. MATERIALS AND METHODS

2.1 Collection of samples

Between September 2024 and January 2025, a total of 276 samples (120 vaginal samples, 120 mouth samples, and 36 blood samples) from patients ranging in age from 3 to 70 years old, depending on the type of sample, were obtained from Rusafa hospitals (Imam Ali Hospital, Fatima Zahraa Maternity Hospital, Ibn Al Baladi Hospital for Women and Children, and Alawiya Women's Hospital). The samples were collected from patients following a clinical diagnosis by the pathologist in each of the aforementioned hospitals. Before culturing, the samples were stored at 4℃.

2.2 Isolation and identification of *Candida* species from clinical samples

The culture medium used in this study, Sabouraud Dextrose Agar (SDA), was prepared according to the manufacturer's instructions. Swabs were collected from the mouth and vaginal areas of the women and then cultured on the prepared culture media. The cultures were incubated at 37℃ for seven days. Then the colonies were identified microscopically at 400x magnification according to the taxonomic keys [1, 16].

2.3 Antifungal susceptibility test

Two antifungal agents (fluconazole and nystatin) were used to test for antifungal susceptibility against 60 isolates of *Candida* spp. The isolates included 29 isolates of *Candida albicans* and 31 isolates of non-*Candida albicans*, including 15 isolates of *Candida tropicalis*, 10 isolates of *Candida glabrata*, and 3 isolates of each of *Candida lusitaniae* and *Candida kefyr*. The isolates were identified phenotypically, microscopically, and using the Vitek-2 device in accordance with the technique by Liu et al. [17].

2.4 DNA and RNA extraction

To detect *ALS* genes in 5 isolates of *C. albicans*, 2 isolates each of *C. tropicalis*, *C. lusitaniae,* and *C. kefyr*, DNA was extracted using the Promega DNA extraction kit (Bioneer, South Korea). The *ALS1* gene family was selected for nitrogenous base sequencing in some of the isolates. Regarding gene expression, two isolates (one resistant and one sensitive to *C. albicans*), one from the mouth and the other from the vagina, were selected to evaluate *ALS1* gene expression following RNA extraction using the TRIzol™ kit.

2.5 Molecular detection of *ALS* genes in *Candida* spp. by the polymerase chain reaction

In this experiment, the primers whose sequences are listed in Table 1 were used to amplify the *ALS* genes using polymerase chain reaction (PCR) following the method described by Sadeq and Ismail [18]. Additionally, the *ALS1, ALS2, ALS3*, and 18S RNA genes were amplified in the real-time reaction according to Ahmed et al. [19]. The forward and reverse primers were used in the PCR procedure to amplify the *ALS* gene and produce numerous copies of it. The reaction was performed with a total volume of 25 µl [18, 20]. After completing the replication process, the products were electrophoresed according to the method described by Green [21]. The PCR products were purified and sequenced (Macrogen, Korea) for molecular detection of *Candida* spp. and analysis of genetic relationships.

Table 1. Nucleotide sequences of the primers used for the study

Primer Name	Seq.	Annealing Temp. (℃)	Product Size (bp)
RTALS1F	GACTAGTGAACCAACAAATACCAGA	58	318
RTALS1R	CCAGAAGAAACAGCAGGTGA		
RTALS2F	CCAAGTATTAACAAAGTTTCAATCACTTAT		366
RTALS2R	TCTCAATCTTAAATTGAACGGCTTAC		
RTALS3F	CCACTTCACAATCCCCAT C		342
RTALS3R	CAGCAGTAGTAGTAACAGTAGTAGTTTCATC		

2.6 *ALS1* gene expression in *Candida albicans* by relative quantification

Using the quantitative real-time polymerase chain reaction (qPCR-PCR) technique and the standard 18S rRNA gene, the expression levels of the *ALS1* gene were assessed in two isolates of *Candida albicans* from the mouth and vagina before and after treatment with fluconazole and nystatin. The procedures followed the reaction conditions as described [19, 22]. Relative quantification was made according to the following equations:

$$\text{Folding} = 2^{-\Delta\Delta CT}$$

$$\Delta CT = CT_{gene} - CT_{\text{House Keeping gene}}$$

$$\Delta\Delta CT = \Delta CT_{\text{Treated or Control}} - \Delta CT_{Control}$$

2.7 Statistical analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS; version 21). Statistical significance was set at $p \leq 0.05$.

3. RESULTS

3.1 Clinical isolates of *Candida* species

Out of 276 samples obtained from patients at Rusafa hospitals (Imam Ali Hospital (AS), Fatima Zahra Hospital, Ibn Al Baladi Hospital for Women and Children, Shahid Al Sadr General Hospital, and Alawiya Women's Hospital), the results of culturing on SDA medium revealed the presence of 135 positive isolates. As indicated in Table 2, the positive clinical samples (48.91% of the total number of samples) comprised samples from the mouth, vagina, and blood in varying proportions (50.37%, 39.26%, and 10.37%, respectively).

Table 2. Percentages of *Candida* spp. isolated from clinical samples

Clinical Samples	Total Number	No. of Positive	% of Infection	No. of Negative	% of Infection
Mouth	120	68	50.37%	52	%36.88
Vagina	120	53	39.26%	67	%47.52
Blood	36	14	10.37%	22	%15.60
Total	276	135	100%	141	%100
% total number of samples		48.91%		51.09%	
p-Value	0.04*	0.01* Person Chi-Square= 0.033* Pearson's R= 0.148**			0.01*

* Significant differences at a probability level of p≤0.05.

Table 3 shows the results of the identification of *Candida* spp. at the species level, where *C. albicans* was the most prevalent species with 63 isolates (46.67%), followed by *C. tropicalis* and C. *glabrata* with 43 (31.85%) and 20 isolates (14.82%), respectively. Meanwhile, *C. lusitaniae* and *C. kefyr* were found with 6 (4.44%) and 3 isolates (2.22%), respectively.

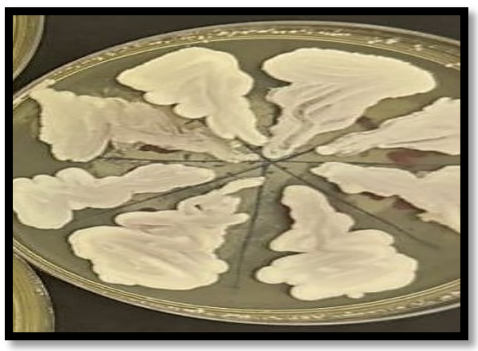
Table 3. Types and percentages of *Candida* spp.

Species	No. of Isolates	%	P-value
C. albicans	63	46.67%	0.001*
C. tropicalis	43	31.85%	
C. glabrata	20	14.82%	
C. kefyr	6	4.44%	
C. lusitaniae	3	2.22%	
Total	135	100%	

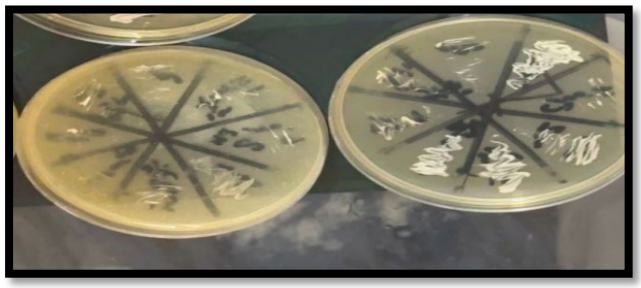
* Significant differences at a probability level of p≤0.05.

3.2 Resistance of *Candida* species to antifungal agents

Figure 1 shows the *Candida* spp. sensitivity testing against fluconazole and nystatin showed that 56.7% of *Candida* spp. were resistant to the antifungal agent, fluconazole, and 15% of *Candida* spp. isolates were resistant to the antifungal agent, nystatin. These isolates included 4, 2, 4, and 1 isolates of *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, *Candida kefyr*, and *Candida lusitaniae*, respectively, as shown in Table 4.



A



B

Figure 1. Susceptibility test of *Candida* species to antifungals, A: Isolations *Candida* Control, B: *Candida* isolates were exposed to fluconazole at a final concentration of 0.6 mg/mL and incubated at 37°C for 24-48 hours

3.3 Molecular detection and sequencing of *ALS* genes in *Candida* species

The molecular detection used to identify the *ALS1, ALS2*, and *ALS3* genes in the *Candida* spp. under study revealed that the molecular sizes of the amplified genes are 318, 366, and 342, respectively (Figure 2). Every isolate of *Candida albicans*, with the exception of one isolate that lacked a gene, and every isolate of *Candida tropicalis*, with the exception of one isolate that lacked the *ALS2* gene, had all three amplified genes. Additionally, the results demonstrated that all isolates of *C. kefyr* and *C. lusitaniae* lacked genes. Following the electrophoresis separation, two isolates from each species of the genus *Candida*, including *C. albicans* (isolates 14 and 19) and C. *tropicalis* (isolates 6 and 18), were chosen for nucleotide sequence analysis of the *ALS1* gene since *ALS* genes were diagnosed with high frequency. Table 4 highlights a comparison of the *ALS1* gene sequences in the *Candida* spp. with reference sequences. Isolate 19 of *Candida albicans* and isolates 6 and 18 of *Candida tropicalis* had the highest nucleotide sequence identity (100%), whereas isolate 14 of *Candida albicans* had the lowest identity (99.62%) because of two mutations (Table 5).

Table 4. Susceptibility of *Candida* species to antifungal agents

Antifungal Agents	*C. albicans* n=29	*Non-albicans Candida* (n=31)				Total (n=60)
		C. tropicalis n=15	*C. glabrata* n=10	*C. kefyr* n=3	*C. lusitaniae* n=3	
Fluconazole	18 (62.07%)	6 (40%)	4 (40%)	3 (100%)	3 (100%)	34 (56.67%)
Nystatin	4 (13.79 %)	2 (13.33%)	2 (20%)	0 (0%)	0 0%	8 (13.33%)
P-value	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
	Person Chi-Square= 0.663** Pearson's R= -0.087***					

Non–*Candida albicans* (n = 31).

Table 5. Nucleotide sequence of the *ALS1* gene in *Candida* spp. showing mutations

ALS1 Gene						
No.	**Type of Substitution**	**Nucleotide**	**Sequence ID with Comparison**	**Country**	**Strain**	**Identities**
14	- Transition	A\ C\T	ID: OR664373.1	USA	*Candida albicans*	99.62%
19	-	-	ID: AF201686.1	USA	*Candida albicans*	100%
6	-	-	ID: AF201686.1	USA	*Candida tropicalis*	100%
18	-	-	ID: LC791630.1	USA	*Candida tropicalis*	100%

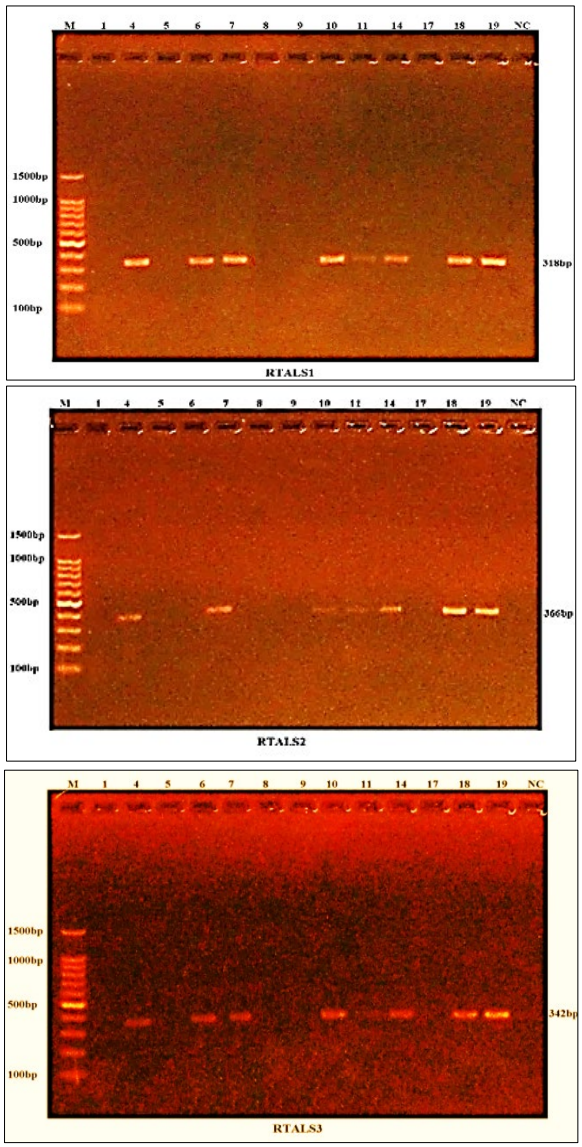


Figure 2. Electrophoretic migration of PCR-amplified *ALS* gene fragments in *Candida* spp.

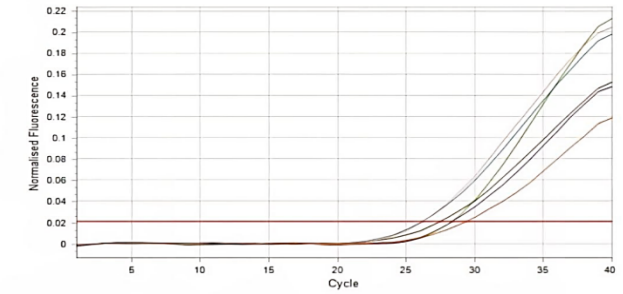
3.4 *ALS* gene expression in *Candida* species

The results of the *ALS1* gene expression analysis in two isolates of *C. albicans* from the mouth (A) and vagina (B) before and after treatment with antifungal agents revealed the doubling of the *ALS1* gene. The standard 18S rRNA gene by qPCR technique showed that the *ALS1* gene doubling curve was observed in the cycler threshold values in all treatments, which indicates the presence of the gene in these treatments. The CT value in control treatment A and B was 28.38 and 26.17, respectively. In the meantime, A1 and B1 received fluconazole treatments at 28.30 and 27.49, respectively. In contrast, the CT values for A and B under the fluconazole therapy were 28.38 and 26.17, respectively, while for A2 and B2 under the nystatin treatment, they were 29.39 and 26.26, respectively. Since all of the values were close to 1, the findings also demonstrated a high level of efficiency, as shown in Figure 3.

The *ALS1* gene's melting curve following qRT-PCR amplification is displayed in Figure 4. All of the samples where the gene was found had melting points between 81.21 and 81.45°C, which is similar to the melting point of the typical 18S rRNA gene, which was 81.36°C.

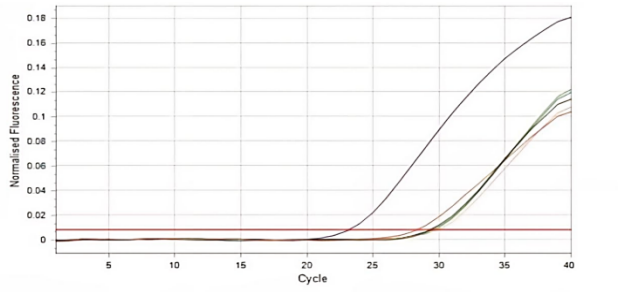
The results of assessing the *ALS1* gene expression in *C. albicans* isolates from the mouth and vagina following treatment with fluconazole and nystatin are displayed in Table 6. These results indicated that the gene expression values for oral samples differ significantly from those for the control treatment (A), where the gene expression value for the mouth isolate (control) was 1.00. The highest gene expression values were obtained with fluconazole (A1) and nystatin (A2), which were 92.82 and 17.52, respectively. The gene expression measurement of vaginal samples (B) revealed that the control had a gene expression value of 1.00, that the fluconazole (B1) treatment had a decrease in expression (0.36), and that the nystatin (B2) treatment had an expression value that was similar to the control treatment (1.35). Regarding the comparison of gene expression between oral isolates (A) and vaginal isolates (B), it was evident that oral isolates had increased gene expression following antifungal treatment, whereas vaginal isolates (B) had decreased gene expression

following the same treatments. This suggests that oral isolates are resistant to antifungals, whereas vaginal isolates are sensitive to them.



Well	Colour	Sample Name	Cq	Efficiency	Efficiency R²	Result
1[C]		A	28.38	0.56	0.98873	
2[B]		A1	28.30	0.63	0.99797	
3[B]		A2	29.39	0.39	0.99495	
4[B]		B	26.17	0.48	0.99139	
5[B]		B1	27.49	0.42	0.99600	
6[B]		B2	26.26	0.56	0.99803	

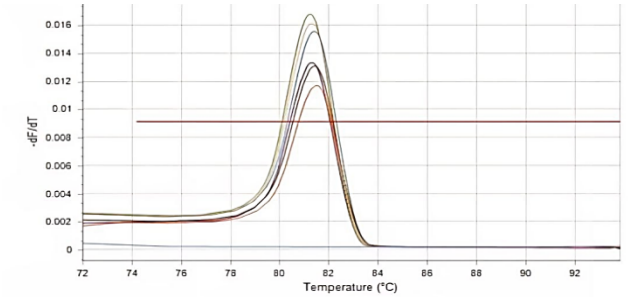
(a)



Well	Colour	Sample Name	Cq	Efficiency	Efficiency R²	Result
1[A]		A	23.17	1.08	0.99436	
2[A]		A1	29.63	1.03	0.98572	
3[A]		A2	28.32	0.86	0.99836	
4[A]		B	29.44	0.89	0.99498	
5[A]		B1	29.29	0.98	0.99544	
6[A]		B2	29.97	0.73	0.99633	

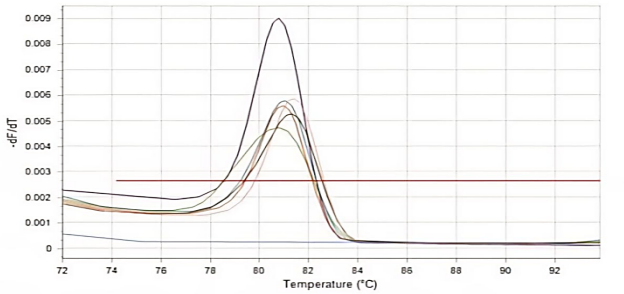
(b)

Figure 3. Real-time polymerase chain reaction (qRT-PCR) amplification curve
a: *ALS1* gene; b: 18S rRNA gene



Well	Colour	Sample Name	Tm (°C)	Genotype
1[C]		A	81.27	
2[B]		A1	81.21	
3[B]		A2	81.45	
4[B]		B	81.37	
5[B]		B1	81.38	
6[B]		B2	81.27	
7[B]		NC		

(a)



Well	Colour	Sample Name	Tm (°C)	Genotype
1[A]		A	80.75	
2[A]		A1	80.70	
3[A]		A2	80.93	
4[A]		B	80.99	
5[A]		B1	81.25	
6[A]		B2	81.36	
7[A]		NC		

(b)

Figure 4. Melting curve of amplified genes after the quantitative real-time polymerase chain reaction amplification
a: *ALS1* gene; b: 18S rRNA gene

Table 6. Determination of *ALS1* gene expression values in *Candida albicans* isolates after antifungal treatment using the Livak method

Sample	18s rRNA	RTALS1	ΔCT	ΔΔCT	Fold Change	Antifungal Conc.
A*	23.17	28.38	5.21	0.00	1.00	-
A1	29.63	28.30	-1.33	-6.54	92.82	0.3
A2	28.32	29.39	1.08	-4.13	17.52	0.1
B	29.44	26.17	-3.27	0.00	1.00	-
B1	29.29	27.49	-1.80	1.47	0.36	0.3
B2	29.97	26.26	-3.70	-0.43	1.35	0.1

A*: *Candida albicans* (control) from the mouth; A1: Fluconazole; A2: Nystatin; B: *Candida albicans* (control) from the vagina; B1: Fluconazole; B2: Nystatin

4. DISCUSSION

The detection and sequencing of *ALS* genes offer important insights into the pathogenicity of *Candida* species. Differences in *ALS* gene sequences indicate potential strain-specific variations in adhesion and biofilm formation. Additionally, the observed variations in *ALS* gene expression highlight their significance in fungal virulence. These findings may contribute to the development of targeted antifungal therapies and diagnostic tools for *Candida* infections. The results of isolation and phenotypic and microscopic characterization are consistent with the findings of Marsaux et al. [23] and Ali and Ewies [24], who isolated and identified different types of *Candida* from clinical samples. Non-culture-based methods, such as PCR, have been shown to rapidly detect and identify pathogenic fungi, offering the possibility of prescribing species-oriented and effective therapy and identifying genetic markers associated with antifungal resistance [13, 25]. These findings were similar to those of Lombardi et al. [13] and emphasize the importance of examining the nitrogenous base

sequences of the genes being studied because of the significance of these genes and their connection to virulence factors through gene expression [26]. They examined these genes' nucleotide sequences and demonstrated that analyzing the sequences in their entirety (by examining all of the nitrogenous bases in the gene), or in their condensed form (a small portion of the gene), and using standardized primers, leads to accuracy. This approach provides a clear picture of the nature of these genes, elucidating their function in the formation of membrane adhesion factors and their role in virulence factors. These findings are comparable to those of Oh et al. [14], who investigated the *C. albicans ALS* gene family due to their role in fungal colonization, cell adhesion, and biofilm formation.

The accessibility of nucleotide sequences from the genomes of pathogenic yeasts and related fungal species offers a comprehensive, comparative, and precise understanding of the *ALS* gene family. These genomic sequences enable researchers to analyze the genetic composition, organization, and evolutionary relationships of *ALS* genes across different fungal species. The stability of the *ALS* gene and possible selected evolutionary forces were highlighted by the fact that one strain of *C. albicans* had 99.62% similarity because of a C to T transition, while other strains remained completely preserved. The 99.62% similarity mutation in the *C. albicans* strain might be the result of adaptive evolution, spontaneous genetic drift, or environmental constraints like exposure to antifungals that favour variants with different adhesion or biofilm-forming characteristics. To find out if this mutation affects *ALS* gene expression or protein function, or if it adds to virulence or antifungal resistance, more functional research would be required. Despite significant advancements in DNA sequencing technologies, such as next-generation sequencing (NGS) and bioinformatics tools for genome analysis, the complete and accurate identification of all *ALS* loci remains challenging. Many *ALS* gene loci still require additional steps, including PCR amplification and Sanger sequencing, to obtain precise gene counts and sequence data. Furthermore, the groundbreaking accuracy of protein structural predictions provided by AlphaFold has revolutionized the study of protein folding, structure, and function, offering unprecedented insights into the molecular architecture of *ALS* proteins. These advancements hold great potential for furthering our understanding of fungal pathogenicity and may aid in the development of targeted antifungal strategies [13, 14].

According to the results of the gene expression measurement, isolates of *Candida albicans* with high gene expression demonstrated resistance to the antifungals used in the experiment. This resistance is caused by the relationship between the *ALS* gene family and the virulence factors of *Candida* spp., particularly biofilm formation [27, 28]. These findings align with those of Abdullah and Mahdi [29] and Roudbarmohammadi et al. [30], who demonstrated that all *C. albicans* isolates exhibiting gene expression of at least one *ALS* gene family gene were fluconazole resistant, while isolates with no or very low gene expression were completely sensitive to fluconazole. This is because *ALS* genes, including the *ALS1* gene, are closely linked to the formation of biofilms, which aid adhesion processes in the human body's mucous membranes and epithelial layers. Additionally, biofilms play a significant role in the isolates' resistance to fluconazole. These findings are also consistent with those of Mohammed et al. [31], who proved that *ALS* genes are associated with virulence factors, especially the *ALS1* gene, as this gene was found 100% in biofilm-forming *C. albicans* isolates, and the same researchers proved that *ALS1* gene expression was significantly elevated in biofilm-forming *C. albicans* isolates. *ALS1* gene expression and sequence variations highlight its strong potential as a diagnostic marker for *Candida* virulence and antifungal resistance.

5. CONCLUSION

The study's findings demonstrated that *Candida albicans* is the most commonly detected species in clinical samples. Additionally, *ALS* genes were detected in the majority of *Candida* species, except for *C. kefyr* and *C. lusitaniae*. One of the study's main findings is that there is a strong correlation between *ALS1* gene expression and antifungal resistance because the expression of the *ALS1* gene is significantly higher in isolates that are resistant to antifungals, while it is significantly lower in isolates that are susceptible to them. The increased expression of *ALS1* in resistant isolates highlights its potential role in fungal pathogenicity and its possible involvement in mechanisms that contribute to antifungal resistance, emphasizing the need for further investigation into its clinical implications. Inhibiting *ALS1* offers a promising strategy to combat drug-resistant *Candida* infections.

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REFERENCES

[1] Muhsen, T.A., Hawar, S.N., Mahdi, T.S., Khaleel, R. (2020). Effect of Eucalyptus and Myrtus extracts identification by gas chromatography-mass spectrometry on some species of Candida as a model of medical plants. Annals of Tropical Medicine & Public Health, 23(S10): 1-11. http://doi.org/10.36295/ASRO.2020.231032

[2] Alqaysi, N.N., Muhsen, T.A., Risan, M.H. (2021). Diagnostic study of candidiasis in the mouth, urine and vagina of diabetic and healthy people. Biochemical and Cellular Archives, 21(2): 4823-4828.

[3] Abdalrazaq, E., Jbarah, A.A.Q., Al-Noor, T.H., Shinain, G.T., Jawad, M.M. (2022). Synthesis, DFT calculations, DNA interaction, and antimicrobial studies of some mixed ligand complexes of oxalic acid and Schiff base trimethoprim with various metal ions. Indonesian Journal of Chemistry, 22(5): 1348-1364. https://doi.org/10.22146/ijc.74020

[4] Jadoa, I.H., Muhsen, T.A. (2024). Study the effect of some physical factors on three isolations of Candida albicans isolated from Iraqi patients. Journal of Biotechnology Research Center (JOBRC), 18(2): 26-37. https://doi.org/10.24126/jobrc.2024.18.2.728

[5] Abdul-Jabbar, M.A., Kadhim, D.J. (2022). Adherence to different treatment modalities among patients on maintenance hemodialysis. Iraqi Journal of Pharmaceutical Sciences, 31(1): 95-101.

[6] Muhsen, T.A.A. (2019). Effect of essential oil extracted from the peels of Citrus paradisi and Citrus sinensis on

some fungi. Biochemical and Cellular Archives, 19: 2679-2684.
[7] Mohammed, N.A., Muhsen, T.A., Risan, M.H. (2020). Isolation and diagnosis of some Candida species from some Baghdad city hospitals with PCR technique and evaluation of the effectiveness of some antifungals. Plant Archives, 20(2): 3895-3900.
[8] Hasan, A.S., Muhsen, T.A., Alabassi, H.M. (2023). Diagnostic study of the most important fungal infections associated with some inflammatory bowel disease in Iraqi patients. Acta Biomed, 94(2): e2023138.
[9] Muhsen, T.A. (2016). The effect of essential oil extracted from yellow peel of Citrus aurantium L. on growth of some fungi. IIbn AL-Haitham Journal for Pure and Applied Sciences, 24(1).
[10] Hamid, G.S., Allawi, A.A., Ghudhaib, K.K. (2021). Correlation of pentosidine with kidney diseases in Iraqi patients with diabetic nephropathy. Iraqi Journal of Science, 62(10): 3436-3442. https://doi.org/10.24996/ijs.2021.62.10.2
[11] Mohammed, B.M., Ali, B.Z., Naef, R.A. (2017). Inhibition of Mico. nazole--resistant mutants o'f Candida al: J, lcalts; by aqueous extract of Matric aria chamomile. Ibn AL-Haitham Journal For Pure and Applied Science, 21(3): 1-9.
[12] Mahdi, S.S., Muhsen, T.A. (1967). The role of fungal infections in the pathogenesis of dialysis-dependent and non-dialysis-dependent patients with kidney. In Obstetrics & Gynaecology Forum, 2024(3): 1967-1975.
[13] Lombardi, L., Zoppo, M., Rizzato, C., Bottai, D., Hernandez, A.G., Hoyer, L.L., Tavanti, A. (2019). Characterization of the Candida orthopsilosis agglutinin-like sequence (ALS) genes. PLoS One, 14(4): e0215912. https://doi.org/10.1371/journal.pone.0215912
[14] Oh, S.H., Schliep, K., Isenhower, A., Rodriguez-Bobadilla, R., Vuong, V.M., Fields, C.J., Hernandez, A.G., Hoyer, L.L. (2021). Using genomics to shape the definition of the agglutinin-like sequence (ALS) family in the saccharomycetales. Frontiers in Cellular and Infection Microbiology, 11: 794529. https://doi.org/10.3389/fcimb.2021.794529
[15] Hardan, S.M., Muhsen, T.A. (2024). Isolation and identification of the most important entomopathogenic fungi and evaluation of their efficiency in Musca domestica L. Assiut Veterinary Medical Journal, 70(183): 401-412.
[16] Deorukhkar, S.C., Saini, S., Mathew, S. (2014). Non-albicans Candida infection: An emerging threat. Interdisciplinary perspectives on infectious diseases, 2014(1): 615958. https://doi.org/10.1155/2014/615958
[17] Liu, C., Xu, C., Du, Y., Liu, J., Ning, Y. (2021). Role of agglutinin-like sequence protein 3 (Als3) in the structure and antifungal resistance of Candida albicans biofilms. FEMS Microbiology Letters, 368(14): fnab089. https://doi.org/10.1093/femsle/fnab089
[18] Sadeq, A.M., Ismail, Z.Z. (2024). Microalgae growth in a biocathode-photosynthesis microbial desalination cell: Molecular characterization, modeling study, and performance evaluation. Iraqi Journal of Chemical and Petroleum Engineering, 25(1): 1-12.
[19] Ahmed, M.A., Goher, H.A., Dwedar, R.A., Nassar, Y., Nada, M.G., Kotb, M.M. (2024). A comparative study between invasive and superficial Candida albicans infections regarding biofilm formation, ALS3 and SAP1-6 genes expression and anti-fungal drug susceptibility. Egyptian Journal of Medical Microbiology, 33(2): 1-9.
[20] Hussain, E.A. (2022). Detection of the aadA1 and aac (3)-1V resistance genes in Acinetobacter baumannii. Archives of Razi Institute, 77(3): 959-966. https://doi.org/10.22092/ari.2022.357271.2010
[21] Green, M.R. (2012). Molecular Cloning: A Laboratory Manual. New York: Cold Spring Harbor Laboratory Press. https://catalog.nlm.nih.gov/permalink/01NLM_INST/m5fc0v/alma9915882953406676.
[22] Mohsin, M.R., AL-Rubaii, B.A.L. (2024). Effect of some drugs (painkiller and anti-inflammatory) on antibiotic resistance genes (vim, imp, ndm, oxa48 and aac (6)) in Proteus mirabilis. Ibn AL-Haitham Journal For Pure and Applied Sciences, 37(3): 19-27.
[23] Marsaux, B., Moens, F., Vandevijver, G., Marzorati, M., Van de Wiele, T. (2024). Candida species-specific colonization in the healthy and impaired human gastrointestinal tract as simulated using the Mucosal Ileum-SHIME® model. FEMS Microbiology Ecology, 100(9): fiae113.
[24] Ali, A.T., Ewies, E.F. (2023). Biosynthesis, characterization, adsorption and antimicrobial studies of zirconium oxide nanoparticles using punica granatum extract. Ibn AL-Haitham Journal For Pure and Applied Sciences, 36(4): 262-273. https://doi.org/10.1093/femsec/fiae113
[25] García-Salazar, E., Acosta-Altamirano, G., Betancourt-Cisneros, P., Reyes-Montes, M.D.R., et al. (2022). Detection and molecular identification of eight candida species in clinical samples by simplex PCR. Microorganisms, 10(2): 374. https://doi.org/10.3390/microorganisms10020374
[26] Jumper, J., Evans, R., Pritzel, A., Green, T., et al. (2021). Highly accurate protein structure prediction with AlphaFold. Nature, 596(7873): 583-589.
[27] Mustafa, M.S., Abdullah, R.M. (2020). Role of oqxA and oqxB genes in the development of multidrug resistant phenotype among clinical Klebsiella pneumoniae isolates from various cases. Iraqi Journal of Science, 61(8): 1902-1912.
[28] Loza, L., Fu, Y., Ibrahim, A.S., Sheppard, D.C., Filler, S.G., Edwards Jr, J.E. (2004). Functional analysis of the Candida albicans ALS1 gene product. Yeast, 21(6): 473-482. https://doi.org/10.1002/yea.1111
[29] Abdullah, R.M., Mahdi, A.A. (2023). Studying the gene expressions of Bla OXA-51Like and Bla OXA-23Like in Acinetobacter baumannii. Ibn AL-Haitham Journal For Pure and Applied Sciences, 36(4): 32-39. https://doi.org/10.30526/36.4.3073
[30] Roudbarmohammadi, S., Roudbary, M., Bakhshi, B., Katiraee, F., Mohammadi, R., Falahati, M. (2016). ALS1 and ALS3 gene expression and biofilm formation in Candida albicans isolated from vulvovaginal candidiasis. Advanced Biomedical Research, 5(1): 105. https://doi.org/10.4103/2277-9175.183666
[31] Mohammed, N.A., Ajah, H.A., Abdulbaqi, N.J. (2021). Determination the gene expression levels of adhesins and extracellular enzymes genes in Candida albicans biofilm producer by quantitative real time PCR technique (qRT-PCR). Indian Journal of Forensic Medicine & Toxicology, 15(2): 1517-1527.