Academic Journal of International University of Erbil

Journal Homepage: https://ojs.cihanrtv.com/index.php/public

PRINT-ISSN: 2519-6928

Online-ISSN: 3080-7174

Genetic Detection of the *ALS1* Virulence Gene in Clinical *Candida albicans* Isolates from Human Gut Samples in Erbil Governorate, Kurdistan Region, Iraq

Hewa A. Mohammed[1], Taha J. Omar Zrary[1]*

[1]Department of Medical Microbiology, Faculty of Science and Health, Koya University,

Koya 44023, Kurdistan Region– F.R. IRAQ

DOI: https://doi.org/10.63841/iue31652

Received 15 Aug 2025; Accepted 24 Aug 2025; Available online 24 Jan 2026

ABSTRACT:

Candida albicans is a common commensal of the human gastrointestinal tract (GIT) but can become an opportunistic pathogen under immunocompromised conditions. The *ALS1* gene, part of the agglutinin-like sequence (ALS) family, encodes an adhesin that facilitates tissue attachment and biofilm formation, contributing significantly to *C. albicans* pathogenicity. This study aimed to determine the prevalence of *C. albicans* in clinical GIT samples and assess the presence of the *ALS1* virulence gene among these isolates, using classical and molecular techniques.

A total of 121 stool samples were collected from patients with GIT symptoms in Koya and Erbil City. Culturing was performed on Sabouraud Dextrose Agar and HiCrome™ for preliminary identification, followed by Gram staining, germ tube testing, and confirmation with the VITEK 2 ID system. Molecular detection of the 18S rRNA and *ALS1* genes was conducted via colony PCR, and selected amplicons were verified by Sanger sequencing and BLAST alignment.

Candida. albicans was isolated in 22.3% (27/121) of samples. Of these, 81.5% (22/27) tested positive for the *ALS1* gene. PCR products were confirmed by sequencing, revealing high identity with reference strains in GenBank. Combining phenotypic and molecular assays ensured robust and accurate identification.

The high prevalence of *ALS1* among GIT-derived *C. albicans* isolates highlights its potential as a molecular marker for virulence and colonization risk.

Key words: *Candida albicans*, Gastrointestinal Candidiasis, *ALS1* virulence gene, PCR, Sequencing

1 INTRODUCTION

Candida albicans is a harmless commensal that typically lives in healthy people oral, vaginal, and gastrointestinal mucosa. Therefore *C. albicans* can be found on the mucosa of the majority of human beings. However, this fungus may trigger outbreaks if the local microbial communities, normal biological barriers, or immune responses are compromised [1],[2].

Candida yeast infections that spread to other areas of the human being usually start in the patient gastrointestinal system. When the host defenses weaken, like after gut barrier disruption, clinical suppression, or taking broad-ranging medicines, systemic infections can develop. A combination of these variables is often required for *C. albicans* to translocate from the gut. Once the microorganism enters the circulatory system [3],[4].

Adhesion thought to be significant in determining host interaction-*Candida albicans*, *Agglutinin-Like Sequence (ALS)* gene family encodes Als proteins function in adhesion which consists of 8 genes *(ALS1* to *ALS7* and *ALS9)* that associated with pathogenicity in *Candida sp.* And mainly *Candida albicans* [5],[6]. *ALS1* in *C. albicans* is a glycoprotein with a large cell surface and best described for its role in facilitating ligand binding and aggregation interactions [7].

*Corresponding author: taha.jalal@koyauniversity.org

Germ Tube testing is a fast way to distinguish *C. albicans* and *C. dubliniensis* from other *Candida* species. Additional special chlamydospore formation tests, sugar assimilation and fermentation tests can perform. However, these tests are time-consuming and costly. Among the latest tests, *Candida* differential agar, VITEK 2ID System, API Systems, Molecular Methods [8],[9],[10], Numerous molecular tests developed based primarily on polymerase chain reaction (PCR), appearing high sensitivity and specificity to distinguish and characterize *Candida spp.*[11].

This article was achieved to determine and provide novel insights into the prevalence of *Candida albicans* in human Gastrointestinal tract clinical isolates and detect *ALS1* gene for the first time among these isolates from gut samples in the Kurdistan Region of Iraq, using classical and molecular methods that lead to deeper understanding of clinical implications of Gastro-intestinal *C. albicans* infections.

2 MATERIALS AND METHODS

2.1 SAMPLE COLLECTION

In this Cross-sectional study, a total of 121 stool samples were collected from GIT patients at Erbil Governorate, Kurdistan Region, Iraq as shown in Study-design in **FIGURE 1** Flow-chart summarizing our original article Study-design.. Patients included in this study were those exhibiting clinical signs such as persistent diarrhea, abdominal pain, bloating, or suspected gastrointestinal infection, and who were referred for stool examination by their attending physicians. While patients with severe systemic diseases unrelated to gastrointestinal infection, and patients who declined to provide informed consent were excluded. Ethical approval for this study was granted by the Ethical Committee of the Faculty of Science and Health at Koya University.

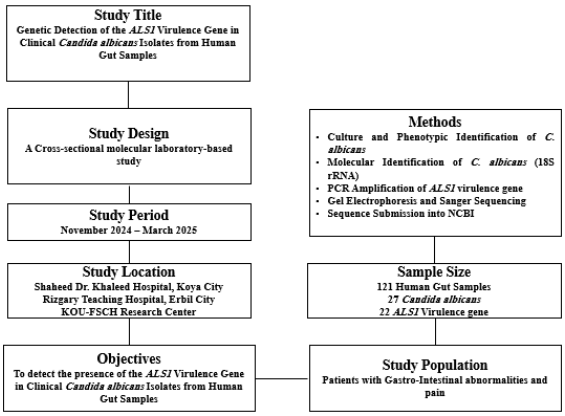

FIGURE 1 Flow-chart summarizing our original article Study-design.

2.2 CULTIVATION AND ISOLATION

To obtain pure fungal colonies, the collected samples undergone a cultivation process on Sabouraud Dextrose Agar (Oxoid - UK) supplemented with chloramphenicol, following inoculation, all SDA plates were incubated at 37°C for a standard duration of 48h. Subsequently, Growing Fungal Colonies were cultured on *Candida* Differential Agar (HiCrome - India) then Incubated for same temperature and duration, The production of color and morphology as outlined by the manufacturer were documented. Light-Green single colonies isolated from each plate, forming the basis for subsequent Gram stain, Germ tube and Vitek 2 ID System (bioMérieux-France) ,[13],[14].

2.2.1 RECOGNITION OF *CANDIDA ALBICANS* APPEARANCE

To ensure reliable confirmation of *C. albicans* presence in the isolates, both Gram stain analysis and the germ tube test were performed. A combination of HiCrome culturing, Gram stain analysis, and the Germ-tube test was employed in a three-step approach to identify and examine *C. albicans* composition within the samples. The selection process is based on distinct colony morphologies and the presence of germ-tube formation, offering a varied representation of *Candida* species. Relying solely on morphological selection cannot fully identify *Candida albicans*, but a positive germ-tube test aids in preparing the sample for genomic DNA extraction. These two methods enhance the identification process and establish a strong foundation for understanding *C. albicans* composition, then confirmatory test was done by Vitek 2 ID System (bioMérieux-France) [15],[16] [17].

2.3 DNA EXTRACTION

Colony PCR method performed [18], Fresh isolated pure colony from each of positive samples subjected into 50 micro-liters of free-nuclease water (ddH2O) which vortexed to make fully mixed then put through thermocycler (BIO RAD – USA) for 20 minutes at 95°C followed by centrifugation for 2.45 minutes at 12500 rpm. The DNA available supernatant was ready and available for PCR run.

2.4 PCR-BASED AMPLIFICATION

The 18S rRNA gene targeted region was amplified using a PCR method to ensure accurate and reliable results. The final reaction mixture had a total volume of 30 µL, which included 15 µL of ready-to-use Mastermix (Genesand Biotech Co. - China), 3 µL of template DNA, 0.9 µL (10 pmol/µL) of each forward primer (18FWD- 5'-CTATCAACTTTCGATGGTAG -3') and reverse primer (18REV-5'-AACCATAACGTCCTATTCTA-3'), and 10.2 µL of nuclease-free water. The same protocol was followed for amplifying the *ALS1* gene, with the forward primer (ALS1FWD-5'-AATTCATTAACTTGGTCCAATG-3')and reverse primer (ALS1REV-5'-TGTACCAGATGTGTAACCAT-3') which was specific primers designed in SnapGene Software to target specific regions of the desired gene, the designated primers with the expected amplicon sizes clearly shown in **Table 1.** Primers used for gene amplifications in *C. albicans.* while the PCR reaction system protocols for the genes were prepared according to the **Table 2.** PCR Reaction System Protocol after Further Analysis (Operated on Ice).

Table 1. Primers used for gene amplifications in *C. albicans.*

Primer	Sequences	Amplicon Size
18FWD	5'-CTATCAACTTTCGATGGTAG -3'	505 bp
18REV	5'-AACCATAACGTCCTATTCTA-3'	
ALS1FWD	5'-AATTCATTAACTTGGTCCAATG-3'	559 bp
ALS1REV	5'-TGTACCAGATGTGTAACCAT-3'	

Table 2. PCR Reaction System Protocol after Further Analysis (Operated on Ice).

Content	30 µL
PCR master-mix (Genesand Biotech Co. – China)	15 µL
FWD-Primer (10 µM)	0.9 µL
REV-Primer (10 µM)	0,9 µL
Template DNA	3 µL
Free Nuclease Water	10.2 µL

The Polymerase Chain Reaction (PCR) process was carried out using a thermal cycler (BIO-RAD), beginning with pre-denaturation step at 95°C for 3 minutes. This was followed by 30 cycles, each consisting of a 25 second denaturation at 94°C, an annealing phase at 56.7°C for 25 seconds for 18S rRNA primers and 52°C for 25 seconds *ALS1* primers, and a primary extension at 72°C for another 1 minute. The procedure concluded with a final extension step at 72°C for 4 minutes as exhibited in

FIGURE 2. The conditions of Polymerase Chain Reaction according to the master mix (Genesand -Biotech Co. – China) guidelines in Thermocycler (BIO-RAD) which amplified the genes for research purposes while the optimal Annealing temperatures T_a obtained was identified through repeated trials.

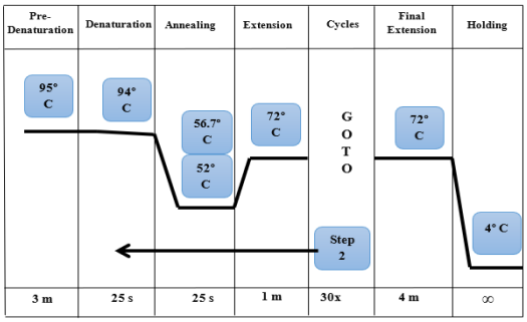

FIGURE 2. The conditions of Polymerase Chain Reaction according to the master mix (Genesand -Biotech Co. – China) guidelines in Thermocycler (BIO-RAD) which amplified the genes for research purposes while the optimal Annealing temperatures T_a obtained was identified through repeated trials.

To assess the effectiveness of the PCR process, the amplicon size was confirmed using gel electrophoresis. A 1% agarose gel was prepared and subjected to an electric field of 85 V for 1 hour and 10 minutes to facilitate this analysis. Ethidium bromide was incorporated into the gel during preparation to enhance DNA band visualization. After electrophoresis, the DNA bands became clearly visible, allowing for the identification of samples with the expected band size of 100 base pairs (bp) ladder (Genesand Biotech Co. - China). Samples exhibiting the correct amplicon size were then selected and forwarded for the next crucial step of the study: DNA sequencing.

2.5 GENE SEQUENCING

A key phase of our study focused on sequencing the nucleotides of the PCR products derived from the selected samples. This task was carried out by Macrogene, a company based in South Korea, utilizing the advanced DNA sequencing system which is Sanger Sequencing method. The sequencing was specifically performed in the reverse direction of the 18S rRNA gene using the 18REV primer and for confirming the specificity of *ALS1* primers using ALS1REV primer, enabling a thorough analysis and detection of the genetic information present in our *C. albicans* samples.

After sequencing was completed, the resulting chromatogram, a visual depiction of the sequencing data was thoroughly analyzed. To verify the accuracy and quality of the obtained DNA sequences. Using Finch TV software, we conducted a systematic evaluation of the chromatogram to remove the noisy ends and ensure the sequenced fragments adhered to high standards of precision and reliability.

The generated sequences were analyzed through a comparative approach by aligning them with the vast genetic database available in the Basic Local Alignment Search Tool (BLAST). This led to identify corresponding sequences from both the species and the gene. By using this method as a reference, it was able to confirm and reinforce the accuracy of the study findings.

3 RESULTS

3.1 SAMPLE COLLECTION

The study cohort consisted of 121 patients, with a gender distribution of 57% male (n= 69) and 43% female (n= 52). The mean age of participants was 24.23 years (±19.38), while *C. albicans* were positive in %22.3 individuals (n= 27), although after isolate differentiation %81.5 had *ALS1* which led to pathogenicity (n= 22). Moreover, the inclusion of distinct and less sourced sample types introduced additional complexity to the study, facilitating a more thorough investigation into the presence of *Candida albicans* across gastrointestinal tract patients.

3.2 ISOLATION AND CONFIRMATION OF *CANDIDA ALBICANS*

Culture on Sabouraud Dextrose Agar: All collected samples were initially cultured on Sabouraud Dextrose Agar, resulting in 110 white, creamy colonies derived from stool swabs then, Gram's Stain, Microscopic Examination, and Germ Tube testing were performed subsequently. CHROMagar Culture: These colonies were then sub-cultured onto CHROMagar medium for the identification of *Candida albicans*. A total of 27(22.3%) light green colonies were observed, indicating the presence of *C. albicans* (**FIGURE 3.** *C. albicans* Colonies on (A) Sabouraud Dextrose Agar (SDA) with Chloramphenicol shows white creamy colonies, (B) *Candida* Differential Agar (HiCrome) exhibits Light-Green colonies.).

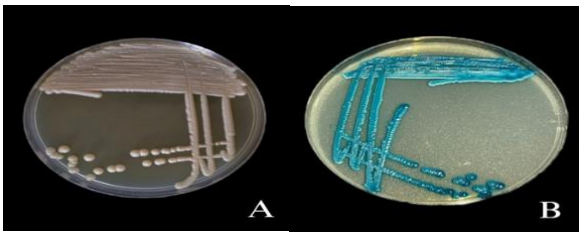

FIGURE 3. ***C. albicans*** **Colonies on (A) Sabouraud Dextrose Agar (SDA) with Chloramphenicol shows white creamy colonies, (B)** ***Candida*** **Differential Agar (HiCrome) exhibits Light-Green colonies.**

3.3 DNA EXTRACTION

Genomic DNA was successfully extracted from the isolates and used as a template for PCR amplification. Amplicons of the expected sizes (~505 bp) for 18S rRNA and (~559 bp) for *ALS1* gene when visualized on a 1% agarose gel. Distinct, sharp bands were observed, with no detectable primer-dimers or non-specific amplification. Negative controls (no-template reactions) showed no contamination, while positive controls (The Isolate that is used for finding Annealing temperature Ta) validated primer specificity. PCR efficiency was further supported by Sanger sequencing of representative amplicons, which matched reference sequence (GenBank accession: [XR_002086442.1]).

3.4 PCR- BASED DETECTION AND SANGER SEQUENCING OF THE 18S RRNA GENE

The additional molecular tests were performed after DNA extraction of the isolates by Colony PCR method and for further and accurate identification of *Candida albicans,* out of the 27 positive isolates tested, all of them were also positive in molecular methods as shown in

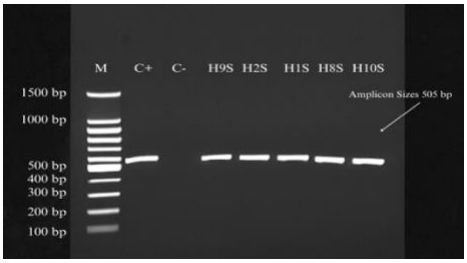

FIGURE 4, The amplified length which then sent to PCR product measured ~505 base pairs in Sequencing.

FIGURE 4. Gel electrophoresis of PCR amplification products. A 100 bp DNA ladder (range: 100 bp–1500 bp) was used as a molecular size marker. PCR products from all samples showed a single, distinct band at approximately 505 bp, consistent with the expected amplicon size of the 18S rRNA gene, confirming successful amplification.

Following PCR analysis, 27 samples tested positive; however, one could not be submitted for sequencing due to technical limitations. Consequently, sequencing was performed on 26 *Candida* isolates, and the resulting chromatograms were analyzed using Finch TV software. To ensure data integrity, sequences were trimmed at both the 5' and 3' ends, specifically at regions where primer binding introduced signal noise, thereby making them suitable for downstream analyses (Figure 5). The refined sequences were subsequently subjected to BLAST analysis and compared against the reference genome of *Candida albicans* strain SC5314 as shown in table 3.

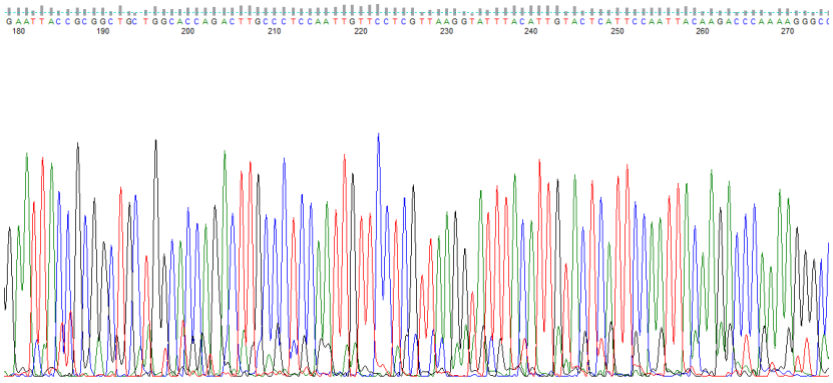

FIGURE 5. Chromatogram representing the V4 region of the 18S rRNA gene. The upper bars indicate the quality scores of individual nucleotide bases. The sequence exhibits clean and evenly spaced peaks with minimal background noise, and the baseline remains consistent throughout, reflecting high-quality sequencing data.

Table 3. Strain ID Identity and accession numbers present compared to gold reference strain of *Candida albicans* which is strain SC5314 with it is accession number XR_002086442.1.

Isolate ID	Accession Number	Percent identity
H1S.T	PV652873	100.00%
H2S.T	PV652874	100.00%
H3S.T	PV652875	100.00%
H4S.T	PV652876	100.00%
H5S.T	PV652877	100.00%
H7S.T	PV652878	99.77%
H8S.T	PV652879	100.00%
H9S.T	PV652880	100.00%
H10S.T	PV652881	100.00%
H11S.T	PV652882	100.00%

H12S.T	PV652883	100.00%
H13S.T	PV652884	100.00%
H14S.T	PV652885	100.00%
H15S.T	PV652886	100.00%
H16S.T	PV652887	100.00%
H17S.T	PV652888	100.00%
H18S.T	PV652889	100.00%
H19S.T	PV652890	100.00%
H20S.T	PV652891	100.00%
H21S.T	PV652892	100.00%
H22S.T	PV652893	100.00%
H23S.T	PV652894	100.00%
H24S.T	PV652895	100.00%
H25S.T	PV652896	100.00%
H26S.T	PV652897	100.00%
H27S.T	PV652898	100.00%

3.5 IDENTIFICATION OF THE *ALS1* GENE

Among the 27 *Candida albicans* isolates examined via PCR, 22 (%81.5) tested showed positive for the presence of the *ALS1* gene. The size of the resulting amplicon size was ~559 base pairs as the primer designed by SnapGene software and the expected result was the same in gel electrophoresis and variation in fluorescence intensity under UV illumination was observed among the PCR products of the DNA isolates during gel electrophoresis, as shown in Figure 6.

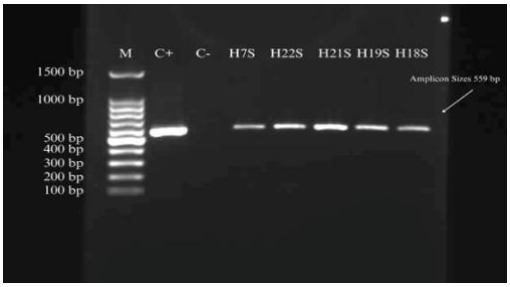

FIGURE 6. Gel electrophoresis of PCR amplification products. A 100 bp DNA ladder (range: 100 bp–1500 bp) was used as a molecular size marker. PCR products from all samples showed a single, distinct band at approximately 559 bp, consistent with the expected amplicon size of the ALS1 gene, confirming successful amplification.

Five random samples of *ALS1* gene amplicon chosen and sent to sequencing to confirm the specificity of the designated pair of primers (ALS1FWD and ALS1REV); after trimming the noisy ends, the identity of the chosen gene detected by BLAST and integrated into NCBI as shown in (**Table 4**).

Table 4. Strain ID Identity and accession numbers present compared to *Candida albicans* strain GFH agglutinin-like protein (als1) gene, partial cds with it is accession number JQ307470.1.

Gene ID	Accession Number	Percent identity
Candida_ALS1.partial.cds.H1A.T.H1	PV654527	99.80%
Candida_ALS1.partial.cds.H2A.T.H2	PV654528	100.00%
Candida_ALS1.partial.cds.H3A.T.H10	PV654529	99.80%
Candida_ALS1.partial.cds.H4A.T.H11	PV654530	99.18%
Candida_ALS1.partial.cds.H5A.T.H13	PV654531	97.57%

4 DISCUSSION

Candida albicans is widely recognized as a normal commensal organism inhabiting the human vaginal and gastrointestinal tracts; however, it also serves as a significant opportunistic pathogen responsible for a broad range of infections globally [19]. The colonization rate (22.3%) detected in our study likely represents a combination of commensal carriage and undiagnosed infections. This aligns with current understanding that *C. albicans* typically exists as a harmless gut resident but can transition to pathogenic states following compromise of host immune defenses [1].

This study aimed to assess the occurrence of *Candida albicans* in gastrointestinal tract (GIT) specimens and to detect the presence of the *ALS1* virulence gene among clinical isolates. The analysis showed that *C. albicans* was present in 22.3% (27 out of 121) of the stool samples, with 81.5% (22 of 27) of these isolates exhibiting *ALS1* gene carriage. These findings

are in agreement with recent literature that emphasizes the pivotal role of *ALS1* in fungal virulence in clinical samples [15]. And also, these findings are comparable to local and regional studies reporting *Candida albicans* prevalence in gastrointestinal or clinical stool samples in Kurdistan Region and Iraq. For example, a study in Diyala reported a colonization rate of 21.5%, while Hamada *et al.* [12] in Kirkuk found 26.57% prevalence among children's diarrheal stool samples. Although no data are available for *ALS1* in gastrointestinal isolates, studies from other sample types show high *ALS1* prevalence; for instance, Ahmadessa and Ali [20] reported 82.2% *ALS1* positivity in vulvovaginal candidiasis (VVC) isolates.

The elevated prevalence of *ALS1*-positive strains in our study likely results from microenvironmental selection pressures within the gastrointestinal tract that favor fungal variants with superior adhesion and invasion potential. This observation aligns with the work of Witchley *et al.* [21], who demonstrated that *C. albicans* dynamically regulates virulence genes (including *ALS1*) to adapt to gut conditions and evade host immunity. Our findings suggest these *ALS1*-positive strains exhibit heightened pathogenic potential, particularly in immunocompromised hosts or those with disrupted gut ecologies [22].

This phased detection system ensured progressively higher specificity and analytical framework combining selective chromogenic culture, phenotypic characterization, and molecular genotyping to achieve optimal discriminatory power for *Candida sp.* identification. This multimodal approach capitalizes on the complementary strengths of each technique: HiCrome™ provides rapid presumptive identification through chromogenic differentiation, while conventional mycological tests verify morphological characteristics. The molecular component, utilizing polymerase chain reaction (PCR) amplification with species-specific primers followed by sequencing, offers definitive confirmation at the genetic level. Contemporary studies have established that such integrated protocols significantly enhance diagnostic precision in clinical mycology compared to conventional methods alone [23],[24].

The analytical validity of our *ALS1* detection methodology was rigorously established through multiple verification steps. Amplicons were subjected to gel electrophoresis and sent to sequencing, with subsequent nucleotide alignments against curated reference sequences in GenBank using BLASTN algorithm analysis. This confirmation pipeline aligns with current best practices for fungal virulence gene characterization [25],[26]. The demonstrated reproducibility of our results underscores the protocol reliability for clinical and research applications in medical mycology.

Critically, this study substantiates the diagnostic relevance of *ALS* detection as a predictive biomarker for evaluating *C. albicans* virulence in gastrointestinal infections. For immunocompromised patients or those with gut dysbiosis [5], routine molecular screening of this adhesin gene could inform early intervention strategies and guide antifungal stewardship.

CONCLUSION

This study highlights the prevalence of *Candida albicans* in gastrointestinal tract samples and underscores the clinical relevance of the *ALS1* gene as a key virulence determinant. The detection of *ALS1* in 81.5% of *C. albicans* isolates suggests a high potential for pathogenicity among colonizing strains, particularly in hosts with compromised gut integrity or immune function. These findings support the role of *ALS1* in mediating adhesion, colonization, and potentially invasive behavior within the gastrointestinal environment.

The application of integrated diagnostic approaches, combining selective culturing, and PCR-based molecular techniques, proved highly effective for the accurate detection and characterization of *C. albicans* and its virulence traits. Importantly, the sequencing and bioinformatic validation of *ALS1* gene amplification further reinforce its utility as a reliable genetic marker in clinical mycology field.

Incorporating molecular screening for *ALS1* into routine diagnostic protocols may enhance early identification of high-risk *Candida* strains and inform antifungal therapy decisions.

RECOMMENDATIONS

Future studies should explore the correlation between *ALS1* expression levels and clinical outcomes, and evaluate the gene's potential as a target for antifungal intervention or vaccine development.

FUNDING

The authors confirm that they did not receive any financial support, grants, or funding of any kind during the development of this manuscript.

ACKNOWLEDGEMENT

The authors express their sincere gratitude to Shaheed Dr. Khaleed and Rizgary Teaching Hospital Staff for their cooperation and support in facilitating the collection of clinical samples. We also extend our appreciation to the staff of the Science and Health Research Center at Koya University for their ongoing assistance and valuable contributions to the researchers.

CONFLICTS OF INTEREST

The author declares no conflict of interest.

AUTHOR CONTRIBUTIONS

Both authors Assisted in the study conception and design. Material preparation, data collection, and analysis were carried out by Hewa Abdulhakeem Mohammed and also first draft of the manuscript was written by the same author, while conceptualization and manuscript review were performed by Assist. Prof. Dr. Taha Jalal Omar Zrary, both authors provided feedback on previous versions. Also, both authors read and approved the final manuscript.

DATA AVAILABILITY

The sequencing data generated for this study have been deposited in the National Center for Biotechnology Information (NCBI) GenBank under accession numbers as listed in Table 3 and Table 4. These data are currently under embargo and will be publicly released upon publication of this article. Access can be provided to reviewers upon request.

REFERENCES

[1] A. A. Mishra and A. Y. Koh, "Adaptation of *Candida albicans* during gastrointestinal tract colonization," *Current clinical microbiology reports,* vol. 5, pp. 165-172, 2018, doi: https://doi:10.1007/s40588-018-0096-8.

[2] J. C. Pérez, "The interplay between gut bacteria and the yeast *Candida albicans*," *Gut Microbes,* vol. 13, no. 1, p. 1979877, 2021, doi: https://doi.org/10.1080/19490976.2021.1979877.

[3] P. G. Pappas, M. S. Lionakis, M. C. Arendrup, L. Ostrosky-Zeichner, and B. J. Kullberg, "Invasive candidiasis," *Nature Reviews Disease Primers,* vol. 4, no. 1, pp. 1-20, 2018.

[4] N. Papon, M.-E. Bougnoux, and C. d'Enfert, "Tracing the origin of invasive fungal infections," *Trends in microbiology,* vol. 28, no. 4, pp. 240-242, 2020, doi: https://doi.org/10.1016/j.tim.2020.01.007.

[5] L. L. Hoyer and E. Cota, "*Candida albicans* agglutinin-like sequence (Als) family vignettes: a review of Als protein structure and function," *Frontiers in microbiology,* vol. 7, p. 280, 2016, doi: https://doi.org/10.3389/fmicb.2016.00280.

[6] G. Marc *et al.*, "New N-(oxazolylmethyl)-thiazolidinedione active against *Candida albicans* biofilm: potential Als proteins inhibitors," *Molecules,* vol. 23, no. 10, p. 2522, 2018, doi: https://doi.org/10.3390/molecules23102522.

[7] X. Zhao, S.-H. Oh, D. A. Coleman, and L. L. Hoyer, "*ALS1* Deletion increases the proportion of small cells in a *Candida albicans* culture population: Hypothesizing a novel role for Als1," *Frontiers in Cellular and Infection Microbiology,* vol. 12, p. 895068, 2022, doi: https://doi.org/10.3389/fcimb.2022.895068.

[8] N. Jain, P. Mathur, M. C. Misra, B. Behera, I. Xess, and S. P. Sharma, "Rapid identification of yeast isolates from clinical specimens in critically ill trauma ICU patients," *Journal of laboratory physicians,* vol. 4, no. 01, pp. 030-034, 2012, doi: https://doi.org/10.4103/0974-2727.98667

[9] M. C. Arendrup and T. F. Patterson, "Multidrug-resistant *Candida*: epidemiology, molecular mechanisms, and treatment," *The Journal of infectious diseases,* vol. 216, no. suppl_3, pp. S445-S451, 2017, doi: https://doi.org/10.1093/infdis/jix131.

[10] N. Rathore, J. Lalwani, P. Singh, and M. Meena, "Speciation of *Candida* species isolated from cutaneous skin lesions using CHROMagar and conventional methods," *Asian Journal of Medical Sciences,* vol. 15, no. 2, pp. 113-117, 2024, doi: https://doi.org/10.3126/ajms.v15i2.59383

[11] E. García-Salazar *et al.*, "Detection and molecular identification of eight *candida* species in clinical samples by simplex PCR," *Microorganisms,* vol. 10, no. 2, p. 374, 2022, doi: https://doi.org/10.3390/microorganisms10020374.

[12] T. A. Hamada, I. H. Saadoon, and N. G. M. Ameen, "Detection of *Candida spp.* in children with diarrhea in Kirkuk province," *Kirkuk J Sci,* vol. 14, no. 1, pp. 56-66, 2019.

[13] S. Kaup, J. Sankarankutty, H. Balasubrahmanya, S. Kulkarni, and M. Nirmala, "Speciation of *Candida* using HiCrome *candida* differential agar," *Int J Curr Microbiol Appl Sci,* vol. 5, no. 7, pp. 267-74, 2016, doi: http://dx.doi.org/10.20546/ijcmas.2016.507.027.

[14] M. Melhem, A. Bertoletti, H. Lucca, R. Silva, F. Meneghin, and M. Szeszs, "Use of the VITEK 2 system to identify and test the antifungal susceptibility of clinically relevant yeast species," *Brazilian Journal of Microbiology,* vol. 44, pp. 1257-1266, 2013, doi: https://doi.org/10.1590/S1517-83822014005000018

[15] A. A. Al-Attraqhchi and S. D. Al-Ahmar, "Molecular detection of *ALS1* virulence gene of *Candida albicans* isolated from groups of Iraqi patients," *infection,* vol. 11, p. 14, 2015, doi: https://doi.org/10.32007/jfacmedbagdad.571317.

[16] R. Shrief, M. E. S. Zaki, E. M. El-Sehsah, S. Ghaleb, and M. Mofreh, "Study of antifungal susceptibility, virulence genes and biofilm formation in *Candida albicans*," *The Open Microbiology Journal,* vol. 13, no. 1, 2019, doi: 10.2174/1874285801913010241.

[17] S. Lamsal *et al.*, "Antifungal susceptibility and biofilm formation of *Candida albicans* isolated from different clinical specimens," *Tribhuvan University Journal of Microbiology,* pp. 53-62, 2021, doi: https://doi.org/10.3126/tujm.v8i1.41195.

[18] K. Mohammed and T. Zrary, "Molecular identification of *Candida spp.* isolated from women withvulvovaginal candidiasis in Koya District, Iraq," *Journal of Zankoy Sulaimani-Part A,* vol. 25, no. 2, pp. 12-12, 2023.

[19] R. Alonso-Monge, M. S. Gresnigt, E. Román, B. Hube, and J. Pla, "*Candida albicans* colonization of the gastrointestinal tract: A double-edged sword," *PLoS pathogens,* vol. 17, no. 7, p. e1009710, 2021, doi: https://doi.org/10.1371/journal.ppat.1009710.

[20] S. M. Ahmadessa and S. J. Ali, "Molecular Characterization of Biofilm-related Virulence and Resistance genes in *Candida albicans* Isolates from Women with Vulvovaginitis," *Kurdistan Journal of Applied Research,* vol. 10, no. 2, pp. 68-76, 2025.

[21] J. N. Witchley, P. Penumetcha, N. V. Abon, C. A. Woolford, A. P. Mitchell, and S. M. Noble, "*Candida albicans* morphogenesis programs control the balance between gut commensalism and invasive infection," *Cell host & microbe,* vol. 25, no. 3, pp. 432-443. e6, 2019, doi: DOI:10.1016/j.chom.2019.02.008

[22] J. P. Lopes and M. S. Lionakis, "Pathogenesis and virulence of *Candida albicans*," *Virulence,* vol. 13, no. 1, pp. 89-121, 2022, doi: https://doi.org/10.1080/21505594.2021.2019950.

[23] B. Uppal, P. S. Panda, S. Kishor, S. Sharma, and F. H. Farooqui, "Speciation of *Candida* isolates obtained from diarrheal stool," *The Egyptian Journal of Internal Medicine,* vol. 28, pp. 66-70, 2016, doi: https://doi.org/10.4103/1110-7782.193888

[24] E. García-Salazar *et al.*, "Detection and Molecular Identification of Eight *Candida* Species in Clinical Samples by Simplex PCR. Microorganisms 2022, 10, 374," ed, 2022.

[25] A.-K. M. Taqi, "Molecular Identification of *Candida albicans* and *C. dubliniensis* Using Small Subunit rRNA Gene Sequence in Kerbala, Iraq," *Archives of Razi Institute,* vol. 78, no. 3, p. 1035, 2023, doi: https://doi.org/10.22092/ARI.2022.360086.2546

[26] A. Y. Khudhair, Z. K. Taiban, and S. H. K. AL-Huchaimi, "DNA Sequencing Detection of *Candida albicans CALB1* Gene Isolated from Oral Candidiasis Patients in Al-Najaf Governorate," *Journal of Science in Volume,* vol. 2, no. 11, 2024.