Content available at: https://www.ipinnovative.com/open-access-journals

IP International Journal of Medical Microbiology and Tropical Diseases

Journal homepage: https://www.ijmmtd.org/

Original Research Article

Identification and characterization of virulence factor genes in coastal Odisha isolates of *Candida albicans*

Sushree Swagatika Subhadarsini[1], Gopal Krishna Purohit[2], Monali Priyadarsini Mishra[1], Debasmita Dubey[3], Subrat Kumar Tripathy[4]*

[1]School of Allied and Healthcare Sciences, Centurion University of Technology and Management, Jatani, Khurda, Odisha, India
[2]Heredity Biosciences, Mayfair Lagoon Road, Jayadev Vihar, Bhubaneswar, Odisha, India
[3]IMS & SUM Hospital, Siksha 'O' Anusandhan University, Deemed to be University, Bhubaneswar, Odisha, India
[4]Dept. of Biochemistry, IMS & SUM Hospital, Siksha 'O' Anusandhan University, Deemed to be University, Bhubaneswar, Odisha, India

Abstract

Introduction: *Candida albicans* is a ubiquitous and opportunistic fungal pathogen that can cause various infections from superficial mucosal lesions to invasive systemic disease. Its virulence is mainly due to important virulence genes which allow adhesion, formation of biofilm, immune evasion.
Aims and Objectives: The present study is to detect and characterize the existence of the four major and invasive virulence genes such as ALS1, HWP1, SAP2, and PLB1 from the clinical isolates of *Candida albicans* involved in the infected patients from coastal Odisha, India. In addition, the relationship between gene expression and phenotypic traits, like biofilm formation, is also analyzed.
Materials and Methods: A total of 50 clinical isolates of *Candida albicans* obtained from patients with clinically diagnosed candidiasis at IMS Sum Hospital, Bhubaneswar were used. All isolates were tested for the presence of the target virulence genes by PCR after extracting genomic DNA. Phenotypic biofilm-formation assay was carried out and results were compared with the pattern of genes detected.
Conclusion: The incidence of ALS1 and HWP1 genes was quite high among the isolates, highly associated with the increased biofilm production. These results indicate that the genes play a central role in increasing fungal virulence. The study highlights the genetic heterogeneity as well as phenotypic diversity displayed by the *Candida albicans* isolates in coastal Odisha and complements the current understanding regarding its molecular pathogenesis. It also demonstrates that specific approaches targeting biofilm inhibition are necessary for better control of infections caused by *Candida*.

Keywords: *Candida albicans*, Virulence factors, Coastal Odisha, Molecular characterization, Biofilm formation.

Received: 17-05-2025; **Accepted:** 19-06-2025; **Available Online**: 04-09-2025

1. Introduction

Candida albicans is a major contributor to fungal infections globally, ranging from surface-level mucosal infections like oral thrush and vulvovaginal candidiasis to more serious systemic infections, especially in individuals with weakened immune systems.[1-4] The pathogenic nature of *Candida albicans* is supported by various virulence factors, such as its capacity to adhere to host tissues, develop biofilms, and release hydrolytic enzymes, including proteases, phospholipases, and lipases.[5,6] These virulence factors are encoded by specific genes that are essential for the organism's survival and pathogenic capabilities within host environments.[7]

Virulence factor genes, such as *ALS1* (agglutinin-like sequence 1), *HWP1* (hyphal wall protein 1), *SAP2* (secreted aspartyl proteinase 2), and *PLB1* (phospholipase B1), have been widely studied for their roles in adhesion, biofilm formation, and tissue invasion.[8-15] The *ALS* gene family, particularly *ALS1*, is critical for adhesion to epithelial cells, a primary step in colonization and infection.[8] The *HWP1* gene encodes a cell wall protein that plays a pivotal role in biofilm

*Corresponding author: Subrat Kumar Tripathy
Email: subrattripathy@soa.ac.in

https://doi.org/10.18231/j.ijmmtd.v.11.i.3.3

development, an important virulence trait associated with antifungal resistance.[16] Secreted aspartyl proteinases, encoded by the *SAP* gene family, degrade host proteins, facilitating tissue invasion and immune evasion. Phospholipases, particularly *PLB1*, contribute to membrane disruption and host cell damage, further enhancing the pathogenicity of *Candida albicans*.[15,17]

Despite the global recognition of the *Candida albicans* as a major pathogen, there is limited data on the prevalence and the genetic characterization of its virulence factors in specific regions such as coastal Odisha, India. The unique climatic conditions of coastal Odisha, characterized by high humidity and temperature, may influence the prevalence and virulence of *Candida albicans* in this area.[18] Previous studies have indicated that environmental factors can significantly impact the expression of virulence genes and the overall pathogenic potential of fungal pathogens.[19] Therefore, understanding the distribution of these virulence factors in local *Candida albicans* isolates is essential for developing targeted treatment strategies and mitigating the impact of infections in this region.

This study aims to detect and characterize key virulence factor genes (*ALS1, HWP1*, *SAP2,* and *PLB1*) in clinical isolates of *Candida albicans* from coastal Odisha. By examining the molecular and phenotypic profiles of these isolates, this research seeks to provide insights into the virulence mechanisms that drive *Candida albicans* infections in this region, thereby contributing to improved diagnostic and therapeutic approaches for managing candidiasis.

2. Materials and Methods

2.1. Sample collection and isolation of Candida albicans

The *Candida albicans* samples used in this study were obtained from IMS and SUM Hospital, Bhubaneswar, between January 2023 and December 2024. A total of 50 isolates were collected from patients exhibiting clinical symptoms of candidiasis. These samples were preserved in the hospital's data repository files. Isolation and identification of the yeast were carried out using standard microbiological procedures. Since the study did not involve direct human participation, ethical clearance was not required.

2.2. Phenotypic identification of Candida albicans

The presumptive identification of the *Candida albicans* was initially conducted using classical phenotypic methods. Gram staining was performed on smears prepared from colony growth on Sabouraud Dextrose Agar (SDA). Under microscopic examination, *Candida albicans* appeared as Gram-positive, oval, or budding yeast cells, sometimes with pseudohyphal structures. This primary observation was crucial for distinguishing yeast morphology from bacteria forms.

The germ tube test was carried out to differentiate *Candida albicans* from non-albicans species. A yeast colony was inoculated into the 0.5 mL of sterile human serum and incubated at 37°C for 2–3 hours. Formation of germ tubes—tube-like outgrowths without constriction at the origin—was regarded as a definitive marker for *Candida albicans* identification.

To further support species-level identification, CHROMagar *Candida* medium was utilized. Upon incubation at 37°C for 48 hours, *Candida albicans* developed characteristic green-colored colonies.

In addition, biochemical tests such as carbohydrate assimilation and fermentation profiles were assessed using standardized commercial kits (Himedia, India). These biochemical assays confirmed the organism's metabolic profile, supporting identification based on sugar utilization patterns typical of *Candida albicans*. Once identified, *Candida albicans* isolates were preserved in 20% glycerol stocks at liquid nitrogen for subsequent molecular analysis. This ensured the viability and genetic stability of the strains for extended laboratory investigations (**Figure 1**).

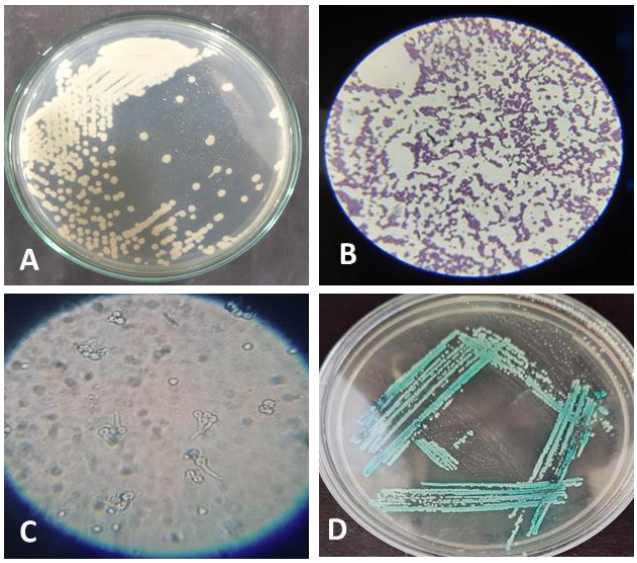

Figure 1: Phenotypic characterization of *Candida albicans*; **A:** Cultivation on sabouraud dextrose agar; **B:** Gram-stained slide displaying budding yeast cells; **C:** Germ tube test showing positive development; **D:** Green colonies on CHROMagar *Candida* verifying species-specific identification.

2.3. Molecular detection of virulence genes

PCR was utilized to identify the virulence factor genes ALS1, HWP1, SAP2, and PLB1 in the *Candida albicans* isolates. Primers specific to each gene were sourced from a previously published study.[13, 22]

Table 1: Primer sequences used for PCR amplification of *Candida albicans* rRNA and virulence genes (5′ to 3′ Orientation).

Gene	Primer Direction	Primer Sequence (5′ → 3′)	Reference
rRNA	Forward (F)	TTTATCAACTTGTCACACCAGA	[20]
	Reverse (R)	ATCCCGCCTTACCACTACCG	
ALS1	Forward (F)	CCATCACTGAAGATATCACCACA	[20]
	Reverse (R)	TGGAGCTTCTGTAGGACTGGTT	
SAP2	Forward (F)	AACAACAACCCACTAGACATCACC	[13]
	Reverse (R)	TGACCATTAGTAACTGGGAATGCTTTA GGA	
HWP1	Forward (F)	CCATGTGATGATTACCCACA	[20]
	Reverse (R)	GCTGGAACAGAAGATTCAGG	
PLB1	Forward (F)	GGTGGAGAAAGATGGCCAAAA	[20]
	Reverse (R)	AGCACTTACGTTACGATGCAACA	

2.4. RNA isolation and cDNA synthesis

2.4.1. RNA extraction, cDNA synthesis, and PCR amplification

RNA was isolated from *Candida albicans* samples using the TRIzol reagent (Genes to Me, India) as per the manufacturer's guidelines. The RNA's concentration and purity were measured with a NanoDrop spectrophotometer (Thermo Scientific, USA), and its integrity was verified through electrophoresis on a 1% (w/v) agarose formaldehyde gel with 16% formaldehyde. The RNA samples were divided into aliquots and kept at −80°C for future use. To remove any remaining genomic DNA, the total RNA underwent treatment with DNase I (NEB, UK) following the supplier's instructions.

Next, 1 µg of total RNA was converted into complementary DNA (cDNA) using M-MLV reverse transcriptase (Genes to Me, India) according to the manufacturer's protocol. PCR amplification was conducted in a 25 µL reaction mixture containing 10 ng of first-strand cDNA, 12.5 µL of 2X PCR Master Mix (Genei, India), and 10 pM of each gene-specific forward and reverse primer. The PCR process was carried out in a gradient thermal cycler (Takara, Japan) with the following settings: an initial denaturation at 95°C for 3 minutes; 35 cycles of denaturation at 95°C for 30 seconds, annealing at gene-specific temperatures (**Table 1**) for 30 seconds, and extension at 72°C for 1 minute; concluding with a final extension at 72°C for 10 minutes.

2.5. Sequencing of Candida albicans

PCR products were separated on 1.5% agarose gels, and the appropriately sized bands were extracted using the QIAquick Gel Extraction Kit (Qiagen, USA). The purified PCR products were sent to Heredity Biosciences in Bhubaneswar for sequencing via Sanger's dideoxy method (ABI 3730 XL) using ITS1 and ITS4 primers. Partial gene sequences were submitted to GenBank, with the accession number PP197343. The sequencing results were analyzed with bioinformatics tools to verify the identity of the *Candida albicans* isolates. Multiple sequence alignment was conducted to compare the obtained sequences with reference strains from GenBank.

2.6. Quantitative real-time PCR analysis of virulence gene expression

To evaluate the expression levels of virulence-related genes (ALS1, HWP1, SAP2, and PLB1) in clinical isolates of *Candida albicans*, Quantitative real-time PCR (qPCR) was utilized. The primer sequences for these genes were sourced from existing literature. The experiments were performed using a TATA MD Check Express Real-Time PCR system (TATA, India) at Heredity Biosciences. Each reaction mixture, with a total volume of 20 µL, included SYBR® Premix Ex Taq™ (Takara, Japan), 1 µL of both forward and reverse primers (final concentration of 0.3 µM), 1 µL of cDNA template (100 ng), and nuclease-free water to reach the final volume.

The rRNA gene was used as an endogenous control for normalization across samples. The qPCR thermal cycling conditions included an initial denaturation step at 95°C for 20 seconds, followed by 40 amplification cycles comprising denaturation at 95°C for 1 minute, and a combined annealing and extension phase at 60°C for 20 seconds, during which fluorescence was recorded. Specificity of amplification was verified by melt curve analysis, initiated at 65°C and incrementally increased to 95°C at a rate of 0.2°C/sec, with continuous fluorescence acquisition every 0.5°C. All reactions were performed in duplicate to ensure reproducibility and reliability of the data.

2.7. Phenotypic characterization of virulence traits

To correlate genotypic findings with phenotypic traits, biofilm formation and proteinase activity assays were performed.

2.8. Biofilm formation assay

Biofilm formation was assessed using a crystal violet staining method on 96-well polystyrene microtiter plates. Briefly, *Candida albicans* cells were inoculated in yeast nitrogen base (YNB) medium supplemented with 50 mM glucose and incubated at 37°C for 48 hours. Non-adherent cells were removed by washing with phosphate-buffered saline (PBS),

and biofilms were fixed with methanol, stained with 0.1% crystal violet, and solubilized with 33% acetic acid. The optical density (OD) was measured at 570 nm using a microplate reader.

2.9. Statistical analysis

Data analysis was conducted using SPSS software version 25.0. The prevalence of virulence genes was expressed as percentages, and the association between gene presence and phenotypic traits was assessed using the Chi-square test. A p-value of <0.05 was considered statistically significant.

3. Results

3.1. Prevalence of Candida albicans isolates

A total of 50 *Candida albicans* isolates were collected from clinical samples, including 20 (40%) from oral swabs, and 30 (60%) from vaginal swabs. The isolates were confirmed as *Candida albicans* based on morphological characteristics, germ tube formation, and biochemical tests. The distribution of isolates across different clinical sources highlights the pathogen's versatility in colonizing various anatomical sites.

3.2. Molecular detection of virulence genes

The presence of key virulence genes (ALS1, HWP1, SAP2, and PLB1) in *Candida albicans* isolates was analysed using qPCR-based detection. These genes play crucial roles in adhesion, biofilm formation, proteolytic activity, and membrane disruption, contributing to the pathogenic potential of fungi.

Quantitative real-time PCR analysis confirmed the presence and expression of key virulence-associated genes in *Candida albicans* isolates obtained from both oral and vaginal swabs. Among the 50 clinical collected tested, expression of *SAP2* was the most prevalent, detected in 88% of the strains, corroborating its central role in proteolytic activity and host tissue invasion. The *HWP1* gene, which facilitates adhesion and hyphal formation, was expressed in 84% of the isolates, indicating a strong potential for mucosal colonization and biofilm development. Expression of the *ALS1* gene, involved in epithelial adherence, was detected in 80% of isolates, with a balanced distribution across both sample types. *PLB1*, linked to phospholipase activity and membrane disruption, was expressed in 78% of isolates (**Table 2**).

These findings are consistent with phenotypic traits observed in preliminary screening and emphasize the widespread molecular basis for pathogenicity in clinical *Candida albicans* strains. Melt curve analysis confirmed the specificity of the amplification products, with single, sharp peaks observed for each target gene, indicating the absence of primer-dimer artifacts or non-specific amplification.

Table 2: Expression of Virulence Genes in *Candida albicans* Isolates from Vaginal Swabs (n=50)

Gene	Positive Isolates (n=50)	Percentage (%)
ALS1	40	80%
HWP1	42	84%
SAP2	44	88%
PLB1	39	78%

3.3. Phenotypic characterization of virulence traits

3.3.1. Biofilm formation

The ability of *Candida albicans* collected to form biofilms was assessed using the crystal violet staining method. Among the 50 isolates, 40 (80%) were classified as strong biofilm formers (OD570 > 0.5), 7 (14%) were moderate formers (OD570 between 0.2 and 0.5), and 3 (6%) showed weak or no biofilm formation (OD570 < 0.2)(**Table 3**). Notably, isolates harbouring both *ALS1* and *HWP1* genes exhibited significantly higher biofilm formation capacity compared to those lacking these genes ($p < 0.05$).

Table 3: Biofilm Formation Ability of *Candida albicans* Isolates and It's Correlation with the Presence of ALS1 and HWP1 Genes (n=50)

Biofilm Formation Category	OD₅₇₀ Range	Number of Isolates	Percentage (%)	ALS1 & HWP1 Positive (n)
Strong	> 0.5	40	80%	36
Moderate	0.2 – 0.5	7	14%	4
Weak/None	< 0.2	3	6%	0

Table 4: Association between the presence of virulence genes and phenotypic characteristics in *Candida albicans* Samples (n=50)

Virulence Gene Profile	No. of Isolates	Biofilm Formation (Mean Biomass*)
ALS1, *HWP1*, *SAP2*, *PLB1* (All)	30	High (+++)
ALS1, *HWP1* only	10	Moderate (++)
SAP2 only	5	Low (+)
PLB1 only	3	Low (+)
≤1 virulence gene	2	Very Low (-/+)

3.4. Correlation between genotype and phenotype

A significant correlation was found between the presence of virulence factor genes and corresponding phenotypic traits. Isolates possessing multiple virulence genes (*ALS1*, *HWP1*, *SAP2*, and *PLB1*) displayed enhanced biofilm formation compared to isolates with fewer virulence genes. Specifically, the co-presence of *ALS1* and *HWP1* was strongly associated with increased biofilm biomass, while the presence of *SAP2* was linked to elevated protease activity (**Table 4**). These findings suggest that the genetic makeup of *Candida albicans* isolates directly influences their pathogenic potential.

3.4. Interpretation of biofilm biomass scores

The biofilm production of *Candida albicans* isolates was evaluated using a crystal violet mirochemistry assay. The cell mass was quantified by measuring the turbidity of the cultures at a wavelength of 595 nm (OD595). An additional score system was used to classify biofilm production level according to the mean absorbance values. The categories were defined as follows: Very Low (-/+) for OD595 values less than 0.2, Low (+) for OD595 ranging from 0.2 to 0.4, Moderate (++) for OD595 between 0.4 and 0.6, and High (+++) for OD595 values exceeding 0.6. This classification allowed for a standardized assessment of biofilm production intensity. This semi-quantitative method also facilitates comparison of biofilm formation ability among isolates. Analysis of the results reveals that the simultaneous occurrence of multiple virulent genes, especially ALS1 and HWP1, is significantly associated with increased biofilm biomass, a main factor involved in the pathogenesis of *Candida albicons*.

4. Discussion

The current research focused on identifying and analysing key virulence factor genes in clinical isolates of *Candida albicans* from coastal Odisha, aiming to link these genetic characteristics with their phenotypic expressions of virulence. The study's results indicate a significant presence of virulence genes (ALS1, HWP1, SAP2, and PLB1), underscoring the pathogenic potential of *Candida albicans* isolates in this area. These findings align with earlier research that highlights the essential role of these genes in the adhesion, biofilm formation, and invasive abilities of *Candida albicans*.[6]

4.1. High prevalence of adhesion-related genes and implications for pathogenicity

The research revealed that the ALS1 gene, which codes for a crucial adhesin involved in attaching to host tissues, was found in 78% of the isolates. This significant prevalence highlights the critical role of adhesion as an initial step in the colonization and infection process of *Candida albicans*.[21-23] Similar results have been observed in studies from various regions, indicating that genes related to adhesion are conserved among clinical isolates, thereby enhancing their ability to cause infections in different host environments.[24] The strong link between the presence of ALS1 and the ability to form biofilms further underscores its importance in promoting persistent infections, especially on indwelling medical devices and epithelial surfaces.[25]

4.2. Role of HWP1 in biofilm formation and antifungal resistance

The HWP1 gene was identified in 84% of the isolates, and its presence was closely linked to increased biofilm formation. Biofilms are intricate microbial communities that create a protective environment for *Candida albicans*, aiding in antifungal resistance and the persistence of chronic infections.[26] The formation of biofilms poses a major clinical challenge because cells within biofilms can be up to 1,000 times more resistant to antifungal treatments than planktonic cells.[16,27] The robust biofilm-forming ability seen in HWP1-positive isolates is consistent with earlier research that highlights HWP1 as a vital component of the biofilm matrix, promoting cell-cell adhesion and resistance to the host's immune defenses.[28]

4.3. Secreted aspartyl proteinase (SAP2) and Its contribution to virulence

The SAP2 gene, identified in 64% of the isolates, is crucial for the pathogenicity of *Candida albicans* by breaking down host proteins and avoiding immune responses.[29] The link between SAP2 expression and protease activity observed in this study underscores its importance in tissue invasion and increasing virulence. Earlier studies have shown that SAP enzymes are vital for colonizing mucosal surfaces and spreading systemically, making them significant targets for treatment strategies.

4.4. Contribution of PLB1 to membrane disruption and host damage

The detection of the PLB1 gene in 58% of the isolates suggests its significant role in boosting the pathogenic capabilities of *Candida albicans* through its phospholipase activity, which compromises host cell membranes and aids in tissue invasion. Phospholipases are instrumental in the organism's capacity to breach epithelial and endothelial barriers, facilitating systemic dissemination. The results of this study are consistent with those of other researchers, who have identified PLB1 as a crucial element in the virulence of *Candida albicans*, especially in invasive infections.[14]

4.5. Environmental and therapeutic impact on virulence gene expression

Genes linked to virulence in *Candida albicans* express in a complex way, influenced by environmental signals. The coastal climate of Odisha, with high humidity, elevated temperatures, and salty air, may affect genetic and phenotypic traits of microbial populations, including *Candida albicans* . These environmental factors impact

fungal physiology and can increase expression of virulence factors like ALS1, HWP1, SAP2, and PLB1.[30] (Soriano-Abarca et al 2024).

Temperature fluctuations between 30 and 37°C, typical of coastal conditions, Favors *Candida albicans* transformation from yeast to hyphal filamentous form. This morphological change is crucial for tissue penetration and biofilm development. Under thermal stress, expression of HWP1 and ALS1 genes, involved in hyphal growth and adhesion, is upregulated. Environmental pH, influenced by host or external exposure, affects genes related to protease activity and membrane integrity.[31] (Véronique et al 2024). Acidic conditions may enhance SAP2 induction, increasing proteolytic activity and tissue damage.

The elevated virulence gene expression observed can be attributed to these regional environmental conditions, which support growth of more virulent strains. This environmental selection might lead to higher baseline virulence of *Candida albicans* populations in Odisha, causing more severe clinical diseases. The therapeutic environment is also significant. Antifungal misuse can have an evolutionary impact, favouring more virulent strains[32] (Andrzej Kazimierz Jaworek et al 2024). Exposure of *Candida albicans* to sub-therapeutic antifungal levels could trigger overexpression of virulence-related genes and increase biofilm resistance to antimicrobial agents and host immune clearance.

4.6. Clinical implications and future directions

The notable presence of virulence factor genes and the strong correlation between genotype and phenotype underscore the need for tailored antifungal approaches to address *Candida albicans* infections in coastal Odisha. The antifungal resistance patterns found in biofilm-forming isolates suggest that conventional treatments may be inadequate, necessitating the development of new therapeutic strategies that focus on biofilm-related pathways.[19] Future studies should focus on exploring how environmental factors affect gene expression and on identifying alternative antifungal agents that can effectively disrupt biofilm integrity. These findings also highlight the importance of routine genotypic and phenotypic screening of *Candida albicans* isolates in clinical settings to guide treatment decisions. Implementing molecular diagnostic techniques could help identify high-risk strains and predict potential antifungal resistance, allowing for more targeted and effective therapies. Additionally, further research into the specific environmental conditions of coastal Odisha may reveal unique selective pressures that contribute to the virulence and antifungal resistance profiles observed in local *Candida albicans* populations.

5. Conclusion

This research offers important insights into the prevalence and function of key virulence factor genes in clinical isolates of *Candida albicans* from coastal Odisha, emphasizing the pathogen's genetic and phenotypic diversity in this distinct geographic area. The high occurrence of genes linked to adhesion (ALS1), biofilm formation (HWP1), protease activity (SAP2), and phospholipase production (PLB1) highlights the complex mechanisms that contribute to *Candida albicans* pathogenicity. The strong association between these genes and increased virulence traits, such as enhanced biofilm formation and heightened protease activity, indicates that these genetic elements are crucial in determining infection severity and persistence.

The results underscore the importance of ongoing surveillance of *Candida albicans* virulence factors, especially in regions like coastal Odisha, where environmental conditions might affect the expression and impact of these genes. The connection between biofilm formation and antifungal resistance further underscores the clinical challenges posed by virulent *Candida albicans* strains, necessitating the creation of targeted antifungal strategies that address both planktonic and biofilm-associated cells.

In summary, this study enhances the understanding of the molecular and phenotypic characteristics of *Candida albicans* in coastal Odisha, laying the groundwork for future research aimed at developing better diagnostic tools, therapeutic interventions, and infection control measures. As antifungal resistance continues to increase, particularly among biofilm-forming strains, targeted approaches that consider local pathogen profiles will be crucial in effectively managing candidiasis and reducing the burden of this opportunistic pathogen.

6. Authors' Contributions

All authors contributed to the conception, design, and writing of the manuscript. All authors read and approved the final manuscript.

7. Source of Funding

None.

8. Conflicts of Interest

None.

9. Acknowledgement

The authors extend their sincere appreciation to the Dean of IMS and SUM Hospital, Bhubaneswar, for the provision of advanced research facilities at the Medical Research Laboratory, which were crucial to this study. We also express our gratitude to Dr. Debasmita Dubey of the MRL Lab, IMS and SUM Hospital, Siksha ‘O’ Anusandhan University, for her invaluable support and for facilitating access to essential laboratory resources. Furthermore, the authors acknowledge the contribution of Heredity Biosciences for their technical assistance and support in conducting the qPCR experiments,

which significantly enhanced the molecular aspects of this research.

References

1. Talapko J, Juzbasic M, Matijevic T, Pustijanac E, Bekic S, Kotris I et al. *Candida albicans* —the virulence factors and clinical manifestations of infection. *J Fungi*. 2021;7(2):79.
2. Tamo SB. Candida Infections: clinical features, diagnosis and treatment. *Infect Dis Clin Microbiol*. 2020;2(2):91-102.
3. Parambath S, Dao A, Kim HY, Zawahir S, AlastrueyIzquierdo A, Tacconelli E, et al. *Candida albicans* —A systematic review to inform the World Health Organization Fungal Priority Pathogens List. *Med Mycol*. 2024;62(6):myae045.
4. Calderone RA, Fonzi WA. Virulence factors of *Candida albicans*. *Trends Microbiol*. 2001;9(7):327-35.
5. Macias Paz IU, Perez Hernandez S, Tavera Tapia A, Luna Arias JP, Guerra Cardenas JE, Beltran ER. *Candida albicans* el principal hongo patógeno oportunista en humanos. *Rev Argent Microbiol*. 2023;55(2):189-98.
6. Mayer FL, Wilson D, Hube B. *Candida albicans* pathogenicity mechanisms. *Virulence*. 2013;4(2):119–28.
7. Ahmad Khan MS, Alshehrei F, Al-Ghamdi SB, Bamaga MA, Al-Thubiani AS, Alam MZ. Virulence and biofilms as promising targets in developing antipathogenic drugs against candidiasis. *Future Sci OA*. 2020;6(2): FSO440.
8. Hoyer LL, Green CB, Oh SH, Zhao X. Discovering the secrets of the *Candida albicans* agglutinin-like sequence (ALS) gene family--a sticky pursuit. *Med Mycol*. 2008;46(1):1–15.
9. Inci M, Atalay MA, Ozer B, EvirgenO, Duran N, KOC AN et al. Investigations of ALS1 and HWP1 genes in clinical isolates of *Candida albicans*. *Turk J Med Sci*. 2013;43(1):125–30.
10. Hoyer LL, Cota E. *Candida albicans* agglutinin-like sequence (Als) family vignettes: a review of Als protein structure and function. *Front Microbiol*. 2016;7:280.
11. Abastabar M, Hosseinpoor S, Hedayati MT, Shokohi T, Valadan R, Mirhendi H et al. Hyphal wall protein 1 gene: A potential marker for the identification of different Candida species and phylogenetic analysis. *Curr Med Mycol*. 2016;2(4):1–8.
12. Naglik JR, Challacombe SJ, Hube B. *Candida albicans* secreted aspartyl proteinases in virulence and pathogenesis. *Microbiol Mol Biol*. 2003;67(3):400–28.
13. Shrief R, Zaki ME, El-Sehsah EM, Ghaleb S, Mofreh M. Study of antifungal susceptibility, virulence genes and biofilm formation in *Candida albicans. Open Microbiol J*. 2019;13(1):241.
14. Mukherjee PK, Seshan KR, Leidich SD, Chandra J, Cole GT, Ghannoum MA. Reintroduction of the PLB1 gene into *Candida albicans* restores virulence in vivo. Microbiology. 2001;147(9):2585–97.
15. Mohammadi F, Charkhchian M, Mirzadeh M. Phenotypic and genotypic characterization of virulence markers and antifungal susceptibility of oral Candida species from diabetic and non-diabetic hemodialysis patients. *BMC Oral Health*. 2023;23(1):261.
16. Staab JF, Bradway SD, Fidel PL, Sundstrom P. Adhesive and mammalian transglutaminase substrate properties of *Candida albicans* Hwp1. *Science*. 1999;283(5407):1535–8.
17. Ghannoum MA. Potential role of phospholipases in virulence and fungal pathogenesis. *Clin Microbiol Rev*. 2000;13(1):122–43.
18. Gadre A, Enbiale W, Andersen LK, Coates SJ. The effects of climate change on fungal diseases with cutaneous manifestations: A report from the International Society of Dermatology Climate Change Committee. *J Clim Change Health*. 2022;6:100156.
19. Nobile CJ, Johnson AD. *Candida albicans* biofilms and human disease. *Annu Rev Microbiol*. 2015;69(1):71–92.
20. Monroy-Perez E, Paniagua-Contreras GL, Rodriguez-Purata P, Vaca-Paniagua F, Vázquez-Villasenor M, Diaz-Velasquez C, et al. High virulence and antifungal resistance in clinical strains of *Candida albicans*. *Can J Infect Dis Med Microbiol*. 2016;2016(1):5930489.
21. Mayer FL, Wilson D, Hube B. *Candida albicans* pathogenicity mechanisms. *Virulence*. 2013;4(2):119–28.
22. Hoyer LL, Green CB, Oh SH, Zhao X. Discovering the secrets of the Candida albicans agglutinin-like sequence (ALS) gene family–a sticky pursuit. *Med Mycol*. 2008;46(1):1–15.
23. Sundstrom P. Adhesins in *Candida albicans*. *Curr Opin Microbiol.* 1999;2(4):353–7.
24. Gulati M, Nobile CJ. *Candida albicans* biofilms: development, regulation, and molecular mechanisms. Microbes Infect. 2016;18(5):310–21.
25. Fanning S, Mitchell AP. Fungal biofilms. *PLoS Pathog*. 2012;8(4):e1002585.
26. Ramage G, Rajendran R, Sherry L, Williams C. Fungal biofilm resistance. *Int J Microbiol*. 2012;2012(1):528521.
27. Samrot AV, Abubakar Mohamed A, Faradjeva E, Si Jie L, Hooi Sze C, Arif A, et al. Mechanisms and impact of biofilms and targeting of biofilms using bioactive compounds—A review. *Medicina*. 2021;57(8):839.
28. Naglik JR, Rodgers CA, Shirlaw PJ, Dobbie JL, Fernandes-Naglik LL, Greenspan D, et al. Differential expression of *Candida albicans* secreted aspartyl proteinase and phospholipase B genes in humans correlates with active oral and vaginal infections. *J Infect Dis*. 2003;188(3):469–79.
29. Schaller M, Borelli C, Korting HC, Hube B. Hydrolytic enzymes as virulence factors of *Candida albicans*. *Mycoses*. 2005;48(6):365–77.
30. Soriano-Abarca M, Tapia JC, Cáceres-Valdiviezo MJ, Morey-León G, Fernández-Cadena J, Díaz-Cevallos L, et al. Virulence-related genes expression in planktonic mixed cultures of *Candida albicans* and non-albicans Candida species. *Microb Physiol*. 2024;34(1):243–54.
31. Véronique A, Guillaume C, Jean-Michel C, Françoise A. *Candida albicans* cells exhibit media specific proteomic profiles during induction of filamentation. *BMC Microbio*. 2024;24(1):500.
32. Jaworek AK, Hałubiec P, Sroka D, Grabarczyk I, Kachnic N, Wojas-Pelc A, et al. Demographic and Pathogen Profiles of Superficial Fungal Infections—A Single-Centre Observational Study in Poland. *Mycoses*. 2024;67(12):e70009.

Cite this article: Subhadarsini SS, Purohit GK, Mishra MP, Dubey D, Tripathy SK. Identification and characterization of virulence factor genes in coastal Odisha isolates of *Candida albicans*. *IP Int J Med Microbiol Trop Dis*. 2025;11(3):257-263.