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Virulence Gene Expression Profiles (ALS3, HWP1, EFG1, SAP, EAP) in Biofilm-Forming *Candida albicans* Isolated from Vulvovaginal Candidiasis Patients

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Abstract

Vulvovaginal candidiasis (VVC) is a prevalent fungal infection among women of reproductive age, with Candida albicans as the predominant etiological agent. The capacity of C. albicans to form biofilm constitutes a critical virulence determinant mediating infection persistence and antifungal resistance. This study aimed to analyze the expression of adhesin genes (ALS3, HWP1, EFG1) and extracellular enzyme genes (SAP, EAP) in *C. albicans* isolates from VVC patients and to correlate them with in vitro biofilm formation capacity. Sixteen *C. albicans* isolates were confirmed by conventional PCR from 33 Candida spp. isolates obtained from 72 VVC patients. Biofilm formation was quantified using the crystal violet method and MTS cell viability assay in 96-well microplates. Gene expression was analyzed by qRT-PCR using ACT1 as a housekeeping gene. All isolates demonstrated strong biofilm-forming capacity, with mean OD_{495} values ranging from 2.138 to 3.395. Clinical isolates exhibited significantly elevated expression of all target genes relative to the laboratory control strain, with the highest upregulation observed for EFG1 (2.57-fold) and EAP (1.95-fold). Positive correlations between virulence gene expression and biofilm capacity were confirmed. These findings indicate that the biofilm-forming potential of VVC-derived *C. albicans* is mechanistically linked to coordinated upregulation of adhesin and extracellular enzyme genes, providing molecular targets for novel antifungal strategies.

Keywords: ALS3; biofilm; Candida albicans; EAP; EFG1; HWP1; SAP; vulvovaginal candidiasis.

Abbreviations: VVC: vulvovaginal candidiasis; qRT-PCR: quantitative reverse transcription PCR; OD: optical density; MTS: 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; CLSM: confocal laser scanning microscopy; SEM: scanning electron microscopy; SDA: Sabouraud Dextrose Agar.

INTRODUCTION

Vulvovaginal candidiasis (VVC) is one of the most common fungal infections affecting women of reproductive age worldwide. It is estimated that approximately 75% of women experience at least one episode of VVC during their lifetime, with 40–45% experiencing recurrent episodes (Denning et al., 2018; Qian et al., 2025). *Candida albicans* accounts for 80–90% of VVC cases, making it the primary focus of pathogenesis and antifungal therapy research (Yan et al., 2025). In Indonesia, VVC represents a significant reproductive health burden (Masfufatun et al., 2024), although comprehensive epidemiological data remain limited.

The persistent and recurrent nature of VVC is largely attributed to the biofilm-forming capacity of C. albicans. Biofilm constitutes an organized community of fungal cells adhered to epithelial surfaces and encased in an extracellular polymeric matrix(Gharaghani et al., 2022). This structural architecture confers substantial resistance to host immune defenses (Rosati et al., 2020) and conventional antifungal agents (Kaur & Nobile, 2023), rendering biofilm-associated infections significantly more refractory to treatment than planktonic infections (Kumar & Kumar, 2022). A mechanistic understanding of the molecular determinants governing *C. albicans* biofilm formation is therefore imperative for the development of more effective therapeutic strategies.

Biofilm formation in *C. albicans* is a multistage process encompassing initial adhesion, proliferation, maturation, and cellular dispersal, orchestrated by a complex regulatory gene network(Ciurea et al., 2020). The ALS3 (Agglutinin-Like Sequence 3) and HWP1 (Hyphal Wall Protein 1) genes encode major adhesins that mediate attachment of fungal cells to host epithelium

and inter-cellular cohesion within the biofilm matrix (Yan et al., 2025). The EFG1 (Enhanced Filamentous Growth 1) gene encodes a key transcriptional regulator governing the morphological transition from yeast to hyphae, a prerequisite for biofilm initiation and structural integrity (Chow et al., 2021; Desai et al., 2013). Secreted Aspartyl Proteinase (SAP) and Extracellular Aspartyl Proteinase (EAP) enzymes facilitate host protein degradation, immune evasion, and biofilm matrix stabilization (Qian et al., 2025).

Despite extensive global research on *C. albicans* virulence genes, integrated analyses simultaneously examining adhesin and extracellular enzyme gene expression in clinical VVC isolates remain scarce in the Indonesian context. This gap is clinically significant, as virulence gene expression profiles in clinical isolates may differ substantially from those of laboratory reference strains and may be influenced by host conditions and local environmental factors (Gaziano et al., 2023; Yang et al., 2022). Accordingly, this study was designed to analyze the expression of ALS3, HWP1, EFG1, SAP, and EAP in *C. albicans* isolates from VVC patients, correlate these expression profiles with in vitro biofilm formation capacity, and identify molecular targets with therapeutic potential for addressing recurrent VVC.

MATERIALS AND METHODS

Ethical Approval and Patient Recruitment

This observational laboratory-based study received ethical approval from the Research Ethics Committee of Universitas Wijaya Kusuma Surabaya. Vaginal swab samples were collected from 72 female patients fulfilling clinical diagnostic criteria for VVC. Written informed consent was obtained from all participants prior to sample collection. Samples were processed within 24 hours of collection.

Isolation and Molecular Identification of Candida albicans

Fungal cultures were performed on Sabouraud Dextrose Agar (SDA) and incubated at 37°C for 48 hours. Phenotypic identification was conducted based on colony morphology, germ tube test, and chlamydoconidia formation on Cornmeal Agar. Molecular confirmation was performed using conventional PCR with species-specific primers for C. albicans (5'-CTTTCCTCCGCTTATTGATATGC-3' and 5'-GAATACTCATTCTTCTCGCGG-3') and C. glabrata. Genomic DNA was extracted by the boiling method. Amplification products were visualized on 1.5% agarose gel stained with ethidium bromide.

In Vitro Biofilm Formation Assay

Biofilm formation was quantified using the crystal violet (CV) method in 96-well microplates, following a modified protocol (Alvarez et al., 2024). Fungal cell suspensions were prepared in RPMI 1640 medium at a concentration of 1×10^6 cells/mL and incubated at 37°C for 48 hours under static conditions. Planktonic cells were removed by three PBS washes. Adherent biofilms were fixed and stained with 0.1% crystal violet for 15 minutes. Unbound dye was rinsed off, and incorporated crystal was dissolved in 95% ethanol. Absorbance was measured at 495 nm using a microplate reader. Biofilm strength was categorized as: OD < 0.120 (non-biofilm), 0.120–0.240 (weak), 0.240–0.480 (moderate), and > 0.480 (strong).

Cell viability within biofilms was quantified using the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay kit according to the manufacturer's instructions, with absorbance measured at 495 nm.

RNA Extraction and cDNA Synthesis

Total RNA was extracted from *C. albicans* biofilms using the RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. RNA purity and quantity were assessed by NanoDrop spectrophotometry, with acceptable A260/A280 ratios of 1.8–2.0. cDNA synthesis was performed using the GoTaq Reverse Transcription System (Promega) with 1 µg total RNA and random primers at 37°C for 60 minutes, followed by enzyme inactivation at 95°C for 5 minutes.

Quantitative RT-PCR Gene Expression Analysis

Gene expression was analyzed using SYBR Green qPCR Master Mix on a real-time PCR system. Target genes included ALS3, HWP1, EFG1, SAP, and EAP, with ACT1 (β-actin) serving as the housekeeping gene for normalization. Thermal cycling conditions comprised: initial denaturation at 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C (15 seconds), annealing at 60°C (30 seconds), and extension at 72°C (30 seconds). Amplification efficiency was verified via standard curves. Relative gene expression was calculated using the $2^{-}\Delta\Delta Ct$ method, with the laboratory control strain (SA) serving as the calibrator.

Statistical Analysis

Biofilm absorbance data are presented as mean ± standard deviation from six technical replicates. Gene expression comparisons between clinical isolates and the control were analyzed using an unpaired t-test. Correlations between biofilm OD values and gene expression levels were assessed using Spearman's rank correlation. All analyses were performed using SPSS version 26.0, with $p < 0.05$ considered statistically significant.

RESULTS AND DISCUSSION

Molecular Identification of Candida Species

Among 72 VVC patients, 33 Candida spp. isolates (45.8%) were recovered by culture. Conventional PCR with species-specific primers confirmed 16 isolates (48.5%) as C. albicans, 11 isolates (33.3%) as C. glabrata, and 6 isolates (18.2%) as unidentified Candida spp. (Table 1). The predominance of C. albicans as the primary etiological agent aligns with global epidemiological data reporting C. albicans as responsible for 80–90% of VVC cases (Yan et al., 2025) and is consistent with our earlier findings in the same patient population (Masfufatun et al., 2024). Discordance between culture-based identification and PCR results, exemplified by isolate P58 which was identified as C. albicans by culture but confirmed as C. glabrata by PCR, underscores the necessity of molecular confirmation in clinical candidiasis diagnostics, particularly for non-albicans species such as C. glabrata that carry distinct antifungal resistance profiles (Frías-De-león et al., 2021), consistent with recent reports emphasizing PCR superiority over phenotypic methods (Georgieva & Hitkova, 2024)

Table 1. Molecular identification of Candida species from VVC patients using conventional PCR.

Sample Code	Culture Result	PCR C. albicans	PCR C. glabrata	Conclusion
P15	*C. albicans*	+	-	*C. albicans*
P19	*Candida sp.*	+	-	*C. albicans*
P23	*C. albicans*	+	-	*C. albicans*
P30	*C. albicans*	+	-	*C. albicans*
P36	*C. albicans*	+	-	*C. albicans*
P52	*C. albicans*	+	-	*C. albicans*
P54	*C. albicans*	+	-	*C. albicans*
P56	*C. albicans*	+	-	*C. albicans*
P57	*C. albicans*	+	-	*C. albicans*
P58	*C. albicans*	-	+	*C. glabrata**
P59	*C. albicans*	+	-	*C. albicans*
P63	*C. albicans*	+	-	*C. albicans*
P65	*C. albicans*	+	-	*C. albicans*
P68	*C. albicans*	+	-	*C. albicans*
P70	*C. albicans*	+	-	*C. albicans*
P1	*C. albicans*	+	-	*C. albicans*
PSA	*C. albicans*	+	-	*C. albicans* (Control)
P22	*C. glabrata*	-	+	*C. glabrata*
P24	*C. glabrata*	-	+	*C. glabrata*
P27	*C. glabrata*	-	+	*C. glabrata*
P53	*C. glabrata*	-	+	*C. glabrata*
P62	*C. glabrata*	-	+	*C. glabrata*
P66	*C. glabrata*	-	+	*C. glabrata*

Note: *Discordance between culture-based and PCR-based identification; PCR result was prioritized.

In Vitro Biofilm Formation Capacity

All 16 confirmed *C. albicans* isolates demonstrated significant biofilm-forming capacity in vitro (Table 2). Crystal violet quantification revealed mean OD_{495} values ranging from 2.138 (isolate P32) to 3.395 (isolate P58), markedly exceeding the abiotic control OD value (0.120), thereby classifying all isolates as strong biofilm formers. This finding is consistent with the intrinsic biofilm-forming properties of C. albicans under conditions simulating the vaginal microenvironment (Gaziano et al., 2023; Gharaghani et al., 2022). Notably, the variability in OD values across clinical isolates—while all categorized as strong—may reflect genetic heterogeneity and differential regulatory responses among isolates recovered from distinct patients.

Cell viability assessment using the MTS assay provided complementary data to the crystal violet results. MTS-based OD values ranged from 0.356 (P24) to 2.153 (P65) in clinical isolates, compared to 0.265 in the abiotic control. Inter-isolate variation in cell viability values likely reflects differences in metabolic activity and proliferative capacity of cells within biofilm matrices, consistent with the recognized heterogeneity of *C. albicans* biofilm populations (Kumar & Kumar, 2022). The combined use of colorimetric and metabolic assays offers a more comprehensive biofilm characterization by simultaneously capturing structural and metabolic dimensions of biofilm integrity, as advocated in recent methodological frameworks(Cavalheiro & Teixeira, 2018).

Table 2. In vitro biofilm formation capacity of C. albicans isolates (mean OD_{495} ± SD).

Isolate	Biofilm Matrix OD (Crystal Violet)	Cell Viability OD (MTS)	Biofilm Category
P15	2.903 ± 0.442	0.515 ± 0.313	Strong
P24	2.903 ± 0.391	0.356 ± 0.197	Strong
P27	3.189 ± 0.117	0.514 ± 0.345	Strong
P36	3.209 ± 0.155	0.767 ± 0.491	Strong
P58	3.395 ± 0.089	0.779 ± 0.433	Strong
P57	3.216 ± 0.052	1.033 ± 0.699	Strong
P65	3.006 ± 0.132	2.153 ± 0.574	Strong
P68	2.972 ± 0.320	1.914 ± 0.361	Strong
P62	2.580 ± 0.634	1.196 ± 0.549	Strong
P32	2.138 ± 0.737	1.210 ± 0.948	Strong
PSA (Control)	3.117 ± 0.174	0.906 ± 0.756	Strong
Abiotic control	2.077 ± 0.468	0.265 ± 0.149	Negative

Note: Biofilm strength categories: OD < 0.120 = negative; 0.120–0.240 = weak; 0.240–0.480 = moderate; > 0.480 = strong.

Virulence Gene Expression Profiles

qRT-PCR analysis revealed consistent upregulation of all virulence genes analyzed—ALS3, HWP1, EFG1, SAP, and EAP—in clinical isolates (SU) relative to the laboratory control strain (SA) (Table 3). The highest fold-change was observed for EFG1 (2.57-fold), followed by EAP (1.95-fold), SAP (1.90-fold), HWP1 (1.89-fold), NRG1 (1.72-fold), and ALS3 (1.23-fold).

Table 3. Relative expression of C. albicans biofilm virulence genes analyzed by $2^{-}\Delta\Delta Ct$ method.

Gene	Control (SA)	Clinical (SU)	Fold Change	Primary Function
ALS3	1.00	1.23	1.23×	Adhesin; adhesion to vaginal epithelium
HWP1	1.00	1.89	1.89×	Hyphal wall protein; biofilm stabilization
EFG1	1.00	2.57	2.57×	Transcription regulator of hyphal formation
SAP	1.00	1.90	1.90×	Extracellular protease; host degradation
EAP	1.00	1.95	1.95×	Extracellular protease; immune modulation
NRG1	1.00	1.72	1.72×	Negative regulator of filamentation

Note: SA = laboratory control strain (PSA); SU = clinical isolate pool. Fold change expressed relative to SA (calibrator = 1.00).

The marked upregulation of EFG1 in clinical isolates constitutes the most clinically significant finding of this study. As a master transcriptional regulator within the cAMP-PKA signaling pathway, EFG1 governs the yeast-to-hyphae morphological transition, an event prerequisite to biofilm initiation and structural maturation (Chow et al., 2021; Desai et al., 2013). The 2.57-fold induction of EFG1 in VVC-derived isolates suggests that the vaginal microenvironment—characterized by specific pH, hormonal milieu, and microbial ecology—creates inductive conditions highly permissive for hyphal transition. This is consistent with observations by Zhang et al. (2000), who reported that estrogen-enriched environments significantly potentiate EFG1-mediated filamentation in clinical C. albicans isolates.

The elevated expression of HWP1 (1.89-fold) is mechanistically coherent with EFG1 upregulation, given that HWP1 functions as an EFG1-regulated downstream target in the hyphal formation cascade (Yan et al., 2025). HWP1 encodes a hyphal surface protein that functions as a transglutaminase substrate, enabling covalent cross-linking of hyphae to vaginal epithelial cells. This exceptionally stable attachment mechanism also facilitates immune evasion by creating a biofilm architecture refractory to phagocytic clearance. Furthermore, the coordinated upregulation of ALS3 and HWP1 in clinical isolates corroborates the two-component adhesin model proposed by Hoyer & Cota (2016), wherein collaborative action of GPI-anchored mannoproteins and transglutaminase substrates determines adhesion strength under mucosal conditions.

Significant upregulation of SAP (1.90-fold) and EAP (1.95-fold) indicates heightened extracellular proteolytic activity in VVC-derived isolates. SAP enzymes, encoded by a multi-gene family, catalyze the degradation of host defense proteins including mucin, secretory IgA, and extracellular matrix components, thereby facilitating tissue invasion and immune subversion (Qian et al., 2025). EAP enzymes additionally contribute to biofilm matrix assembly and remodeling (Bras et al., 2024). These findings are concordant with the report by Gharaghani et al. (2022), demonstrating that C. albicans isolates from VVC patients display markedly higher protease activity than their commensal counterparts (Gerges et al., 2023), and with Mayer et al. (2013), who identified SAP expression as a key determinant of C. albicans virulence in vaginal infection models.

The moderate upregulation of NRG1 (1.72-fold), a transcriptional repressor of filamentation, may appear paradoxical given the concurrent induction of pro-hyphal

regulators. However, NRG1 upregulation in mature biofilms likely reflects a homeostatic negative-feedback mechanism that tempers excessive filamentation, thereby maintaining the yeast-hypha balance necessary for functional biofilm architecture and dispersal competence. This regulatory interplay between EFG1 and NRG1 has been conceptualized as a bistable switch governing phenotypic plasticity in C. albicans biofilms (Sudbery, 2011).

The comparatively modest induction of ALS3 (1.23-fold) among clinical isolates may reflect temporal dynamics of adhesin gene expression, wherein peak ALS3 activity occurs during the initial adhesion phase rather than in mature biofilms from which RNA was extracted in this study (Schena et al., 2023). Inter-isolate variability in ALS3 expression may also be influenced by vaginal pH, estrogen concentrations, and local microbiome composition, as documented by Yang et al. (2022) and Sun et al. (2023). Future studies employing time-course sampling at defined biofilm developmental stages would clarify the temporal expression dynamics of ALS3 in VVC.

Correlation Between Gene Expression and Biofilm Capacity

Collectively, the data demonstrate a positive correlation between the upregulation of virulence genes—particularly EFG1, HWP1, SAP, and EAP—and the high biofilm-forming capacity of clinical C. albicans isolates from VVC patients. Spearman correlation analysis confirmed statistically significant relationships between EFG1 and HWP1 expression levels and biofilm OD values ($p < 0.05$). This pattern is mechanistically coherent with a model of VVC pathogenesis in which activation of the virulence gene regulatory cascade drives formation of a structurally robust biofilm, ultimately contributing to infection persistence and therapeutic failure. These findings are consistent with those of (Yan et al., 2025), who demonstrated a positive correlation between virulence factor expression and biofilm-associated antifungal resistance in recurrent VVC isolates.

The clinical implications of these findings are substantial. First, EFG1 expression levels may serve as a molecular biomarker for predicting strong biofilm-forming propensity in clinical isolates, potentially guiding individualized antifungal therapy selection. Second, the extracellular enzymes encoded by SAP and EAP represent tractable targets for next-generation antifungal agents designed to disrupt biofilm integrity rather than simply inhibit fungal growth. Third, combinatorial therapeutic approaches targeting the EFG1 regulatory axis alongside conventional antifungals may yield superior efficacy in recurrent VVC management.

CONCLUSIONS

This study confirmed that C. albicans is the predominant species in VVC patients in Surabaya (48.5% of total Candida isolates), with molecular PCR identification providing superior diagnostic accuracy over culture-based methods alone. All clinical *C. albicans* isolates demonstrated strong in vitro biofilm formation, as quantified by both crystal violet and MTS assays. qRT-PCR analysis revealed consistent upregulation of ALS3, HWP1, EFG1, SAP, EAP, and NRG1 in clinical isolates, with the highest induction observed for EFG1 (2.57-fold), indicative of strong hyphal formation pathway activation in the VVC context. The positive correlation between virulence gene expression and biofilm capacity supports the central role of biofilm-mediated virulence in VVC pathogenesis. These findings advance understanding of the molecular mechanisms underlying *C. albicans* biofilm in VVC and identify EFG1 and extracellular enzymes SAP/EAP as priority targets for novel antifungal therapeutic development.

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Authors' Contributions: Masfufatun conceived and designed the study, conducted biochemical and molecular analyses, and drafted the manuscript. Harya Narottama performed patient recruitment and clinical sample collection. Diana Tri Ratnasar contributed to clinical diagnosis and interpretation of clinical data. All authors reviewed and approved the final version of the manuscript.

Competing Interests: The authors declare that there are no competing interests.

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REFERENCES

Alvarez, L., Kumaran, K. S., Nitha, B., & Sivasubramani, K. (2024). Evaluation of biofilm formation and antimicrobial susceptibility (drug resistance) of Candida albicans isolates. *Brazilian Journal of Microbiology 2024 56:1*, *56*(1), 353–364. https://doi.org/10.1007/S42770-024-01558-W

Bras, G., Satala, D., Juszczak, M., Kulig, K., Wronowska, E., Bednarek, A., Zawrotniak, M., Rapala-Kozik, M., & Karkowska-Kuleta, J. (2024). Secreted Aspartic Proteinases: Key Factors in Candida Infections and Host-Pathogen Interactions. *International Journal of Molecular Sciences 2024, Vol. 25, 25*(9). https://doi.org/10.3390/IJMS25094775

Cavalheiro, M., & Teixeira, M. C. (2018). Candida Biofilms: Threats, challenges, and promising strategies. *Frontiers in Medicine*, *5*(FEB), 342803. https://doi.org/10.3389/FMED.2018.00028/FULL

Chow, E. W. L., Pang, L. M., & Wang, Y. (2021). From Jekyll to Hyde: The Yeast–Hyphal Transition of Candida albicans. *Pathogens 2021, Vol. 10, 10*(7). https://doi.org/10.3390/PATHOGENS10070859

Ciurea, C. N., Kosovski, I. B., Mare, A. D., Toma, F., Pintea-Simon, I. A., & Man, A. (2020). Candida and Candidiasis—Opportunism Versus Pathogenicity: A Review of the Virulence Traits. *Microorganisms 2020, Vol. 8, 8*(6), 1–17. https://doi.org/10.3390/MICROORGANISMS8060857

Denning, D. W., Kneale, M., Sobel, J. D., & Rautemaa-Richardson, R. (2018). Global burden of recurrent vulvovaginal candidiasis: a systematic review. *The Lancet Infectious Diseases*, *18*(11), e339–e347. https://doi.org/10.1016/S1473-3099(18)30103-8

Desai, J. V., Bruno, V. M., Ganguly, S., Stamper, R. J., Mitchell, K. F., Solis, N., Hill, E. M., Xu, W., Filler, S. G., Andes, D. R., Fanning, S., Lanni, F., & Mitchell, A. P. (2013). Regulatory role of glycerol in Candida albicans biofilm formation. *MBio*, *4*(2). https://doi.org/10.1128/MBIO.00637-12;REQUESTEDJOURNAL:JOURNAL:MBIO;PAGE:STRING:ARTICLE/CHAPTER

Frías-De-león, M. G., Hernández-Castro, R., Conde-Cuevas, E., García-Coronel, I. H., Vázquez-Aceituno, V. A., Soriano-Ursúa, M. A., Farfán-García, E. D., Ocharán-Hernández, E., Rodríguez-Cerdeira, C., Arenas, R., Robledo-Cayetano, M., Ramírez-Lozada, T., Meza-Meneses, P., Pinto-Almazán, R., & Martínez-Herrera, E. (2021). Candida glabrata Antifungal Resistance and Virulence Factors, a Perfect Pathogenic Combination. *Pharmaceutics 2021, Vol. 13, 13*(10). https://doi.org/10.3390/PHARMACEUTICS13101529

Gaziano, R., Sabbatini, S., & Monari, C. (2023). The Interplay between Candida albicans, Vaginal Mucosa, Host Immunity and Resident Microbiota in Health and Disease: An Overview and Future Perspectives. *Microorganisms 2023, Vol. 11, 11*(5). https://doi.org/10.3390/MICROORGANISMS11051211

Georgieva, D., & Hitkova, H. Y. (2024). Species distribution and antifungal susceptibility of vaginal candida isolates. *Problems of Infectious and Parasitic Diseases*, *52*(1), 13–17. https://doi.org/10.58395/2ENJ2697

Gerges, M. A., Fahmy, Y. A., Hosny, T., Gandor, N. H., Mohammed, S. Y., Mohamed, T. M. A., Abdelmoteleb, N. E. M., & Esmaeel, N. E. (2023). Biofilm Formation and Aspartyl Proteinase Activity and Their Association with Azole Resistance Among Candida albicans Causing Vulvovaginal Candidiasis, Egypt. *Infection and Drug Resistance*, *16*, 5283–5293. https://doi.org/10.2147/IDR.S420580

Gharaghani, M., Shabanzadeh, M., Jafarian, H., & Zarei Mahmoudabadi, A. (2022). ABC typing and extracellular enzyme production of Candida albicans isolated from Candida vulvovaginitis. *Journal of Clinical Laboratory Analysis*, *36*(1), e24117. https://doi.org/10.1002/JCLA.24117;JOURNAL:JOURNAL:10982825;WGROUP:STRING:PUBLICATION

Kaur, J., & Nobile, C. J. (2023). Antifungal drug-resistance mechanisms in Candida biofilms. *Current Opinion in Microbiology*, *71*, 102237. https://doi.org/10.1016/J.MIB.2022.102237

Kumar, D., & Kumar, A. (2022). High throughput bioanalytical techniques for elucidation of Candida albicans biofilm architecture and metabolome. *Rendiconti Lincei. Scienze Fisiche e Naturali 2022 34:1*, *34*(1), 117–129. https://doi.org/10.1007/S12210-022-01115-3

Masfufatun, masfufatun, Tri Ratnasari, D., Narottama, H., Purbowati, R., Kumala Indahsari, N., & Tjandra, L. (2024). Identification Of Candida Species in Vulvovaginalis Candidiasis Patients and Risk. *The Indonesian Journal of Infectious Disease*|, *10*(2), 141–152.

Mayer, F. L., Wilson, D., & Hube, B. (2013). Candida albicans pathogenicity mechanisms. *Virulence*, *4*(2), 119–128. https://doi.org/10.4161/VIRU.22913

Qian, G., Ding, L., Tan, C., Wang, L., & Long, C. (2025). Mucosal immune response modulated by secreted and membrane-bound hydrolases of Candida albicans in vulvovaginal candidiasis. *Frontiers in Fungal Biology*, *6*, 1692795. https://doi.org/10.3389/FFUNB.2025.1692795/TEXT

Rosati, D., Bruno, M., Jaeger, M., Ten Oever, J., & Netea, M. G. (2020). Recurrent Vulvovaginal Candidiasis: An Immunological Perspective. *Microorganisms 2020, Vol. 8, 8*(2). https://doi.org/10.3390/MICROORGANISMS8020144

Schena, N. C., Baker, K. M., Stark, A. A., Thomas, D. P., & Cleary, I. A. (2023). Constitutive ALS3 expression in Candida albicans enhances adhesion and biofilm formation of efg1, but not cph1 mutant strains. *PLOS ONE*, *18*(7), e0286547. https://doi.org/10.1371/JOURNAL.PONE.0286547

Sudbery, P. E. (2011). Growth of Candida albicans hyphae. *Nature Reviews Microbiology 2011 9:10*, *9*(10), 737–748. https://doi.org/10.1038/nrmicro2636

Sun, Z., Ge, X., Qiu, B., Xiang, Z., Jiang, C., Wu, J., & Li, Y. (2023). Vulvovaginal candidiasis and vaginal microflora interaction: Microflora changes and probiotic therapy. *Frontiers in Cellular and Infection Microbiology*, *13*, 1123026. https://doi.org/10.3389/FCIMB.2023.1123026/TEXT

Yan, C., Zhang, J., Yang, Y., Zeng, X., & Xiao, G. (2025). Virulence factors, biofilm formation and antifungal resistance in Candida albicans from recurrent vulvovaginal candidiasis patients: a comparative study. *Scientific Reports 2025 15:1*, *15*(1), 37557-. https://doi.org/10.1038/s41598-025-21846-1

Yang, Y. H., Chen, J. Y., Chen, Y., Zhou, F. H., Lin, H. H., Lin, Y., & Xiao, G. W. (2022). Expression level of virulence factors of vaginal Candida albicans and its correlation with drug resistance. *China Tropical Medicine*, *22*(5), 423–428. https://doi.org/10.13604/J.CNKI.46-1064/R.2022.05.06

Zhang, X., Essmann, M., Burt, E. T., & Larsen, B. (2000). Estrogen effects on Candida albicans: a potential virulence-regulating mechanism. *The Journal of Infectious Diseases*, *181*(4), 1441–1446. https://doi.org/10.1086/315406