Experimental and Applied Veterinary Research Journal
Volume 1, Issue No. 1, 2025

Molecular identification of non-albicans candida isolated from vulvovaginitis of cows

Nabaa Ali Hasan[1]*, Zainab Abdulzahra Abbas Al-haddad[1]

[1]*Zoonotic Diseases Research Unit, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.*

***Corresponding Author**
Nabaa Ali Hasan
naba.ali2024m@covm.uobaghdad.edu.iq
Received: 5 July 2025
Accepted: 22 July 2025
Published: 28 December 2025

Abstract

Background: Vulvovaginitis in cows is an important reproductive issue that can affect fertility, cause discomfort, and lead to complications during breeding or calving. This research aims at the molecular identification of non-albicans Candida (NAC) species isolated from cows with vulvovaginitis in Baghdad City, highlighting their genetic diversity and potential clinical implications. **Methodology:** A total of 150 vaginal swab samples were collected from cows suffering from vaginal infection. Identification was done through the VITEK 2 system, and the molecular detection by the PCR assay targeting the internal transcribed spacer (ITS) region was also performed followed by sequencing. Then, the multiplex PCR was used to amplify two key genes associated with biofilm formation in various NAC species, these genes included Agglutinin-Like Sequence 1(ALS1) and Hyphal wall protein 1 (HWP1). Moreover, the PCR assay was used to determine the presence of anti-fungal resistance by targeting the multidrug resistance gene (MDR1). **Results:** Using the Vitek2 system, a yeast-like growth was observed in 17% (26/150) of the samples. Candida tropicalis was the most prevalent species (30.7%), followed by *Candida krusei* (19.2%). Other NAC species included *C. parapsillosis* (7.6%), C. utilis (2.6%), *C. pelluiculosa*, and *C. albicans* (2.6% each). PCR amplification of the ITS region followed by sequencing confirmed the genetic diversity of the isolates, with species-level identification achieved through high nucleotide sequence identity (99–100%) to GenBank references. The findings highlighted the utility of molecular techniques in distinguishing closely related species. The research also identified the presence of the biofilm-associated genes and the multidrug resistance gene in several NAC species, emphasizing their virulence and anti-fungal resistance. Biofilm formation was particularly prominent in *C. tropicalis*, correlating with treatment challenges. **Conclusions:** The findings underscore the rising prevalence and pathogenic potential of NAC species in vulvovaginitis, emphasizing the need for advanced molecular diagnostics to guide effective treatment strategies.

Keywords: Non-albicans Candida, vulvovaginitis, molecular identification, biofilm formation, antifungal resistance

Cite: Hasan, N. A. & Al-haddad, Z. A. (2025). Molecular identification of non-albicans candida isolated from vulvovaginitis of cows. *Experimental and Applied Veterinary Research Journal, 1*(1).

Introduction

Vulvovaginitis, a common gynecological condition, is frequently associated with fungal pathogens, particularly Candida species (Willems *et al*., 2020). While *Candida albicans* has traditionally been recognized as the primary causative agent (Talapko *et al*., 2021), recent studies indicate a growing prevalence of non-albicans Candida (NAC) species in vulvovaginal infections (Makanjuola *et al*., 2018). These NAC species, including *Candida glabrata*, *Candida tropicalis*, *Candida krusei*, and others, often exhibit unique pathogenic mechanisms, higher biofilm-forming capacities, and increased antifungal resistance, posing significant diagnostic and therapeutic challenges (Malinovská *et al*., 2023).

The emergence of NAC species as predominant or co-pathogens reflects a shifting trend in vulvovaginitis etiology (Othman *et al*., 2018), influenced by factors such as overuse of antifungal agents, changes in host immune status, and alterations in the vaginal microbiome (Alwan & Aziz, 2012; Makanjuola *et al*., 2018; Willems *et al*., 2020). This shift necessitates advanced diagnostic approaches to identify fungal pathogens accurately, as conventional methods may lack the sensitivity and specificity to differentiate between Candida species (Pham *et al*., 2024). Molecular techniques, including polymerase chain reaction (PCR) and the internal transcribed spacer (ITS) region sequencing, have proven to be invaluable tools in overcoming these limitations, offering precise and reliable species-level identification (Khot & Fredricks, 2009; Tsui *et al*., 2011). Furthermore, the role of virulence factors, such as biofilm formation and multidrug resistance, in complicating treatment outcomes underscores the importance of studying genetic markers associated with these traits (Satala *et al*., 2021; Kumar *et al*., 2023). Genes such as ALS1, HWP1, and MDR1 have been implicated in enhancing fungal adhesion, biofilm development, and antifungal resistance, particularly in NAC species (Abastabar *et al*., 2016; Jahanshiri *et al*., 2023). The detection and analysis of these genes provide critical insights into the pathogenic potential of NAC species and inform targeted therapeutic strategies (Mohammadi *et al*., 2021).

This research focuses on the molecular identification of NAC species isolated from cows with vulvovaginitis in Baghdad City, highlighting their prevalence, genetic diversity, and pathogenic characteristics. By combining phenotypic methods with molecular diagnostics, this research aims to elucidate the complex role of NAC species in vulvovaginal infections and contribute to developing more effective diagnostic and treatment protocols.

Materials and Methods

Ethics approval

This study was approved by the ethical and research committee of the College of Veterinary Medicine, University of Baghdad, Ministry of Higher Education and Scientific Research (issue No.1928/H.E. on 17/6/2025).

Collection of samples

A total of 150 vaginal swab samples were collected from cows suffering from vaginal infection by a trained veterinarian during routine examinations in Baghdad City using sterile cotton swabs. Informed consent was obtained from all owners. The owners were instructed to avoid using any antifungal medication or douching for at least 48 hours before the sample collection. All swabs were placed in sterile amies transport media (containing agar and balanced salt solution with inorganic phosphates for buffering, sodium thioglycolate to create a reduced environment, and charcoal to neutralize toxic substances) and transferred to the laboratory within 2 hours of collection (Stelzner, 1990; Balows, 2003).

Culture and isolation

All 150 swab samples were inoculated onto Sabouraud Dextrose Agar (SDA) plates (HiMedia, India) supplemented with chloramphenicol (0.05 g/L) to inhibit bacterial growth. The plates were incubated at 37°C for 48-72 hours under aerobic conditions to promote the growth of yeast-like fungi (Balows, 2003).

During the incubation period, the plates were monitored and examined for the presence of yeast-like fungal colonies, which were identified based on morphological characteristics. Suspicious colonies were repeatedly subcultured onto fresh SDA plates to obtain pure isolates. The resulting pure yeast colonies were subjected to a series of morphological and biochemical tests for preliminary identification, including microscopic examination of cell morphology and germ tube testing (Larone, 1994; Deorukhkar *et al*., 2014**)**

VITEK 2 system identification

The VITEK 2 system (bioMérieux, France) was used to identify Candida species from yeast-like isolates obtained from vaginal swab samples of cows with vulvovaginitis. The isolates were suspended in a sterile normal saline to be compared with a McFarland standard tube of 2.0 and inoculated into VITEK 2 YST identification cards containing preloaded biochemical substrates. The inoculated cards were analyzed using the VITEK 2 compact instrument, which matched biochemical profiles with an integrated database for species-level identification. This method enabled the accurate identification of both common and rare Candida species, including *C. tropicalis*, *C. parapsillosis*, and others.

DNA extraction

Genomic DNA was extracted from 17 yeast cultures using the FavorPrep Fungi/Yeast Genomic DNA Extraction Mini Kit (Cat. No. FAFYG 001). The process was conducted according to the manufacturer's instructions (Intron, Korea).

Determination of the DNA quality and concentration

The quality of the extracted DNA was evaluated using agarose gel electrophoresis. 1% agarose gel was prepared by dissolving 1 g of the agarose powder in 100 ml of 1x TBE buffer. The mixture was heated using a hot block until the agarose was completely dissolved, resulting in a clear solution. The agarose solution was then allowed to cool to approximately 50–55°C with intermittent swirling to ensure even cooling. Subsequently, 3 µl of Red Stain was added to the warm gel solution to facilitate visualization of DNA bands. Once prepared, the ends of a gel-casting tray were sealed with two layers of tape, and combs were positioned to create wells. The agarose solution was carefully poured into the casting tray and allowed to solidify at room temperature. After the gel solidified, the combs were gently removed, and the tape was peeled off. The gel was then transferred to an electrophoresis chamber, which was filled with 1x TBE buffer. DNA samples were prepared by mixing 5 µl of each DNA sample with 3 µl of DNA loading buffer. The samples were loaded into the wells of the agarose gel, and electrophoresis was performed at 70V and 65 mA for 1 hour. Upon completion, the DNA bands were visualized using a UV transilluminator.

Primer's preparation

The lyophilized primers (Macrogen/ Korea) used in this research were reconstituted with nuclease-free double-distilled water (ddH2O) to achieve a stock concentration of 100 pmol/µl. The stock solution was stored at -20°C. For PCR amplification, a working primer solution was prepared by diluting 10 µl of the stock solution with 90 µl of ddH2O, resulting in a final concentration of 10 pmol/µl.

Two sets of primers targeting the ITS region designed by White *et al*. (1990) were utilized for PCR amplification. Multiplex PCR was employed to detect and differentiate *Candida* species by amplifying the ALS1 and HWP1 (Mohammadi *et al*., 2021). Primers specific for the MDR1 gene (Jin *et al*., 2018) were amplified by the conventional PCR. The sequences of the primers, along with their properties and expected product sizes, are summarized in Table 1.

Table 1: The sequence and properties of primers used in this study

Primer		Sequence	Tm (°C)	GC%	Size of Product (bp)
ITS1	F	5′- TCCGTAGGTGAACCTGCGG -3′	60.30	50.00	525-879
ITS4	R	5′- TCCTCCGCTTATTGATATGC-3′	57.80	41.00	
ALS1	F	5′- ACCAGAAGAAACAGCAGGTG-3′	58.03	50.00	319
	R	5′- GACTAGTGAACCAACAAATACCAG-3′	57.52	41.67	
HWP1	F	5′- ATGACTCCAGCTGGTTC-3′	53.08	52.94	503
	R	5′- TAGATCAAGAATGCAGC-3′	48.30	41.18	
MDR1	F	5′GGGTGCATCATTCCAGCCTA-3′	59.82	55.00	189
	R	5′ GGGATGGCAATCATCACGAG-3′	58.77	55.00	

PCR Reaction components

The PCR reaction mix was prepared with a final volume of 25 µl. Table 2outlines the components and their respective volumes used for each PCR reaction.

Table 2: Reaction components for PCR amplification

Component	Volume (Final)
Taq PCR PreMix (5x)	5.00 µl
Forward primer (10 pmol/µl)	1.00 µl
Reverse primer (10 pmol/µl)	1.00 µl
DNA template	1.50 µl
Distilled water	16.5 µl
Total Volume	25.0 µl

PCR conditions

The PCR amplification was performed using the conditions listed in Table 3.

Table 3: Thermal cycling conditions for PCR

Step	Temperature (°C)	Time	Cycles
Initial Denaturation	95	5 minutes	1
Denaturation	95	45 seconds	35
Annealing	54	1 minute	35
Extension	72	1 minute	35
Final Extension	72	5 minutes	1

ITS region sequencing and data analysis

Seventeen samples were sent to Macrogen/Korea and been analyzed and registered by the National Center for Biotechnology Information (NCBI).

Results

Isolation and identification of yeast-like fungi

Yeast-like growth was observed in 17% (26/150) of the cow vaginal swab samples. The colonies after incubation at 37°C for 48 hours, were characterized by the appearance of creamy and robust, shiny, and smooth colonies which were consistent with yeast morphology, Figure 1(a) strongly suggests the presence of yeast-like fungal growth and this agrees with Hadi & AlSultany (2020), Risan (2022) and Jarallah & Al-Haddad (2022). The consistency in yeast-like growth indicates the stability and viability of the isolated strain (Figure 1 b). This consistency across subcultures is crucial, as it confirms the isolate's identity and ensures its reliability for further analysis (Hadi & AlSultany, 2020).

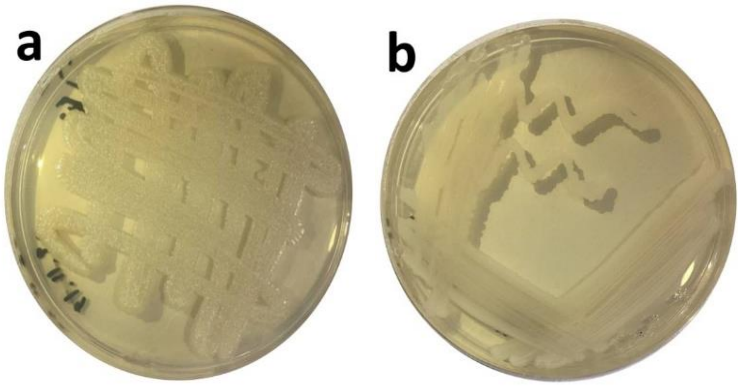

Figure 1: Yeast-like growth, a- after inoculating vaginal swab on SDA for 48 hours at 37ºC, b- after subculturing for 3 times

VITEK 2 system identification of NAC

In this research, the VITEK 2 system confirmed the isolation of yeast species from 26 swabs from cows with vulvovaginitis. The results revealed a diverse range of Candida and other yeast species, highlighting the complexity of vulvovaginitis etiology (Eleutério *et al*., 2023). The most prevalent species identified was *C. tropicalis*, accounting for eight isolates out of 26 samples (30.77%) (Table 4). This observation aligns with the typical characteristics of *C. tropicalis*, which are known to cause vulvovaginal candidiasis (Habib *et al*., 2016). These findings are consistent with recent literature indicating an increasing incidence of NAC species in vulvovaginitis cases (Makanjuola *et al*., 2018; Mushi *et al*., 2022). Such percentages may indicate their responsibilities (virulence and pathogeny) for the occurrence of vulvovaginitis (Sardi *et al*., 2013).

Table 4: Results of the VITEK 2 System for identification of yeast-like fungi in cows

Species	No. and percentage of the isolates	Percentage%
	No.	
Candida tropicalis	8	30.7
Candida krusei	5	19.2
Candida parapsilosis	2	7.6
Candida utilis	1	2.6
Candida pelliculosa	1	2.6
Candida albicans	1	2.6
Rhodotorula	2	5.2
Millerozyma farinose	6	15.6
Total	26	100
P-value	0.0266 *	

* (P<0.05).

Molecular identification of NAC

The gel electrophoresis for the PCR products is shown in Figure 2, which illustrates the molecular identification of various NAC species isolated from vulvovaginitis of cows in Baghdad City. The DNA fragments amplified by PCR using primers targeting the ITS region showed product sizes of 525–618 bp, which were determined following nucleotide sequencing performed for these PCR products. The resulting product sizes corresponded to species-specific bands.

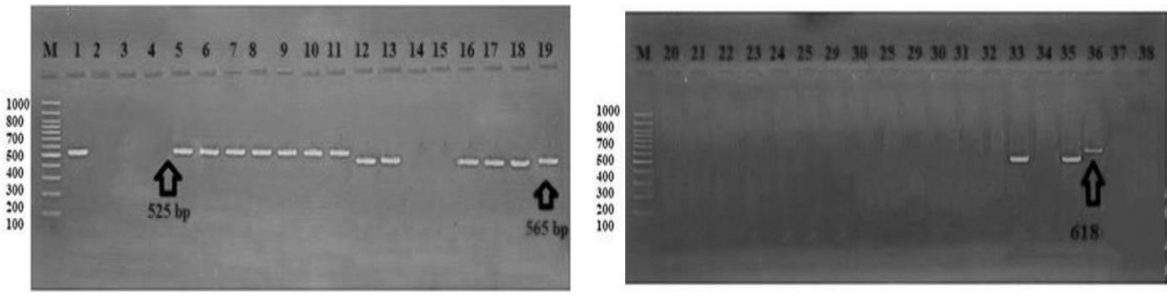

Figure 2: Gel electrophoresis of the PCR products using primers targeting the ITS region shows bands of 525–618 bp of NAC species isolated from cow vulvovaginitis. The upper gel (lanes 1, 5–11) represent *C. tropicalis*. Lanes (12,13,16-18) correspond to *C. krusei*. Lane 19 corresponds to C. utilis. Lanes 33 and 35 correspond to *C. parapsilosis*. Finally, lane 36 corresponds to *C. pelliculosa*

These findings observed for *C. tropicalis*, with a product length of 525 bp, align closely with previous research, such as the work of Maikan *et al*. (2022) in Iraq, who reported a similar length. Also, Mirhendi *et al*. (2006) reported approximately 524 bp for *C. tropicalis*. This consistency reinforces the reliability of this research methodology and supports the accuracy of *C. tropicalis* identification in clinical settings. Similarly, the results for *C. krusei* and *C. parapsilosis*, with product lengths of 509 bp and 523 bp, respectively, show strong agreement with existing literature. Fujita *et al*. (2001) using ITS1-ITS4 primer regions reported approximately 496–503bp for *C. krusei* and about 511–521bp for *C. parapsilosis*. Mirhendi *et al.* (2006) reported approximately 510 bp for *C. krusei* and 520 bp for *C. parapsilosis*. These correlations across different studies underscore the robustness of PCR-based identification for these species (Yang & Rothman, 2004).

The results demonstrate efficient molecular identification of various NAC species using PCR, with amplicon sizes specific to each species. Key investigation findings were that ITS region identification result was 100% consistent

with the Vitek2 results. This molecular approach is crucial for distinguishing between Candida species that may present similarly in clinical infections but require different treatment approaches due to varying antifungal susceptibilities (García Salazar *et al*., 2022; Ortiz *et al*., 2018).

Biofilm gene detection in NAC isolates

The gel electrophoresis results demonstrate the successful amplification of two key genes associated with biofilm formation in various NAC species, these genes included ALS1 (319 bp) and HWP1 (503 bp) (Figure 3). This aligns with Mohammed *et al.* (2021). This duplex PCR approach effectively identified these genes across multiple Candida species, including *C. tropicalis* (lanes 1-8) and *C. parapsilosis* (lane 17). The presence of both ALS1 and HWP1 genes in these isolates is significant, as these genes play crucial roles in biofilm formation and adherence to host tissues (Roudbarmohammadi *et al*., 2016; Mohammadi *et al.,* 2021; Gerges *et al*., 2023).

Figure 3: Duplex PCR analysis of biofilm-related genes, including ALS1 (319 bp) and HWP1 (503 bp) in NAC Species. M: DNA ladder (100-1000 pb) (Kapa/USA). Lanes 1-8 are 1*C. tropicalis*, lane 17 is *C. parapsilosis*

The ALS1 (Agglutinin-Like Sequence 1) gene is part of the ALS gene family, which encodes cell-surface glycoproteins involved in adhesion to host surfaces (Hoyer *et al*., 2008), while HWP1 (Hyphal Wall Protein 1) is particularly important in developing hyphal and biofilm (Abastabar *et al*., 2016). The detection of these genes across multiple NAC species isolated from vulvovaginitis cases in cows highlights the potential for cross-species transmission and its importance in epidemiological studies. This finding aligns with previous research suggesting that NAC species are becoming increasingly prevalent in vulvovaginitis cases and may possess enhanced virulence factors contributing to antifungal resistance (Deorukhkar *et al*., 2014; Czechowicz *et al*., 2022).

The consistent presence of both ALS1 and HWP1 in these isolates suggests a possible correlation between biofilm formation capability and antifungal resistance (Gerges *et al*., 2023). This relationship has been observed in other research, where biofilm-forming Candida isolates often exhibit higher levels of antifungal resistance compared to their planktonic counterparts (Silva *et al*., 2017; Ajetunmobi *et al*., 2023). Furthermore, the identification of these genes in *C. tropicalis* (lanes 1-8), is particularly noteworthy, as this species is inherently more resistant to azole antifungals and has been associated with recurrent infections, and this is compatible with what Rodrigues *et al*. (2014) found in their research about *C. glabrata*. The presence of biofilm-related genes in *C. tropicalis* isolates may contribute to its persistence and treatment challenges in vulvovaginitis cases (Boahen *et al*., 2022) caused by NAC. The presence of ALS1 and HWP1 genes across multiple species underscores the importance of considering biofilm formation as a virulence factor in these infections (Corbu *et al*., 2024).

Additionally, Figure 4 demonstrates the electrophoresis results of PCR amplification of the Multidrug Resistance1 (MDR1) gene in biofilm-producing *C. tropicalis* isolates. This gene encodes a membrane-bound efflux pump that belongs to the major facilitator superfamily (MFS) (Jahanshiri *et al*., 2023). This gene product was roughly 189 bp, as shown in Figure 4. These results indicate a correlation between biofilm formation and multidrug resistance in C. tropicalis isolates. The detection of this gene in *C. tropicalis* aligns with the study of Jahanshiri *et al.* (2023) that associated biofilm formation with multidrug resistance in NAC species. Jahanshiri *et al*. (2023) found that biofilm-producing *C. tropicalis* isolates exhibited enhanced expression of the MDR1 efflux pump gene, contributing to resistance against azoles and other antifungal agents.

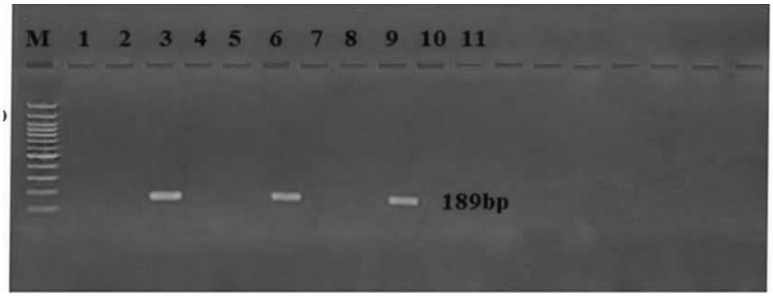

Figure 4: Agarose gel electrophoresis shows bands of the PCR amplified MDR1 gene in C. tropicalis. The electrophoresis was conducted for 1.5% agarose gel at 5 volt/cm2 for 1:30 hours. M: DNA ladder (100-1000 bp). The expected bands of 189 bp are shown in lanes 3, 6, and 9

ITS region sequencing for some NAC isolates

The sequencing results of the ITS region in five isolates of NAC from vulvovaginal infection of cows provided molecular evidence for the identification of several Candida species, emphasizing their genetic variability (Shirvani & Fattahi, 2021). Each sequence comparison revealed a high identity (99-100%) with established GenBank references, indicating reliable species-level identification Table 5.

Table 5: Summary of the molecular identification and nucleotide substitutions in NAC isolates based on the ITS region sequencing

Sample No.	Type of substitution	Location	Nucleotide substitution	Source	Compared sequence ID	Accession No.	Identities
1	Transvertion	236	A/T	*C. tropicalis*	LC317536.1	PQ288699,1	0.99
2	Transvertion	290	G/C	*C. krusei (pichia kudriavzevii)*	MT366877.1	PQ288702.1	0.99
3	Transvertion	398	T/A	*C. utilis (Cyberlindnera jadinii)*	MT192797.1	PQ288700.1	0.99
4	Transition	336	A/G	*C. parapsilosis*	MT153735.1	PQ288703.1	0.99
5	Transition	396	G/A	*C. pelliculosa (Wickerhamomyces anomalus)*	KJ527050.1	PQ288701.1	0.99

The application of ITS region sequencing in the molecular identification of NAC species has proven to be a highly reliable and precise approach, particularly in differentiating closely related species (Cornet *et al*., 2011). This study highlights the genetic variability and species-specific nucleotide substitutions observed in various Candida isolates, providing critical insights into their molecular profiles and clinical significance. The ITS region is widely recognized for its interspecies variability and intraspecies conservation, making it an ideal target for fungal identification (Qi *et al*., 2024). In this study, the sequencing results revealed high identity percentages (99-100%) with established GenBank references for the most prominent isolates, confirming the robustness of ITS sequencing in species-level identification. The observed nucleotide substitutions, including transitions and transversions, emphasize the genetic variability within the ITS region that facilitates the differentiation of closely related Candida species. These findings align with previous reports that underscore the ITS region's utility in resolving taxonomic ambiguities among fungi (Nilsson *et al*., 2019).

Conclusions

In conclusion, this study underscores the growing clinical significance of NAC species in vulvovaginitis, with C. tropicalis emerging as the most prevalent pathogen. Molecular identification through ITS sequencing not only confirmed species diversity but also revealed genetic variations contributing to antifungal resistance and biofilm formation, highlighting potential therapeutic challenges. The detection of virulence and multidrug resistance genes further emphasizes the pathogenic potential of NAC species. These findings call for the integration of molecular diagnostics into routine clinical practice to enhance species-level identification and optimize treatment strategies. Continued research into NAC resistance mechanisms and ecological dynamics is essential to mitigate their impact, particularly in resource-constrained environments.

Acknowledgements

This work was supported by the College of Veterinary Medicine, Department of Veterinary Public Health, Zoonoses Research Unit, University of Baghdad, Iraq.

Conflicts of Interest

The authors declare no conflict of interest.

References

Abastabar, M., Hosseinpoor, S., Hedayati, M. T., Shokohi, T., Valadan, R., Mirhendi, H., Mohammadi, R., Aghili, S. R., Rahimi, N., Aslani, N., Haghani, I., & Gholami, S. (2016). Hyphal wall protein 1 gene: A potential marker for the identification of different Candida species and phylogenetic analysis. *Current Medical Mycology, 2*(4), 1–8. https://doi.org/10.18869/acadpub.cmm.2.4.

Ajetunmobi, O. H., Badali, H., Romo, J. A., Ramage, G., & Lopez-Ribot, J. L. (2023). Antifungal therapy of *Candida* biofilms: Past, present and future. *Biofilm, 5*, 100126. https://doi.org/10.1016/j.bioflm.2023.100126

Alwan, M. J., & Aziz, M. M. (2012). Isolation and identification of Candida species from urogenital tract infections of women in Baghdad city. *The Iraqi Journal of Veterinary Medicine*, *36*(0E), 115–119. https://doi.org/10.30539/iraqijvm.v36i0E.404

Balows, A. (2003). Manual of clinical microbiology (8th ed.; P. R. Murray, E. J. Baron, J. H. Jorgenson, M. A. Pfaller, & R. H. Yolken, Eds.). ASM Press. *Diagnostic Microbiology and Infectious Disease, 47*(4), 625–626. https://doi.org/10.1016/S0732-8893(03)00160-3

Boahen, A., Than, L. T. L., Loke, Y. L., & Chew, S. Y. (2022). The antibiofilm role of biotics family in vaginal fungal infections. *Frontiers in Microbiology, 13*, 787119. https://doi.org/10.3389/fmicb.2022.787119

Corbu, V. M., Georgescu, A.-M., Marinas, I. C., Pericleanu, R., Mogos, D. V., Dumbravă, A. Ș., Marinescu, L., Pecete, I., Vassu-Dimov, T., Czobor Barbu, I., Csutak, O., Ficai, D., & Gheorghe-Barbu, I. (2024). Phenotypic and genotypic characterization of resistance and virulence markers in Candida spp. isolated from community-acquired infections in bucharest, and the impact of agnps on the highly resistant isolates. *Journal of Fungi, 10*(8), 563. https://doi.org/10.3390/jof10080563

Cornet, M., Sendid, B., Fradin, C., Gaillardin, C., Poulain, D., & Nguyen, H. V. (2011). Molecular identification of closely related Candida species using two ribosomal intergenic spacer fingerprinting methods. *Journal of Molecular Diagnostics, 13*(1), 12–22. https://doi.org/10.1016/j.jmoldx.2010.11.014

Czechowicz, P., Nowicka, J., & Gościniak, G. (2022). Virulence factors of Candida spp. and host immune response important in the pathogenesis of vulvovaginal candidiasis. *International Journal of Molecular Sciences, 23*(11), 5895. https://doi.org/10.3390/ijms23115895

Deorukhkar, S. C., Saini, S., & Mathew, S. (2014a). Virulence factors contributing to pathogenicity of *Candida tropicalis* and its antifungal susceptibility profile. *International Journal of Microbiology, 2014*, 456878. https://doi.org/10.1155/2014/456878

Deorukhkar, S. C., Saini, S., & Mathew, S. (2014b). Non-albicans Candida infection: An emerging threat. *Interdisciplinary Perspectives on Infectious Diseases, 2014*, 615958. https://doi.org/10.1155/2014/615958

Eleutério, J. Jr., Campaner, A. B., & de Carvalho, N. S. (2023). Diagnosis and treatment of infectious vaginitis: Proposal for a new algorithm. *Frontiers in Medicine (Lausanne), 10*, 1040072. https://doi.org/10.3389/fmed.2023.1040072

Fujita, S. I., Senda, Y., Nakaguchi, S. & Hashimoto, T. (2001). Multiplex PCR using internal transcribed spacer 1 and 2 regions for rapid detection and identification of yeast strains. *Journal of Clinical Microbiology, 39*(10):3617-22. https://doi.org/10.1128/JCM.39.10.3617-3622.2001

García-Salazar, E., Acosta-Altamirano, G., Betancourt-Cisneros, P., Reyes-Montes, M. D. R., Rosas-De-Paz, E., Duarte-Escalante, E., Sánchez-Conejo, A. R., Ocharan Hernández, E., & Frías-De-León, M. G. (2022). Detection and molecular identification of eight Candida species in clinical samples by simplex PCR. *Microorganisms, 10*(2), 374. https://doi.org/10.3390/microorganisms10020374

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

Habib, K. A., Najee, E. N., & Abood, M. S. (2016). Identification of Candida species isolated from vulvovaginal candidiasis patients by Chromgen agar and PCR-RFLP method. *Baghdad Science Journal, 13*(2), 12. https://doi.org/10.21123/bsj.2016.13.2.0291

Hadi, H. S., & AlSultany, S. J. (2020). Isolation and identification of Candida species among renal failure Iraqi patients. *Drug Invention Today, 14*(6), 1182–1188.

Hoyer, L. L., Green, C. B., Oh, S. H., & Zhao, X. (2008). Discovering the secrets of the *Candida albicans* agglutinin-like sequence (ALS) gene family—a sticky pursuit. *Medical Mycology, 46*(1), 1–15. https://doi.org/10.1080/13693780701435317

Jahanshiri, Z., Manifar, S., & Hatami, F. (2023). Evaluation of virulence factors and azole resistance mechanisms of *Candida tropicalis* isolates from head and neck cancer patients with OPC. *Iranian Journal of Microbiology, 15*(1), 163–173. https://doi.org/10.18502/ijm.v15i1.11933

Jarallah, M. A., & Al-Haddad, Z. A. (2022). Isolation and molecular detection of *Candida albicans* from human and domesticated small animals in Baghdad, Iraq. *Biochemical and Cellular Archives, 22*(1), 2085-2092. https://connectjournals.com/03896.2022.22.2085

Jin, L., Cao, Z., Wang, Q., Wang, Y., Wang, X., Chen, H., & Wang, H. (2018). MDR1 overexpression combined with ERG11 mutations induce high-level fluconazole resistance in *Candida tropicalis* clinical isolates. *BMC Infectious Diseases, 18*, 162. https://doi.org/10.1186/s12879-018-3082-0

Khot, P. D., & Fredricks, D. N. (2009). PCR-based diagnosis of human fungal infections. *Expert Review of Anti-infective Therapy, 7*(10), 1201–1221. https://doi.org/10.1586/eri.09.104

Kumar, H., Mukherjee, D., Banerjee, S., Upadhyay, P., Sharma, V., & Akilimali, A. (2023). *Candida auris*: An emerging multidrug-resistant fungal pathogen in the United States and the urgent call for action. *Microbiology Insights, 16*, 11786361231200836. https://doi.org/10.1177/11786361231200836

Larone, D. H. (1994). Medically important fungi. *Revista do Instituto de Medicina Tropical de São Paulo, 36*(4):362. https://doi.org/10.1590/S0036-46651994000400019

Maikan, H. K., Jabbar, S., & Al-Haishawi, H. (2022). Isolation and identification of *Candida* tropicalis as a cause of cutaneous candidiasis in Kalar District, Iraq. *Archives of Razi Institute, 77*(4), 1377–1382.

Makanjuola, O., Bongomin, F., & Fayemiwo, S. A. (2018). An update on the roles of non-albicans Candida species in vulvovaginitis. *Journal of Fungi,* 4(4), 122. https://doi.org/10.3390/jof4040121

Malinovská, Z., Čonková, E., & Váczi, P. (2023). Biofilm formation in medically important Candida species. *Journal of Fungi, 9*(10), 955. https://doi.org/10.3390/jof9100955

Mirhendi, H., Makimura, K., Khoramizadeh, M., & Yamaguchi, H. (2006). A one-enzyme PCR–RFLP assay for identification of six medically important Candida species. *Nihon Ishinkin Gakkai Zasshi, 47*(3), 225–229. https://doi.org/10.3314/jjmm.47.225

Mohammadi, F., Hemmat, N., Bajalan, Z., & Javadi, A. (2021). Analysis of biofilm-related genes and antifungal susceptibility pattern of vaginal Candida albicans and non-*Candida* albicans species. *BioMed Research International, 2021*, 5598907,9. https://doi.org/10.1155/2021/5598907

Mohammed, N. A., Ajah, H. A., & Abdulbaqi, N. J. (2021). Detection the prevalence of adhesins and extracellular hydrolytic enzymes genes in *Candida albicans* biofilm formation. *Iraqi Journal of Science, 58*(2C), 988–1000. https://ijs.uobaghdad.edu.iq/index.php/eijs/article/view/5931/1870

Mushi, M. F., Olum, R., & Bongomin, F. (2022). Prevalence, antifungal susceptibility and etiology of vulvovaginal candidiasis in sub-Saharan Africa: A systematic review with meta-analysis and meta-regression. *Medical Mycology, 60*(7), myac004. https://doi.org/10.1093/mmy/myac037

Nilsson, R. H., Larsson, K. H., Taylor, A. F. S., Bengtsson-Palme, J., Jeppesen, T. S., Schigel, D., Kennedy, P., Picard, K., Glöckner, F. O., Tedersoo, L., Saar, I., Kõljalg, U., & Abarenkov, K. (2019). The UNITE database for molecular identification of fungi: Handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research, 47*(D1), D259–D264. https://doi.org/10.1093/nar/gky1022

Odds, F. C., & Bernaerts, R. (1994). CHROMagar Candida, a new differential isolation medium for presumptive identification of clinically important Candida species. *Journal of Clinical Microbiology, 32*(8), 1923–1929. https://doi.org/10.1128/jcm.32.8.1923-1929.1994

Othman, K. I., Abdullah, S. M., Ali, B., & Majid, M. (2018). Isolation and identification of Candida spp. from urine and antifungal susceptibility testing. *Iraqi Journal of Science, 59*(4B), 1981–1988. https://doi.org/10.24996/ijs.2018.59.4B.3

Ortiz, B., Pérez-Alemán, E., Galo, C., & Fontecha, G. (2018). Molecular identification of Candida species from urinary infections in Honduras. *Revista Iberoamericana de Micología, 35*(2), 73–77. https://doi.org/10.1016/j.riam.2017.07.003

Pham, D., Sivalingam, V., Tang, H. M., Montgomery, J. M., Chen, S. C., & Halliday, C. L. (2024). Molecular diagnostics for invasive fungal diseases: current and future approaches. *Journal of Fungi, 10*(7), 447. https://doi.org/10.3390/jof10070447

Qi, G., Hao, L., Xin, T., Gan, Y., Lou, Q., Xu, W., & Song, J. (2024). Analysis of whole-genome facilitates rapid and precise identification of fungal species. *Frontiers in Microbiology, 15*, 1336143. https://doi.org/10.3389/fmicb.2024.1336143

Risan, M. H. (2022). Molecular identification of yeast *Candida glabrata* from candidemia patients in Iraq. *Iraqi Journal of Science, 57*(2A), 808–813.

Rodrigues, C. F., Silva, S., & Henriques, M. (2014). *Candida glabrata*: A review of its features and resistance. *European Journal of Clinical Microbiology & Infectious Diseases, 33*(5), 673–688. https://doi.org/10.1007/s10096-013-2009-3

Roudbarmohammadi, S., Roudbary, M., Bakhshi, B., Katiraee, F., Mohammadi, R., & Falahati, M. (2016). ALS1 and ALS3 gene expression and biofilm formation in *Candida albicans* isolated from vulvovaginal candidiasis. *Advances in Biomedical Research, 5*, 105. https://doi.org/10.4103/2277-9175.183666

Sardi, J. C. O., Scorzoni, L., Bernardi, T., Fusco-Almeida, A. M., & Mendes Giannini, M. J. S. (2013). Candida species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. *Journal of Medical Microbiology, 62*(Pt 1), 10–24. https://doi.org/10.1099/jmm.0.045054-0

Satala, D., Gonzalez-Gonzalez, M., Smolarz, M., Surowiec, M., Kulig, K., Wronowska, E., Zawrotniak, M., Kozik, A., Rapala-Kozik, M., & Karkowska-Kuleta, J. (2021). The role of *Candida albicans* virulence factors in the formation of multispecies biofilms with bacterial periodontal pathogens. *Frontiers in Cellular and Infection Microbiology, 11*, 765942. https://doi.org/10.3389/fcimb.2021.765942

Shirvani, F., & Fattahi, M. (2021). Molecular identification of Candida species isolated from candiduria and its risk factors in neonates and children. *Current Medical Mycology, 7*(3), 9–12. https://doi.org/10.18502/cmm.7.3.7799

Silva, S., Rodrigues, C. F., Araújo, D., Rodrigues, M. E., & Henriques, M. (2017). Candida species biofilms' antifungal resistance. *Journal of Fungi, 3*(1), 8. https://doi.org/10.3390/jof3010008

Stelzner, A. (1990). Review of the book Candida and Candidosis: A review and bibliography (2nd ed.), by F. C. Odds. *Journal of Basic Microbiology, 30*(5), 367. https://doi.org/10.1002/jobm.3620300522

Talapko, J., Juzbašić, M., Matijević, T., Pustijanac, E., Bekić, S., Kotris, I., & Škrlec, I. (2021). *Candida albicans* the virulence factors and clinical manifestations of infection. *Journal of Fungi, 7*(2), 79. https://doi.org/10.3390/jof7020079

Tsui, C. K., Woodhall, J., Chen, W., Lévesque, C. A., Lau, A., Schoen, C. D., Baschien, C., Najafzadeh, M. J., & de Hoog, G. S (2011). Molecular techniques for pathogen identification and fungus detection in the environment. *IMA Fungus, 2*(2), 177–189. https://doi.org/10.5598/imafungus.2011.02.02.09

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, p. 315–322. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (ed.), PCR protocols: a guide to methods and applications. Academic Press, San Diego, Calif.

Williams, D. W., Wilson, M. J., Lewis, M. A. O., & Potts, A. J. (1995). Identification of Candida species by PCR and restriction fragment length polymorphism analysis of intergenic spacer regions of ribosomal DNA. *Journal of Clinical Microbiology, 33*(9):2476-2479. https://doi.org/10.1128/jcm.33.9.2476-2479.1995

Willems, H. M. E., Ahmed, S. S., Liu, J., Xu, Z., & Peters, B. M. (2020). Vulvovaginal candidiasis: A current understanding and burning questions. *Journal of Fungi, 6*(1), 27. https://doi.org/10.3390/jof6010027

Yang, S., & Rothman, R. E. (2004). PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings. *The Lancet Infectious Diseases, 4*(6), 337–348. https://doi.org/10.1016/S1473-3099(04)01044-8.

التعريف الجزيئي للمبيضات غير البيضاء المعزولة من التهاب الفرج والمهبل في الابقار

نبأ علي حسن[1]، زينب عبد الزهرة عباس الحداد[1]

[1]وحدة بحوث الامراض المشركة، فرع الصحة العامة البيطرية، كلية الطب البيطري، جامعة بغداد، العراق

المستخلص

الخلفية: التهاب الفرج والمهبل في الأبقار هو مشكلة تناسلية مهمة من الممكن أن تؤثر الخصوبة مسببة عدم الراحة وتؤدي إلى مضاعفات خلال الإنتاج، هذا البحث يهدف إلى التشخيص الجزيئي لأنواع المبيضات غير البيضاء المعزولة من أبقار مصابة بالتهاب الفرج والمهبل في مدينة بغداد، وتنوعها الجيني ومضاعفاتها السريرية المحتملة. **المنهجية:** جمعت 150 مسحة مهبلية، من أبقار مصابة بالتهاب المهبل. جرى التشخيص باستخدام جهاز Vitek2وجرى التشخيص الجزيئي باستخدام تقنية PCR عن طريق استهداف منطقة ITS وأيضا أجري التحري عن تسلسل النيوكليوتيدات، وبعدها استخدمMultiplex PCR لتضخيم جينين رئيسين مرتبطان بتكوين الأغشية الحيوية بمختلف أنواع NAC هذه الجينات تضمنت (ALS1) و. (HWP1) أيضا استخدمت تقنية ال PCR للتحري عن وجود مقاومة لمضادات الفطريات عن طريق استهداف جين .MDR1 **النتائج:** أظهرت نتائج VITEK 2أن 17% من العينات (150/26) تحوي على نمو الخمائر. النوع الأكثر شيوعاً هو C. tropicalis بنسبة 30.7% تلتها *C. krusei* بنسبة 19.2% وشملت الأنواع الأخرى من المبيضات غير البيضاء كلاً من(7.6%) *C. parapsilosis*، (2.6%) *C. utilis*، *C. pelliculosa*و C. albicansبنسبة (2.6%) لكل منهما. تم تأكيد التشخيص الجزيئي باستخدام تقنية PCR لمنطقة ITS، متبوعة بتسلسل الحمض النووي، مما أظهر تنوعًا جينيًا ملحوظًا بين العزلات. وقد أُنجزت عملية التشخيص على مستوى النوع من خلال وجود تطابق عالي في تسلسل النيوكليوتيدات (90-100%) مع التسلسلات المرجعية في قاعدة بيانات GenBank وُثقت حصول استبدالات مهمة في النيوكليوتيدات. تُبرز هذه النتائج فاعلية التقانات الجزيئية في التمييز بين الأنواع المتقاربة من حيث البنية الوراثية. كما كشف البحث عن وجود جينات مرتبطة بتكوين الأغشية الحيوية ALS1 وHWP1، بالإضافة إلى جينات مقاومة متعددة للأدوية (MDR1) في عدد من أنواع NAC، مما يؤكد على ضراوتها ومقاومتها للعلاجات الفطرية. وكان تكوين الأغشية الحيوية أكثر وضوحًا في *C. tropicalis*، ما يرتبط بصعوبات في العلاج. **الاستنتاجات:** تؤكد هذه النتائج على الانتشار المتزايد والقدرة الممرضة المرتفعة لأنواع المبيضات غير البيضاء في حالات التهاب الفرج والمهبل، مما يستدعي الحاجة إلى اعتماد تقنيات تشخيص جزيئية متقدمة لتوجيه الاستراتيجيات العلاجية بفعالية.

الكلمات المفتاحية: المبيضات غير البيضاء، التهاب الفرج والمهبل، الاغشية الحيوية، تقنيات جزيئية، مقاومة المضادات الفطرية