International Journal of Microbiology and Mycology | IJMM |
pISSN: 2309-4796
http://www.innspub.net
Vol. 19, No. 4, p. 1-7, 2024

Open Access RESEARCH PAPER

Prevalence of molecular markers of virulence in *Candida albicans* strains from *Vulvovaginal* infections in Abidjan, Côte d'Ivoire

Akpa Paterne Gnagne[*1], Abibatou Konaté-Touré[1,2], Valérie Akoua Bédia-Tanoh[1,2], Fulgence Kondo Kassi[2,3], Estelle Gnanyo Massafoma Koné[2,3], Hervé Eby Ignace Menan[2,3], William Yavo[1,2]

[1]*Malaria Research and Control Centre, National Institute of Public Health, Abidjan, Côte d'Ivoire*

[2]*Department of Parasitology, Mycology, Animal Biology and Zoology, Felix Houphouët-Boigny University, Abidjan, Côte d'Ivoire*

[3]*Laboratory of Parasitology and Mycology of the Diagnosis and Research Centre on AIDS and the others infectious diseases/Teaching Hospital of Treichville, Abidjan, Cote d'Ivoire*

Keywords: *Candida albicans,* HWP1 and ALS1, Virulence, *Vulvovaginal candidiasis*

Publication date: October 08, 2024

Abstract

Candidiasis is the term for disseminated, visceral, and mucocutaneous infections. The majority of these infections are caused by the species *Candida albicans*. *C. albicans* can infect such diverse host niches because it is supported by a variety of virulence factors and fitness attributes. These factors, particularly biofilm production, may explain treatment failures; for example, biofilm-producing strains show increased resistance to antifungal drugs and host immunity. Several genes are thought to promote biofilm formation, including those for hyphal wall protein 1 *(hwp1),* agglutinin-like sequence 1 (*ALS1*), and agglutinin-like sequence 3 *(ALS3)*. This research aimed to detect the prevalence of virulence markers (*hwp1* and *ALS1*) of *C. albicans* that were isolated from patients with vulvovaginal candidiasis. DNA extracts of *C. albicans* were obtained from the conserved strains. These strains were then confirmed through the application of molecular biology. The *hwp1* gene was observed in 95.8% of *C. albicans*, and the *ALS1* gene in 97.9%. The simultaneous presence of *ALS1* and *hwp1* genes was observed in 94.4% of *C. albicans* species. As the *hwp1* and *ALS1* genes play important roles in biofilm formation, which adds to the virulence of *C. albicans,* they could be targets for vulvovaginal candidiasis therapy.

* **Corresponding Author:** Akpa Paterne Gnagne ✉ gap75m@yahoo.fr

Introduction

The term candidiasis is used for a wide range of deep and superficial mycoses that are caused by the fungus *Candida*. Candidiasis occurs as disseminated, visceral, or mucocutaneous infections. These infections can occur at any age and are risk factors for pathogenic and opportunistic infections (Diekema *et al*., 2012; Segal and Frenkel, 2018). Approximately 20 of 200 known *Candida* species are associated with human infections (Berkow and Lockhart, 2017). The majority of these infections are caused by the species *Candida albicans* (Djohan *et al*., 2012).

C. albicans infects such diverse host niches because it is supported by a variety of virulence factors and fitness attributes. Several attributes, including morphological transition between yeast and hyphal forms, cell-surface expression of adhesins and invasins, thigmotropism, biofilm formation, phenotypic switching and the secretion of hydrolytic enzymes, are considered virulence factors (Mayer *et al*., 2013). These factors, particularly biofilm production, may explain treatment failures. In support of this hypothesis, biofilm-producing strains show increased resistance to antifungal drugs and host immunity (Mathé and Van Dijck, 2013; Mohammadi *et al.*, 2021). Several genes are thought to promote biofilm formation, such as hyphal wall protein 1 (*hwp1*), agglutinin-like sequence 1 (*ALS1*), and agglutinin-like sequence 3 (*ALS3*). In addition, the release of extracellular hydrolytic enzymes increases the virulence of the fungus (Dawoud *et al*., 2024).

It is essential to know the antifungal susceptibility profiles of *Candida* species to select appropriate treatment for vulvovaginal candidiasis. HWP1 is a mannoprotein that is linked to glycosylphosphatidylinositol (GPI). Similar to the protein encoded by the *ALS* gene family, it plays an important role in *Candida* adhesion. This protein may be important in the pathogenicity and virulence of *C. albicans* (Mohammadi *et al.*, 2021). Several studies have shown that the gene that produces HWP1 is expressed during the early stages of biofilm formation. There is little extant data on virulence factors associated with strains of *C. albicans* isolated in Côte d'Ivoire.

This research aimed to detect the prevalence of the virulence markers *HWP1* and *ALS1* of *C. albicans* isolated from patients in the Côte d'Ivoire who had vulvovaginal candidiasis.

Materials and methods

Study material

The biological material used in this work was DNA extracted from *C. albicans* clusters. These had been obtained from vaginal swabs and stored at -20°C in the laboratory of the Centre de Diagnostic et de Recherche sur le SIDA et les autres maladies infectieuses (CeDReS) after culture on fresh Sabouraud chloramphenicol agar and incubation at 37°C for 24 to 72 hours for morphological identification.

Patient selection

The colony from which DNA was extracted came from vaginal swabs taken from patients who had attended the gynaecology departments of the General Hospital of Adjamé in Abidjan) and the Anti-Venereal Dispensary. These were patients who had lesions suggestive of vulvovaginitis.

Sample collection

Sterile cotton swabs that had been wetted with sterile physiological saline were used by the physician to collect vaginal specimens. The posterior fornix was swabbed after speculum placement. At the laboratory, the swab was used to inoculate the culture medium.

DNA extraction

Before analysis, one to two drops of stored strains of *C. albicans* were transferred back under strict aseptic conditions onto fresh Sabouraud

chloramphenicol agar and incubated at 37°C for 24 to 72 hours. All successful subcultures were extracted.

Approximately 100mg of each strain, which had been resuspended in sterile phosphate-buffered saline solution, was used to extract parasitic DNA using the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, California, USA) according to the manufacturer's instructions.

Molecular identification of Candida albicans

The species from the isolates was confirmed through use of the polymerase chain reaction (PCR) species-specific approach according to the protocol described by Theill *et al*., 2016 (Table 1). The conditions for amplification were as follows: 95°C for 5min; 30 cycles at 94°C for 45s, 58°C for 40s and 72°C for 55s; and a final 10 min extension step at 72°C. After amplification, electrophoresis enabled visualisation of PCR products on a 1.5% agarose gel. Electrophoresis was performed at 100mV for two hours to enable better dissociation of the fragments. The amplicon were analysed by use of system gel documentation VWR gel electrophoresis in 0.5% agarose gel that contained GelRed® Nucleic Acid Gel Stain (Biotium). The expected size was 941bp for *C. albicans*.

Table 1. *HWP1* oligonucleotide sequences used for the identification of *Candida albicans* species

Primer	5'-sequence-3'	Length of PCR product (bp)
Candida hwp1	Forward GCT ACC ACT TCA GAA TCA TCA TC Reverse GCA CCT TCA GTC GTA GAG ACG	850 - 951

Table 2. Sequences of the oligonucleotides used for PCR assays for the virulence genes study

Primer	5'-sequence-3'	Length of PCR product (bp)
C. albicans ALS1	Forward GAC TAG TGA ACC AAC AAA TAC CAG Reverse CCA GAA GAA ACA GCA GGT GA	318
C. albicans hwp1	Forward CCATGTGATGATTACCCACA Reverse GCTGGAACAGAAGATTCAGG	572

Molecular detection of virulence markers in Candida albicans

To analyse the virulence genes, we employed PCR methodology with specific primers. The conditions for amplification of the genes *ALS1* and *hwp1* were used as previously reported by Goulart *et al.,* 2018 (Table 2). The final volume of the reaction was 25µl for each sample, which contained: 4µl of DNA, 12.5µl of GoTaq Hot Start Green Master Mix (Promega, Madison, Wisconsin, USA) and 0.5µl (10µmol/µl) of oligonucleotides (Eurofins Genomics LLC, Germany).

The *ALS1* gene was amplified through the use of the following conditions: initial denaturation at 94°C for 5min, 40 cycles of denaturation at 94°C for 30s, annealing at 58°C for 30s, extension at 72°C for 30s and final extension at 72°C for 7min. The PCR conditions for the *hwp1* gene were as described here: initial denaturation at 94°C for 3min, 30 cycles of denaturation at 94°C for 30s, annealing at 60°C for 30s, extension at 72°C for 30s and final extension at 72°C for 10min. The PCR products were analysed through the application of system gel documentation VWR gel electrophoresis in 0.5% agarose gel that contained GelRed® Nucleic Acid Gel Stain (Biotium).

Ethical considerations

The study was approved by the National Committee of Ethics and Life and Health Sciences of Côte d'Ivoire (certificate number: 059-23/MSHPCMU/CNESVS-km). This study was conducted in accordance with the principles of the Helsinki Declaration. Freely written informed consent was obtained from the patients, parents or legal guardians prior to enrolment.

Data analysis

All statistical analyses were performed through the use of SPSS Statistics version 21 (IBM, Armonk, New York, USA).

Results

Candida species identification

A total of 151 DNA extracts of *C. albicans* were obtained from the conserved strains. These strains were then confirmed through the application of molecular biology. The success rate of molecular testing for the *hwp1* gene was 96.03% (145/151). All 145 strains that were identified as *C. albicans* by mycological analysis were confirmed as such by molecular analysis. The majority of isolates (95%, 144/151) were from patients older than 18 years.

Detection of virulence gene markers

The *hwp1* and *ALS1* genes were observed at 572bp and 318bp, respectively (Fig. 1&2).

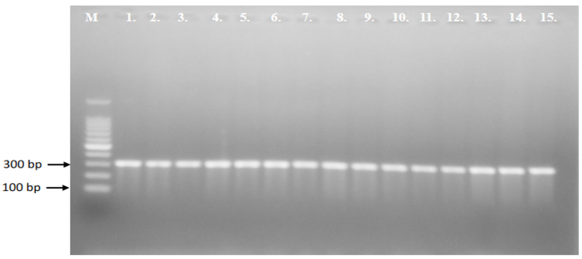

Fig. 1. Agarose gel electrophoresis of *ALS1* gene PCR products

M: Molecular weight marker, Lane 1 to 15: isolates

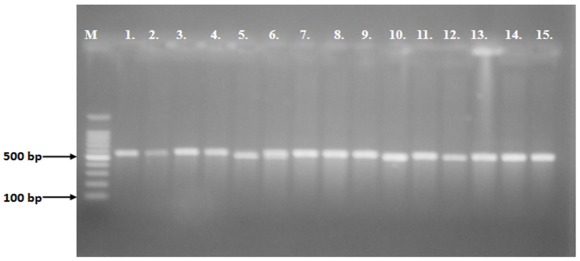

Fig. 2. Agarose gel electrophoresis of *hwp1* gene PCR products

M: Molecular weight marker, Lane 1 to 15: isolates

The *hwp1* gene was observed in 95.8% (138/144) of the *C. albicans*, while the prevalence of the *ALS1* gene was 97.9% or 141/144. Only one isolate expressed neither *hwp1* nor *ALS1*. This was an isolate from a patient with a single partner whose symptoms had lasted for three weeks and who had previously taken an antibiotic. Seven isolates expressed one or the other of the genes studied. These were isolates from patients with single partners whose symptoms had lasted less than one month in each case and who were not receiving antibiotic treatment when the swabs were taken.

The simultaneous presence of *ALS1* and *hwp1* genes was observed in 94.4% of *C. albicans* species. These were patients whose symptoms had lasted one month or more (38.5%), who were not receiving antibiotic treatment (82.5%) and who each had one sexual partner (96.5%).

Discussion

To assess the prevalence of *C. albicans* in vaginal specimens that had been collected in Abidjan, we identified 151 incoming isolates (previously identified as *C. albicans* by phenotypic methods) through the amplification of the *hwp1* gene with a single primer pair.

The chosen methodology was designed to obtain highly purified DNA through the use of a specific extraction kit for simple and rapid isolation of DNA from difficult-to-lyse fungi, such as *Aspergillus fumigatus*, *C. albicans*, *Neurospora crassa*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and Gram (+/-) bacteria. Fungal samples were lysed rapidly and efficiently through the use of state-of-the-art ultra-high-density "Bashing Beads". A Zymo-Spin column was then used to isolate DNA. This column is ideal for downstream molecular applications including PCR, quantitative PCR and next-generation sequencing. By combining these two methods, 145 *C. albicans* species were confirmed from 151 isolates. In this study, *C. albicans* was isolated from patients who were

between 15 and 45 years old, with a 95% predominance in patients who were over 18 years old. These results are consistent with those found by Amal *et al.*, 2018 between June 2022 and January 2024 at the Faculty of Medicine and National Liver Institute of Menoufia University, Egypt (Dawoud *et al.*, 2024).

The use of PCR enabled the rapid discrimination of *C. albicans* species that were involved in vulvovaginal infections; conventional methods require three to five days to obtain a result (Codreanu and Ciurea, 2023). Taqi Al-Khazali *et al*., 2023 used non-structural protein 1 (NS1) and NS8, which were different from those we used, to differentiate between two *Candida* species, *C. albicans* and *C. dubliniensis,* in Iraq. However, other authors (Eghtedar Nejad *et al*., 2020) have used real-time PCR with high-resolution melting analysis to identify several clinical yeast species such as *Candida* spp.

Our data showed that the majority of isolates (> 95%) were biofilm producers. They were positive for biofilm-related genes (*hwp1* and ALS1). The frequency of occurrence of these genes was 97.9% and 95% for *ALS1* and *hwp1*, respectively. These data are consistent with the findings of (Brunetti *et al*., 2019; Treviño-Rangel *et al*., 2018) that all the *Candida* species that caused bloodstream infections were biofilm producers, with the highest intrinsic production among *C. albicans* and *C. tropicalis*. Additionally, these results are similar to those found by Ardehali *et al.*, 2019 in Tehran, Iran, between March 2016 and February 2017. In a similar study that was conducted in Brazil, the authors found that the prevalence of *ALS1* and *hwp1* in vulvovaginal swab specimens was 73.7% and 21.1%, respectively (Goulart *et al*., 2018).

HWP1 is the first cell-surface protein of *C. albicans* required for biofilm formation *in vivo*(Nobile *et al.*, 2006). Biofilm formation plays an essential role in the pathogenicity of *C. albicans*. Biofilm is a population of cells surrounded by an extracellular matrix that consists of yeast cells and filaments; it has a relationship with the surface and exhibits phenotypic characteristics that are different from those of planktonic cells (İNcï *et al*., 2013). The initiation of biofilm formation is dependent on the attachment of yeast cells to a substrate before the yeast cells aggregate (Granger *et al.*, 2005). The *ALS* gene family is the largest among known adhesin gene families in *C. albicans*. The ALS1 protein is a GPI-anchored adhesin that is involved in *C. albicans* biofilm formation, cell-cell and cell-surface interactions, and interactions with the host epithelium (Martorano-Fernandes *et al.*, 2023).

The ALS proteins belong to a family of adhesins that are known to play a role in adhesion and the early formation of biofilms (Du *et al*., 2012). *C. albicans* proteins in the *ALS1* family are among the best-studied fungal adhesions. *ALS* genes seem to be responsible for fluconazole resistance through biofilm formation, which contributes to drug resistance (Roudbarmohammadi *et al*., 2016). Two studies show that the suppression of *ALS1* reduces *C. albicans* virulence (Alberti-Segui *et al*., 2004; Fu *et al.*, 2002). These profiles suggest that the *hwp1* and *ALS1* genes could be therapeutic targets for the development of antifungal drugs that target *C. albicans* infections.

Conclusion

Our data show that the majority of the isolates that were obtained from patients in Côte d'Ivoire who had vulvovaginal candidiasis were biofilm producers. The *hwp1* and *ALS1* genes play an important role in biofilm formation, which is involved in the virulence of *C. albicans*. Investigation of other genes in the *ALS* family may provide further insights into the mechanism of biofilm production. The different profiles of the *hwp1* and *ALS1* genes show that they could be targets for the

development of therapies against vulvovaginal candidiasis.

Acknowledgments

We would like to thank all the patients for their contribution to this work.

References

Alberti-Segui C, **Morales AJ**, **Xing H**, **Kessler MM**, **Willins DA**, **Weinstock KG**, **Cottarel G**, **Fechtel K**, **Rogers B**. 2004. Identification of potential cell-surface proteins in *Candida albicans* and investigation of the role of a putative cell-surface glycosidase in adhesion and virulence. Yeast **21**, 285–302. https://doi.org/10.1002/yea.1061

Ardehali SH, **Azimi T**, **Fallah F**, **Aghamohammadi N**, **Alimehr S**, **Karimi AM**, **Azimi L**. 2019. Molecular detection of *ALS1*, *ALS3*, *HWP1* and *SAP4* genes in *Candida* Genus isolated from hospitalized patients in Intensive Care Unit, Tehran, Iran. Cell Mol Biol (Noisy-le-grand) **65**, 15–22. https://doi.org/10.14715/cmb/2019.65.4.3

Berkow EL, **Lockhart SR**. 2017. Fluconazole resistance in *Candida* species: a current perspective. Infect Drug Resist **10**, 237–245. https://doi.org/10.2147/IDR.S118892

Brunetti G, **Navazio AS**, **Giuliani A**, **Giordano A**, **Proli EM**, **Antonelli G**, **Raponi G**. 2019. *Candida* bloodstream infections observed between 2011 and 2016 in a large Italian University Hospital: A time-based retrospective analysis on epidemiology, biofilm production, antifungal agents consumption, and drug-susceptibility. PLoS One **14**, e0224678. https://doi.org/10.1371/journal.pone.0224678

Codreanu SI, **Ciurea CN**. 2023. *Candida* spp. DNA Extraction in the Age of Molecular Diagnosis. Microorganisms **11**, 818. https://doi.org/10.3390/microorganisms11040818

Dawoud AM, **Saied SA**, **Torayah MM**, **Ramadan AE**, **Elaskary SA**. 2024. Antifungal susceptibility and virulence determinants profile of *Candida* species isolated from patients with candidemia. Sci Rep **14**, 11597. https://doi.org/10.1038/s41598-024-61813-w

Diekema D, **Arbefeville S**, **Boyken L**, **Kroeger J**, **Pfaller M**. 2012. The changing epidemiology of healthcare-associated candidemia over three decades. Diagn Microbiol Infect Dis **73**, 45–48. https://doi.org/10.1016/j.diagmicrobio.2012.02.001

Djohan V, **Angora KE**, **Vanga-Bosson AH**, **Konaté A**, **Kassi FK**, **Yavo W**, **Kiki-Barro PC**, **Menan H**, **Koné M**. 2012. Sensibilité in vitro des souches de *Candida albicans* d'origine vaginale aux antifongiques à Abidjan (Côte d'Ivoire). Journal de Mycologie Médicale **22**, 129–133. https://doi.org/10.1016/j.mycmed.2011.11.005

Du H, **Guan G**, **Xie J**, **Sun Y**, **Tong Y**, **Zhang L**, **Huang G**. 2012. Roles of *Candida albicans* Gat2, a GATA-Type Zinc Finger Transcription Factor, in Biofilm Formation, Filamentous Growth, and Virulence. PLOS ONE **7**, e29707. https://doi.org/10.1371/journal.pone.0029707

Eghtedar Nejad E, **Ghasemi Nejad Almani P**, **Mohammadi MA**, **Salari S**. 2020. Molecular identification of *Candida* isolates by Real-time PCR-high-resolution melting analysis and investigation of the genetic diversity of *Candida* species. J Clin Lab Anal **34**, e23444. https://doi.org/10.1002/jcla.23444

Fu Y, **Ibrahim AS**, **Sheppard DC**, **Chen YC**, **French SW**, **Cutler JE**, **Filler SG**, **Edwards JE Jr**. 2002. *Candida albicans* Als1p: an adhesin that is a downstream effector of the EFG1 filamentation pathway. Molecular Microbiology **44**, 61–72. https://doi.org/10.1046/j.1365-2958.2002.02873.x

Goulart LS, **Lima JS de**, **Souza WWR de**, **Vieira CA**, **Crestani J**, **Araújo C**. 2018. Analysis of the *ALS1* and *HWP1* genes from clinical isolates of *Candida albicans* / Análise de genes *ALS1* e *HWP1* em isolados clínicos de *Candida albicans*. Brazilian Journal of Health Review **1**, 112–119.

Granger BL, **Flenniken ML**, **Davis DA**, **Mitchell AP**, **Cutler JE**. 2005. Yeast wall protein 1 of *Candida albicans*. Microbiology **151**, 1631–1644. https://doi.org/10.1099/mic.0.27663-0

İNcï M, **Atalay MA**, **Özer B**, **EvïRgen Ö**, **Duran N**, **Motor VK**, **Koç AN**, **Önlen Y**, **Kilinç Ç**, **Durmaz S**. 2013. Investigations of *ALS1* and *HWP1* genes in clinical isolates of *Candida albicans*. Turkish Journal of Medical Sciences **43**, 22. https://doi.org/10.3906/sag-1205-90

Martorano-Fernandes L, **Goodwine JS**, **Ricomini-Filho AP**, **Nobile CJ**, **Del Bel Cury AA**. 2023. *Candida albicans* Adhesins Als1 and Hwp1 Modulate Interactions with *Streptococcus mutans*. Microorganisms **11**, 1391. https://doi.org/10.3390/microorganisms11061391

Mathé L, **Van Dijck P**. 2013. Recent insights into *Candida albicans* biofilm resistance mechanisms. Curr Genet **59**, 251–264. https://doi.org/10.1007/s00294-013-0400-3

Mayer F, **Wilson D**, **Hube B**. 2013. *Candida albicans* pathogenicity mechanisms. Virulence **4**. https://doi.org/10.4161/viru.22913

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. https://doi.org/10.1155/2021/5598907

Nobile CJ, **Nett JE**, **Andes DR**, **Mitchell AP**. 2006. Function of *Candida albicans* Adhesin Hwp1 in Biofilm Formation. Eukaryot Cell **5**, 1604–1610. https://doi.org/10.1128/EC.00194-06

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. Adv Biomed Res **5**, 105. https://doi.org/10.4103/2277-9175.183666

Segal E, **Frenkel M**. 2018. Experimental In Vivo Models of Candidiasis. Journal of Fungi **4**, 21. https://doi.org/10.3390/jof4010021

Taqi AL-Khazali M, **Mousa Hassan B**, **Ahmed AbedIbrahim S**. 2023. Molecular Identification of *Candida albicans* and *C. dubliniensis* Using Small Subunit rRNA Gene Sequence in Kerbala, Iraq. Arch Razi Inst **78**, 1035–1040. https://doi.org/10.22092/ARI.2022.360086.2546

Theill L, **Dudiuk C**, **Morano S**, **Gamarra S**, **Nardin ME**, **Méndez E**, **Garcia-Effron G**. 2016. Prevalence and antifungal susceptibility of *Candida albicans* and its related species *Candida dubliniensis* and *Candida africana* isolated from vulvovaginal samples in a hospital of Argentina. Rev Argent Microbiol **48**, 43–49. https://doi.org/10.1016/j.ram.2015.10.003

Treviño-Rangel RDJ, **Peña-López CD**, **Hernández-Rodríguez PA**, **Beltrán-Santiago D**, **González GM**. 2018. Association between *Candida* biofilm-forming bloodstream isolates and the clinical evolution in patients with candidemia: An observational nine-year single center study in Mexico. Revista Iberoamericana de Micología **35**, 11–16. https://doi.org/10.1016/j.riam.2017.01.005