



MOLECULAR CHARACTERIZATION OF *Candida albicans* IN WOUNDS OF DIABETIC PATIENTS IN SELECTED HEALTH FACILITIES IN NORTH CENTRAL-NIGERIA

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Abstract

Molecular study shows that the Internal Transcribed Spacer 1 (*ITS-1*) gene region is a highly variable ribosomal DNA sequence used as a primary molecular marker for identifying *Candida* species in clinical samples. By exploiting *ITS-1* size polymorphism, PCR-based methods was used to identify genus *Candida*. This study was a cross-sectional study carried out in selected health facilities in North Central Nigeria among adult diabetic foot ulcer patients over a period of one year. A total of 33 samples was used for the study. Specie specific identification involved phenotypic methods such as chromogenic character on CHROMagar *Candida* species medium, germ tube examination, and microscopic characteristics. Data were analysed using WHONET and SPSS. Fungal DNA was extracted using Qiagen DNA extraction kit and identified molecularly using *ITS-1* genus specific *Candida* primers also focusing on detecting virulence gene such as *HWP-1* and Resistance genes such as *ERG-1* and *MDR1* gene using PCR techniques. Results for *ITS-1* gene for genus specific *Candida* was at bp 525bp on 100bp ladder. All the *Candida* isolates harbored virulence gene on chromosomal DNA. Specifically, (75%) carried the *HWP-1* gene at 572bp ($P < 0.001$ no significant difference). *Candida albicans* harbored resistant genes on chromosomal DNA. Specifically, (45%) carried the *ERG-1* gene at 510bp and no band for the *MDR1* gene. The highest *HWP-1* (75%) encodes a major surface adhesin for virulence, specifically mediating the binding of hyphae to host epithelial cells. A Maximum Likelihood phylogenetic tree was constructed using the IQTREE and the generated file was viewed. The proportion of trees (1000 bootstrap) in which the corresponding taxa clustered. Phylogenetic analysis confirmed their genetic affiliation showing minimal intra-specie variation. Non-*albicans* *Candida* (NAC) contained the *ERG-1* gene in *C. tropicalis* at 380bp and *C. glabrata*. This study reports a concerning prevalence of virulence and resistant genes in *Candida albicans* at 480bp. The genomic presence of virulence and resistant genes underscores the importance of molecular surveillance, improved water and sanitation infrastructure and urgency of effective antifungal strategies in combating diabetic foot ulcer infections in vulnerable populations.

Keywords: Genotypic, virulence, resistance, sequencing, phylogeny.

INTRODUCTION

Fungal wound infections pose a special challenge and cause substantial morbidity among burn patients (Ladhani *et al.*, 2021). The negative consequences of these fungi cause large healthcare burdens, including chronic wounds or nonhealing wounds (Guariguata *et al.*, 2015). Chronic wounds can increase morbidity and mortality rates in elderly patients (Mihai *et al.*, 2018). For example, diabetic foot ulcers contribute to 80% of nontraumatic lower-

extremity amputations, and their 5-year mortality rate is approximately 43%–55%, which is higher than those of breast cancer, prostate cancer and Hodgkin’s disease. Non-pathogenic species are able to collaborate and interact together with synchronization to create a “functionally equivalent pathogroup” (FEP) responsible for the infection chronicity and the maintenance of the pathogenic biofilm.

Chronic wounds are a major healthcare burden worldwide (Mihai *et al.*, 2018). Healthy individuals normally heal quickly, while some people, such as patients with diabetes, have factors that can impair the wound healing process. (Sharma *et al.*, 2011). Wound swabs, cleaning, dressing and debridement of the wound bed are the current standard of care for chronic wounds. In addition, topical antiseptics are recommended as a first-line therapy by recent consensus guidelines for the management and intervention of chronic wounds with multimicrobial biofilms (Sharma *et al.*, 2011).

Biofilm makes wound healing and infections very difficult to resolve, as local access for antimicrobial agents and the host’s immune system is hampered. Colonization is defined as the presence of multiplying bacteria with no overt host immunological reaction. (Boyko *et al.*, 2018). In contrast, infection results when invading organisms overwhelm the host’s defenses. Several factors are thought to be involved, including the bacterial load within the wound. It has been suggested that bacterial concentration greater than 10^5 colony-forming units (CFUs) per gram of tissue indicates the presence of a “critical” degree of colonization at which the host defenses are no longer able to contain it. However, many individuals with diabetes mellitus have an impaired inflammatory response and may not show the classical signs of infection in a wound with a high bacterial load.

MATERIALS AND METHODS

Study Area

A cross-sectional laboratory-based study was performed on wounds of diabetic patients attending health care services in selected health facilities in north central Nigeria. North Central Nigeria (also known as the Middle Belt) encompasses six states—Benue, Kogi, Kwara, Nasarawa, Niger, and Plateau—plus the Federal Capital Territory (FCT). Located between latitudes 7°00’ and 11°30’ North and longitudes 4°00’ and 11°00’ East, the zone features the Guinean forest-savanna mosaic, tropical climates, and the vital Niger-Benue river basin.

Inclusion Criteria

Patients presenting with diabetic wounds and foot ulcer(s) and those admitted to the surgical wards with diabetic wounds were recruited.

Exclusion Criteria

Patients without visible wounds and diabetic foot ulcer and/or superficial lesions or declined consent to participate were excluded from the study.

Ethical Considerations

An ethical approval for the study was obtained from the Ethical Clearance Committee of Federal Medical Centre, Makurdi Benue State.

Sample Size Determination

The sample size was determined using the following equation as described by (Shapiro *et al.*, 2014).

$$n = \frac{Z^2 P (1-P)}{d^2}$$

Where;

n= sample size

Z= statistics for a level of 95% confidence interval =1.96

p= prevalence rate at 55% (0.55) Nasarawa State (Shapiro *et al.*, 2014).

d= precision (allowable error) = 5% = 0.05

Therefore;

$$n = \frac{1.96^2 * 0.55 (1 - 0.55)}{0.05^2}$$

$$n = \frac{0.38 * 0.55 * 0.45}{0.0025}$$

$$n = \frac{0.0957}{0.0025}$$

$$n = 38.28$$

Sampling Procedure

Genomic *Candida albicans* DNA was extracted in a 5 ml double-strength Mueller Hinton broth, resistant isolates was cultured for 72 h at 37°C. The cultures was collected into clearly marked Eppendorf tubes and centrifuged for 10 minutes to produce pellets in a microcentrifuge (Biofuge, Biotra Bio-trade Hecrus Sepatech Co. Ltd. USA). After gently decanting the supernatant, the cell pellets will undergo a 5-minute vortexing process. Then, to lyse the bacteria cell pellet, 300 µl of Tris EDTA (TE) buffer and 150 µl of 3.0 M aqueous sodium acetate was added at pH 5.2. After another 10-minute centrifugation of the samples, the supernatant was transferred to a new Eppendorf tube and thoroughly mixed with 0.9 ml of 70% ethanol that had been cooled to -20°C in order to precipitate DNA. The supernatant was discarded after it underwent a second centrifugation for 10 minutes. The pellet was washed twice with 1 ml of 70% ethanol, air-dried for 10 minutes, resuspended in 20–40 µl of TE buffer, and kept at 40°C pending use (Soares *et al.*, 2021).

Molecular Identification of *Candida albicans*

Optimization of primer set and reaction conditions was done. DNA Extraction was done. Pure colonies of Genus *Candida* were harvested into sterile 1.5 µl Eppendorf tubes containing 200 µl of 1× phosphate-buffered saline. Fungal genomic DNA was extracted using the Qiagen *QIAamp* DNA extraction kit (Hilden, Germany), following the manufacturer's instructions, for pure high-quality DNA suitable for amplification.

Molecular Detection of *ITS-1* gene by Polymerase Chain Reaction (PCR)

The complete region of *ITS-1* gene of Genus *Candida* isolates was amplified through conventional PCR using genus specific primers in Table 1. The extracted DNA served as the template for PCR assays designed to amplify specific target genes of *ITS-1* for the molecular identification of *Candida ITS1* F: (5'-TCC GTA GGT GAA CCT GCG G-3'), *ITS4* R: (5'-TCC TCC GCT TAT TGA TAT GC-3'). The Internal Transcribed Spacer 1 (*ITS-1*) gene region is a highly variable ribosomal DNA sequence used as a primary molecular marker for identifying *Candida* species in clinical samples. By exploiting *ITS-1* size polymorphism, PCR-based methods was used to distinguish between species like *C. albicans*, *C. glabrata*, and *C. tropicalis*. Fungal *ITS-1* gene was amplified using genus specific primer pair designed in Inqaba biotec (Pretoria, South Africa). The PCR master mix consisted of 2 µl of DNA template, taq 5x, 1.5 µl each of forward and reverse primers, and nuclease-free water NFW 15.0 µl PCR-reagents 1x and 13x was added to achieve a total reaction volume of 25 µl. The protocol began with an initial denaturation step at 95 °C for 1 minute, at 40 cycles. Primer annealing occurred at 60°C for 1 minute after which DNA synthesis was initiated. The extension step was carried out at 72°C for 1 minute, and the reactions concluded with a final extension phase at 72°C for 10 minutes (Shemer *et al.*, 2017). Exactly 1.5% agarose gel was prepared and dissolved in TBE for electrophoresis at 100v for 35min. Amplicons were visualized with a GENESnap with expected band size (EBS) of 525bp for Genus *Candida* identification

Molecular Detection of Virulence Gene *HWP-1*

Optimization and reconstitution of primers and reaction conditions was done by using a known positive control of Genus *Candida*. PCR amplification was employed to detect virulence-associated genes, including *HWP-1*, which are commonly used as molecular markers for specie characterization of *Candida albicans*. Primer sets specific to these genes (are shown in Table 1) were used under optimized thermal cycling conditions, and the amplicons were visualized using agarose gel electrophoresis to determine the presence or absence of the target genes in the isolates. The PCR master mix consisted of 1 µl of DNA template, taq 5x, 1.5 µl each of forward and reverse primers, and nuclease-free water NFW 15.0µl PCR-reagents 1x and 13x was added to achieve a total reaction volume of 23 µl. Amplification was performed using a Bio-Rad thermocycler under optimized cycling conditions. 1.5% agarose gel was prepared and dissolved in TBE for electrophoresis at 95v for 35min. Amplicons were visualized with a GENESnap with EBS of 572bp for *Candida albicans* (Singh *et al.*, 2017). *HWP-1* Gene: F: 5'-ATG ACT CCA GCT GGT TC- 3', R: 3'-TAG ATC AAG AAT GCA GC- 5.

Molecular Detection of Resistant Genes

Optimization and reconstitution of primers and reaction conditions. PCR amplification was employed to detect resistant genes including *ERG-1* and *MDR1*. These genes were targeted due to their clinical relevance in conferring resistance. Primer sets specific to these genes were used under optimized thermal cycling conditions, and the amplicons were visualized using agarose gel electrophoresis to determine the presence or absence of the target genes in the isolates. The PCR master mix consisted of 2µl of DNA template, taq 5x 10µl, 1.5 µl each of forward and reverse primers, and nuclease-free water NFW 5.0µl PCR-reagents 1x and 13x was added to achieve a total reaction volume of 20µl. Amplification was performed using a Bio-Rad thermocycler under optimized cycling conditions. Exactly 1.5% agarose gel was prepared and dissolved in TBE for electrophoresis at 95v for 50min. Amplicons was visualized with a GENESnap with EBS of 510bp for *ERG1* and 520bp for *MDR1* gene (Soll *et al.*, 2014). *ERG1* Gene F: (5'-CAA GAA GAT CAT AAC TCA AT-3') R: (3'-AGA ACA CTG AAT CGA AAG-5'). *MDR1* Gene F: 5- AGG TGA ACC CAA TTC AAG TC-3. R: 5-ACA ACT GGT TCC ATA ACG GT-3. PCR conditions for the *ERG-1* gene comprised an Initial denaturation at 95°C for 10 minutes, denaturation at 94°C for 45 seconds of 35 cycles, annealing at 60°C for 45 sec of 25 cycles, extension at 72°C for 15 sec. of 25 cycles and final extension at 72°C for 3 minutes. PCR conditions for the *MDR1* gene comprised an Initial denaturation at 95°C for 5 minutes, denaturation at 95°C for 45 seconds of 35 cycles, annealing at 60°C for 30 sec of 35 cycles, extension at 72°C for 3 minutes of 35 cycles and final extension at 72°C for 2 minutes. The primer pairs used in the above-mentioned reactions amplified the DNA fragments correspond to 510bp for *ERG-1* and 520bp for *MDR1* gene (Singh *et al.*, 2019). A known positive *Candida* isolate was used as positive and NFW as negative controls, respectively.

Preparation of Gel for Electrophoresis

The gel casting apparatus was set up as instructed in the product manual. The comb was placed straight, and was few millimetres of clearance between the bottom of the comb and the bottom of the gel tray. A 1.0% gel was created by dissolving 0.5 g of agarose in 50 ml of Tris Borate EDTA buffer (TBE). After the agarose was completely melted, the agarose solution was gently swirled, and any translucent “flecks” of agarose that had not completely melted and kept an eye out for. The heat was then be kept on after that until all flecks was removed. Before pouring, the agarose solution was allowed to cool to a temperature of between 50 and 55°C. Then, gently adding 2 µl of a 10 mg/ml EtBr solution (per 50 ml gel), the liquid was swirled to mix, and then it was carefully placed into a gel tray. The comb was gently be taken out of the opaque gel after it had solidified for 20 minutes. In the gel electrophoresis tank, the gel carrier was inserted after being withdrawn from the pouring tray. The electrophoresis tank was filled with 250 ml of 1x TBE until the gel was completely be submerged. Air bubbles trapped in the sample well was carefully dislodged with a pipette as air bubbles was exclude the buffer and make the wells challenging to fill with the sample (Smith *et al.*, 2018).

DNA Sample Electrophoresis

A 50 µl sample of DNA and 3 µl of loading dye was combined using a micropipette, and the solutions was then gently mixed by pipetting the solutions up and down . One sample per well was carefully inserted into the gel wells, which was then be placed on the gel box at the electrophoresis machine’s negative charge end. The agarose containing the sample DNA was sealed by the addition of buffered water, which also served as an electrolyte by directing the current and the sample Genomic DNA towards the positive end for 2 h at a voltage of 63 Volts. The standards and sample DNA was be taken from the agarose and allowed to drain off. A photo displaying the size and movement of the sample plasmid DNA was captured using a GeneSnap UV light, UV-certified safety glasses, and a camera. *Candida albicans* is one specific species of *Candida*, while non-*albicans Candida* (NAC) refers to all the other *Candida* species that can cause infection. Table 1 presents the major differences between *Candida albicans* and non-*albicans Candida* species based on their common species, frequency, virulence characteristics, morphology, diagnostic features, and antifungal susceptibility.

Table 1. Difference between *Candida albicans* and Non-*albicans Candida*

Feature	<i>Candida albicans</i>	Non- <i>albicans Candida</i>
Common species	<i>C. albicans</i>	<i>C. glabrata</i> , <i>C. tropicalis</i> , <i>C. parapsilosis</i> , <i>C. krusei</i>
Frequency	Most common cause of candidiasis	Less common overall, but increasingly important
Virulence	Generally more virulent	Variable depending on species
Morphology	Can form germ tubes, pseudohyphae and true hyphae	Many have limited or different filamentation ability
Germ-tube test	Positive	Usually negative
Chlamydospores	Can form chlamydospores	Generally absent
Fluconazole susceptibility	Usually susceptible	More variable; resistance is more common
Important example	<i>C. albicans</i>	<i>C. glabrata</i> may have reduced azole susceptibility; <i>C. krusei</i> is intrinsically resistant to fluconazole
Clinical significance	Common in oral, vaginal and invasive candidiasis	Particularly important in healthcare-associated and recurrent infections

The most clinically important difference is antifungal susceptibility. Non-*albicans* species are more likely to show reduced susceptibility or resistance to commonly used azole antifungals, so species identification and susceptibility testing can be important, especially in recurrent or invasive infections.

DNA Sequencing

Exactly 50 µl of the amplified fragments was sequenced using the Nimagen, Brilliant Dye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000 according to manufacturer's instructions. The labelled products was then cleaned with the ZR-96 DNA Sequencing Clean-up Kit (Catalogue No. D4053). The cleaned products were injected on the Applied Biosystems ABI 3500XL Genetic Analyser with a 50cm array, using POP7 and sequence data collected. The sequencing was done at a final volume of 10µl, the components included 0.25 µl BigDye® terminator v1.1/v3.1, 2.25µl of 5 x BigDye sequencing buffer, 10µl Primer PCR primer, and 2-10ng PCR template per 100bp. The sequencing conditions was as follows 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4min (Soll *et al.*, 2014).

The generated sequences were trimmed for ambiguous peaks or 'noise' in the chromatogram using FinchTV software version 1.4.0. The trimmed forward and reverse complement sequences were joined using EMBL:merger (accessed on the 16th October, 2025). The trimmed sequences were blasted using the NCBI BLAST (MEGABLAST), comparing generated sequences with available sequences in the GenBank. Sequences with a percentage similarity of 90-100%, query cover of 90-100%, and e- value $\leq 1e-5$ from the BLAST result were obtained for multiple sequence alignment in MAFFT version 7 online server <https://mafft.cbrc.jp/alignment/server/index.html> (accessed on the 16th October, 2025).

Phylogenetic Analysis

The Tamura 3-parameter model and a Maximum Likelihood phylogenetic tree was constructed using the IQTREE online server <http://iqtree.cibiv.univie.ac.at/> and the generated file was viewed <https://itol.embl.de/> (accessed on the 16th oct., 2025). The displayed tree has the highest log likelihood (-110.62). Next to the branches, the proportion of trees (1000 bootstrap) in which the corresponding taxa clustered together was displayed.

Instrumentation

Disruptor Genie® (Scientific Industries, Inc., USA), NanoDrop One Microvolume UV-Vis Spectrophotometer (Thermo Scientific™, USA), Eppendorf Mastercycler Nexus Gradient 230, Gel Documentation System (E-BOX, Vilber Lourmat, Italy). Applied Biosystems ABI 3500XL Genetic Analyser.

Statistical Analysis

Data were expressed in percentages, analyzed using Statistical Product and Service Solutions (SPSS) version 29 and Pearson's Chi Square analysis test for comparison at $P>0$. Table 2 shows the primer sequences, amplicon sizes, and references used for the amplification of *Candida* identification, virulence (HWP-1), and resistance-associated (ERG-1 and MDR1) genes.

Table 2: Primers used for ITS-1 Gene in Genus Specific *Candida*, Amplification of Virulence and Resistance Genes.

Target Gene	Primer Sequence (5' – 3')	Amplicon Size (bp)	References
ITS-1	Forward (F): 5'-TCC GTA GGT GAA CCT GCG G-3'	525	Shapiro <i>et al.</i> , 2014
	Reverse (R): 5'-TCC TCC GCT TAT TGA TAT GC-3'		
HWP-1	Forward (F): 5'-ATG ACT CCA GCT GGT TC-3'	572	Sohn <i>et al.</i> , 2017
	Reverse (R): 3'-TAG ATC AAG AAT GCA GC-5'		
ERG-1	Forward (F): 5'-CAA GAA GAT CAT AAC TCA AT-3'	510	Shareck <i>et al.</i> , 2011
	Reverse (R): 3'-AGA ACA CTG AAT CGA AAG-5'		
MDR1	Forward (F): 5'-AGG TGA ACC CAA TTC AAG TC-3'	520	Shen <i>et al.</i> , 2015
	Reverse (R): 5'-ACA ACT GGT TCC ATA ACG-3'		

RESULTS

Table 3 shows the distribution of virulence and resistance genes among *Candida* species plays a central role in disease severity and treatment outcomes. The prevalence of these genes varies among *Candida* species. *Candida albicans* generally possesses the widest range of virulence genes, while non-*albicans* *Candida* species such as *Candida tropicalis*, *Candida glabrata* exhibit different virulence gene profiles. PCR primers commonly used to amplify the internal transcribed spacer (ITS) region of *Candida albicans* ribosomal DNA was 6 (80%) for fungal identification and phylogenetic studies *ITS1*: A forward primer that binds near the end of the 18S (small subunit) rRNA gene. *ITS4*: A reverse primer that binds near the beginning of the 28S (large subunit) rRNA gene. Using the *ITS-1* and *ITS4* primers amplifies a DNA fragment that includes: *ITS-1* region, 5.8S rRNA gene. *HWP-1* (Hyphal Wall Protein 1) 10 (75%): Promotes adhesion and hyphal development, especially in *Candida albicans*. *ERG-1* 9 (45%) plays a role in mutations alter the target enzyme of azole drugs, reducing drug binding. *MDR1* protein 6 (30%): Encodes a major facilitator superfamily (MFS) efflux pump associated with fluconazole resistance.

Plate 1 shows agarose gel electrophoresis image showing PCR amplification of the *ITS* (Internal Transcribed Spacer) region for genus specific molecular identification of *Candida*. *ITS-1* and *ITS-4* amplify the *ITS-1*–5.8S–*ITS2* region. Different *Candida* species often produce PCR products of similar size (500–600 bp), agarose gel electrophoresis confirms amplification but usually does not distinguish species. Species identification often requires sequencing, PCR-RFLP, or species-specific primers.

Plate 2 shows agarose gel electrophoresis image showing PCR amplification of the *HWP-1* gene in *Candida albicans*: Description of the gel: M = DNA molecular weight marker (ladder) Lane 1 = *Candida albicans* reference strain (approximately 572 bp *HWP-1* amplicon, Lane 3 = Clinical isolate of *Candida albicans* (approximately 569 bp). This *HWP-1* PCR assay is commonly used to differentiate members of the *Candida albicans* complex because each species produces a characteristic amplicon size.

Plate 3 shows typical agarose gel for PCR detection of the *ERG-1* gene in *Candida albicans* would appear similar to this: The gel shows distinct band at the expected amplicon size (determined by the primer pair used) with no band in the negative control.

Plate 4 shows no bands for *MDR1* gene. The *MDR1* gene (Multidrug Resistance 1) in *Candida* species encodes a transport protein in the cell membrane. It acts as an efflux pump. It pumps toxic drugs out of the cell. This action lowers the drug level inside the fungus and causes drug resistance. Table 3 shows the distribution and frequency of occurrence of identification, virulence-associated, and resistance genes among *Candida* species isolates.

Table 3: Distribution of Virulence and Resistant Genes in *Candida* species Isolates

Target Gene	Frequency of Occurrence (n)	Frequency (%)	Description
ITS-1	8	60	Internal Transcribed Spacer 1

Target Gene	Frequency of Occurrence (n)	Frequency (%)	Description
HWP-1	10	75	Hyphal Wall Protein 1
ERG-1	9	45	Ergosterol Gene 1
MDR1	0	0	Multidrug Resistance Gene 1

Key:

- ITS-1: Internal Transcribed Spacer 1
- HWP-1: Hyphal Wall Protein 1
- ERG-1: Ergosterol Gene 1
- MDR1: Multidrug Resistance Gene 1

Sample size (n) = 33

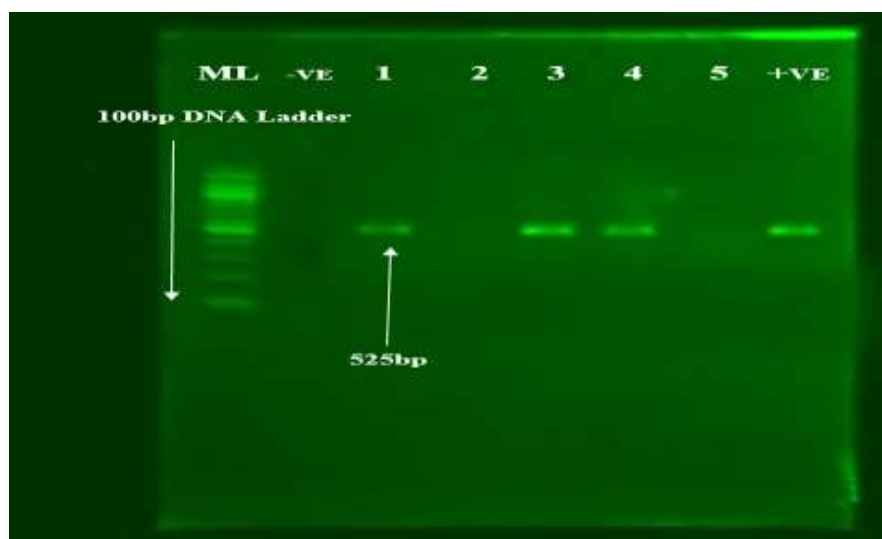


Plate 1: Agarose gel electrophoresis picture of *ITS-1* gene detected in genus specific *Candida*

ML: Molecular Ladder at 100bp

well 1, 3 and 4: Positive bands of *ITS-1* gene at 525bp

-ve: Negative Control, +ve: Positive Control

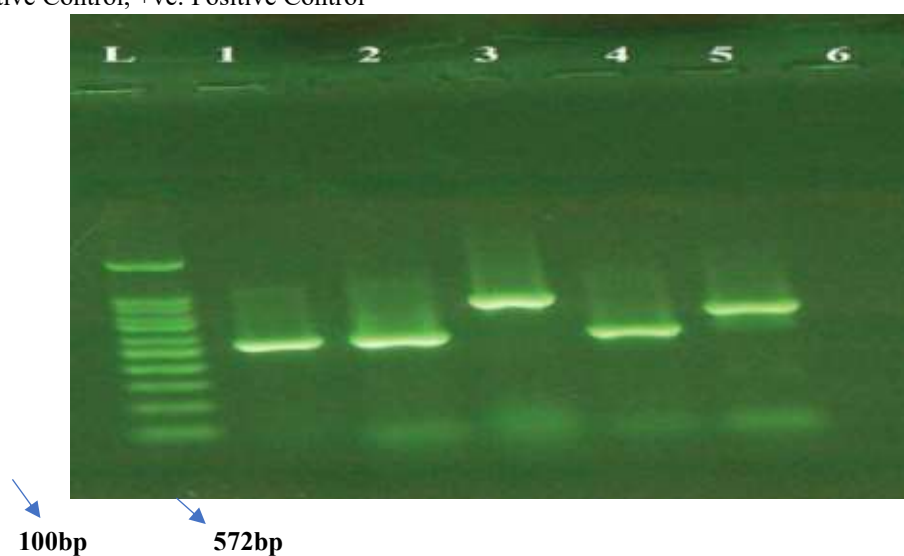


Plate 2: Agarose gel electrophoresis picture of *HWP-1* gene detected in genus specific *Candida albicans*

L: Molecular Ladder at 100bp

well 1 to 5: Positive bands of *HWP-1* gene at 572bp

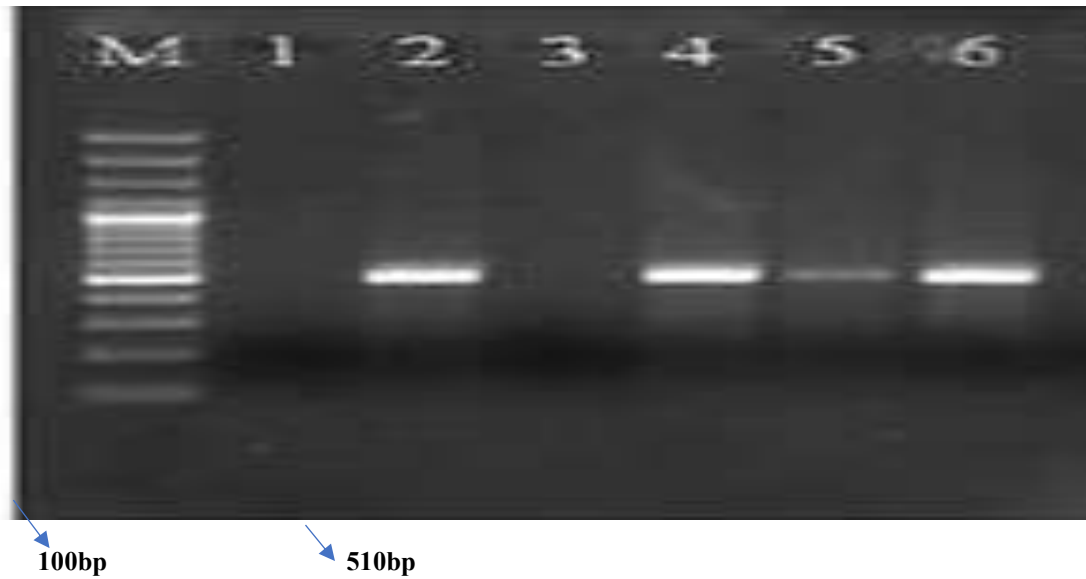


Plate 3: Agarose gel electrophoresis picture of *ERG1* gene detected in genus specific *Candida albicans*

ML: Molecular Ladder at 100bp

well 2, 4 to 6: Positive bands of *ERG-1* gene at 510bp



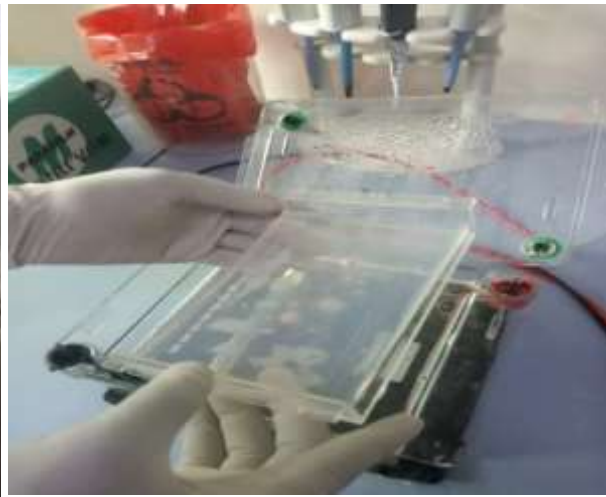
Plate 4: Agarose gel electrophoresis picture of *MDR1* gene detected in genus specific *Candida albicans*

ML: Molecular Ladder at 100bp

well 1 to 3: no bands for *MDR1* gene



Qiagen DNA Extraction kit



Gel Electrophoresis Tank

Phylogenetic Analysis

The phylogenetic analysis in this study showed that the sequences generated in this study shared the same ancestral roots as the one previously reported in Nigeria with accession number PV163171.

The blue-coloured taxa are the sequences generated in this study; the grey-coloured taxa are the reference sequences obtained from the GenBank. The partial sequence with accession number PX395408 from the GenBank, which was used as an out group.

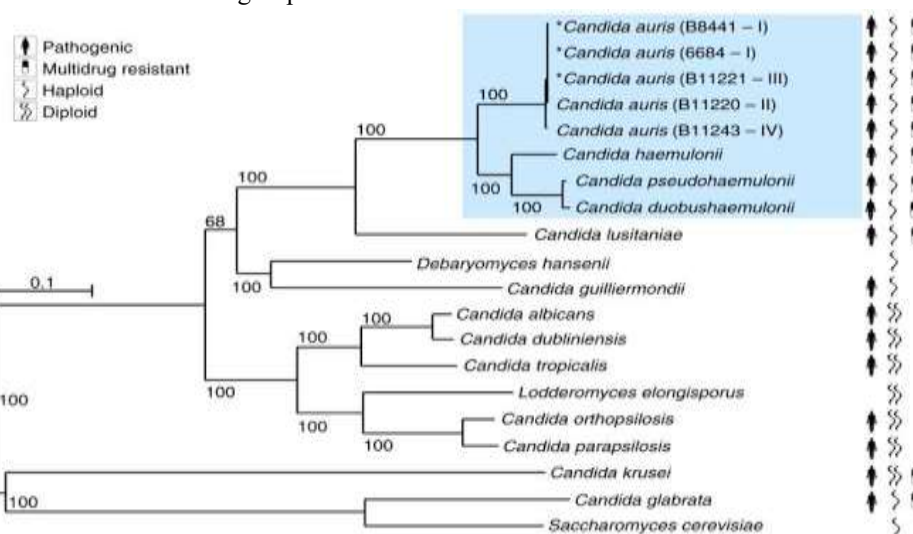


Figure 1: Maximum Likelihood Phylogenetic Tree in *Candida albicans*

Discussion

The *ITS-1* (Internal Transcribed Spacer 1) is a non-coding spacer DNA sequence located between the 18S and 5.8S ribosomal RNA (rRNA) genes in the fungal genome. Across the *Candida* genus, *ITS-1* is considered one of the most reliable and heavily utilized genetic markers for species identification and fungal taxonomy. The *ITS-1* gene is highly conserved among Genus *Candida*, with inter spersed hypervariable regions that allow discrimination at the genus level and, in some cases, species level (Soares, 2019). The *ITS-1* region sits alongside the 5.8S rRNA gene and the *ITS-2* region, which separate the highly conserved 18S and 28S rRNA genes. High Variability unlike the highly conserved flanking rRNA genes, *ITS-1* accumulates mutations at a much faster rate. This makes it hyper-variable between species but relatively stable within a single species, allowing for highly accurate species-level differentiation and length polymorphism: The base-pair length of *ITS-1* varies significantly across *Candida* species, allowing for initial identification through simple amplicon sizing (e.g., agarose gel electrophoresis). The results of PCR amplification to specific *ITS-1* gene indicated that 3(65%) of *Candida* species

isolates gave positive result at 525bp when compared with allelic ladder. This result align with results of Mihai (2018). They reported that PCR product was visible of *ITS-1* gene which was 525bp. The findings of this study are consistent with previous reports conducted by Sorber *et al.* (2021) and Sigurgeirsson *et al.* (2014) demonstrated clear 525bp amplicons for *Candida* species isolates obtained from diabetic wounds. Similar molecular studies by Shemer (2012) conducted in Nigeria have reported comparable results that documented successful amplification of the *ITS-1* gene of *Candida* species isolates at 525bp.

A study by Sorber (2019) successfully amplified the *ITS-1* gene of *Candida* species isolates from diabetic wound samples, observing bands around 525bp, consistent with the results obtained in this study. Several isolates clustered closely with established reference sequences such as NR 044370.1, NR 044371.1, and NR 044372.1, suggesting a high degree of sequence homology. This close association implies that these isolates likely belong to the same species or closely related taxa.

The PCR amplification of the *HWP-1* gene in *Candida albicans* at 572bp is considered a critical virulence determinant, encodes a major surface adhesin crucial for virulence, specifically mediating the binding of hyphae to host epithelial cells. Expressed in filamentous forms, *Hwp1* is a substrate for host transglutaminases, enabling covalent attachment to host tissues and promoting biofilm formation (Shintani, 2020). Molecular studies by Shimono (2010) show that *HWP-1* is highly prevalent in *Candida* strains isolated from diabetic wounds, often found in 100% of analyzed clinical isolates. Studies conducted by Shimono *et al.* (2010) shows *Candida albicans* virulence found that the *HWP-1* gene at 572bp is nearly universally present in diabetic wounds. Studies conducted by Shapiro *et al.* (2011) shows that *Candida* is more frequently isolated in diabetic wounds compared to *P. vulgaris*, both species exhibit similar urease-mediated virulence mechanisms, including related accessory genes. The *HWP-1* gene is consistently found in nearly all *C. albicans* strains with studies conducted by Shimono (2010) reporting 94.17% to 100% prevalence in clinical samples. PCR amplification of the *HWP-1* gene allows for the differentiation of *C. albicans* (producing a 540 to 650 bp band) from related species like *C. dubliniensis* (570 bp) and *C. africana* (600 bp). While highly prevalent, the gene shows heterozygosity (differing alleles in the same strain) in roughly 71% of cases, and homozygosity (identical alleles) in roughly 29% of cases (Sigurgeirsson *et al.*, 2014).

In the genus *Candida*, the *ERG-1* genes (specifically *ERG-1*) are critical components of the ergosterol biosynthesis pathway. They are best known in the medical and scientific community for their direct role in antifungal drug resistance. A study conducted by Sigurgeirsson (2014) indicated that 80% of specific *C. albicans* isolates expressed *ERG-1* gene on mRNA. This is the most famous gene in the pathway; it encodes the enzyme lanosterol 14- α -demethylase. This enzyme is the primary target for azole antifungal medications like fluconazole, itraconazole, and voriconazole. Azole drugs work by binding to the *ERG-1* protein, thereby shutting down the production of ergosterol and destroying the fungal cell membrane. However, *Candida* can develop resistance to these life-saving drugs through two primary genetic mechanisms related to *ERG-1*. The clinical implication of *ERG-1* is also expressed in some cancers, and the *ERG-1* protein is involved in transcriptional regulation. However, the strongest established inherited clinical association is cardiac arrhythmia. This prevents azole drugs from properly attaching, rendering the medication ineffective. Gene overexpression in *Candida* cells can heavily upregulate (over-express) the *ERG-1* gene. By producing a massive amount of the target enzyme, the fungus outnumbers and dilutes the effects of the antifungal drug. These resistance mechanisms are monitored globally across multiple *Candida* species responsible for mucosal and invasive candidiasis, including: *Candida albicans*, *Candida glabrata* (now often reclassified as *Nakaseomyces glabratus*) *Candida tropicalis* and *Candida auris*. The *MDR1* (multidrug resistant) gene, encoding a major efflux pump, is highly prevalent in *Candida albicans*, particularly in clinical isolates showing resistance to fluconazole (Sigurgeirsson *et al.*, 2014). Studies reported by Singh (2019) shows that *MDR1* gene detection rates ranging from approximately 58% to over 80% in resistant strains of *Candida albicans* with significant overexpression associated with persistent, recurrent, and drug-resistant infection. The *MDR1* is often detected alongside other resistance genes, with one study finding it in 58.43% of tested, often drug resistant, *Candida* isolates (Ladhani *et al.*, 2021). A study conducted by Singh (2016) indicated that 80% of specific *C. albicans* isolates expressed *MDR1* gene on mRNA. While basal expression may be low, *MDR1* is significantly overexpressed in fluconazole-resistant *C. albicans*, contributing to the efflux of azole drugs. Overexpression of *MDR1* gene is frequently linked to recurrence in diabetic wounds (Smijts *et al.*, 2011).

The overall topology of the phylogenetic tree reveals clear grouping into major clades, supporting the notion of a shared evolutionary ancestry among the isolates while highlighting intra-species variability. The radial representation used in this study is appropriate for illustrating genetic relatedness without implying a strict evolutionary timeline, which aligns Smith (2018) with best practices in molecular phylogenetics. The phylogenetic relationships in fig. 1 observed in this study provide molecular support for phenotypic and antifungal susceptibility findings. Integrating phylogenetic data with phenotypic characteristics strengthens bacterial classification and enhances understanding of evolutionary and epidemiological patterns. Similar integrative findings have been recommended by Soll (2014) for improving the accuracy of microbial identification and tracking bacterial transmission

Conclusion

Evidence in the study demonstrates the coexistence of key virulence gene (*HWP-1*) and resistance genes (*ERG-1* and *MDR1*) genes among *Candida* species isolates in this study. The successful amplification of *ITS-1* gene in all tested isolates provides a strong molecular basis for subsequent sequencing and phylogenetic analysis. The phylogenetic analysis demonstrates that, the bacterial isolates share a common evolutionary origin, they exhibit varying levels of genetic relatedness. This highlights both conservation and diversity within the population and underscores the importance of molecular phylogenetics in bacterial taxonomy, evolutionary studies, and public health surveillance.

Recommendations

- i. Non-*Candida albicans* species with potential biofilm forming should be eradicated because of their ability to act as emerging predominant cause of diabetic foot ulcers.
- ii. A vivid understanding of the mechanisms and variations of biofilm formation in *Candida* species will make the advent of methods to curb their formation possible
- iii. There should be a continuous antifungal resistance surveillance and strengthening of antifungal stewardship programmes which is imperative to enhance patient care and management
- iv. Further studies in this area will contribute towards the identification of new targets for future therapeutics against these recently emerged pathogens.

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