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Internal Transcribed Spacer Characterization and Molecular Detection of ERG2 gene Mutation in Clinical Isolates of Suspected *Candida species* at a Tertiary Hospital in Port Harcourt, Nigeria

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Abstract

Internal transcribed spacer (ITS) is a nonfunctional RNA located between structural ribosomal RNAs (rRNA) of a common precursor transcript. ITS sequence analysis provides species-level identification when routine testing is inadequate for optimal patient care. Studies in Nigeria have focused on the molecular characterization of *Candida albicans* and their resistance mechanisms, including ERG2 gene mutations. Clinical specimens were collected from various clinics and cultured on Sabouraud dextrose agar. After molecular characterization using PCR and sequencing, the results showed that females (91.4%) predominated among the 221 subjects analyzed, with the highest prevalence in high vaginal swabs (33.5%). Antifungal drug resistance was highest in the 21-30 years age group, with females showing greater resistance (72.1%) compared to males (8.5%). Molecular studies showed 20.7% of females harbored the ERG gene, while no males did. Molecular characterization was more significant than phenotypic, as not all phenotypically confirmed isolates were *C. albicans*. The study highlighted the importance of ITS testing for prompt diagnosis.

Keywords: Internal transcribed spacer (ITS); *Candida albicans*; ERG 2; Molecular; Port Harcourt

Introduction

The polymorphic fungus *Candida albicans* is part of the normal human microbiome. In most people, *C. albicans* exists as a lifelong, harmless commensal. It is found in the gastrointestinal tract and mouth in 40-60% of healthy individuals (Meyer et al., 2013). *Candida albicans* can become a pathogenic yeast in immunocompromised individuals under various conditions. It does not multiply outside the human body (Gow, 2017). This species causes the human infection candidiasis, ranging from super-

ficial skin infections to life-threatening systemic infections due to an overgrowth of the fungus (Erdogan & Rao, 2015; Martins et al., 2014). Recent studies suggest that *C. albicans* can cross the blood-brain barrier (Wu et al., 2019).

The internal transcribed spacer (ITS) is a nonfunctional RNA piece located between structural ribosomal RNAs (rRNA) of a common originator transcript. ITS regions offer the highest chance of successful identification for a wide range of fungi due to a clear barcode gap between inter- and intraspecific variation. The nuclear ribosomal large subunit, another phylogenetic marker, has superior species resolution in some groups like early diverging lineages and ascomycete yeasts (Schoch et al., 2012). When routine testing is inadequate, ITS sequence analysis provides species-level identification in most unresolved cases (Ciardo et al., 2006). ITS analysis can identify most specimens, including non-culturable ones, and can differentiate closely related medically important species, such as *C. albicans* and C. dubliniensis (Nagia et al., 2018). It is essential for characterizing new and emerging pathogens, especially those resistant to current antifungal drugs (Ciardo et al., 2006). ITS analysis is also crucial for constructing phylogenetic trees and establishing evolutionary relationships between related organisms (Chen et al., 2011).

Recent changes in the epidemiology of candidiasis highlight an increase in *non-Candida albicans* species, stressing the need for reliable identification methods. Molecular diagnostics in fungal infections provide advanced species characterization, especially for closely related species in the *Candida albicans* complex (Boerhm et al., 1994). Several molecular approaches target the ribosomal RNA (rRNA) genes, focusing on the D1D2 domain of the 26S rRNA large subunit or the ITS1 and ITS2 regions (Boerhm et al., 1994). With the emergence of non-albicans Candida species, quick and accurate laboratory identification is necessary to help physicians select and dose optimal treatments (Nagia et al., 2018).

In *C. albicans* as well as in other fungi, Ergosterol (ERG) biosynthesis is one of the indispensable processes needed for life and continuity; and ERG2 gene encoded by the enzyme C-8 sterol isomerase is one of the genes involved in the process (Karl et al., 2000). *Candida albicans* species are significant fungal pathogens responsible for various infections, particularly in immunocompromised individuals. The identification and characterization of *Candida albicans* species, alongside their anti-fungal resistance profiles, have become crucial in clinical microbiology. One important aspect of resistance is the role of ERG genes, such as ERG11 and ERG2, which are involved in ergosterol biosynthesis, a key component of the fungal cell membrane. Mutations in these genes can lead to resistance against azole anti-fungal drugs, commonly used to treat *Candida albicans* infections.

Studies conducted worldwide have highlighted the increasing prevalence of azole-resistant *Candida albicans* species, with a notable focus on the detection of mutations in ERG genes. In Nigeria, several studies have focused on the molecular characterization of *Candida albicans* species and their resistance mechanisms. For instance, Ikenyi et al. (2020) investigated the antifungal susceptibility and presence of mutant ERG11 genes in vaginal *Candida albicans* isolates in Uyo, Nigeria, emphasizing the need for molecular surveillance in clinical settings. Similarly, the detection of ERG2 gene mutations, specifically, is also critical as it impacts the efficacy of amphotericin B and other azole antifungals. Mohamed et al. (2020) reported the characterization of clinical isolates of *Candida albicans* species, including the identification of ERG2 mutants with reduced sensitivity to amphotericin B.

In Port Harcourt, molecular studies on *Candida albicans* isolates have focused on identifying species and understanding their resistance profiles. The ongoing surveillance and molecular detection of ERG gene mutations are essential to guide effective anti-fungal therapy and control measures in hospitals. This study aims to extend the understanding of ERG2 gene presence in clinical *Candida albicans* isolates from hospitals in Port Harcourt, providing valuable insights into the molecular epidemiology of anti-fungal resistance in this region.

Methodology

Area of Study

This study was conducted at the University of Port Harcourt Teaching Hospital, Alakahia. Isolates of *Candida albicans* were collected for a period of four months and analyzed using molecular techniques at the Niger Delta University, Amasoma, Bayelsa State. The University of Port Harcourt is a Tertiary Hospital with 500 bed capacity and a hand full of health professionals who are experts in different

fields of medicine. It is a center of excellence in Healthcare for the South-South geopolitical zone of Nigeria. It is situated at Alakahia community, in Obio-Akpo local government area, Rivers State (the sixth-most populous state in Nigeria). It is located 4°45′N 6°50′E / 4.750°N 6.833°/ E. The inland part of Rivers State consists of tropical rainforest; towards the coast the typical Niger Delta environment, features many mangrove swamps. The capital of Rivers State, Port Harcourt, is the largest city and it is economically significant as the focal point of Nigeria's petroleum industry.

Sample Size Calculation

The sample size was computed using the formula

$N= Z^2P(1-P)/d^2$

Where N is sample size, P is expected prevalence (P=0.107) (Aaron et al., 2017).

d is precision (d=0.05) and Z is statistic (1.96 at 95% confidence level (Daniel, 1999).

$N= (1.96)2(0.107)\ (1-0.107)/(0.05)^2$

N=147

Sample Collection

Clinical specimens were collected from Obstetrics and Gynecology Clinic as well as from Out Patients Clinic, Accident and Emergency Clinic and also from the routine samples processed at the Medical Microbiology and Parasitology Department of the University of Port Harcourt Teaching Hospital. The specimens were collected from high vagina swabs, endocervical swabs, throat swabs, eye swabs, ear swabs, wound swabs, urethral swabs, catheter tips and blood cultures.

Culturing Method

Each specimen was aseptically inoculated onto a Saboraud dextrose agar plate and incubated at 37oC overnight. Each suspected colony from the above culture was further sub-inoculated onto another Saboraud dextrose agar plate for purity aseptically and incubated at 37oC overnight. Suspected colonies of *Candida albicans* albicans from the purity plate were identified using: One drop of sterile normal saline was placed on a clean dry grease free slide and a colony of *Candida albicans* isolate was emulsified in it and covered with a cover slip. The smear was examined under the microscope using X10 and X40 objective lens. Gram stain was carried out. A colony of the *Candida albicans* isolate was used to make a smear on a clean dry grease free slide using a drop of sterile normal saline and allowed to air dry. The dried smear was passed through flame three times to fix it. Then, the slide was placed on a plain horizontal staining rack and the smear was flooded with the primary stain crystal violet and allowed to stain for 30 seconds. Then, the stain was washed off with water and the smear was flooded with the mordant, lugol's iodine for 60 seconds and washed off. It was then flooded with the decolorizer, 70% alcohol, washed off with water and was counter stained with neutral red for a further 3 minutes. The stain was washed off with water and the back of the slide was cleaned and allowed to air dry; when dried it was viewed under the microscope using X100 objective. *Candida albicans* species being gram positive appear as purple colored big oval cells.

Germ Tube Test

One colony of each *Candida albicans* isolate was inoculated onto a sterile test tube containing 0.5ml (12 drops) of freshly serum collected blood under aseptic conditions. The test tube was agitated to allow for proper emulsification of the mixture. Then, the tube was incubated at 37°C for 4 hours. Thereafter, a drop of the serum/*Candida albicans* mixture was placed on a clean dry grease free slide, covered with a coverslip and was viewed under the microscope using X10 and X40 objective lens. The test is said to be Positive when a slender tube protrusion is observed from a single cell without any constriction. Otherwise, the test is negative.

Urease Test

One to two colonies of the *Candida albicans* isolates was aseptically inoculated onto a urease agar slant using a sterile wire loop by streaking the surface of the slant, then corked loosely and incubated at 37oC for 24 to 48 hours. The organism is urease negative so does not produce urea making the agar slant remain light orange (that is no color change). It is used to differentiate between the yeasts, *Candida albicans* albicans and *Cryptococcus neoformans*.

Susceptibility Testing of Isolates

The antifungal susceptibility testing was carried out using the Saboraud dextrose agar, sterile cotton wool swab stick, sterile wire loop, antifungal discs from Oxoid diagnostics Ltd, 0.5 McFarland standard, sterile normal saline, Petri dishes, forceps and mm rule. A sterile wire loop was used to touch two pure colonies of the *Candida albicans* isolate from a purity plate and suspended in a 3ml sterile saline and mixed to homogeneity in the bijou bottle. The turbidity was compared with that of 0.5 McFarland standards against a white card containing several horizontal backlines. Then, a sterile swab stick was dipped into the bijou bottle solution and excess fluid drained. Thereafter, the swab was used to streak on the Saboraud dextrose agar plate three times over the entire surface of the plate. The swab was discarded into a waste container; using a sterile forceps the antifungal discs were then applied onto the plates in an aseptic manner with each disc about 24mm apart from each other. Then the plate was incubated at 37oC for 24 hours. The zones of inhibition were then measured using minimal inhibitory concentration by agar dilution method.

Molecular Amplification

Colonies of the *Candida albicans* isolate were inoculated onto a Saboraud dextrose agar slant and were stored at -10°C for molecular studies. DNA extraction employed the boiling method. From Luria Bertani (LB) overnight broth culture of the fungal isolate 0.5 ml of the isolate was transferred into 1.5ml Eppendorf tubes and made up to 1.5 ml by adding 1ml of normal saline and was spun at 12000rpm for 5 min. The supernatant was decanted and the cells re-suspended in 500ul of normal saline and spun at 12000rpm for 5 min. The washing was repeated 3 times after which DNA Elution buffer 500µl (0.5ml) was added and then heated at 95°C for 20 min using Heating Block. The heated fungal suspension was cooled on ice at -20°C for 10 min and spun for 3 min at 12000rpm. The supernatant containing the DNA was transferred to a 1.5ml microcentrifuge tube and stored at -20°C for other downstream reactions.

DNA quantification was carried out using a Nanodrop 1000 spectrophotometer. The equipment was made ready with 2 ul of sterile distilled water, after which it was blanked using 2 ul of DNA Elution buffer. Two microliter (2 ul) of the extracted DNA was loaded onto the lower pedestal; the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured and recorded. The internal transcribed spacer (ITS) region of the rRNA genes of the extracted and quantified fungal genome of the isolates were amplified using the forward primer ITS1F: 5'-CTTGGTCATTTAGAGGAAGTAA-3' and the reverse primer ITS4: 5'- TCCTCCGCTTATTGATATGC-3. The PCR amplification was performed on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and the extracted DNA as template. Each PCR reaction contained: 1 µl template DNA, 0.32 µl each of forward primer and reverse primer, 20 µl of the Master mix (Taq DNA polymerase, deoxyribonucleotide triophosphate solution (dNTPs, MgCl) and water. The PCR conditions were as follows: (step 1) Initial denaturation, 95°C for 5 minutes; (step 2) denaturation, 95°C for 30 seconds; (step 3) annealing, 53°C for 30 seconds; (step 4) extension, 72°C for 30 seconds, steps 2-4 were repeated for 35 cycles and final extension, 72°C for 5 minutes. The amplicones were ascertained on a 1% agarose gel prepared in 1X Tris Acetate EDTA (TAE) buffer at 120V for 30 minutes and visualized on a blue light transilluminator.

ERG2 gene detection (amplification) was carried out using the polymerase chain reaction (PCR). After the extraction and quantification of the whole fungal genome of the isolates, polymerase chain reaction (PCR) analysis was carried out for the detection of ERG2 (1480bp) using the forward primer ERG11-S (5' AGGGGTTCCATTTGTTTACA 3') and the reverse primer ERG11-A (5' CCAAATGATTTCTGCTGGTT 3'). Each PCR reaction contained: 2 µl template DNA, 0.8 µl each of forward primer and reverse primer, 1.25 µl deoxyribonucleotide triophosphate solution (dNTPs) (2.5 mM of each dNTP), 0.1 µl Taq DNA polymerase (5 units/µl), 2.5 µl 10 × PCR

buffer and water to a total volume of 40 µl. The PCR amplification was performed on a Mastercycler gradient thermocycler (Eppendorf AG, North Ryde, Australia). The thermal cycling conditions were (step 1) Initial denaturation 95°C for 5 minutes, followed by (step 2) denaturation 94°C for 45 seconds, (step3) annealing 58°C for 45 seconds, and (step 4) extension 72°C for 90 seconds, steps 2-4 were repeated for 35 cycles with a final extension step at 72°C for 5 minutes. The amplicones were separated on 1% agarose gel prepared in 1X Tris Acetate EDTA (TAE) buffer and visualized under UV illumination after staining with safe stain load dye 120V for 30 minutes to verify amplicon quantity prior to sequence analysis (Wang et al., 2009).

Agarose Gel Electrophoresis

1g of agarose powder was measured into a clean microwavable flask. The powder was mixed with 100ml 1X Tris-Boric EDTA (TBE) and microwaved in pulses, for 1-3 minutes by swirling the flask intermittently until the agarose was completely dissolved. Care was taken not to over boil the solution to prevent reducing the final percentage of the agarose in the gel. After which the agarose solution was allowed to cool to about 500C for 5 minutes. It was then poured slowly into a tray with the well comb in place to avoid bubbles. The freshly poured gel was refrigerated at 4°C for 10-15 minutes until it has completely solidified. To each of the DNA samples loading buffer was added which provided a visible dye and increased the density of samples enabling them to settle. The solidified agarose gel was placed into the gel box of the electrophoretic tank. The box was filled with 1X TBE until the gel was covered. A DNA ladder was carefully loaded into the first lane of the gel. Then the samples were carefully loaded into the additional wells of the gel. The gel was allowed to run at 120V for 30 minutes. After which the power was turned off and the gel was carefully removed from the gel box. Using UV light, the DNA fragments were visualized as bands on the gel with the DNA ladder as a guide.

Internal Transcribed Spacer (ITS) Sequencing

Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10ul; the components included 0.25 ul BigDye terminator v1.1/v3.1, 2.25ul of 5 x BigDye sequencing buffer, 10uM Primer PCR primer, and 2-10ng PCR template per 100bp. The sequencing condition were as follows 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4min.

Phylogenetic Analysis

Obtained sequences were edited using the bio-informatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN. These sequences were aligned using MAFFT. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 (Saitou & Nei, 1987). The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) is taken to represent the evolutionary history of the taxa analysed. The evolutionary distances were computed using the Jukes-Cantor method (Jukes & Cantor, 1969).

Data Analysis

All data were entered into a Microsoft excel 2010 spread sheet and transferred unto SPSS IBM version 21 for statistical analysis using Chi-square at 95% confidence level. $P < 0.05$ was considered as significant.

Results

The study analyzed 221 subjects, with a predominant number being females (91.4%) and a minority being males (8.6%). The age distribution revealed the majority of subjects (53.4%) were between 21-30 years, while the least were in the age brackets 51-60 and 71-80 years (0.9%). No subjects were found in the 61-70 years age range. From 129 isolated *Candida albicans* species, a higher prevalence was found in females (52.5%) compared to males (5.9%). Age-wise distribution showed the highest isolation in the 21-30 years bracket (30.8%). The prevalence of *Candida albicans* species was significantly higher in females aged 21-30 years ($P<0.05$). Specimen analysis revealed the highest prevalence in high vaginal swabs (33.5%), followed by endocervical swabs (13.6%) and throat swabs (5.9%). Resistance to antifungal drugs was highest in the 21-30 years age group (45.0%), with females showing greater resistance

(72.1%) compared to males (8.5%). For molecular studies, 20.7% of females harbored the ERG gene, while no males did. Age brackets 0-10 and 31-40 years showed a higher prevalence of the ERG gene. High vaginal and endocervical swabs exhibited the highest prevalence of the ERG gene (6.9%). The ITS of the isolates showed a percentage similarity to other species at 99-100%. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of ITS of the isolates within the *Candida albicans* and Cryptococcus sp. and revealed a closely relatedness to *Candida albicans* glabrata, *Candida albicans* albicans and *Cryptococcus neofromans* respectively(Fig. 1).

Age Range	*Number Examined (%)*	*Male (%)*	*Female (%)*	X^2
0--10	16 (7.2)	6 (2.7)	10 (4.5)	
11--20	12 (5.4)	0 (0)	12 (5.4)	
21--30	118 (53.4)	8 (3.6)	110 (49.8)	
31--40	59 (26.7)	3 (1.4)	56 (25.3)	
41--50	12 (5.4)	0 (0)	12 (5.4)	
51--60	2 (0.9)	0 (0)	2 (0.9)	
61--70	0 (0)	0 (0)	0 (0)	
71--80	2 (0.9)	2 (0.9)	0 (0)	
Total	221	19 (8.6)	202 (91.4)	0.83, p<0.05

Table 1: Distribution of Subjects by Age and Gender.

Age Range	*Number Examined*	*Male (%)*	*Female (%)*	*Total (%)*	X^2
0--10	16	6 (2.7)	8 (3.6)	14 (6.3)	
11--20	12	0 (0)	6 (2.7)	6 (2.7)	
21--30	118	4 (1.8)	64 (29.0)	68 (30.8)	
31--40	59	3 (1.4)	30 (13.6)	33 (14.9)	
41--50	12	0 (0)	6 (2.7)	6 (2.7)	
51--60	2	0 (0)	2 (0.9)	2 (0.9)	
61--70	0	0 (0)	0 (0)	0 (0)	
71--80	2	0 (0)	0 (0)	0 (0)	
Total	221	13 (5.9)	116 (52.5)	129 (58.4)	20.34, p<0.05

Table 2: Distribution of Candida albicans species by Age and Gender.

Sample Sources	*Number Examined (%)*	*Number Positive (%)*	X^2
High Vaginal Swab	130	74 (33.5)	
Endocervical Swab	54	30 (13.6)	
Throat Swab	17	13 (5.9)	
Ear Swab	4	0 (0)	
Wound Swab	4	2 (0.9)	
Pleural Fluid	4	2 (0.9)	
Eye Swab	2	2 (0.9)	
Urethral Swab	2	2 (0.9)	
Catheter Tip	2	2 (0.9)	
Blood Culture	2	2 (0.9)	
Total	221	129 (58.4)	40.56, p<0.05

Table 3: Distribution of Candida albicans species by Source of Specimen.

Age	***Number***	***FLU***	***CLOT***	***NYS***	***ITRA***	***KET***	***Correlation (r)***
Range	Tested	(% R)	(% R)	(% R)	(% R)	(% R)	
0--10	14	9 (7.0)	9 (7.0)	14 (10.9)	13 (10.1)	7 (5.4)	
11--20	6	6 (4.7)	3 (2.3)	6 (4.7)	6 (4.7)	4 (3.1)	
21--30	68	56 (43.4)	36 (27.9)	56 (43.4)	58 (45.0)	40 (31.0)	
31--40	33	22 (17.1)	15 (11.6)	27 (20.9)	25 (19.4)	15 (11.6)	
41--50	6	3 (2.3)	1 (0.8)	3 (2.3)	5 (3.9)	1 (0.8)	
51--60	2	2 (1.6)	2 (1.6)	2 (1.6)	0(0)	2 (1.6)	
61--70	0	NA	NA	NA	NA	NA	
71--80	0	NA	NA	NA	NA	NA	
Total	129	98 (76.0)	66 (51.2)	108 (83.7)	107 (83.0)	69 (53.5)	0.95, p<0.05 666666666666666663365

Table 4: Susceptibility Pattern of Candida albicans species by Age.

Gender	***Number Tested***	***FLU***	***CLOT***	***NYS***	***ITRA***	***KET***	
		(% R)	***(% R)***	***(% R)***	***(% R)***	***(% R)***	***Correlation (r)***
Male	13	8 (6.2)	7 (5.4)	11 (8.5)	11 (8.5)	5 (3.9)	
Female	116	85 (65.9)	69 (53.5)	93 (72.1)	92 (71.3)	65 (50.4)	
Total	129	93 (72.1)	76 (58.9)	104 (80.6)	103 (79.8)	70 (54.3)	r=0.89, p<0.05

Table 5: Susceptibility Pattern of Candida albicans specie by Gender.

Sample Sources	***Number Tested***	***FLU***	***CLOT***	***NYS***	***ITRA***	***KET***	
		(% R)	***(% R)***	***(% R)***	***(% R)***	***(% R)***	***Correlation (r)***
High Vaginal Swab	74	56 (43.4)	33 (25.6)	61 (47.3)	59 (45,7)	38 (29.5)	
Endocervical Swab	30	19 (14.7)	20 (15.5)	22 (17.1)	23 (17.8)	18 (14.0)	
Throat Swab	13	10 (7.8)	11 (8.5)	11 (8.5)	11 (8.5)	9 (7.0)	
Ear Swab	0	0	0	0	0	0	
Wound Swab	2	2 (1.6)	1 (0.8)	2 (1.6)	2 (1.6)	1 (0.8)	
Pleural Fluid	2	2 (1.6)	1 (0.8)	2 (1.6)	2 (1.6)	1 (0.8)	
Eye Swab	2	2 (1.6)	2 (1.6)	2 (1.6)	2 (1.6)	0(0)	
Urethral Swab	2	0 (0)	0(0)	2 (1.6)	2 (1.6)	0(0)	
Catheter Tip	2	2 (1.6)	2 (1.6)	2 (1.6)	2 (1.6)	2 (1.6)	
Blood Culture	2	2 (1.6)	2 (1.6)	2 (1.6)	2 (1.6)	2 (1.6)	
Total	129	95 (73.6)	72 (55.8)	106 (82.2)	105 (81.4)	71 (55.0)	0.83, p<0.05

Table 6: Susceptibility Pattern of Candida albicans specie by Source of Specimen.

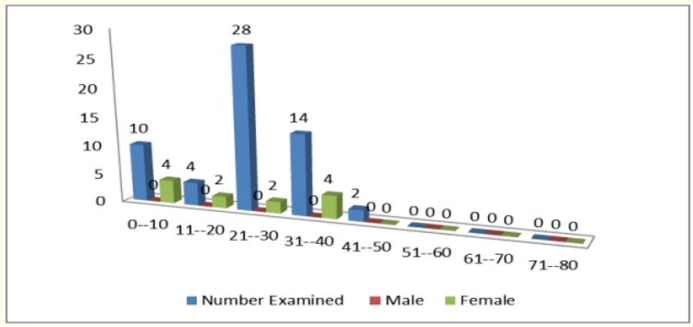

Figure 1: Distribution of ERG2 by Age and Gender.

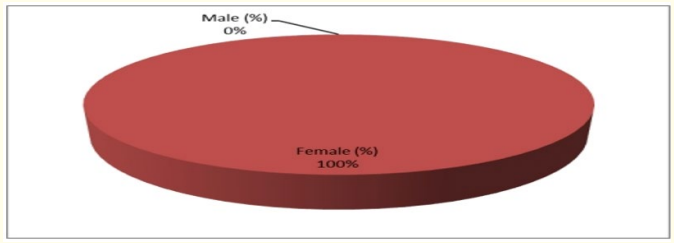

Figure 2: Percentage Sex Distribution of the Total Population of ERG2 Studied.

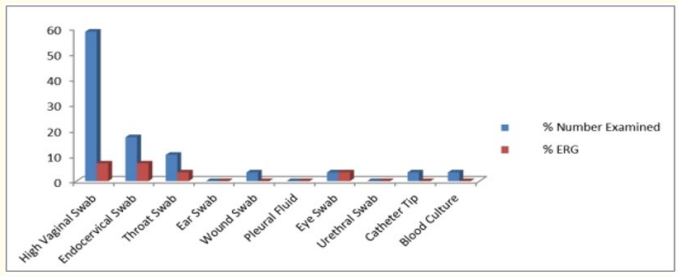

Figure 3: Percentage Distribution of ERG 2 by Source of Specimen.

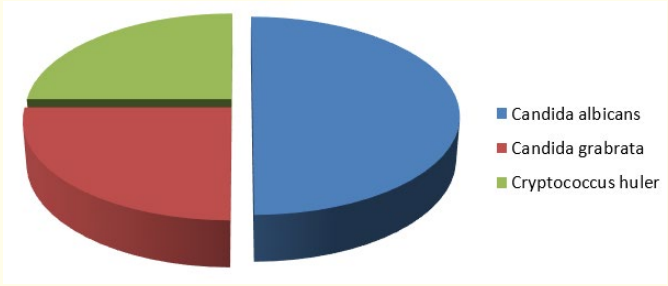

Figure 4: Percentage Distribution of Molecularly Identified Study Isolates.

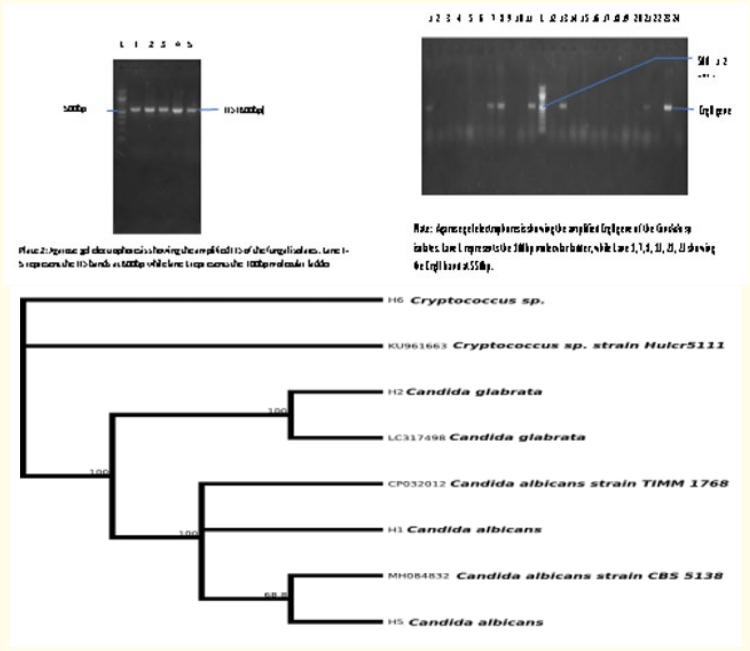

Figure 4: Phylogenetic Tree showing the evolutionary distance between the fungal isolates.

Molecularly Identified Organisms	***Percentage Occurrence (%)***
Candida albicans	50
Candida grabrata	25
Cryptococcus huler	25

Discussion

The study found that men had a significantly lower percentage (8.6%) of *Candida albicans* isolates compared to women, supporting the idea that men's genital anatomical structure offers better protection against *Candida albicans* infections than women's more exposed and fragile genital structure. This finding aligns with previous studies by Jombo et al. (2010) and Aaron et al. (2017) but contrasts with findings by Ga-Yeon et al. (2016) and Shin et al. (2010), which reported no significant gender differences in *Candida albicans* infections.

Age-wise, the highest prevalence of *Candida albicans* infections was among individuals aged 21-30 years (30.8%), followed by those aged 31-40 years (14.9%), with the lowest detection rate in individuals over 50 years. This trend suggests that younger, sexually active individuals are more prone to *Candida albicans* infections, likely due to higher rates of sexually transmitted diseases and antibiotic misuse, leading to vaginal flora disruption. These findings are consistent with studies by Aaron et al. (2017), Al-akeel et al. (2012), Fernandes et al. (2009), and Akinbiyi et al. (2008), but differ from reports by Ga-Yeon et al. (2016), Shin et al. (2010), and Moon et al. (2002), which noted the lowest infection rates among teenagers. The study also highlights the role of iatrogenic factors in nosocomial *Candida albicans* infections and suggests that most young adult women will experience symptomatic urogenital candidiasis at some point (Ringahi et al., 2010).

Statistical analysis of study variables using ChiSquare showed that Age Range and Gender ($\chi 2=60.12, p<0.05$), Age Range and Presence of *Candida albicans* ($\chi 2=20.34, p<0.05$), Source of Specimen and Presence of *Candida albicans* ($\chi 2=40.56, p<0.05$), all indicate significant associations between age range, gender, and the presence of Candida albicans. The majority of cases (53.4%) were in the 21-30 age range, with females (91.4%) being significantly more affected than males (8.6%). This aligns with previous research indicating higher Candida prevalence in females, possibly due to hormonal factors and anatomical differences (Aaron et al., 2017). Also, this is consistent with findings that Candida infections are more common in the vaginal region due to its moist environment, which is conducive to fungal growth (Abuod et al., 2008). *Candida albicans* species were most frequently detected in high vaginal swabs, correlating with the high prevalence of vaginal candidiasis among commercial sex workers as reported by Aaron et al. (2017) and Aboud et al. (2008). This suggests that unsafe sexual activities increase candidiasis prevalence. Mohamadi et al. (2015) found a significant positive relationship between high estrogen contraceptives and vaginal candidiasis due to hormonal and pH level changes in the vagina. In contrast, Ga-Yeon et al. (2016) and Shin et al. (2010) found most *Candida albicans* spp. in urine catheters and sputum.

The study results indicates a significant correlations between age, gender, source of specimen, and susceptibility patterns. The present study showed *Candida albicans* species' highest resistance to nystatin (83.7% and 80.6%) and fluconazole (76% and 72.1%), with the lowest resistance to clotrimazole (51.2%) and ketoconazole (54.3%). This differs from Mohamadi et al. (2015), where fluconazole had the highest resistance (79%) and nystatin and amphotericin B the least (1% and 0%). Aaron et al. (2017) found the highest resistance for itraconazole. Higher resistance levels were seen in the 21-30 and 31-40 age groups, among females, and in high vaginal and endocervical swabs. Resistance rates to fluconazole (FLU) were highest among the antifungal drugs tested, especially in the 21-30 age group (43.4%) and in females (65.9%). The statistical analysis indicated a significant relationship between Age and Susceptibility Pattern ($r=0.95, p<0.05$), Gender and Susceptibility Pattern ($r=0.89, p<0.05$), as well as Source of Specimen and Susceptibility Pattern ($r=0.83, p<0.05$). These results reflect the increasing global concern over antifungal resistance, particularly to fluconazole, which is a commonly used antifungal agent. These findings align with Mohamadi et al. (2015) and indicate that nystatin resistance may result from the overuse of antibiotics and anti-malaria drugs, stressing the need for proper diagnostics in a malaria-endemic society.

Previous studies by Tavakoli et al. (2010) and Henry et al. (2010) demonstrated the role of ERG11 upregulation in fluconazole-resistant clinical isolates of *Candida albicans* spp. and the upregulation in *Candida albicans* krusei by azoles, respectively. In this study, PCR employing ITS was used to detect ERG2 in clinical *Candida albicans* spp. isolates, considering age, gender, and sample sources. Results indicated the presence of the ERG2 gene predominantly in females and sexually active individuals aged 20-40 years, with high prevalence in high vaginal and endocervical swabs. This suggests that ERG2 plays a critical role in antifungal resistance by regulating fungal vacuole integrity in *C. albicans* and other *Candida albicans* spp. High resistance in these demographics can be attributed to ERG2

gene presence. Similarly, Bammert and Fostel (2000), and unpublished data cited by Rosamond and Allsop (2000), reported ERG2 involvement in ERG gene upregulation in response to azole, terbinafine, and amorolfine treatments or mutations. The ERG2 gene was detected predominantly in the 21-30 age group (48.3%). The ERG2 gene is associated with antifungal resistance, and its prevalence in this age group may indicate a higher exposure to antifungal agents, leading to selective pressure and resistance development. This aligns with the conclusions of Rosamond and Allsop (2000).

Nagia et al. (2018) found that molecular characterization using RNA markers provided more specific results than phenotypic biochemical tests, due to the unique internal transcribed spacer (ITS) in fungi DNA. This finding was supported by Ciardo et al. (2006), who used ITS to differentiate *Candida albicans* albicans from *Candida albicans* dubliniensis, showing similar biochemical qualities.

Conclusion

In conclusion, molecular characterization remained more significant than the phenotypic as not all the phenotypically confirmed isolates were *C. albicans*. This findings also revealed that these organisms also harboured ERG2 resistant genes.

Hence, the government and pharmaceutical companies should utilize the findings of this research in production of antifungal drugs since ERG2 gene also confers resistance on *Candida albicans* albicans in this our clime. This research work carried out revealed that *Candida albicans* albicans harbored ERG2 resistant genes in our environment. This study confirmed internal transcribed spacer (ITS) as one of the important marker for fungal identification.

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