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Detection of *ERG2* gene in *Candida albicans* in clinical isolates

Dunga Kingsley Excel[1*], Ofoegbu Nnamdi Jude[1], Tatfeng Y M[2], Njoku J O[3], Okoro Chinyere Ihuarulam[4], Ohalette C N[5], Nnodim Johnkennedy[6]

[1]Department of Medical Laboratory Science, Kingsley, Ozumba University, Ogboko, Ideato, Imo State, Nigeria.
[2]Department of Medical Laboratory Science, Niger Delta University, Amassom, Bayelsa State, Nigeria.
[3]Department of Public Health, Federal University of Technology, Owerri, Nigeria.
[4]Department of Microbiology/Parasitology, Federal Medical Centre, Owerri, Nigeria.
[5]Department of Microbiology, Imo State University, Owerri, Nigeria.
[6]Department of Medical Laboratory Science, Imo State University, Owerri, Nigeria.

[*]**Correspondence**
Dunga Kingsley Excel
kingsleydunga1@gmail.com

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Background: *Candida albicans* is an infectious fungus that infects people with weakened immune systems. The identification of resistance genes such as *ERG2* is important to manage the disease prevalence. This study aimed to detect the *ERG2* gene and internal transcribed spacer (ITS) sequences in *Candida albicans* clinical isolates and evaluate antifungal resistance.

Methods: This study assessed 221 samples for *Candida albicans* using conventional methods like Gram staining, germ tube tests, and biochemical analysis. Molecular techniques, specifically PCR, were employed to detect the *ERG2* gene and amplify internal transcribed spacer (ITS) sequences for accurate identification and antifungal resistance evaluation. Antifungal susceptibility testing was performed using the agar dilution method against a panel of five drugs. The amplified ITS regions were also subjected to sequencing for further molecular characterization of the isolates.

Results: The maximum prevalence of *Candida* species was observed in the female patients (116 out of 129). The age group 21-30 showed a higher prevalence of Candida species also showed higher resistance to antifungal drugs. The maximum resistance observed was for Nystatin (83.7%) and Itraconazole (83.0%) for all the age groups. The *ERG2* gene detection was limited to females' isolates, especially from the vaginal and endocervical swabs.

Conclusion: Our study thus supports the use of molecular techniques, particularly the internal transcribed spacer (ITS), as a key marker for identifying fungi.

Keywords: *ERG2 gene, Candida albicans, teaching hospital, internal transcribed, sequencing profile*

Introduction

Candida albicans, a polymorphic fungus, is a typical component of the human microbiome. The majority of the time, *C. albicans* is a lifelong, benign commensal. In the oral and digestive systems of 40–60% of healthy people (Talapko et al., 2021). *Candida albicans* is an opportunistic pathogenic yeast that can grow in a range of environments in immunocompromised people. Outside of the human body, it does not proliferate (Asao et al., 2023) One of the few species of the genus *Candida* and phylum Ascomycota that causes the human ailment candidiasis, which is brought on by an overgrowth of the fungus, can range from minor skin infections to potentially fatal systemic infections (Peng et al., 2021). Yet considering that fresh research suggests *C. albicans* can penetrate the blood-brain barrier (Hillenbrand et

al., 2022). An internal transcribed spacer (ITS) is a segment of structural ribosomal RNA (rRNA) that is found between two transcripts that share an origin. The internal transcribed spacer (ITS) region of the ribosomal cistron, which has the most clearly defined barcode gap between inter- and intraspecific variation, provides the highest likelihood of successful identification for the broadest variety of fungi (Kim et al., 2019). A well-liked phylogenetic marker in several taxonomic groupings, the nuclear ribosomal large subunit had greater species resolution in the early diverging lineages and the ascomycete yeasts (Maione et al., 2022). The epidemiology of candidiasis infection, however, has recently changed, highlighting a rise in non-*Candida albicans* species and emphasizing the necessity for accurate identification techniques (Waikhom et al., 2020). Advanced species characterisation is provided by molecular diagnostics in fungal infections, typically in cases of closely related species in the *Candida* complexes (Sagar, 2017). The D1D2 domain of the 26S rRNA large subunit or the internal transcribed spacer regions ITS1 and ITS2 are two molecular strategies that have been established and were typically designed for the ribosomal RNA (rRNA) genes (Sah et al., 2019). Ergosterol (ERG) production is one of the essential processes required for life and continuation in *C. albicans* and other fungus, and the *ERG2* gene encoded by the enzyme C-8 sterol isomerase is one of the genes implicated in the process (Chen et al., 2022). The *ERG2* gene also controls the fungal vacuole's ability to function normally in *C. albicans*. However, morpholine antifungals have a negative impact on its action, causing a disruption in the production route for ergosterol (*ERG*) (Kishore et al., 2020). The most significant contributor to *Candida* infections linked to healthcare is now known to be *Candida albicans*. Because it has spread globally, it is much more concerning for public health indicators. To help healthcare professionals find a solution, coordinated efforts must be made to better understand the mechanisms that lead to *Candida albicans* resistance. Increased incidence of resistance to fluconazole and other regularly used antifungals are seen in our clinics. It has been found that *Candida albicans* is resistant to common antifungals. Moreover, the *ERG2* gene has been linked to antifungal resistance in various climates, necessitating research into its relationship to our native strains of *Candida albicans.*

Materials and methods

Area of study

The University of Port Harcourt Teaching Hospital, Alakahia, served as the study's site. At the Niger Delta University in Amasoma, Bayelsa State, *Candida* isolates were gathered over the course of four months and subjected to molecular analysis. With 500 beds and a small number of medical staff who are specialists in several specialties, the University of Port Harcourt is a Tertiary Hospital. It serves as a medical center of excellence for Nigeria's South-South geopolitical region. It is located in the hamlet of Alakahia in the Rivers State local government area of Obio-Akpor (the sixth-most populous state in Nigeria). It is situated at 4.750°N 6.833°E, or 4°45'N, 6°50'E. The inland region of Rivers State is covered in mangrove swamps and tropical rainforests that are similar to the typical Niger Delta environment. Port Harcourt, the capital and largest city of Rivers State is economically significant as the hub of Nigeria's petroleum industry.

Sample collection

The University of Port Harcourt Teaching Hospital's Medical Microbiology and Parasitology Department routinely processes routine samples from the Obstetrics and Gynecology Clinic, Out Patients Clinic, Accident and Emergency Clinic, and other clinics. These routine samples also served as the source of clinical specimens. The samples were taken from catheter tips, blood cultures, endocervical swabs, throat swabs, eye swabs, ear swabs, wound swabs, and high vaginal swabs. For purity testing and gram staining, each isolate was subsequently inoculated onto a Sabouraud dextrose agar plate. Each sample was aseptically injected onto a Sabouraud dextrose agar plate and incubated at 37°C overnight as part of the sample processing and culture process. A second Sabouraud dextrose agar plate was used to sub-inoculate each questionable colony from the aforementioned culture. This plate was incubated at 37°C overnight to ensure purity.

Identifying techniques

Candida albicans colonies from the purity plate were identified by:

Microscopy: A colony of *Candida* isolate was emulsified in one drop of sterile normal saline, which was then applied on a clean, dry slide that was free of oil. The slide was then covered with a cover slip. Under the microscope, the smear was looked at through X10 and X40 objective lenses. Gram Staining Methodology. Using a drop of sterile normal saline and a colony of the *Candida* isolate, a smear was created on a slide that was clean, dry, and free of grease and allowed to air dry. The dried stain was restored by passing it through the flame three times. The principal stain crystal

violet was then applied to the smear and left to stain for 30 seconds while the slide was set up on a simple, horizontal staining rack. When the stain had been removed with water, the smear was drenched with Lugol's iodine for 60 seconds before being wiped off. Afterward, it was submerged in 70% decolorizing alcohol, removed with water, and counter-dyed with neutral red for an additional three minutes. The slide was cleaned, the stain removed with water, and allowed to air dry before being examined under a microscope with an X100 objective. Gram-positive *Candida* species show up as large, purple-colored cells in a germ tube test. Under aseptic conditions, one colony of each *Candida* isolate was inoculated onto a sterile test tube containing 0.5 ml (12 drops) of recently obtained blood serum. To ensure that the mixture was properly emulsified, the test tube was shaken. The tube was then incubated for 4 hours at 37°C. After that, a coverslip-covered slide with a drop of the serum/*Candida* mixture was placed on it, and an X10 or X40 objective lens was used to see it under the microscope. When a thin tube protrudes from a single cell without being constricted, the test is said to be positive. The test is negative if anything else.

Urease test: A sterile wire loop was used to streak one to two colonies of the *Candida* isolate onto a urease agar slant. The slant was then loosely corked and incubated at 37°C for 24 to 48 hours. Because the bacterium lacks urease, it cannot make urea, which keeps the agar slant's color bright orange (that is no color change). It is used to distinguish between the yeasts *Cryptococcus neoformans* and *Candida albicans*.

Testing for susceptibility in isolates

Assay for antifungal

The Sabouraud dextrose agar, sterile cotton wool swab stick, sterile wire loop, antifungal discs from Oxoid Diagnostics Ltd, 0.5 McFarland standard, sterile normal saline, forceps, and mm ruler were used to conduct the antifungal susceptibility tests. Two pure colonies of the *Candida* isolate were touched with a sterile wire loop, suspended in 3 ml of sterile saline, and homogenized in the bijou bottle. The turbidity was measured on a white card with several horizontal backlines and compared to 0.5 McFarland standards. The extra fluid was then drained after dipping a sterile swab stick into the bijou bottle solution. The Sabouraud dextrose agar plate was then streaked three times with the swab across its whole surface. The swab was deposited into a garbage receptacle, and the antifungal discs were then aseptically attached to the plates, spacing them apart by roughly 24 mm using sterile forceps. The plate was then incubated for 24 hours at 37°C. The zones of inhibition were then quantified using the agar dilution method at the lowest inhibitory dose.

Molecular studies' storage

For molecular research, pure colonies of the *Candida* isolates were inoculated onto Sabouraud dextrose agar slants and kept at -10°C.

DNA extraction for molecular identification (boiling method)

The fungal isolate was obtained using Luria Bertani (LB) overnight broth culture. 0.5 ml of the isolate was put into a 1.5 ml Eppendorf tube, and the volume was increased to 1.5 ml by adding 1 ml of ordinary saline. The fungus suspension was thoroughly combined with a vortex mixer and spun for five minutes at a speed of 12000 rpm. The cells were re-suspended in 500/µl of normal saline and spun at 12000 rpm for 5 minutes after the supernatant had been decanted into a garbage container. After three rounds of washing, DNA Elution buffer SOUL (0.5 ml) was added and heated using a heating block at 95°C for 20 minutes. The heated fungal suspension was spun at 12000 rpm for three minutes after cooling on ice for 10 minutes at -20°C. After that, 200 µl of the DNA-containing supernatant was transferred to a 1.5ml microcentrifuge tube and kept at -20°C for further reactions that would take place later.

Quantity of DNA

The Nanodrop 1000 spectrophotometer was used to measure the amount of DNA that was extracted. 2 ml of sterile distilled water was used to prepare the equipment, and 2 µl of DNA Elution buffer was used to blank it. The top pedestal was lowered into contact with the extracted DNA on the lower pedestal after two microliters (2µl) of the extracted DNA were put onto the lower pedestal. The DNA concentration reading was then obtained and recorded. PCR Amplification of the Internal Transcribed Spacer (ITS). The extracted and quantified fungal genomes of the isolates' internal transcribed spacer (ITS) region rRNA genes were amplified using the forward primers ITSIF: 5'-CTTGGTCATTTAGAGGAAGTAA-3' and ITS4: 5'-TCCTCCGCTTATTGATATGC-3'. On an Applied Biosystems ABI 9700 thermal cycler, the PCR amplification was carried out 35 times at a final volume of 40 microliters. The X2 Dream Taq Master mix from Inqaba, South Africa, Taq polymerase, DNTPs, and $MgCl_2$ were used in the PCR mix,

along with the primers at a concentration of 0.4 M and the extracted DNA as template. Each PCR reaction contained 1 µl template DNA, 0.32 µl of forward primer and 0.32 µl of reverse primer, 20 µl of the Master mix (Taq DNA polymerase, dNTPs, MgCl, and water), and 1 µl of Taq DNA polymerase. The following were the PCR conditions: (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; (step 5) Final extension, 72°C for 5 minutes. Steps 2-4 were repeated for 35 cycles. The amplicons were then detected on an agarose gel with a concentration of 1% in IX Tris Boric EDTA (TBE) buffer at 120V for 30 minutes and observed under a blue light transilluminator. Polymerase chain reaction (PCR) analysis was performed for the detection of *ERG2* (1480 bp) using the forward primer ERG2-S (5' AGGGGTTCCATTTGTTTACA 3') and the reverse primer ERG2-A (5' CCAAATGATTTCTGCTGGTT 3'). This was done after the extraction and quantification of the entire fungal genome of the isolates. A total of 40 µl of water, 2 µl of template DNA, 0.8 (µ1 each of forward and reverse primers, 1.25 µl of deoxyribonucleotide triphosphate solution (dNTPs), 2.5 µl of PCR buffer, and 0.1 µl of Tag DNA polymerase were used in each PCR experiment. On a Mastercycler gradient thermocycler, the PCR amplification was carried out (Eppendorf AG, North Ryde, Australia). The thermal cycling conditions were as follows: step 1: initial denaturation at 95 °C for 5 minutes, step 2: denaturation at 94 °C for 45 seconds, step 3: annealing at 58 °C for 45 seconds, step 4: extension at 72 °C for 90 seconds, step 5: final extension at 72 °C for 5 minutes. Steps 2-4 were repeated for 35 cycles. The amplicons were then separated on a 1% agarose gel that had been produced in IX Tris Boric EDTA (TBE) buffer, and their quantity was checked for 30 minutes under UV illumination at 120V before sequence analysis (Abbey et al., 2017).

DNA sample loading with agarose gel running

Following internal transcribed spacer (ITS) amplification and *ERG2* identification using polymerase chain reaction (PCR) analysis, the amplicons of each amplification reaction were identified. This was accomplished by adding a loading buffer to each DNA sample, which offered a visible dye and increased sample density, allowing the samples to settle. The electrophoretic tank's gel box was filled with the hardened agarose gel. VIII Tris Boric EDTA (TBE) was added to the box until the gel was completely covered. The first lane of the gel was carefully loaded with a DNA ladder. The materials were then meticulously put into the additional gel wells. For 30 minutes, the gel was left to operate at 120V. The gel was then gently taken out of the gel box and the power was turned off. The DNA ladder served as a reference as UV light was used to see the DNA fragments as bands on the gel.

Sequencing using Internal Transcribed Spacers (ITS)

Inqaba Bioteclinological, Pretoria, South Africa, used a 3510 ABI sequencer to perform the sequencing using the Big Dye Terminator kit. A final volume of l0 µl was used for sequencing, and the following materials were used: 2.25 µl of 5 x Big Dye sequencing buffer, 10 µM Primer PCR primer, and 2–10 ng PCR template per 100 bp. The following were the sequencing requirements: There are 32 cycles of 96°C for 10s, 55°C for 5s, and 60°C for 4 minutes.

Results

The distribution of subjects by gender showed that 202 (91.4%) subjects were females, while 19(8.6%) were males. The distribution of subjects by age revealed that 16 (7.2%) were in the age bracket 0 and 10 years, 12 (5.4%) were in the age brackets 11-20 years and 41-50 years respectively. Subjects within the age bracket 21-30 years were 118 (53.4%), those within the age bracket 31-40 years were 59 (26.7%) and 2 (0.9%) were in the age brackets 51-60 and 71-80 years, respectively. On the other hand, there were no subjects for the age range of 61-70 years (Table 1).

Table 1. Distribution of subjects by age and gender

Age Range	Number Examined (%)	Male (%)	Female (%)
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)

Of the 129 *Candida species* isolated, 13 (5.9%) were from male subjects, while 116 (52.5%) were from female subjects. The distribution of the isolates by age revealed that 14 (6.3%), 6 (2.7%), 68 (30.8%), 33 (14.9%), 6 (2.7%) and 2 (0.9%) were isolated from subjects within age brackets 0-10 years, 11-20 years, 21-30 years, 31-40 years, 41-50 years and 51-60 years respectively. The prevalence of *Candida species* was significantly higher in females within the age bracket 21-30 years when compared to females in other age groups (P< 0.05) (Table 2).

Table 2. Distribution of *Candida* species by age and gender

Age Range	Number Examined	Male (%)	Female (%)	Total (%)
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)

(P< 0.05)

Table 3. Distribution of *Candida* species by source of specimen

Sample Sources	Number Examined (%)	Number Positive (%)
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)

(P< 0.05)

The distribution of *Candida species* by specimen showed that 74 (33.5%), 30 (13.6%) and 13(5.9%) were from high vaginal swab, endocervical swab and throat swab respectively; whereas 2(0.9%) were from wound swab, pleural fluid, eye swab, urethral swab, catheter tip and blood culture respectively and none from ear swab. The prevalence of *Candida species* was significantly higher in high vaginal swabs than what was obtained in other specimens (P< 0.05) (Table 3). The susceptibility pattern of *Candida* species isolated from the 129 subjects by age showed that resistance to antifungal drugs was observed across all age groups.

Table 4. Susceptibility pattern of *Candida* species by age

Age Range	Number Tested	FLU (% R)	CLOT (% R)	NYS (% R)	ITRA (% R)	KET (% 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)

(P< 0.05)

The susceptibility pattern (Table 4) of *Candida species* isolated from the 129 subjects by age showed that, the *Candida species* to antifungal drugs was significantly higher within the age bracket 21-30 years when compared to other age groups.

Table 5. Susceptibility pattern of *Candida* species by gender

Gender	Number tested	FLU (%R)	CLOT (%R)	NYS (%R)	ITRA (%R)	KET (%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)

Table 6. Susceptibility pattern of *Candida* species by source of specimen

Sample Sources	Number Tested	FLU (% R)	CLOT (% R)	NYS	ITRA (% R)	KET (% 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	NA	NA	NA	NA	NA
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)

(P<0.05)

FLU: Fluconazole; CLOT: Clotrimazole; NYS: Nystatin; ITRA: Itraconazole; KET: Ketoconazole; R: Resistance; NA: Not Applicable

The susceptibility pattern of the *Candida species* isolated from the 129 subjects by gender revealed that the highest resistance prevalence of 93 (72.1%) was seen in females while 11 (8.5%) in males respectively to Nystatin and Itraconazole antifungal drugs. However, the prevalence of *Candida species* to antifungals drugs was significantly higher in females when compared to males (P< 0.05) (Table 5) Of the 129 *Candida* isolates subjected to antifungal studies the susceptibility pattern showed that, 61 (47.3%), 23 (17.8%) and 11 (8.5%) were from high vaginal swab, endocervical swab and throat swab respectively; whereas 2 (1.6%) were from wound swab, pleural fluid, eye swab, urethral swab, catheter tip and blood culture respectively and none from ear swab. The prevalence of *Candida species* was significantly higher in high vaginal swabs than what was obtained in other specimens (P< 0.05) (Table 6).

Table 7. Distribution *of ERG2* by age and gender

Age Range	Number Examined (%)	Male (%)	Female (%)
0-10	10(17.2)	0(0)	4 (6.9)
11 –20	4 (6.9)	0(0)	2(3.4)
21-30	28 (48.3)	0(0)	2(3.4)
31-40	14(24.1)	0(0)	4 (6.9)
41-50	2(3.4)	0(0)	0(0)
51-60	0(0)	0(0)	0(0)
61-70	0(0)	0(0)	0(0)
71-80	0(0)	0(0)	0(0)
Total	58	0(0)	12(20.7)

(P< 0.05)

Table 8. Distribution of *ERG2* by source of specimen

Sample Sources	Number Examined (%)	*ERG* (%)
High Vaginal Swab	34(58.6)	4 (6.9)
Endocervical Swab	10(17.2)	4 (6.9)
Throat Swab	6(10.3)	2 (3.4)
Ear Swab	0(0)	0(0)
Wound Swab	2 (3.4)	0(0)
Pleural Fluid	0(0)	0(0)
Eye Swab	2(3.4)	2(3.4)
Urethral Swab	0(0)	0(0)
Catheter Tip	2 (3.4)	0(0)
Blood Culture	2(3.4)	0(0)
Total	58	12 (20.7)

(P< 0.05)

Table 7 shows the distribution of *ERG2* by Age and Gender in which the highest occurred in females 4 (6.9%) and 4 (6.9%) within the ages of 0-10 and 31-40 years respectively. Furthermore, the distribution of *ERG2* by Source of Specimen showed that high vaginal swab 4 (6.9%) and endocervical Swab 4 (6.9%) had a significant *ERG2* (Table 8).

Discussion

Because men have a more protective genital anatomical structure than women, who have an open, receptive, and fragile genital structure, making them more vulnerable to acquiring infections, this study's result of the percentage distribution of the *Candida* species indicated that men are less predisposed to *Candida* infections than women, and 5.9% male percentage frequency distribution of *Candida* isolates (Ueda et al., 2019). On the other hand, it was different from the findings of Aaron et al. (2017); Dhingra & Cramer (2017), which showed that there was no significant difference between the sexes and that the sex ratio was 1:1.1. The age group 21 to 30 years had the highest distribution of *Candida* species, followed by 31 to 40 years (14.9%), and the distribution of *Candida* species decreased as you moved to the left or right of group 21 to 30 years (the highest percentage occurring group). Although the age group over 50 had the lowest detection rate. It was believed that this age group's infection rate was relatively low due to both a weakened immune system brought on by aging and a lack of outdoor activity. However, this was in contrast to the findings of Breazzano et al. (2022), in which teenagers had the lowest detection rate. This implied that young people who were sexually active were more likely to have *Candida* infections, which was in line with other reports (Chouhan et al., 2019; Osset-Trénor et al., 2023; Koehler et al., 2019). Additionally, the high prevalence of candidiasis in these age groups may be explained by the fact that they were more likely to contract STDs and were, therefore, more likely to self-medicate with antibiotics out of fear of social stigma. As a result, antibiotic abuse could result in the destruction of the vagina's normal flora, which would lead to *Candida* overgrowth and symptomatic infections (Aaron et al., 2017). In addition to their sexual habits, this may be due to the use of iatrogenic materials during their clinic visits, which led to nosocomial infections, which allowed opportunistic infections like candidiasis to flourish (Banos et al., 2018). Nonetheless, it is believed that the majority of young adult women will go through a symptomatic episode of urogenital candidiasis at some point in their lives (Chen et al., 2022). These episodes often occur anytime *Candida* spp. has multiplied over a specific threshold in the genital region. When each sample source was taken into account, the highest rates of *Candida* species were found in high vaginal swabs compared to other sample sources from this study. This coincided with the remarkably high prevalence of vaginal candidiasis, which was linked to the activities of commercial sex workers (Arip et al., 2022). Thus, it was also projected how risky sexual behavior would affect the prevalence of candidiasis. The study of Iranian women in Ham-Iran showed a substantial link between the use of high estrogen contraceptives and vaginal candidiasis since these changes in hormone levels and vaginal pH occur as a result (O'Donnell et al., 2022). Although the majority of *Candida* spp. were found in urinary catheters and sputum, there were some minor discrepancies in the detection rates from high vaginal swabs acquired in this investigation. In the current study, the resistance of *Candida* species isolated from age and gender of subjects to five antifungal medications, including four azoles including two imidazoles (ketoconazole and clotrimazole) and two triazoles (fluconazole and itraconazole), and nystatin, indicated that the highest resistance was seen for nystatin with 83.7% and 80.6% respectively while fluconazole was 76% and 72.1% respectively. However, in comparison to other age groups, as well as among females and from high vaginal and endocervical swabs in comparison to other sample sources, a higher resistance level was observed in the age groups 21–30 years and 31–40 years. This finding suggested that the high nystatin resistance across age, gender, and sample sources could be attributed to the overgrowth of *Candida* species as a result of the consumption of antibiotics and anti-malaria drugs due to stress, the misdiagnosis of malaria and typhoid fever due to their nonspecific signs and symptoms, and the fact that malaria is endemic in our society as a developing African country (Ramírez-Soto & Bonifaz, 2022). Furthermore, the majority of these disorders may have become more prevalent as a result of empiric therapy without an accurate laboratory diagnosis of a malaria infection or candidiasis (Wu et al., 2019). The role of *ERG 11* overexpression in fluconazole-resistant clinical isolates of *Candida* spp. and upregulation in *Candida krusei* by azoles, respectively, had been demonstrated in earlier research (Esfahani et al., 2022; Spivak & Hanson, 2017). However, a PCR using ITS was used in this study to detect *ERG2* in clinical *Candida* spp. isolates in relation to age, gender, and sample sources. The results showed that the gene was present in females and in those between the ages of 20 and 40 who were sexually active, with a high prevalence in vaginal and endocervical swabs. Results suggested that *ERG2*, by its involvement in the regulation of the functional integrity of the fungal vacuole in *C. albicans* and other *Candida* spp., also plays crucial roles in the antifungal resistance of yeast. As a result, the existence of the *ERG2* gene may also be associated with the high resistance seen in the aforementioned age groups, genders, and sample sources. Similar to this, it was observed that *ERG2* was involved in the elevation of ERG genes in response to azole, terbinafine, and amorolfine therapy or mutations among the other four ERG genes employed in their investigations. Among cancer patients at the Isotope and Radiation Center in Khartoum, Sudan, the results of this study's phylogenetic analysis did not match those of the phenotypic biochemical tests (Kim et al., 2019). This could suggest that molecular characterization using RNA markers is more accurate due to the internal transcribed spacer

(ITS), which is specific to fungi because it involves the DNA of the fungi isolates. The quality of the biochemical tests and the interpreter's prejudice could both be contributing factors to the discrepancy. Nonetheless, this phylogenetic analysis conclusion is consistent with Banos et al. (2018), which employed molecular characterization to distinguish *Candida albicans* from *Candida dubliniensis*, which had many of the same biochemical characteristics.

Conclusion

In conclusion, not all of the phenotypically confirmed isolates were *C. albicans*, indicating that molecular characterisation remained more important than phenotypic. This discovery also demonstrated the presence of *ERG2* resistance genes in these organisms.

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Author contributions

Dunga Kingsley Excel was responsible for conceptualisation of the study and writing the protocol. Ofoegbu Nnamdi Jude handled the preparation of the materials, data collection, and experimental analysis. Tatfeng Y.M wrote the first draft of the manuscript, Njoku JO, Okoro Chinyere Ihuarulam and Ohalette CN provided the literature searches. The final part of the manuscript, as well as the references and citations were handled by Nnodim Johnkennedy. All authors read and approved the final manuscript.

Conflict of interest

The authors declared no conflict of interest.

Ethics approval

The ethical approval was obtained from the University of Port Harcourt Teaching Hospital.

AI tool usage declaration

No AI tool was used in manuscript preparation.

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