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Prevalence and Antifungal Susceptibility of *Candida* species from Patients attending Rivers State University Teaching Hospital, Nigeria

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Abstract

The development of medical therapy and patients profile has led to a rise in the incidence of nosocomial fungal infection. The frequency of candidiasis has surged worldwide, and the prevalent of healthcare diseases are now *Candida* species. *Candida* species causes a range of human infections known as Candidiasis. The non-albicans *Candida* (NAC) species have recently superseded *Candida albicans* as significant opportunistic pathogens. The study was conducted to determine the prevalence and antifungal susceptibility of *Candida* species isolated from various Clinical samples in Rivers State University Teaching Hospital, Port Harcourt, Nigeria. A total of 206 clinical specimens from male and female patients of all ages were sampled in the Department of Microbiology, Rivers State University Teaching Hospital, Port Harcourt, to investigate suspected *Candida* infections. The isolation and identification of *Candida* species was done by culture on SDA, Gram stain, sugar fermentation and phylogenetic profiling. Antifungal susceptibility pattern was done by Disc Diffusion method using Fluconazole, Ketoconazole, Miconazole, Nystatin and Itraconazole. The results showed that out of 206 specimens, 44 isolates (21.4%) were identified, with the majority (56.82%) from high vaginal swabs (HVS), followed by urine (31.82%) and oral swabs (11.36%). The age of patients ranged from four months to 73 years giving a Mean Age 1.86± 0.344, with females (85.4%) outnumbering males (13.6%). Prevalence of *Candida* spp revealed C*andida albicans* (50%), *Candida krusei* (18.2%), *Candida parapsilosis* (11.4%), *Candida glabrata* and *Candida tropicalis* (9.1%) respectively and *Candida pelliculosa* (2.2%), with *C. albicans* being the most prevalent. The antifungal susceptibility testing among the azoles showed that Fluconazole (79.5%) and Ketoconazole (77.3%) were most sensitive agents against isolates from HVS, urine and oral swabs respectively and Itraconazole (34.1%) was most resistant especially to those from oral swabs. This study highlights the increasing prevalence of NAC species over *Candida albicans* and the growing resistance of *Candida* isolates to commonly used antifungal drugs. Diagnosis of these species of *Candida* and sensitivity to antifungal agents are critical components to

treatment, particularly for patients with severe underlying illnesses who are hospitalized.

Introduction

In a worldwide scale, *Candida* species are the most common cause of fungal infections in humans. There are around 200 species in the *Candida* genus, with over 20 known to be significant agents of hospital-acquired infections, contributing to about 8-10% of all nosocomial infections [1]. *Candida* species are responsible for a wide range of human infections known as candidiasis, including superficial ones like oral thrush and vulvovaginal candidiasis, as well as more severe infections such as candidemia and stomatitis which are said to be ventures [2]. They can be found in various parts of the body like the mouth, throat, intestines, and genital and urinary tracts, making them one of the most common fungal pathogens causing diseases in humans [3, 4]. *Candida* infections can manifest in various chronic forms, with *Candida albicans* being the main culprit behind most superficial and systemic *Candida* infections as documented by [5]. The majority of *Candida* infections according to [6] affect the body's epithelial surfaces. Gastrointestinal candidiasis has been identified as a common cause of peptic ulcers, as *Candida* yeasts naturally inhabit the mouth and can lead to gastroesophageal inflammation, affecting up to 88% of patients. *Candida* yeast infections in the cardiovascular system are rare, except when they are in specific materials like blood, cerebrospinal fluid, or specialized culture media [6].

Candidiasis is considered one of the most widespread opportunistic fungal infections affecting humans globally. The organism mainly responsible for these infections is *C. albicans*, which naturally resides in the human intestinal system [1]. *C. albicans* accounts for approximately 70% of all *Candida* infections in humans with the remaining 30% caused by non-albicans *Candida* (NAC) species such as *C. tropicalis, C. glabrata, C. parapsilosis*, and *C. krusei.* These non-albicans *Candida* species are frequently identified in various sources like soil, animals, hospitals, and foods, and their prevalence has been noted [7; 3]. It is estimated that more than 30% of the global population, mostly women over the age of twelve, are affected by *Candida* infections [8]. *Candida albicans* plays a significant role in systemic candidiasis (45.3%), organ dysfunction, and mortality linked to the use of polyene antifungals as stated by [9].

The increase in predisposing conditions such as age, poor oral hygiene, HIV/AIDS, malnutrition, smoking, immune suppression, endocrine-related diseases, and changes in the epithelial cells have led to the rise in *Candida* infections [4]. Hospitalized patients worldwide are frequently affected by healthcare-associated infections, which significantly contribute to morbidity and mortality. Fungal nosocomial infections, especially candidemia, have been described as epidemic and a major factor the morbidity and mortality of critically ill patients [10]. Research by [11], has shown a decrease in the prevalence of *Candida albicans* as the causative agent of candidiasis, with a simultaneous increase in non-albicans *Candida* (NAC) species due to the extensive use of antifungal medications for longer treatment durations. Various classes of antifungals, including azoles, polyenes and echinodins like Fluconazole, have been used in the treatment of *Candida* infections. Additionally, Amphotericin B and Nystatin have become preferred medications for treating superficial fungal infections caused by *Candida* species [12; 4]. Antifungal susceptibility testing is crucial for selecting the most effective antifungal treatment for a specific fungal infection by detecting antifungal resistance. This testing is essential for optimizing treatment and improving patient outcomes, making it a valuable tool in medical mycology [13].

The incidence of candidiasis has been on the rise in recent years due to various risk factors like

prolonged catheterization, extensive surgical procedures, use of immune-suppressive medications, HIV infections, and other conditions affecting immunocompromised individuals. The increase in candidemia poses a severe threat , particularly to critically ill patients. The emergence of non-albicans *Candida* species as both colonizers and pathogens causing nosocomial fungal bloodstream infections has complicated the overall rise in candidemia. Different Candida species, such as *C. albicans* and *C. krusei* have been identified globally with varying antifungal susceptibility profiles. Though antifungal medications are available, *Candida* remains a significant medical challenge, exacerbated by the emergence of non-albicans *Candida* species and their resistance to existing antifungal drugs, making treatment increasingly difficult. Since the treatment of candidiasis if often empiric, selecting antifungal agents should be based on the likelihood of the pathogen and their resistance pattern anticipated in a specific region. Therefore, regular surveillance of causative agents of candidiasis and their resistance patterns in a given locality is crucial. In Rivers State, there is inadequate research on the resistance patterns of *Candida* species causing candidiasis in different clinical samples. No data has been published regarding yeast resistance in Otomycosis, Candiduria and Vulvovaginal candidiasis at the Rivers State University Teaching Hospital in Port Harcourt city Rivers State metropolitan area. This study aims to investigate the prevalence patterns and antifungal susceptibility among patients at the facility.

Materials and Methods

This research was conducted as a cross-sectional observational study in the Department of Microbiology Rivers State University Teaching Hospital (RUSTH) Port Harcourt, Nigeria. It is a State Government-owned Teaching Care Hospital. The study was carried out with a period of three months, from July to September, 2023. Approval of Ethical clearance was obtained from the Institutional Ethical Committee (IEC) NHREC No. RSUTH/REC/2023326, dated: the 24th July, 2023.

Specimens' collection

Specimen collection in this study was done following strict aseptic precautions from clinically suspected cases of candidiasis. Three types of clinical Specimens- High vaginal swabs (HVS), Urine, and Oral Swabs (OSs) were collected in this study using sterile swabs and bottles for appropriate specimens. The specimens were collected from inpatients and outpatients from the Departments of Gynecology, Pediatrics and Intensive Care unit (ICU), as well as Department of Medicine and Surgery of RSUTH. Comprehensive clinical history of all patients in this study was recorded but only age and sex were considered as demographic data in this study.

The following guidelines as described by [14] for the collection of clinical specimens were duly followed during the study.

1. Sterile collection devise and containers were used to collect the specimens
2. Specimens were collected under strict aseptic precautions
3. Sufficient specimens were collected
4. Specimens were collected from an active lesion containing viable organisms
5. The specimens were labeled appropriately

The methods employed in collecting these specimens from various sources were as follows:

1. **High vaginal Swabs (HVSs):** As speculum examination was done and the vaginal discharge was

collected with the aid of sterile swabs from the posterior fornix.

2. **Urine sample:** Fresh mid-stream and clean catch urine of about 20-50ml was collected in sterile screw-capped containers.
3. **Oral (Mouth) Swabs:** With the aid of a tongue depressor, the lesions were visualized and the specimens were collected using a sterile swab. All specimens were transported immediately to the laboratory for analysis.

Sample size

A total of 206 clinical samples with suspected cases within the period of July to September, 2023 made up the sample size for this investigation.

Inclusion criteria

Patients (male and female) of all age group were included in the study. All specimen showing *Candida* isolation from samples were also included in the study

Exclusion Criteria

Patients who were on antifungal treatment were excluded from the study

Culturing of *Candida* species from clinical sample

Candida species in this study were cultured using the streak method. All the clinical samples of HVS and oral swabs were streaked on the sterile surface of Saboraud Dextrose Agar (SDA) (Titen Biotech Ltd India) amended with Tetracycline. For the urine samples, a sterile swab stick was dipped into the urine before it was streaked onto the surface of the sterile SDA. They were labeled accordingly and incubated at room temperature for 2 – 5 days and examined daily for growth [15, 14]. Following incubation, colonial examinations of each isolate with respect to the clinical sample were identified for colour, shape, size and texture [16].

Isolation, Purification and Preservation of Isolates

Yeast cultures were isolated by streaking using a sterile wire loop on freshly prepared SDA and incubated for 3 days at room temperature so as to obtain pure isolates. Following incubation, the discrete single colony of each isolate that were grown along the line of streak was further grown on SDA using a sterile cotton wool. The pure isolate was then preserved in 15% sterile glycerol solution for further use.

Identification and Characterization of colonies

The colonies of yeast isolates were identified by the colony characters (cultural characteristics) as describe by [16], Gram staining and sugar fermentation.

Gram stain

Smears of each colony were made on a clean grease free slide, then heat fixed after air drying by passing the reverse side of the slide over a flame of Bunsen burner. The smears were stained by Gram's method using crystal violet (60 sec) Lugol's iodine (60 seconds), and decolorized with 95% alcohol till no colour appeared to ooze out, then counterstained for 40 seconds using Safranin, while washing was done after each stain was used. The smears were blot dried and observed under oil immersion for shapes and cream reactions [17].

Sugar Fermentation

Three carbohydrate broths were used in this study for the sugar fermentation test, each containing 1%

glucose, Lactose and sucrose separately with 1% peptone and 0.005% phenol red indicator. Inverted Durham's tubes were immersed for gas detection. All the broths were sterilized by autoclaving at 121°C for 15 minutes. On cooling the yeast colonies were inoculated into each, broth and incubated at 25oC for 2-3 days and examined at 24 hour intervals for acid (yellow colouration) and gas (space in Durham tubes) production.

Preparation of antifungal Agents

The antifungal agents used were 200mg Fluconazole capsule BP (Mancare Pharmaceuticals PVT LTD, India), 200mg Ketoral (Ketoconazole) Tablet USP (Laborate Pharmaceuticals LTD, India) Microzol-Plus containing 200mg Micronazole Nitrate and 750mg Metronidazole Tablet (Drugfield Pharmaceuticals LTD, Nigeria); 500000IU Nystatin Tablet (Mekophar Chemical Pharmaceutical Joint- Stock Company, Vietnam) and 250mg Itracap (Itraconozole)(Genix Pharma PVT, LTD, Pakistan). A twofold dilution of each stock solution of antifungal agent was prepared by diluting from the stock to the least concentration. Concentrations of 100µg/ml, 50µg/ml, 25µg/ml, 12.5µg/ml, 6.25µg/ml, 3.124µg/ml and 1.56µg/ml were prepared and were used to impregnate the sterile perforated WhatmanNo. 1 filter paper discs

Preparation of Fungal Suspension for Agar Diffusion Test

The inoculums of yeast isolates were grown in Tryptic Soy broth (TSB) medium for 18-24hrs, and the turbidity of the medium was adjusted by adding sterile normal saline until the turbidity matches that of 0.5 McFarland standard [18].

Inoculation of Agar Plates for Agar Diffusion Test

With a sterile swab was dipped into the already prepared inoculums and rotated firmly against the upper inside wall of the test tube for many times in order to remove excess fluid, the test yeast isolates were inoculated onto the dried sterile surface of SDA amended with tetracycline by streaking until the entire surface was covered. The plates were left for about 5 minutes in order for the surface moisture to be absorbed before applying the drug impregnated discs.

Antifungal Susceptibility Testing

In compliance with Clinical and Laboratory Standards Institute [19] guidelines the agar disc diffusion techniques were employed in the antifungal susceptibility testing. The drug impregnated discs were taken out form the containers using a pair of sterile forceps, and they were then placed onto of the yeast infected Saboraud Dextrose Agar. The discs were tightly pressed down to make contact with the SDA's surface using the sterile forceps. All plates were incubated at 37^0C for 48-72hrs [14].

Reading of the Plates and Measurement of Diameter of Zone of Inhibitions

After the incubation period, the plates were evaluated. Using a meter rule, the diameter of the zone of complete inhibition was measured and the Mean values were recorded in millimeters, rounding to the nearest whole number. The susceptibility (sensitivity), Intermediate or Susceptible-Dose Dependent (S-DD), and Resistance to the antifungal drugs to the test isolates were measured and compared to the standard zone interpretive breakpoints provided by the CLSI M44-A2 recommendations [14; 15].

Statistical Analysis

Patients' information was collected through oral questionnaires. The risk factors of infection type and the data obtained in this study were in this study were statistically analyzed using IBM SPSS statistic for windows version 26/IBM Corp Armonk. NY. USA. Descriptive statistics such as Mean, Standard

Table 1. Interpretive categories: breakpoint zone diameter (mm) for *Candida* species

Antifungal Agents	Disc Content (µg)	Sensitive (S)	Susceptible-Dose Dependent (S-DD)	Resistant (R)
Fluconazole	25	≥19mm	15 – 18mm	≤ 14mm
Ketoconazole	10	≥20mm	10 – 19mm	≤9mm
Miconazole	10	≥20mm	17 – 19mm	≤ 16mm
Nystatin	50	≥15mm	10 – 14mm	≤ 9mm
Itraconazole	10	≥17mm	14 – 16mm	≤ 13mm

Sources: (14; 15).

Deviation (SD), Tables and Proportional Annova were used to describe the data. Difference between propositions was analyzed using X^2 tests, if the sample sizes were small or unbalanced. A two tailed p-value< 0.05 was considered statistically significant.

Results

An uneven distribution of patient ages was seen in this study as shown in Table 2, with a large number of patients (82.0%) falling into the 11-40 years age group, with a mean age of 1.86± 0.344. The range of ages among the patients was 4 months old to 73 years. The age range of 11 to 30 has the greatest frequency.

The distribution of patients in this investigation based on gender is presented in Table 3. The results showed a higher percentage (86.4%) for female patients as compared to the male patients (13.6%) and this implies that the females outnumbered the males in this study. At p = 0.000, the patients' gender distribution showed a statistically significant difference of 36.59, p≤0.01.

Table 2. The Patients Age Distribution

	Gender		
Age	Female	Male	Total
0.10	7	2	9
11-20	51	5	56
21-30	63	3	66
31-40	43	6	49
41-50	8	9	17
51 and above	6	3	9
Total	178	28	206

Mean Age 1.86± 0.344

Table 3. Patients Distribution based on Gender

Gender	No of patients	Percentage
Female	178	86.4
Male	28	13.6
Total	206	100

$X^2 = 36.59$, $p = 0.000$

The total of 44 samples out of the 206 specimens collected, tested positive in this study for *Candida* species giving a prevalence of 66.1% ($p<0.513$), as shown in Table 4. Out of these 44 positive cases from the various specimens, 25(23.8%) were positive for HVS, 14(17.3%) from urine and 5(25.0%) from oral swabs. There was no statistically significance difference at $p\leq0.513$ among the specimens.

Table 4. Distribution of Culture Positive Cases according to Clinical specimen

Clinical Samples	No. of Sample Screened	Total number of isolates	Percentage
HVS	105	25	23.8
Urine	81	14	17.3
Oral swabs	20	5	25.0
Total	206	44	66.1

$X^2 = 1.334$; $p< 0.513$, significant

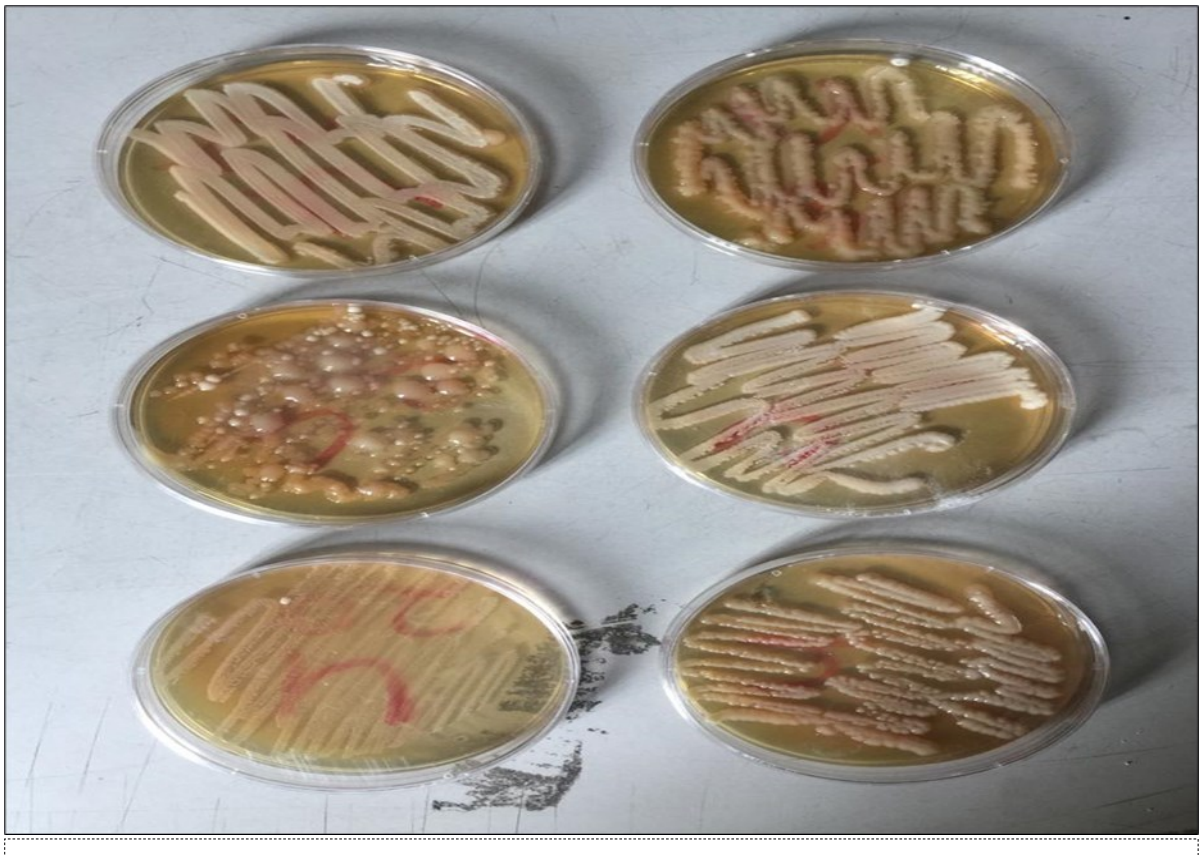

Figure 1. Plates showing Culture Positive Cases

Table 5. Culture positive cases according to Age and Gender

Age Groups	Female (%)	Male (%)	Total
0-10	0	1 (33.3%)	1
11-20	5 (12.2%)	1 (33.3%)	6
21-30	14 (34.1%)	0	14
31-40	20 (48.7%)	0	20
41-50	1 (2.4%)	1 (33.3%)	2
51 and above	1 (2.4%)	0	1
Total	41 (93.2%)	3 (6.8%)	44

X^2 – 25.112, p≤0.000

The results of the culture positive cases of *Candida* species according to age and gender in this study is shown in Table 5. Out of the 44 isolates obtained from various clinical specimens, 20 (48.7%) were isolated from the female patients within the age group of 31-40 years and this was the highest prevalence of *Candida* species observed in this study. Following that, 14 (34.1%) positive cases were isolated from female patients in the age group of 21-30 years while 5 (12.2%) were isolated from those of the age 11-20 years of age as the least 1 (2.4%) were found within the age group of 41 years and above for the females. For the male patients, 1 (33.3%) positive case was observed for those within the age group of 0-10 years, 11-20 years and 41-50 years. The prevalence percentage of positive cases in this investigation showed that females (93.2%) were greater than males (6.8%) with statically significant difference of 25.112 at p=0.000.

The prevalence of *Candida* species according to Clinical samples as shown in Table 6 showed a total of 44 isolates with 25 (56.8%) from HVS, 14 (31.8%) from urine and 5 (11.4%) from oral swabs. *Candida albicans* in this study was the most prevalent species accounting 22 (50%) whereas the non-albicans *Candida* (NAC) made up the other 50% of the total isolates. The prevalence rate of *Candida albicans* were 13 (59.1%), 7 (31.8%) and 2 (9.1%) for HVS, urine and oral swabs respectively, *C. glabrata* was 2 (50%), 1 (25%) and 1 (25%), *C. krusei* was 4 (50%), 3 (37.5%) and 1 (12.5%); *C. parapsilosis* 4

Table 6. Prevalence of *Candida* species according to Clinical Samples

Candida species	Specimens Hvs	Urine	Os	Total number of isolate	Percentage (%)
C. albicans	13(50%)	7(31.8%)	2(9.1%)	22	50
C. glabrata	2(50%)	1(25%)	1(25%)	4	91
C. krusei	4(50%)	3(37.5%)	1(12.5%)	8	18.2
C. parapsilosis	4(80%)	1(20%)	0	5	11.4
C. pelliculosa	1(100%)	0	0	1	2.2
C. tropicalis	1(25%)	2(50%)	1(25%)	4	9.1
Total	**25(36.8%)**	**14(31.8%)**	**5(11.4%)**	**44**	**100**

Table 7. Antifungal Susceptibility Pattern of *Candida* species

***Candida* Species**	**Fluconazole**			**Ketoconazole**			**Miconazole**			**Nystatin**			**Itraconazole**		
	S	**SDD**	**R**	**S**	**SDD**	**R**	**S**	**SDD**	**R**	**S**	**SDD**	**R**	**S**	**SDD**	**R**
C. albicans	19 (86.4%)	0	3 (13.6)	18 (81.9%)	1 (4.5%)	3 (13.6%)	15 (68.2%)	4 (18.2%)	3 (13.6%)	13 (59.1%)	6 (27.3%)	3 (13.6%)	11 (50%)	6 (27.3%)	5 (22.7%)
C. glabrata	3 (75%)	0	1 (25%)	3 (75%)	0	1 (25%)	2 (50%)	1 (25%)	1 (25%)	2 (50%)	1 (25%)	1 (25%)	0	3 (75%)	1 (25%)
C. krusei	7 (87.5%)	0	1 (12.5)	6 (75%)	1 (12.5%)	1 (12.5%)	5 (62.5%)	2 (25%)	1 (12.5%)	3 (37.5%)	4 (50%)	1 (12.5%)	1 (12.5%)	4 (50%)	3 (37.5%)
C. parapsilo-sis	4 (80%)	0	1 (20%)	4 (80%)	0	1 (20%)	2 (40%)	2 (40%)	1 (20%)	3 (60%)	1 (20%)	1 (20%)	1 (20%)	2 (40%)	2 (40%)
C. pelliculosa	0	0	1 (100%)	0	0	1 (100%)	0	0	1 (100%)	0	0	1 (100%)	0	0	1 (100%)
C. tropicalis	2 (50%)	1 (25%)	1 (25%)	3 (75%)	0	1 (25%)	2 (50%)	1 (25%)	1 (25%)	2 (50%)	1 (25%)	1 (25%)	0	1 (25%)	3 (75%)
Total	35 (79.5%)	1 (2.3%)	8 (18.2%)	34 (77.3%)	2 (4.5%)	8 (18.2%)	26 (59.1%)	10 (22.7%)	8 (18.2%)	23 (52.3%)	13 (29.5%)	8 (18.2%)	13 (29.5%)	16 (36.3%)	15 (34.1%)

Key: S= Sensitivity; SDD= Susceptible Dose Dependent; R= Resistant

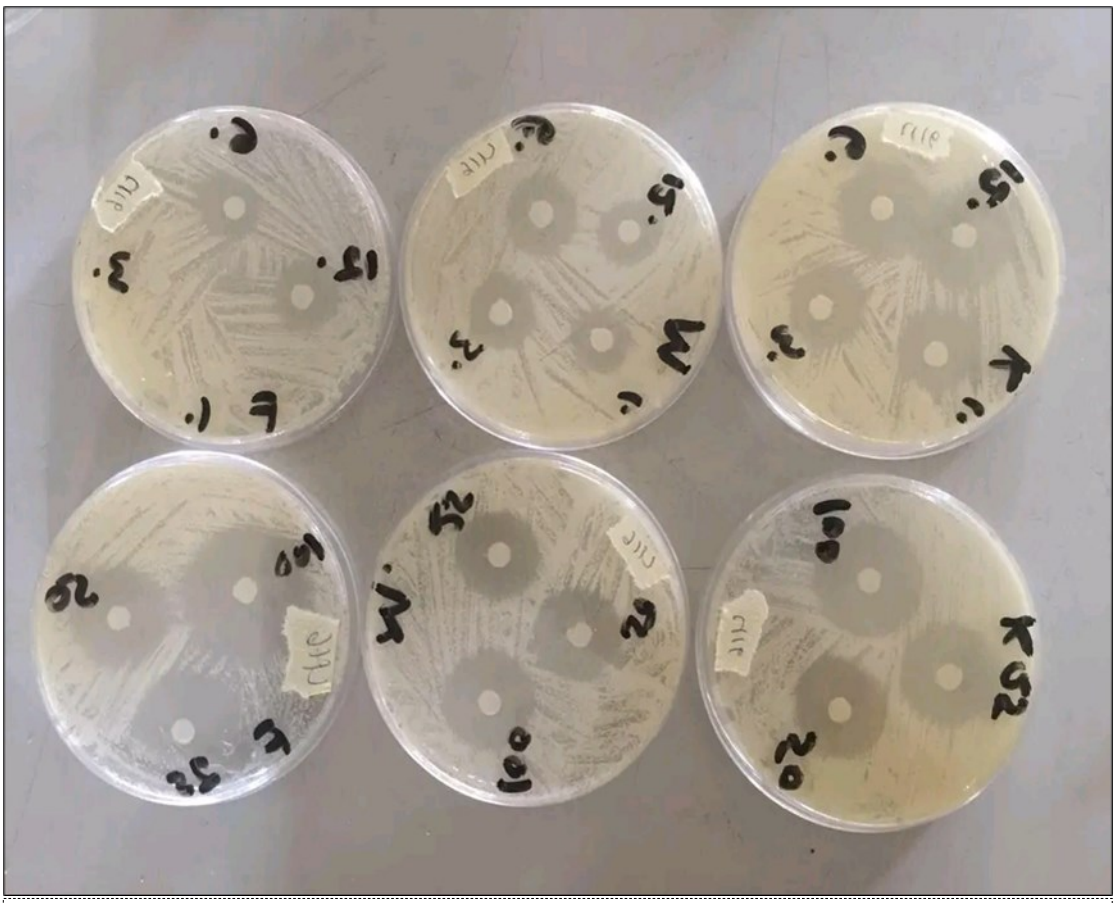

Figure 2. Plates showing zone of inhibitions of Antifungal agents to Test Isolates.

(80%) and 1 (20%) from HVS and urine respectively; *C. pelliculosa* 1 (100%) from HVS only whereas *C. tropicalis* was 1 (25%), 2 (50%) and 1 (25%) from HVS, urine and oral swabs, respectively.

The results of the Antifungal Susceptibility Pattern of *Candida* species in this study is presented in Table 7. In the present investigation, out of the 44 *Candida* isolates tested, Itraconazole demonstrated resistance in 34.1% cases studied. This was observed to be the most resistant among the antifungal agents. Miconazole and Nystatin showed resistance of 18.2%. The susceptible –Dose dependent was high in Itraconazole (36.4%), followed by Nystatin (29.3%) and Miconazole (22.7%). The highest sensitivity was noted in this study for Fluconazole (79.5%) and Ketoconazole (77.3%), 18.2% showed resistance and 2.3% and 4.5% showed Susceptible-Dose Dependent to Fluconazole and Ketoconazole respectively. Among the *Candida* species examined, *C. albicans* was most sensitive to all the antifungal agents followed by *C. krusei, C. glabrata* and *C. parapsilo*

Discussion

Distribution of patients according to Age

The current investigation revealed that among the patients who visited Rivers State University Teaching Hospital, Port Harcourt, both sexes and all ages can develop candidiasis as the study identified the particular types of *Candida* that results infections. The oldest participant in our study was 73 years old, and the youngest was a four-month – old baby giving a mean age of Mean Age 1.86± 0.344 years. The age range of 11-40 years old accounted for bulk of the patients in this study, which may be explained by the fact that this is the age group with the highest rates of pregnancy, sexual

activity and hormonal volatility. This results is however, lower when compared to the study of [20] who in their study reported the mean age group of 43.4 years. The result of this study is consistent with other studies as many investigations noted high rate in the age group of 20-49 years [14; 21]. Patients' age has a major influence on how susceptible they are to candidiasis especially if they are older than 65 years. *Candida* infections are more likely to cause death in the elderly especially in those over 65 years. Individuals in the reproductive age group are particularly susceptible to immune system and hormone variations. The distribution of *Candida* infections is influenced by these variables as well as physiological changes associated with ageing [22]

Distribution of patients according to gender

The study revealed that female patients 178 (86.4%) were higher when compared to male patients 28 (13.6%), which indicated that the females surpassed in number the males. This high distribution of females as noticed in this study has proven that they are mostly infected than the males. This finding is in conformity with the report of [14]. Similarly, the study also agrees with the report of [23], and [24], who had stated high distribution of females than males in the study but disagrees with the study of [25], who stated that males are commonly infected than females with an incidence of 94 (62.6%) and 56 (37.3%), respectively. Gender has a major role in the clinical research of candidiasis in different age groups, male and female exhibit different infection rates and yeast growth intensities [26]. This emphasizes how gender differences in the epidemiology and clinical symptoms of candidiasis must be taken into account.

Distribution of *Candida* species according to clinical samples.

The prevalence of *Candida* species with respect to the clinical specimens showed high distribution of 66.1%. The prevalence rate observed in this study is said to be higher compared to those reported by [27], who reported 26%. [28] and [29] had reported the prevalence rate of 30.7% and 30% in Jamaica and Nigeria respectively. This finding is however higher than the above results. The prevalence of *Candida* isolates in this study fluctuates with respect to the clinical specimens, and these results is in agreement with the report of [24], who documented in Ghana that high prevalence of *Candida* infection was isolated from HVS compared to urine. The high frequency of HVS and Oral Swabs observed in this research could be attributed to the vaginal and oral environment which could be influenced by hormonal changes, pH level, and the presence of glycogen.

Distribution of Culture Positive Cases according to Age and Gender

Age and sex are two main factors influencing candidiasis, with older people more prone to infections and invasive candidiasis is more common in women. These pre-disposition are further influenced by variation in the strength of yeast growth in males and females [30]. Comprehending these variables is imperative for proficient handling and avoidance of candidiasis. In our study, the prevalence of positive cases of *Candida* species with respect to gender and their age showed an inequitable distribution. The high prevalent of *Candida* species among the age group of 21-40years conforms with the study earlier reported by [25], that the majority of patients were in the age group of 21-60 years. [31], had similarly reported high incidence of *Candida* species in women in the age range of 17-44 years, while [14], reported high incidence of *Candida* species in the age group of 20-49 years.

Our investigation revealed that both sexes and all ages can get candidiasis. High infection rate in the age group as observed in this study for the females may be probably due to the indiscriminate drug usage, especially contraceptives among females; decreases levels of protection cervical antibodies in the reproductive tract, practice poor hygiene, history of antibiotics or drug misuse, hormonal factors,

anatomical differences and diabetes.

In the research we conducted, substantial gender disparities were seen when the number of yeast colonies in the entire group were analyzed with respect to age. The number of yeast colonies in female group varies with age and this agrees with the study of [30]. Rural women had an elevated rate of infections owing mostly to conditions of poor medical care, lack of health education, scarce economic resources and difficulty in timely medical treatment as documented by [32] and this also may contribute to the high prevalence of *Candida* species in this study.

Distribution of *Candida* species according to clinical samples

In our study we identified 44 species of *Candida*, with *Candida albicans* 22 (50%) being the most prevalent. Among the non-albicans *Candida* species it was observed in this study that *C. krusei* 8 (18.2%) demonstrated increased prevalence proceeded by *C. parapsilosis* 5 (11.4%), *C. glabrata*and *C. tropicalis* 4 (9.1%), respectively and the least was *C. pelliculosa* 1 (2.2%) which aligns with the publicly available reports from various global location [14; 1; 16., 15; and 33]. The high prevalence of *Candida* species from HVS in this study is concordant with the study of [34]. *Candida* species has been stated to be the fifth most frequent nosocomial infections in hospitals with *C. albicans* accounting for 76-89% in VVC and 25% in Urine, followed by non-albicans *Candida* and fourth most frequently isolated pathogens from blood stream [35; 25; 4. and 14). *C. albicans* being the most prevailing *Candida* species in this study has been reported for several decades to be the primary cause of invasive infections that can be fatal. Globally, it is the most frequent cause of mucosal and systemic infections that accounts for over 70% fungal infection. *C. albicans* is the primary cause of candidemia [36].

Antifungal Susceptibility Pattern of *Candida* species

The *in vitro* susceptibility of *Candida species* recovered from different clinical samples was determined in this study. Fluconazole, Ketoconazole, Miconazole, Nystatin and Itraconazole are the antifungal medications that were examined. Based on their widespread availability in the local market and their status as hospital prescription medications, the antifungal agents utilized in this investigation were selected. The results of our study indicated that 34.1% of all the *Candida* species were resistant to Itraconazole, whereas 18.2% were resistant to Fluconazole, Ketoconazole, Miconazole and Nystatin respectively. This is in conformity with the result of [25], and [15]. Among the species that infect humans most frequently is *Candida albicans*. The non-albicans *Candida* in this study corresponds with *Candida albicans* and their identification is vital since non-albicans *Candida* are more resistant to azoles than *C.albicans*. Despite their similarities, *Candida albicans and* non-albicans *Candida* (NAC) species differ in terms of their virulence traits, susceptibility to antifungal agents and incidence studies [14].

All the isolates of *Candida* species in this study displayed susceptibility to Fluconazole, Ketoconazole, Miconazole and Nystatin at varying degrees except *C. pelliculosa* which showed 100% resistant to all agents. The percentage susceptibility of antifungal agents to the isolates showed 79.5% for Fluconazole, 77.3% for Ketoconazole which were the highest susceptible antifungal agents. Following this agent were Miconazole (59.1%) and Nystatin (52.3%) and this agrees with the document of [14] and [31], but was in contrast to the findings of [4 and 15]. In this study, *Candida albicans* showed 86.4% and 81.2% to Fluconazole and Ketoconazole respectively and this agreed with the report of [31 and 37], but in contrast to the study of [38] and [25] .*Candida albicans* in this study showed 22.7% resistance to Itraconazole and this conform with the study of [14].

Increase in the resistance to fluconazole had been documented to rise from 2.4% to 55.4% within

2006- 2012, but the rate dropped to 8.9% in 2013. In the same vein, there has been considerable increase in the resistance of Miconazole and Itraconazole from 2.4% and 7.1% respectively, from 2006 -2023 [39]. Despite the similarities in the disease spectrums between *Candida* species, their susceptibilities to agents and degrees of severity vary, which helps to explain their epidemiology and manner of transmission [40].

Many potential reasons have been attributed to the high resistance rates observed in some *Candida* species in this study. Amongst these reasons are:

Adaptability of Genes: As documented by [41], one of the major reasons for high resistance rates in some Candida species is the adaptability of their genes. The genetic adaptability of *Candida* yeasts enables them to swiftly adapt to new surroundings due to their great chromosomal fluidity. By causing genetic modifications, this adaption may cause resistance populations to arise.

Secondly, antifungal use is another potential reason for high resistance rates observed in some *Candida* species in this study and this agrees with the study of [42]. The accumulation of resistance in some *Candida* species has been facilitated by the overuse and neglect of antifungal medications, such as azoles. This happens as a result of the medications' ongoing exposure, which favours mutant alleles that provides higher resistance.

Again, heterogeneity loss is another reason observed in this study that is responsible for high resistance rates observed in some *Candida* species. This has also been reported by [41]. Alleles with point mutations in diploid fungi that have undergone genetic variability can become homozygotes. This may results in resistance building and infectious persistence.

The production of biofilm as seen in many of the *Candida* species in this study also contributed to their high resistance rates. *Candida* yeasts have the ability to produce biofilms, which let them to thrive when contacted by antifungal medications. This conforms to the study of [43]. The development of resistance may be triggered by the capacity of *Candida* to build biofilms.

Furthermore, the techniques of Charles Darwin's theory termed "Evolutionary Mechanism" in some *Candida* species also promote high resistance rates in some yeast in this study. Unlike bacteria, where resistance is frequently results from the transmission of DNA migratory components, the resistance in *Candida* often emerges through genetic changes within an evolutionary branch. This is in agreement with the study of [44], in their study of Evolutionary Emergence of Drug Resistance in *Candida* Opportunistic Pathogens

Moreso, variations in physiology has been reported by [44], as another key reason for the high resistance rates in some *Candida* species as seen in this study. When compared to other species of *Candida*, several species show clear variations in glycerolipids, sphingolipids, cell wall content, and sterol composition. The innate resistance in these *Candida* species isolated in this study may be influenced by these distinctions.

Species-Unique susceptibility also contributes to the high resistance in some of the studied *Candida* species and this agrees with the study of [44]. When it come to antifungal medications, *Candida pelliculosa* , for example, shows different susceptibility profiles than other *Candida* species. This implies that various species may employ various strategies to avoid being resistant to antifungal drugs.

Demographic variables are other contributory factors of high resistance rates of some *Candida* species in this study and this agrees with the report of [45]. The durability and dissimilation of resistance isolates may be influenced by the region of distribution, severity and circulation characteristics of the

isolates.

Conclusion

The isolation of *Candida* species in this study indicated that non-albicans *Candida* species (NAC) corresponds with *Candida albicans* and that candidiasis could be associated with both gender at all ages. The study revealed that *C. albicans* was the most prevalent isolate. The current investigation concluded by demonstrating that non-albicans *Candida* (NAC) species were more common in a variety of clinical specimens, while *Candida albicans* were the most prevalence across all specimens. The infection of *Candida* is rising globally as a result of a rise in the predisposing circumstances. Some non -albicans species of *Candida* have an innate resistance to Itraconazole while most sensitivity of *Candida* species was noted with Fluconazole and Ketoconazole. Therefore, early classification of *Candida* isolates in hospital settings and their antifungal susceptibility testing will limit the direct application use of antifungal medications and have a significant impact on physician's treatment options, which will benefit patients. This study highlights the importance of routinely monitoring the antifungal susceptibility pattern of common *Candida* species, as this will help patients utilize antifungal medications wisely and prevent the establishment of new infections.

Conflict of Interest:

The authors declare that there was no conflict of interest.

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