Characterization and Antifungal Susceptibility Patterns of *Candida* Species Isolated from a Tertiary Hospital in Benin City, Nigeria

Running title: Characterization and antifungal susceptibility pattern of *Candida* Isolates

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Abstract

Introduction: *Candida* species are known to be the most frequently encountered fungal pathogens in humans. There has been a noticeable rise in the occurrence of human infections caused by *Candida* over the past few decades. This cross-sectional study aimed to identify different species of *Candida* and determine the antifungal susceptibility patterns of *Candida* species isolated from clinical specimens in a tertiary hospital in Benin, Edo state, Nigeria.

Methods: A total of 104 *Candida* isolates were obtained from various clinical specimens using a simple random sampling technique. The isolates were cultured on Sabouraud dextrose agar and were later sub-cultured on CHROMagar *Candida* after morphological characteristics observation and identification of *Candida* species were confirmed based on characteristic color production on CHROMagar. Antifungal susceptibility testing for *Candida* isolates was conducted following the Clinical and Laboratory Standards Institute M44-A recommendations for Amphotericin B, Ketoconazole, Fluconazole, and Nystatin. Statistical analysis was conducted using SPSS version 20.0.

Results: *Candida albicans* was the most prevalent species, accounting for 72.1% of isolates, followed by *Candida krusei* (17.3%), *Candida tropicalis* (7.7%), and *Candida glabrata* (2.9%). High vaginal swabs showed the highest frequency of *Candida* isolates (46.2%). Females exhibited a higher preponderance of candidiasis (81.3%) compared to males. With p-values of 0.007* and 0.028* respectively, *Candida albicans and Candida glabrata* exhibited significant differences in susceptibility to amphotericin B and fluconazole antifungal drugs. The antifungal susceptibility testing indicated variations in resistance patterns among different *Candida* species.

Conclusion: The study revealed a predominance of *Candida albicans* in clinical specimens, with emerging cases of non-*albicans* species. Antifungal resistance to clinically available agents raised concerns, necessitating continuous surveillance and monitoring of susceptibility patterns. The results underscore the importance of developing targeted strategies to combat the challenges posed by antifungal resistance.

Keywords: Candidiasis, *Candida* infection, Fungal infection, Susceptibility patterns

Introduction

Candidiasis can be caused by various types of *Candida* yeast, which belong to the natural microorganisms found on the skin, mucous membranes, and gastrointestinal tract (1). Following birth, *Candida* species establish themselves on the mucosal surfaces of all individuals, creating a constant risk of endogenous infection. Among systemic fungal infections, candidiasis is the most widespread, with *Candida albicans, Candida parapsilosis*, *Candida glabrata, Candida tropicalis, Candida guilliermondii*, and *Candida dubliniensis* being the most commonly identified agents (2). *Candida* species are the primary opportunistic fungal pathogens that have an impact on patients receiving medical care in healthcare settings. Among newborns, *Candida* species rank as the third most isolated microorganism in cases of invasive infections (3). In critical neonates, the morbidity and mortality rates associated with invasive candidiasis can be as high as 59%. Furthermore, around 60% of neonates who survive such infections may experience neurodegenerative complications. Newborns who are admitted to a neonatal intensive care unit (NICU) possess an underdeveloped immune system due to factors such as low birth weight, prematurity, and external risk factors (such as the use of central venous catheters and parenteral nutrition). These factors increase their vulnerability to invasive infections (4). Chromagar *Candida* is a specialized medium used to isolate and distinguish the primary clinical species of *Candida*. It exhibits a high degree of specificity and sensitivity for three major *Candida* species: *Candida albicans, Candida tropicalis, and Candida krusei*. *Candida* isolates were cultured on Chromagar *Candida* and incubated aerobically at 37°C for 36 to 48 hours. The identification of *Candida* species was based on the production of characteristic colors on the medium (5).

The infection caused by *Candida* species occurs when there is an imbalance in the parasite-host relationship, a consequence of the association between the inefficiency of individual defenses and the expression of virulence factors by microorganisms. Although *C. albicans* remains the main causative pathogen, the increasing isolations of non-*Candida* albicans spp. resistance to first- and second-line antifungals in nosocomial infections is concerning (6).

Candida species' resistance to antifungal medicines is often attributed to mutations in genes or other alterations in the drug target. The prevalence and impact of *Candida* species infections in Nigeria, including their resistance to antifungal drugs, remain poorly understood. There is a need to investigate the epidemiology, antifungal susceptibility patterns, risk factors, and clinical outcomes associated with *Candida* infections in Nigerian populations to develop effective strategies for prevention, diagnosis, and treatment. This study aims to specify different species of *Candida* isolates and perform antifungal susceptibility testing from different clinical specimens of the In-and-Out patients attending Edo State Specialist Hospital in Benin, Edo State, Nigeria.

Methods

Study settings and design: A simple random sampling technique was used in selecting participants in a cross-sectional and prospective survey to characterize and perform antifungal susceptibility testing on *Candida* isolates from clinical specimens of patients attending the Edo State specialist, Nigeria. The sample size of 346 was calculated by a formula given by (7), with a 95% confidence level, a 5% margin of error and a prevalence of *Candida* in Asaba, Nigeria, of 34.25% (8). A total of 104 *Candida* isolates from various clinical specimens were included in this study.

Data and sample collection: The bar data of the patient was collected from the laboratory register. The data obtained includes socio-demographic characteristics. *Candida* isolates of each patient were collected and subcultured onto a Sabouraud dextrose agar (SDA) slant and incubated at 37℃. Data collected from the fungal analysis of the collected samples were collated and analyzed statistically.

Isolate Identification and Antifungal Susceptibility Test: Every isolate diagnosed as a *Candida* species was included in this study. These isolates were inoculated on modified SDA slants that had gentamycin and chloramphenicol added to them. They were incubated for a duration of 24 to 48 hours at 37°C in an aerobic environment. In the case of blood culture, 5mls of blood was withdrawn aseptically and subsequently cultured in Brain Heart Infusion (BHI) broth. The cultures were then incubated for 96 hours at 37°C. Species identification was done on the isolates that had obvious growth on the SDA slant. This required performing tests on a colony recovered from the sample using macroscopic assessment, Gram staining technique, urea hydrolysis, and germ tube testing, and their morphological characteristics were observed. Colonies that were creamy and yeasty, showed Gram-positive budding yeast cells with pseudohyphae under a microscope, and produced negative urea hydrolysis test results, were then subcultured on CHROMagar for the identification of *Candida* species (9).
CHROMagar™ *Candida* (HiMedia, Mumbai, India), a selective and differential medium, was employed to specifically inhibit the growth of microorganisms other than *Candida* (10). The subcultured CHROMagar plate was incubated at 37°C for 24-48 hrs. After 24 hours of incubation, colonies produced different hues, with *Candida albicans* colonies producing a green color, *Candida tropicalis* colonies producing a metallic blue color, *Candida krusei* colonies producing a pink color, and *Candida glabrata* producing a mauve color (11). Using the disc diffusion method, antifungal susceptibility testing for *Candida* isolates was conducted following the Clinical and Laboratory Standards Institute (CLSI) M44-A recommendations. 3-5 colonies of growth were suspended in five milliliters of sterile saline, which had been adjusted to match the turbidity of a 0.5 McFarland Standard. This inoculum was streaked on Mueller-Hinton agar and the susceptibility of fungal growth was evaluated. Following a 24-hour incubation period at 37°C, the zone of inhibition surrounding the discs was assessed (9,12).
Statistical Analysis: All data were encoded into Excel spreadsheets, and the data were analyzed using IBM-Statistical Package for Social Sciences (SPSS) version 20.0. The variables were expressed in means and standard deviations. Student's t-test and Analysis of Variance (ANOVA) were the statistical methods used. Levels of significance were considered at $P<0.05$.
Ethical Considerations: Before the commencement of the study, permission was also obtained from the Chief Medical Director of Edo State Specialist Hospital. Ethical approval was sought and obtained from the Ethics Committee, Edo State University, Uzairue, Nigeria, with the reference number EDSU/AHS/ERC/VOL.1/25/2022

Results

In Table 1, a total of 104 *Candida* species were isolated from the specimens. The distribution of *Candida* revealed that High vaginal swabs had the highest frequency for *Candida* species 48(46.2%), followed by Urine 23(22.1%), Endocervical swab 12(11.5%), Catheter tip 11(10.6%), Wound swab 4(3.8%), Ear swab 3(2.9%) and the least, Abdominal drain efflux 1(1.0%). The distribution of *Candida* species presented *Candida albicans* with the highest frequency of 75(72.1%), followed by *Candida krusei* 18(17.3%), *Candida tropicalis* 8(7.7%) and *Candida glabrata* 3(2.9%), respectively (Table 1). The age distribution of *Candida* species among the participants in the study showed that *Candida albicans* had the highest frequency among ages 30-44, with 31(41.3%). *C. krusei* had its highest frequency, 7(38.9%), among the age group 30- 44 years, *C. tropicalis* had 3(37.5%) among the age group 30-44 years; and *C. glabrata* had a frequency of 1(33.3%) among the age groups 30-44 years, 45-59 years and 60-74 years, respectively. There was no statistically significant difference ($P =0.065$) between age and *Candida* species distribution among the participants, as revealed in Table 2.
The gender distribution of *Candida* species in this study revealed that females had the highest frequency of *Candida albicans,* 61(81.3%), and 14(18.7%) in males. *Candida glabrata* had its only frequency in female 3(100%), *Candida krusei* had the highest frequency of 16(88.9%) in

females and 2(11.1%) in males, and *Candida tropicalis* had the highest frequency in females. There was no statistically significant relationship at (P =0.67) between gender and *Candida* species distribution, respectively, as shown in Table 2. *C albicans* had the highest frequency of 37(49.3%) in High Vagina Swab. *C. krusei* had its highest frequency 8(44.4%) in High vaginal swab. *C. tropicalis* had its highest frequency in urine samples 4(50%). *C. glabrata* had 1(33.3%) each in catheter tips, ear swab, and urine samples, respectively. There was a statistically significant association (P=0.05) between the type of specimen and *Candida* species distribution, respectively. *Candida albicans* had its highest frequency, 39(52%), among the inpatients. *Candida glabrata* among the In-patients 2(66.7%), *Candida krusei* among the In-patients 11(61.7%), while *Candida tropicalis* had its highest frequency 5(62.5%) among the out-patients (Table 2). There was no statistically significant relationship (P=0.61) between patients' admission status and *Candida* species distribution. The antimicrobial susceptibility pattern of the *Candida* species to antifungal agents revealed that *C. albicans* was more susceptible to ketoconazole [47], Nystatin [40], and fluconazole [39], while most were resistant to Amphotericin B [72]. *C. glabrata* were more susceptible to fluconazole [3]. *C. krusei* were most resistant to Amphotericin B [16], while *C. tropicalis* were most susceptible to fluconazole [8]. There was a statistically significant association between Amphotericin B (P=0.007) and fluconazole (P=0.028) with *Candida* species. There was no statistically significant association between other antibiotics and *Candida* species distribution, as shown in Table 3.

Discussion

C. albicans and non-albicans candidiasis have been prevalent etiological agents causing candidiasis of human origin, ranging from localized infections to systemic colonization. This study investigated the characterization, speciation and antibiogram of *Candida* species from clinical specimens of human origin from all age groups and genders. A total of 104 *Candida* isolates from various clinical specimens were included in this study. *Candida* was mainly isolated from high vaginal swab 48(46.2%), corresponding with previous works (13,14). *C. albicans* was the most common species isolated from this study (72.1%). This is due to its widespread prevalence in clinical practices and ubiquity in the human body, which agrees well with some studies (5,11) as well as disagrees with previous work stating *C. glabrata* to be the most common isolated species (14,15). The prevalence of *Candida* species varies due to factors like study populations, geographic areas, healthcare settings, and identification methods. Additionally, antifungal medication uses and resistance patterns may impact the frequency of *Candida* species in clinical settings (16).

This finding reaffirms the high prevalence of *Candida albicans* and a gradual shift towards *non-Candida albicans* species, suggesting the fact that non-*Candida albicans* are emerging as important pathogens in human infection (17). The identification of different *Candida* strains holds significant importance in epidemiological research and laboratory diagnosis, particularly due to the increasing prevalence of antifungal resistance and the shifting trends in resistance patterns observed in both *C. albicans* and non-*Candida* albicans species (18). Though *Candida* species can be found causing varying infections that can occur at all ages, this study showed the highest prevalence among the age group 30-44 years at 41.3%, followed by 15-29 years at 25.3%, which is in line with the findings of studies (5,19,20) that reported high prevalence of candidiasis in the age group 21-40 years. This suggests the fact that the 30-44 years age range represents the peak of childbearing in Nigerian societies due to so many socio-demographic factors delaying young couples from settling down early (21). Advancement in age, on the other hand, reduces the effect of the estrogen hormone in women, which could lead to lower infection rates as women advance in age. Most women aged over 50 years might have reached menopause and are less or not sexually active, suggesting the reason why there is a decrease in the prevalence rate of *Candida* among these older age groups (22). Numerous factors, including

immune status, hormonal changes, underlying medical conditions, lifestyle choices, sexual activity, personal hygiene practices, and genetic predisposition, can be linked to the varying prevalence of candidiasis among different age groups. It is a complex phenomenon that is influenced by several elements that work together to determine the risk of candidiasis in particular age groups (23).

A high female preponderance of candidiasis of 86 out of 104 was found in this study, with a species distribution of 61(81.3%) *Candida albicans,* which agrees well with Omosigho *et al.,* 2019 (5). Among females, vulvovaginal candidiasis represents the most prevalent form of genital candidiasis. Females are more likely to develop candidiasis due to various factors, including *Candida* fungus, hormonal fluctuations, contraceptive use, weakened immune responses, sexual activity, personal hygiene, tight clothing, and certain medications. Genetic susceptibility and differences in female anatomy also contribute to the higher incidence of candidiasis in women (24,25). A higher frequency of high vaginal swabs (49.3%) with *Candida* isolates confirms higher prevalence of vulvovaginal candidiasis among females of reproductive age in Benin, which is consistent with previous studies (5,26). High vaginal swabs are more likely to detect candidiasis due to their focus on the vaginal region, a common site for *Candida* colonization, making them the suitable specimen of choice for diagnosis (27,28).

The study illustrates how different *Candida* species, and antifungal medications have varying degrees of resistance. Overall, *Candida albicans* exhibited significant resistance to Amphotericin B and Fluconazole, while other species showed mixed susceptibility patterns. In some species, drug resistance was statistically significant, suggesting a higher potential pathogenicity for those medications. The drug resistance pattern and overall pathogenicity of *Candida* species are dependent on the antifungal drugs as well as the species. The mixed and resistance patterns to antibiotics among *Candida* species can be attributed to intrinsic and acquired resistance mechanisms, alterations in drug targets, efflux pumps, and biofilm formation (29). The overuse of antifungal drugs, impaired host immunity, and cross-resistance also play significant roles (30). Antifungal susceptibility among *Candida* species varies; this could be due to differences in resistance, local resistance patterns, drug-resistant strains, and widespread use of antifungal medications. Patient-specific factors like health conditions and prior treatment also impact susceptibility. This complex interplay contributes to the diversity in antifungal susceptibility observed among *Candida* species (29,31). Comprehending the drug resistance patterns of *Candida* species is crucial for the betterment of patient treatment, public health, and upcoming drug research initiatives. It lowers the possibility of epidemics, improves patient outcomes, and enables more focused and efficient treatment of fungal diseases (32). Some of the limitations of this study include a small sample size and a single-center design. Additionally, the study focused on a limited set of antifungal agents, and the underlying mechanisms of antifungal resistance were not investigated. Incomplete patient data, such as comorbidities and treatment history, further restricts the comprehensive analysis of *Candida* infections and resistance patterns. There are drawbacks to using CHROMagar to speciate *Candida* species, such as the possibility of misidentification and the inability to reliably identify new or unusual species. It is advised that future research consider molecular techniques, which provide more accuracy and specificity in species identification, to overcome these constraints. Addressing these limitations in future research is essential to gain a more comprehensive understanding of *Candida* infections and to develop effective strategies for prevention and treatment.

Conclusion

Understanding the *Candida* species and their susceptibility to antifungal drugs is crucial for selecting effective treatment. The rising rates of antifungal resistance in *Candida* isolates are concerning, necessitating continuous monitoring of susceptibility patterns and investigating

resistance mechanisms. Instances of breakthrough infections underscore the importance of staying vigilant in addressing this issue. The growing occurrence of less common *Candida* species that show resistance to currently available antifungal drugs, along with the emergence of new resistance mechanisms, underscores the significance of conducting both local and global surveillance studies. These studies are essential for monitoring the changing epidemiology of *Candida* infections and guiding the development of effective strategies to combat the challenges posed by antifungal resistance.

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Ethical approval

Ethical approval was collected from the ethics committee, Edo State University, Uzairue, with the reference number EDSU/AHS/ERC/VOL.1/25/2022

Conflict of interest

The authors declare no conflict of interest.

Author contributions:

Pius Omoruyi Omosigho, Ugiagbe Victory Osayekewmen, Guobadia Precious Oghogho, Okesanya Olalekan John, Oladejo Janet Mosunmola and Uyigue Paulinus Osarodion conceived and designed the research, reviewed, analysed, performed the research, interpreted the data, wrote the paper, supervised, reviewed, edited, and proofread. All authors have read and approved the final draft of this paper.

Data availability statement

The dataset generated during this study is available with the corresponding author upon reasonable request.

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Table 1. Distribution of clinical specimens and frequency of *Candida* isolates

Type of Specimen	Frequency (N)	Percentage (%)
Abdominal drain efflux	1	1.0
Catheter tip	11	10.6
Ear swab	3	2.9
Endocervical swab	12	11.5
High vaginal swab	48	46.2
Throat swab	2	1.9
Urine	23	22.1
Wound swab	4	3.8
Total	**104**	**100**
***Candida* Isolate**		
C.albican	75	72.1
C. glabrata	3	2.9
C. krusei	18	17.3
C. tropicalis	8	7.7
Total	**104**	**100**

Table 2. Distribution of *Candida* species by clinical variables and associated statistical significance

Variable	*C. albican* (%)	*C. glabrata* (%)	*C. krusei* (%)	*C. tropicalis* (%)	P-Value	Remark
Age					**0.065**	**NS**
0-14 yrs	8 (10.7%)	0	2 (11.1%)	0		
15-29 yrs	19 (25.3%)	0	6 (33.3%)	1 (12.5%)		
30-44 yrs	31 (41.3%)	1 (33.3%)	7 (38.9%)	3 (37.5%)		
45-59 yrs	7 (9.3%)	1 (33.3%)	0	1 (12.5%)		
60-74 yrs	9 (12%)	1 (33.3%)	2 (11.1%)	2 (25%)		
75-99 yrs	1 (1.3%)	0	1 (5.6%)	1 (12.5%)		
Gender					**0.067**	**NS**
Female	61 (81.3%)	3 (100%)	16 (88.9%)	6 (75%)		
Male	14 (18.7%)	0	2 (11.1%)	2 (25%)		
Specimen					**0.05**	**S**
Abdominal drain efflux	1 (1.3%)	0	0	0		
Catheter tip	7 (9.3%)	1 (33.3%)	3 (16.7%)	0		
Ear swab	0	1 (33.3%)	2 (11.1%)	0		
Endocervical swab	9 (12%)	0	3 (16.7%)	0		
High vaginal swab	37 (49.3%)	0	8 (44.4%)	3 (37.5%)		
Throat swab	1 (1.3%)	0	1 (5.6%)	0		
Urine	17 (22.7%)	1 (33.3%)	1 (5.6%)	4 (50%)		
Wound swab	3 (4%)	0	0	1 (12.5%)		
Admission status					**0.610**	**NS**
In-patients	39 (52%)	2 (66.7%)	11 (61.7%)	3 (37.5%)		
Out-patients	36 (48%)	1 (33.3%)	7 (38.9%)	5 (62.5%)		
Total	**75 (100%)**	**3 (100%)**	**18 (100%)**	**8 (100%)**		

NS: Non-Significant, S: Significant

Table 3. Antifungal susceptibility patterns of *Candida* isolates

Antifungal	***Candida* isolate N (%)**			
	C. albican	*C. glabrata*	*C. krusei*	*C. tropicalis*
Amphotericin B				
R	72 (96)	2 (66.7)	16 (88.9)	5 (62.5)
S	3 (4)	1 (33.3)	2 (11.1)	3 (37.5)
Fluconazole				
R	36 (48)	0 (0)	8 (44.5)	0 (0)
S	39 (52)	3 (100)	10 (55.5)	8 (100)
Nystatin				
R	35 (46.7)	1 (33.3)	9 (50)	3 (37.5)
S	40 (53.3)	2 (66.7)	9 (50)	5 (62.5)
Ketoconazole				
R	28 (37.5)	1 (33.3)	8 (44.5)	3 (37.5)
S	47 (62.7)	2 (66.7)	10 (55.5)	5 (62.5)
Total = 104	75 (100)	3 (100)	18 (100)	8 (100)

R: Resistant, S: Sensitive