

SJMLS-9(3)-024

Catheter associated *Candiduria* among In-patients accessing care in a Tertiary Health Institution in Calabar, Nigeria.Ogba, Ofonime Mark*¹, Bullem-Odu Davina Chidinma¹, Inah, Inah Onete², Ogba, Rejoice Oghenovo³, Akpan, Nseobong Godwin⁴, Nyambi, Edim Sunday², Ugar Louisa Ifu¹.Department of Medical Bacteriology, Virology and Mycology, Faculty of Medical Laboratory Science, University of Calabar, Nigeria¹, Department of Microbiology, Faculty of Science, University of Cross River State, Nigeria², Department of Medicine and Surgery, Faculty of Clinical Science, University of Calabar, Nigeria³, Institute of Biomedical Research and Innovations, College of Health Sciences, University of Uyo, Akwa Ibom State, Nigeria⁴.Author for Correspondence*: ofonimemark@yahoo.com; ofonimemark@unical.edu.ng/ +234-803-540-4728/ ORCID Number: 0000-0002-5194-7574/ <https://dx.doi.org/10.4314/sokjmls.v9i3.24>**Abstract**

Urinary tract infections caused by *Candida* species are becoming increasingly common in hospital settings. The association is higher in patients with urinary catheterization. The aim of this study was to determine the prevalence of *Candida* species causing urinary infection in catheterized patients in University of Calabar Teaching Hospital and the ability of isolates to form biofilm. The study was a cross-sectional prospective study which ran for five months, from March to August, 2021. Catheterized patients from surgical ward and orthopedic wards were recruited for the study. Purposive sampling method was used because the subjects were few. Urine samples were obtained from the catheter port and subjected to microscopy, culture and susceptibility testing. *Candida* isolates were characterized and tested for antifungal susceptibility and biofilm production. The study recorded (25.7%) prevalence of candiduria among subjects. Most of the *Candida* isolates (55.5%) were non- albicans *Candida* species. *Candida albicans* accounted for (44.4%) infections. The infection rates among female subjects 7(77.7%) was higher than males 2(22.2%) but there was no significant association between gender and infection rates ($\chi^2 = 3.88$; p 0.05). Patients with highest *Candida* infection (55.6%) were aged 41-50 years while subjects in their first to third decade of life had no infection. *Candida glabrata* was the highest occurring non-albicans *Candida* species (33.3%). In this study biofilm production was seen in (66.7%) of the *Candida* isolates and the isolates were highly susceptible to Itraconazole (100.0%) and Fluconazole (77.3%). Only *Candida glabrata* was

resistant to Amphotericin B (0.0%). *Candiduria* is a health problem in the study setting among catheterized subjects. *Candida albicans* was the most isolated *Candida* species with high rates of biofilm production. There was high antifungal resistance among isolates which calls for better antifungal stewardship for better patients' management and outcome.

Keywords: Candiduria, Catheterized patients, Antifungal resistance, Biofilm production

Introduction

The presence of *Candida* in urine samples of hospitalized patients is a common clinical finding. Catheter-associated urinary tract infection is one of the most common device-associated nosocomial infections developed frequently by patients who are hospitalized. About 75% of patients admitted to hospitals undergo urinary catheterization in the course of their hospital stay, posing challenges in catheter associated UTI prevention and control (Bizuayehu *et al.*, 2022). *Candida* yeasts colonize the mucosal surfaces and external genitalia of both men and women, especially in premenopausal women's urethral meatus area. In general population, >1% of urine samples contain *Candida* in measurable quantity; however, in hospitalized patients, this rate is 5-10 times higher. The *Candida* may enter the bladder during insertion, manipulation of catheter or the drainage system around the catheter (Lo *et al.*, 2008). *Candida* species account for 10-15% of nosocomial urinary tract infections in catheterized patients and the clinicians face the diagnostic dilemma as to whether the presence of candiduria in a patient is as a result of contamination, colonization, or true infection.

The predisposing factors associated with *Candida* infection include; urinary tract instrumentation, diabetes mellitus, prolonged hospital stay, prolonged antibiotic use, young and old age, female gender and the use of immunosuppressive therapy. *Candida albicans* is the most common cause of catheter-associated candiduria worldwide. Other non-*albicans* species includes; *Candida glabrata*, *Candida tropicalis*, *Candida krusei* and *C. parapsilosis* (Tumbarello *et al.*, 2007). In a study carried out in North American medical centers, a predominance of non-*albicans* species was observed although *C. albicans* was the most frequently isolated species (Gajdacs *et al.*, 2019). This study is the first of its kind to be conducted in University of Calabar Teaching Hospital, Calabar, Nigeria. The aim of this study was to determine the prevalence of catheter-associated candiduria and the antifungal susceptibility profile of the *Candida* isolates associated with the disease.

Materials and methods

The study was carried out in the University of Calabar Teaching Hospital (UCTH), surgical and orthopedic ward, with bed space capacity of 30 and 17 for males and females respectively in the two wards. The study was a cross-sectional prospective study which ran for six months, from March to August, 2021. Ethical approval with Registration Number: NHREC/07/10/2012 and Protocol Assigned Number: UCTH/HREC/33/526 was obtained from the Ethics Research Committee of the University of Calabar Teaching Hospital Calabar, Nigeria. Thirty-five catheterized patients from surgical and orthopedic wards were recruited for the study. Subjects who did not give consent were excluded from the study. Purposive sampling method was used because the subjects were few. About 10 ml of urine sample was aspirated from an indwelling catheter near the urethral site from all patients into sterile urine container by trained Nurses or attending Physicians and immediately transported to the Medical Microbiology Laboratory, UCTH for analysis.

The samples were subjected to macroscopy, culture and microscopy. Uncentrifuged and homogenized urine samples were cultured on

Sabouraud Dextrose agar (SDA), Chocolate agar, and Cysteine lactose electrolyte deficient agar (CLED) media using standard wire loop method that delivers 0.001ml of urine in a biosafety cabinet. The culture plates were incubated aerobically at 37°C for 24-48 hours. Identification of the *Candida* species was based on Gram staining, germ tube test and growth on CHROMagar *Candida* medium, (Biomérieux, France) following the manufacturer's instruction. Plates were read daily and colony counts of yeast growth 10^4 were considered significant for candiduria (Rosenthal *et al.*, 2014).

Antimicrobial susceptibility testing.

Candida antifungal susceptibility pattern was determined using Kirby-Bauer disk diffusion method. Five antifungal agents (Fluconazole, Amphotericin B, Nystatin, Voriconazole and Itraconazole) were tested. A loop full of *Candida* isolates from the plates was emulsified in sterile peptone water and the turbidity of the inoculum adjusted to 0.5 McFarland standards ($1-5 \times 10^6$ cells/mL). This was inoculated on Mueller-Hinton agar plates using sterile swab sticks. The antifungal discs were placed on the plates which were incubated at 37°C for 24-48 hours aerobically. The antifungal susceptibility was evaluated by the zones of inhibition of the yeasts on the media containing the antimycotics (Jalalpure *et al.*, 2008; Edim *et al.*, 2022)

Biofilm Formation

Biofilm production was determined using Congo red agar. Brain heart infusion agar was used as the agar base with Congo red. Congo red agar was prepared as concentrated aqueous solution and autoclaved at 121°C for 15 minutes. It was added to the agar and cooled at 55°C. *Candida* isolates were sub-cultured on Sabouraud dextrose agar 48 hours prior to the study and then inoculated on Congo red agar plates. Plates were incubated aerobically for 24 to 48 hours at 37°C. Positive results were indicated by black colonies on the Congo red agar plates. Biofilm negative strains yielded white- or pink-coloured colonies.

Statistical analysis

Data generated in the research study was analyzed using the statistical package for social sciences (SPSS) version 23. Descriptive

statistics were carried out. Frequencies were calculated for categorical variables. Interactions between specific categorical clinical variables were tested for significance using the Chi square test. A p value 0.05 was considered statistically significant.

Results

Out of 35 catheterized subjects sampled, 9(25.7%) had candiduria (Figure 1). *Candida albicans* was the most encountered isolate (44.4%) followed by *Candida glabrata* (22.2%) while *Candida tropicalis* was the least encountered isolate (22.2%) (Fig. 2). Females 7(77.7%) suffered more candiduria than males 2(22.3%) but there was no significant association between gender and infection rates ($\chi^2 = 3.88$; $p > 0.05$) (Table 1). Subjects aged 41-50 years had the highest infection rates 7(77.7%), while subjects aged 31 -40 years and 51 years and above had the lowest infection rate 1(11.1%). Subjects in their first to third decade of life had no infection. There was no statistically

significant effect of age on infection rates ($\chi^2 = 3.88$; $p > 0.05$) (Table 2). Table 3 shows the biofilm production by *Candida* species in the study. Out of the 9 *Candida* isolates in the study, 6(66.7%) were biofilm producers. *Candida albicans* was the highest biofilm forming isolates 3(50.0%) while *Candida tropicalis* was the lowest biofilm producing isolates 1(16.7%). The susceptibility pattern of *Candida* isolates to selected antifungal agents in the study is shown in Table 4. The isolates were most susceptible to Itraconazole followed by Fluconazole and least susceptible to Voriconazole. *Candida albicans* was most susceptible to itraconazole 4(100%) followed by Amphotericin B 3(75.0%). *Candida glabrata* was most susceptible to Fluconazole and Itraconazole (75.0%) and completely resistant to Amphotericin B. However, *Candida tropicalis* was least susceptible to Amphotericin B and Voriconazole 1(25.0%) respectively. Figure 3 shows *Candida* biofilm formation on Congo red agar indicated by blackening of the colonies on the plate.

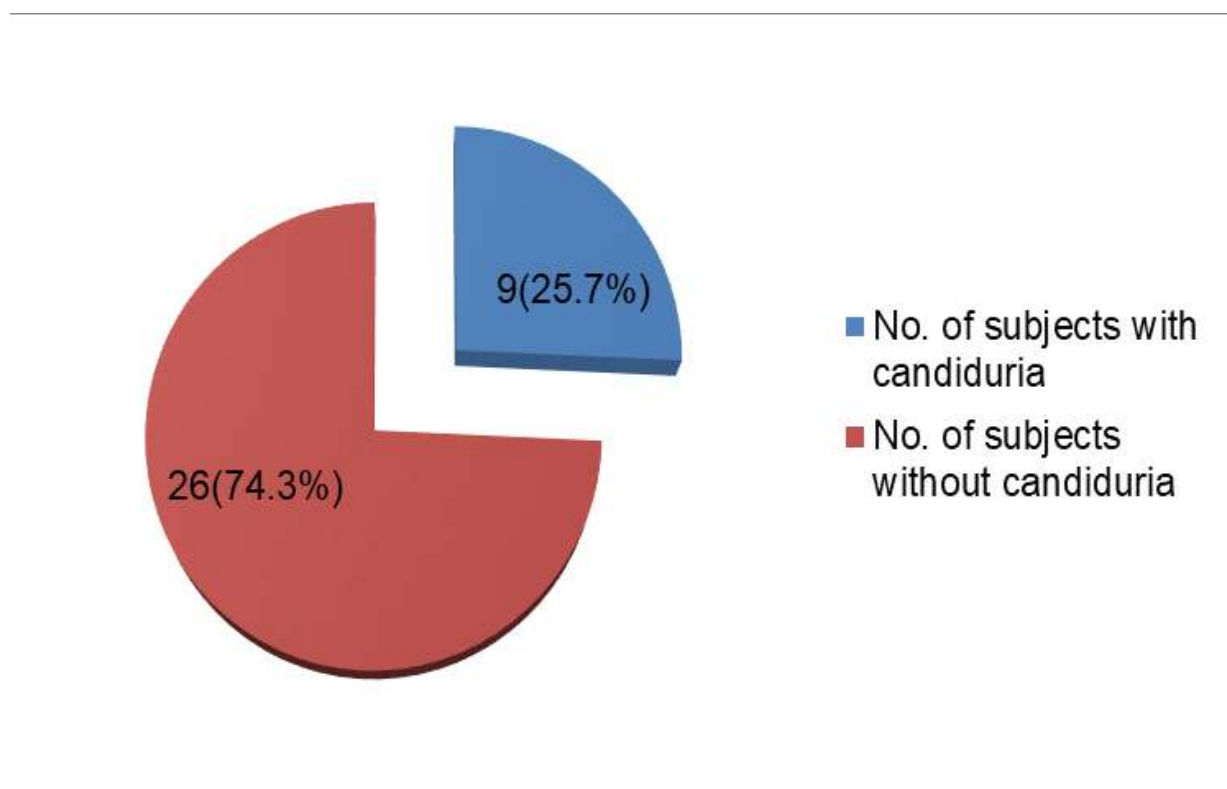


Figure 1: Prevalence of *Candiduria* among subjects

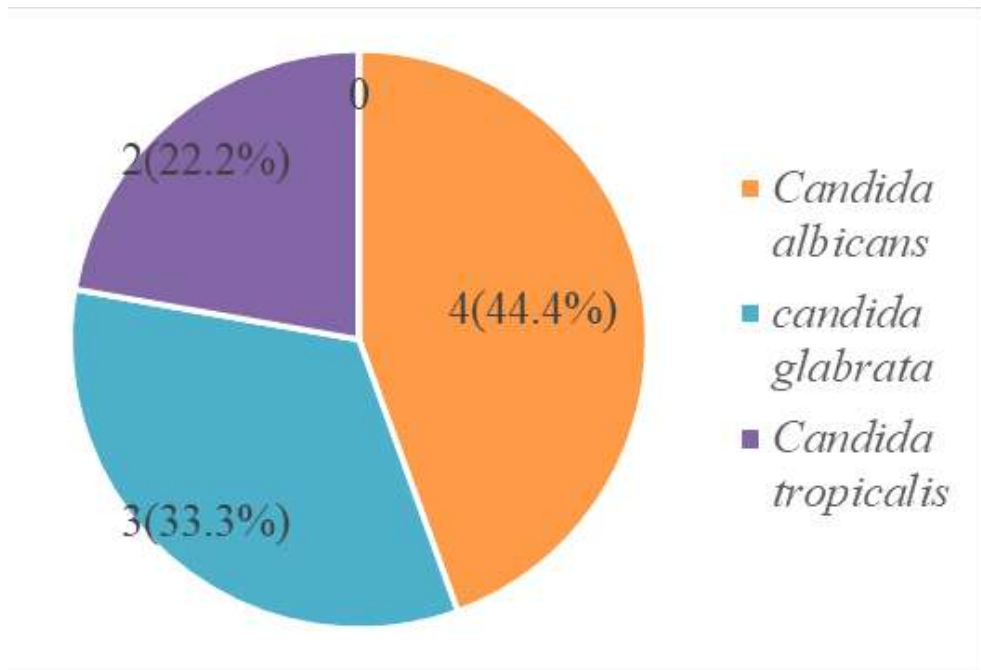


Figure 2: Distribution of *Candida* isolates among subjects

Table 1: Distribution of *Candida* infection by gender of subjects

| Gender | No examined | No. (%) with <i>Candida</i> infection | X^2 | P-value |
|---------|-------------|---------------------------------------|-------|---------|
| Males | 14 | 2(22.3) | 3.88 | >0.05 |
| Females | 21 | 7(77.7) | | |
| Total | 35 | 9(25.7) | | |

Table 2: Distribution of *Candida* infection based on the age subjects

| Age (years) | No. examined | No. (%) with <i>Candida</i> infection | X^2 | P-Value |
|-------------|--------------|---------------------------------------|-------|---------|
| 11- 20 | 1 | 0(0.0) | 3.88 | >0.05 |
| 21- 30 | 4 | 0(0.0) | | |
| 31- 40 | 8 | 1(11.1) | | |
| 41- 50 | 18 | 7(77.7) | | |
| ≥ 51 | 4 | 1(11.1) | | |
| Total | 35 | 9(25.7) | | |

Table 3: Biofilm production by *Candida* species in the study

| <i>Candida</i> species | No. (%) of isolates tested | No. (%) positive for biofilm production |
|------------------------|----------------------------|---|
| <i>C. albicans</i> | 4(44.4) | 3(50.0) |
| <i>C. glabrata</i> | 3(33.3) | 2(33.3) |
| <i>C. tropicalis</i> | 2(22.2) | 1(16.7) |
| Total | 9 | 6(66.7) |

Table 4: Susceptibility pattern of *Candida* isolates in the study

| <i>Candida</i> species | No tested | Types of antifungal agent (No and (%) susceptibility) | | | | |
|------------------------|-----------|---|----------------|----------|--------------|--------------|
| | | Fluconazole | Amphotericin B | Nystatin | Voriconazole | Itraconazole |
| <i>C. albicans</i> | 4 | 2(50.0) | 3(75.0) | 2(50.0) | 2(50.0) | 4(100) |
| <i>C. glabrata</i> | 3 | 3(75.0) | 0(0.0) | 3(75.0) | 1(25.0) | 3(75.0) |
| <i>C. tropicalis</i> | 2 | 2(50.0) | 1(25.0) | 2(50.0) | 1(25.0) | 2(50.0) |
| Total | 9 | 7(77.7) | 4(44.4) | 7(77.7) | 4(44.4) | 9 |

Keys: FLU (Fluconazole); AMPB (Amphotericin B); NYS (Nystatin); VAR (Voriconazole) ; ITRA (Itraconazole) and 0(0.0) denotes not susceptible.

**Figure 3: *Candida* biofilm formation on Congo red**

Discussion

Urinary tract infections caused by *Candida* species are becoming increasingly common in hospital settings. In this study (25.7%) of catheterized patients had candiduria. This is similar to the 23.0% and 26.0 % reported in Karnataka, India by Sahai and Kumar (2018) and Rishpanal and Kabbin (2015) respectively. The finding that 25.7% of catheterized patients in this study developed candiduria reflects a significant and persistent issue in hospital settings, particularly among patients with prolonged hospital stays. This prevalence aligns closely with the rates reported in Karnataka, India, by Sahai and Kumar (2018) and Rishpanal and Kabbin (2015), who found candiduria rates of 23.0% and 26.0%, respectively, among catheterized patients. The consistency of these figures across different geographic locations suggests that candiduria is a common complication associated with catheter use in hospitalized patients, regardless of regional differences. The high prevalence of candiduria in this study, particularly in patients with extended hospital stays, points to the critical role of catheterization as a risk factor. Prolonged catheter use can lead to the development of biofilms, which provide a protective environment for *Candida* species, making infections more likely and harder to treat. This is particularly concerning in settings where patients are hospitalized for extended periods, as the risk of developing healthcare-associated infections, including candiduria, increases with the length of stay.

Globally, the challenge of managing candiduria in catheterized patients is compounded by factors such as antibiotic usage, which can disrupt normal microbial flora and predispose patients to fungal overgrowth, and the growing issue of antifungal resistance. The data suggest that more stringent infection control measures, including regular assessment of the necessity of catheterization and timely removal when no longer needed, are essential in reducing the incidence of candiduria. This finding highlights the importance of continuous monitoring and early intervention strategies for patients at risk of candiduria, particularly those with prolonged hospitalizations. By addressing the underlying risk factors, healthcare systems can better manage and potentially reduce the prevalence of this infection,

improving patient outcomes and reducing the burden on healthcare resources globally.

In this study most of the *Candida* isolates (55.5%) were non- *albicans* species. *Candida albicans* accounted for (44.4%) infections. This agrees with the work of Rishpanal and Kabbin (2015) who reported non- *albicans Candida* species as the most prevalent species (61.5%) causing candiduria in catheterized patients. The observed shift in the prevalence of *Candida* species in infections, particularly the rise in non-*albicans Candida* (NAC) species, is a significant trend in global healthcare. The findings of this study, where non-*albicans Candida* species accounted for 55.5% of infections, align with the observations by Rishpanal and Kabbin (2015), who reported a higher prevalence of NAC species (61.5%) in catheterized patients. This shift towards NAC species is noteworthy because these species often display different antifungal resistance profiles, which can complicate treatment strategies.

Globally, the increasing incidence of NAC species represents a broader trend in clinical mycology, reflecting changes in environmental, therapeutic, and patient-related factors. For instance, the use of broad-spectrum antibiotics, immunosuppressive therapies, and medical devices such as catheters can selectively favor NAC species, which might be less susceptible to commonly used antifungals. However, it's important to note that this trend is not universally observed. Weinberger *et al.* (2003) and Petah *et al.* (2008) in Paris, France reported a higher prevalence of *Candida albicans* of 50% to 70% of *Candida in* urinary isolates. These discrepancies might be attributed to regional differences, variations in patient populations, or the methodologies used in these studies. The increasing prevalence of NAC species has important implications for the management of candidiasis, necessitating the development of more targeted diagnostic tools and surveillance for the detection of factors that drive this shift.

Candida glabrata was the highest occurring non- *albicans Candida* species (33.3%) in this study. This is in concordance with the 10-year study done in Hungary between (2008-2017),

which revealed *Candida glabrata* (20-30%) as an emerging aetiologic agent among catheterized patients. In another study carried out in major North American Medical centers, a predominance of non-*albicans* species was observed although *C. albicans* was the most frequently isolated species, it was followed by *C. glabrata* and other non-*albicans* species. (Tortorano *et al.*, 2006).

Candida infections were seen to occur more amongst the female subjects 7(77.8%) than male subjects 2(22.3%). The higher prevalence of *Candida* infections among female subjects, with 77.8% of infections occurring in women compared to 22.3% in men, is consistent with global epidemiological trends. This disparity is often attributed to the female reproductive anatomical structure, which predisposes women to ascending infections. The shorter urethra in women, combined with the proximity of the genital and urinary tracts, creates a conducive environment for pathogens like *Candida* species to ascend and cause infections such as candiduria and vulvovaginal candidiasis. Hormonal fluctuations associated with the menstrual cycle, pregnancy, and the use of hormonal contraceptives can disrupt the normal microbial balance in the female genital tract, promoting the overgrowth of *Candida* species (Sobel, 2007). Additionally, the use of broad-spectrum antibiotics, which can eliminate protective bacterial flora, further increases the susceptibility of women to *Candida* infections (Achkar & Fries, 2010).

Globally, this pattern is observed across various populations and highlights the need for targeted public health strategies. Preventive measures, such as promoting proper genital hygiene, cautious use of antibiotics, and education on risk factors, can play a significant role in reducing the incidence of *Candida* infections among women. Furthermore, understanding the role of gender-specific factors in the pathogenesis of these infections can inform the development of more effective treatment protocols. The global burden of *Candida* infections, particularly among women, underscores the importance of ongoing research into the biological and environmental factors that contribute to this disparity.

Patients with highest *Candida* infection (55.6%) were aged 41-50 years. The observed concentration of *Candida* infections in patients aged 41-50 years highlights a critical demographic trend in healthcare, particularly concerning the susceptibility of middle-aged adults to infections like candiduria. This age group, representing the 4th and 5th decades of life, may be increasingly vulnerable to physiological changes in the urinary system, such as reduced renal function or bladder control, which can predispose them to conditions requiring catheterization. Catheter use is a well-documented risk factor for catheter-associated urinary tract infections, including those caused by *Candida* species. This finding is consistent with previous research by Jain *et al.* (2011) in India, which also reported a higher incidence of *Candida* infections among middle-aged individuals. The alignment between these studies suggests that age-related physiological changes, along with potential co-morbidities such as diabetes or hypertension, might contribute to the increased risk of urinary system infections in this demographic.

As populations age, the prevalence of age-related health issues, including urinary system dysfunctions, is likely to rise, potentially leading to a higher incidence of *Candida* infections. This trend calls for the need for targeted preventive strategies, such as early intervention in at-risk populations, improved catheter management protocols, and regular monitoring for signs of infection. Furthermore, the association between age and infection risk calls for more multifaceted clinical approaches that consider the unique vulnerabilities of middle-aged and older adults which may improve patient outcomes on a global scale.

In this study biofilm production was seen in (66.7%) of the *Candida* isolates. This finding is higher than the work of Rishpanal and Colleague (2015) who reported (53.8%) biofilm production rates in their *Candida* isolates. This may be due to the fact that non-*albicans* *Candida* species were the highest biofilm producers in their study when compared to the *Candida albicans* (50.0%) being the highest biofilm producer in this study. Biofilm formation is associated with virulence and resistance to antifungal agents. The findings

in this study point to the need for continuous surveillance of Health care associated infections (HCAIs). The purpose of surveillance of HCAIs is to reduce the incidence of nosocomial infections, which in turn will reduce the associated morbidity, mortality and costs. The primary aim of surveillance of nosocomial infections is to establish its baseline rates in a particular health care Institutions in order to effectively monitored (Deorukhkar *et al.*, 2012).

Antifungal susceptibility pattern of *Candida* isolates in this study to Fluconazole, Nystatin, Amphotericin B, Voriconazole, and Itraconazole revealed that isolates were highly susceptible to Itraconazole and Fluconazole. Only *Candida glabrata* was resistant to Amphotericin B. The findings highlight the variability in susceptibility among species, which is crucial for guiding appropriate antifungal therapy. The finding is in concordance with the study carried out by Tobudic *et al.* (2010) in Vienna, Austria, who also reported high resistance against Amphotericin B by *Candida glabrata*.

The isolates of *C. albicans* showed a high susceptibility to Itraconazole (100%) and Amphotericin B (75%), which indicates that these antifungal agents remain highly effective against this species. However, the lower susceptibility to Fluconazole (50%) and Nystatin (50%) suggests that some strains of *C. albicans* may exhibit resistance to these commonly used antifungals, a trend that was reported by Pfaller & Diekema (2012) in their global review of perspective on antifungal resistance and Ogba *et al.* (2013) in Nigeria. The 50% susceptibility to Voriconazole aligns with findings of Ruhnke (2006) in Berlin, Germany who reported that although effective, Voriconazole may not be universally reliable against *C. albicans*.

Candida glabrata isolates displayed significant resistance to Amphotericin B, with 0% susceptibility. This is consistent with global trends that show *C. glabrata* as a species often resistant to Amphotericin B, which complicates treatment options (Tobudic *et al.*, 2010). Conversely, these isolates were largely susceptible to Fluconazole (75%) and Nystatin (75%), although the reduced susceptibility to Voriconazole (25%) suggests

emerging resistance. The high susceptibility to Itraconazole (75%) is notable, offering a potentially effective treatment alternative for *C. glabrata* infections. Globally, the emergence of antifungal resistance, particularly among non-albicans *Candida* species like *Candida glabrata*, poses a significant challenge to healthcare providers. It complicates treatment options and necessitates the use of more potent or combination antifungal therapies, which may come with increased toxicity and cost. The high susceptibility to Itraconazole and Fluconazole observed in this study suggests that azoles remain valuable, but reliance on a limited number of antifungal classes could drive further resistance.

The *C. tropicalis* isolates demonstrated a mixed susceptibility pattern. They were most susceptible to Itraconazole (50%) and Fluconazole (50%) but showed lower susceptibility to Amphotericin B (25%) and Voriconazole (25%). These findings align with global data indicating that *C. tropicalis* can exhibit resistance to multiple antifungals, particularly in regions with high antifungal usage (Ogba *et al.*, 2015; Pappas *et al.*, 2018). The susceptibility to Nystatin (50%) suggests that it remains a viable option, but with caution due to potential resistance. The findings emphasize the importance of routine antifungal susceptibility testing in clinical practice to guide appropriate therapy. On a global scale, these efforts are crucial for maintaining the efficacy of current treatments and ensuring that healthcare systems are equipped to manage the growing threat of antifungal-resistant *Candida* infections.

Conclusion

Candiduria associated with urinary catheter is a health problem in the study setting. Candida albicans was the most isolated *Candida* species with high rates of biofilm production. There was high antifungal resistance among isolates which calls for better antifungal stewardship for better patients' management and outcome.

Conflict of interest: The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work

presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Funding/support

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Author's Contributions

OOM conceived the study. OOM and BDC contributed to the design of the study. OOM, BDC and IIO performed the laboratory studies. OMO, BDC, ORO, ANG, NES and ULI analyzed the data and drafted the manuscript. OMO is the guarantor of the paper. All authors read and approved the final version.

Acknowledgements: We acknowledge the management of the University of Calabar for an enabling environment for research and the University of Calabar Teaching Hospital for the use of their facility for laboratory work.

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Citation: Ogba, Ofonime Mark, Bullem-Odu Davina Chidinma, Inah, Inah Onete, Ogba, Rejoice Oghenovo, Akpan, Nseobong Godwin, Nyambi, Edim Sunday, Ugar Louisa Ifu. Catheter associated *Candiduria* among In-patients accessing care in a Tertiary Health Institution in Calabar, Nigeria. *Sokoto Journal of Medical Laboratory Science*; **9(3)**: 227 – 236. <https://dx.doi.org/10.4314/sokjmls.v9i3.24>

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