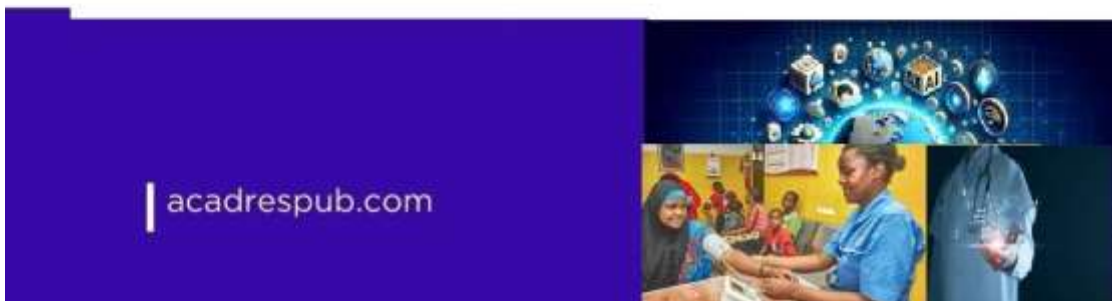


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SHIFTING PATTERNS IN CANDIDA SPECIES AND RESISTANCE AMONG HEALTHY CARRIERS: INSIGHTS FROM A UNIVERSITY COHORT IN SOUTHERN NIGERIA

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ABSTRACT

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Background: Yeast infection caused by overgrowth of *Candida* species, remains a significant global health concern due to increasing antifungal resistance and the emergence of non-albicans species.

Aim: This study aimed to determine the prevalence, species distribution, and antifungal susceptibility of *Candida* isolates among apparently healthy female students in Okada, Edo State, Nigeria.

Method: A cross-sectional study was conducted involving 120 undergraduate students aged 17-28 years selected through systemic random sampling. High vaginal swabs were collected and cultured on Sabouraud dextrose agar. Identification of isolates was based on cultural characteristics, microscopy, and germ tube test, while antifungal susceptibility was determined using broth dilution technique to establish minimum inhibitory concentrations (MICs).

Results: The overall prevalence of *Candida* carriage was 33.3%, with the highest occurrence observed among individuals aged 21–24 years (44.2%), showing a statistically significant association ($p = 0.017$). Non-albicans *Candida* species predominated (60%) over *Candida albicans* (40%), indicating an epidemiological shift. Antifungal susceptibility testing revealed complete sensitivity to fluconazole (100%), while high resistance rates were observed for ketoconazole (81.2%) and nystatin (56.2%). Metronidazole showed moderate activity (75%) but is not considered a standard antifungal agent.

Conclusion: The findings highlight a substantial burden of candida carriage and a growing dominance of resistant non-albicans species in the study population. These trends underscore the need for routine species identification and susceptibility testing to guide effective treatment. Enhanced surveillance and rational use of antifungal agents are essential to mitigate the spread of resistant *Candida* strains.

Keywords: Antifungal susceptibility testing, broth dilution, *Candida* species, Fluconazole, Minimum inhibitory concentration.

Introduction

Candidiasis, an infection caused by the genus *Candida*, represents a significant and growing clinical concern worldwide, particularly as *Candida* species continue to adapt to and develop resistance against commonly used antifungal agents (Dos Santos & Ishida, 2023). While superficial candidiasis, such as oral thrush and vulvovaginal candidiasis, remains a relatively common and manageable condition, more severe forms, such as invasive candidiasis and candidemia, present a considerable challenge, especially in immunocompromised patients. These infections can lead to life-threatening complications, including sepsis and multi-organ failure, and are often associated with high mortality rates when not promptly diagnosed and treated. Over the past few decades, the epidemiology of candidiasis has evolved, with an increase in the frequency of invasive infections and the emergence of multidrug-resistant *Candida* species, including *Candida auris*, which has posed a significant threat to global health (Gavilanes-Martinez *et al.*, 2021).

The clinical management of candidiasis involves the use of antifungal agents, including azoles, polyenes, echinocandins, and pyrimidine analogs, each with distinct mechanisms of action against *Candida* species (Dawoud *et al.*, 2024). The appropriate selection of antifungal therapy is critical to successful outcomes, and therapy must be tailored to the specific species of *Candida* involved, the site of infection, the severity of the disease, and the patient's underlying risk factors. With the emergence of resistance, particularly to azoles and echinocandins, clinicians are increasingly turning to combination therapies and novel antifungal agents to overcome treatment failures. Furthermore, biofilm-associated infections and the rising prevalence of multidrug-resistant organisms complicate treatment regimens, underscoring the need for a more nuanced approach to managing these infections (Freitas *et al.*, 2023).

In light of the increasing burden of candidiasis globally, with a rise in invasive forms of the disease and the emergence of resistant strains, effective management has become more complex. This complexity is compounded by the diversity of *Candida* species and their varying susceptibility profiles to antifungal drugs. Given the persistent threat posed by *Candida* infections, the need for effective therapeutic strategies remains more important than ever (Schwarz *et al.*, 2022). This

study aimed to isolate *Candida* species from apparent healthy female individuals in Okada, Edo State, and determine their susceptibility to antifungal agents.

Methodology

Study Area

The research was carried out in Okada, precisely participants were students drawn from various department of the Institution (Igbinedion University, Okada). Igbinedion University, located in Okada, Ovia North-East Local Government Area of Edo State, Nigeria, with geographic coordinates approximately 6.7302° N latitude and 5.3958° E longitude. The university lies centrally in Okada town and the town is known for its bolstering activities with social life being fair.

Study Population

The participants were undergraduate students of Igbinedion University. The age group of the participants is of the range 17 to 28 years. Consent was sought for in simple understanding language and assured of strict confidentiality.

Sample Size

Sample size was based on systemic sampling. Participants were enrolled using the class enrollment list and every Third name on the list of female students were registered for the study. A total of 120 female students were thus selected.

Administration of Questionnaire

A well-structured questionnaire was administered to all participants. Questionnaire filled and retrieved from participants after answering questions There-in Questionnaires were kept by the researcher for further study.

Ethics committee approval

Approval for the research project was obtained from the ethical committee, Igbinedion University Teaching Hospital, Okada (Ref: IUTH/R.24/VOL.1/50).

Sample Collection

High vaginal swabs were collected from all participants; swab sticks were aseptically placed in

its tube and sent to the laboratory immediately after collection.

Microbiology Analysis

Swab sticks were cultured on sabouraud dextrose agar supplemented with chloramphenicol at a concentration of 5mg/ml. This is to ensure selective tendency of the media, the primary focus was fungus isolates.

All inoculated plates in duplicate were incubated at room temperature and at 37°C. Isolates was identified after 18 hours incubation.

Identification of Fungi Isolates

Pure isolates of the cultured fungus was identified based on cultural characteristics, production of fermented odor, wet preparations microscopy and Germ tube procedure as described by cheesebrought, (2006).

Procedure

All isolates were examined for yeast-like odor and evaluated further by undergoing a wet preparation using Normal saline.

Isolates producing budding cells were confirmed to be yeast by carrying out a Gram stain and subculturing into chromogenic media.

Confirmation using chromogenic agar

All isolates conforming to yeast on examination using normal saline wet preparation were subcultured into well prepared chromogenic agar (CHROMagar) and incubated at 37°C overnight were identified based on color production exhibited on agar plate

Germ Tube Test

All isolates confirming to yeast cells by its morphology were subjected to Germ tube test.

Procedure

1. Using a micro tube, 2-3 drops of serum was added.
2. One to two colonies were emulsified onto the serum and incubated at 37°C for about 45 minutes.

The production of true hyphae on examination at 40x objective denotes isolates to be *Candida albicans*.

Susceptibility Testing

The sensitivity profile of all candida species was carried out using broth dilution technique using Fluconazole (50mg), Nystation (500,000 iu) and Ketoconazole (200mg)

Broth Dilution Technique

Using the method described by (Wiegand et al., 2008) and (Owuama, 2015) different concentration of the antifungal drugs was made using Muller Hinton broth.

Preparation of the Concentrate

1. Fluconazole (50mg)

- i. 50mg of Fluconazole tablet was dissolved in 10ml of sterile normal saline, to obtain a concentration of 5mg/ml. This solution serves as the stock solution. On to series of tubes labeled 1-6, one ml of sterile Muller Hinton broth was added to all tube.
- ii. One ml of the stock solution was aseptically added to tube 1 and carefully mixed.
- iii. One ml from tube 1 was transferred to tube 2, and mixed carefully.
- iv. This procedure was repeated for all tubes, and 1ml from tube 6 was discarded.
- v. Final concentrates obtained are 2.5mg/ml, 1.25 mg/ml, 0.625mg/ml, 0.3125 mg/ml, 0.1663mg/ml and 0.0831mg/ml.

2. Nystatin (500,000iu).

Preparation of 10-fold serial dilutions.

- i. Into tubes labeled 1-6, 0.9ml of Muller Hinton broth was added to all tubes.
 - ii. 0.1 ml of the stock antibiotic was added to tube 1 and mixed thoroughly.
 - iii. 0.1 ml from tube 1 was added to tube 2, and mixed thoroughly.
 - iv. This process was repeated for all subsequent tubes.
 - v. 0.1ml from last tube was discarded.
- Final concentration obtained are 50,000iu/ml, 5,00 iu/ml, 50iu/ml and 5iu/ml.

3. Ketoconazole (200mg)

Preparation of 10-fold dilutions.

- i. Preparation of stock solution.
200mg of the fungal tablets was dissolved in 10ml of sterile normal saline and allow to completely dissolve. This 20mg/ml serves as the stock solution for the 10-fold dilution.
- ii. On to series of tube labeled 1-6, 0.9ml of the Muller Hinton broth was added to all tubes.
- iii. 0.1ml of the stock was added to tube 1, and mixed thoroughly.
- iv. 0.1ml from tube 1 was transferred to tube 2, and mixed thoroughly.
- v. This process was repeated to all subsequent tube.

Final concentrations obtained for tubes 1-6 are: 2mg/ml, 0.2mg/ml, 0.02mg/ml, 0.002mg/ml and 0.0002mg/ml, 0.00002mg/ml.

Standardization of Candida Isolates using procedure described by EUCAST

- i. 3-5 fresh overnight colonies of *Candida albicans* grown on Sabouraud Dextrose Agar was emulsified in about 4ml of normal saline, mixed well to obtain an homogenous suspension.
- ii. Using a Spectrophotometer set at a wavelength of 530 nm and blanked with normal saline, the absorbance of the test suspension was read and adjusted with sterile normal saline until an absorbance within the range of 0.12-0.15 was obtained, this corresponds to a 0.5 McFarland standard (Berkow et al., 2020)

Using The Standardized Inoculum For Testing.

- i. Using aseptic procedure, the suspension for each *Candida albicans* strain equivalent to 0.5
- ii. McFarland standard was further diluted 1:100 in broth to have 1×10^6 cfu/ml of isolated, this serves for the MIC

Determination of Minimum Inhibition Concentration

- i. 0.1ml of the standardized inoculum was added onto all tubes labeled 1-6 for each of the antifungal tablets (Fluconazole, Nystatin and Ketoconazole respectively).
 - ii. Tubes were incubated at 37°C for about 16 hours.
 - iii. Tubes were observed for turbidity.
- Lowest Concentration of antifungal that shows no visible growth is recorded for MIC and results recorded as described by EUCAST (Otto et al., 2023).

Data Analysis

The data obtained was subjected to the use of SPSS. The P-value and the mean value were used to compute if data obtained is statistically significant. Likewise frequency distribution table was used to simplify results.

Results

The Prevalence of *Candida* carriage across age groups reveals a clear, age-dependent gradient in candida colonization presented in Table 1. Among the 120 volunteers screened, only 15.8% of adolescents aged 17–20 years (6 of 38) carried *Candida*, whereas carriage more than doubled to

44.2% in the 21-24 year group (23 of 52) and remained elevated at 36.7% among adults aged 25-28 years (11 of 30). When the data are viewed in absolute terms, the middle cohort not only contributed the largest number of positives but also the highest proportion relative to its size. The χ^2 statistic of 8.19 ($p = 0.017$) confirms that these differences are statistically significant rather than random fluctuations, underscoring a heightened susceptibility, or at least a greater exposure, to *Candida* carriage during early adulthood. Negative results mirror this pattern inversely, with 84.2% of the youngest cohort testing negative compared with just 55.8% of the 21-24 year group, suggesting that behavioural, hormonal or immunological factors unique to this age span may facilitate *Candida* overgrowth. Overall prevalence across the population stands at one-third (33.3%), showing that asymptomatic carriage is common, yet intensely concentrated within the 21-24 year bracket.

The Age-specific risk expressed as odds ratios in Table 2 translates the raw prevalence figures into relative risk estimates. Using the 17-20year cohort as the baseline, individuals aged 21-24 years were over four times more likely to harbour *Candida* (OR = 4.23, 95% CI 1.51–11.84, $p = 0.006$), a robust association that remains significant after adjusting for unequal group sizes. The 25-28 year group also faced an elevated risk (OR = 3.09), though the confidence interval narrowly includes unity (0.98–9.71) and the p-value hovers just above the conventional threshold ($p = 0.054$), indicating a trend that might reach significance with larger numbers. These odds-ratio calculations affirm that early adulthood represents a critical window for *Candida* colonization, corroborating the χ^2 outcome and directing attention to the 21-24 year demographic as a prime target for preventive measures.

Candida Species distribution by age as presented in Table 3 dissects the 40 positive cultures to determine whether *Candida albicans* or non-*albicans* *Candida* (NAC) species dominate within each age stratum. NAC organisms predominate overall, accounting for 60% of isolates, an observation consistent with global epidemiological shifts toward NAC predominance, while *C. albicans* constitutes the remaining 40%. Age-wise, NAC species make up two-thirds of isolates in the youngest group, 60.9% in the 21-24 year cohort, and 54.5% among the oldest participants. Despite these numerical variations, the χ^2 test ($\chi^2 = 0.25$, $p = 0.88$) indicates no meaningful

association between age and species type; the distribution of *C. albicans* versus NAC appears remarkably stable across the life stages examined.

The Antifungal susceptibility and MIC ranges presented Table 4 showed the in-vitro pharmacodynamic profile of 16 representative isolates subjected to three commonly used agents. Fluconazole exhibits impeccable activity, inhibiting all isolates at low MICs (0.3125- 0.625 mg L⁻¹) with no resistance detected, a reassuring finding given

fluconazole's frontline status in resource-limited settings. In contrast, nystatin and ketoconazole reveal alarming resistance patterns, with 56.2 % and 81.2 % of isolates, respectively, able to grow at or above the tested concentrations. Nystatin's MIC span of 50-500 IU mL⁻¹ reflects both moderate and high resistance levels, whereas ketoconazole's broad 0.002-2 mg L⁻¹ range masks a sharp dichotomy in which only three isolates remain susceptible

Table 1 Prevalence of Candida carriage by age group

Age group	No. examined	Positive n (%)	Negative n (%)	Prevalence %
17 – 20	38	6 (15.8)	32 (84.2)	15.8
21 – 24	52	23 (44.2)	29 (55.8)	44.2
25 – 28	30	11 (36.7)	19 (63.3)	36.7
Total	120	40 (33.3)	80 (66.7)	33.3

Pearson $\chi^2(2) = 8.19, p = 0.017$

Table 2 Association between age group and risk of candida carriage

Age group	Odds ratio†	95 % CI	p-value
17 – 20 (ref)	1.00	–	–
21 – 24	4.23	1.51 – 11.84	0.006
25 – 28	3.09	0.98 – 9.71	0.054

†Odds ratios compare the odds of being Candida-positive in each age group with the 17-20 year reference category, calculated from a 2 × 2 contingency table.

Table 3: Species distribution among positive samples

Age group	<i>Candida albicans</i> n (%)	Non-albicans <i>Candida</i> * n (%)	Total positives
17 – 20	2 (33.3)	4 (66.7)	6
21 – 24	9 (39.1)	14 (60.9)	23
25 – 28	5 (45.5)	6 (54.5)	11
Total	16 (40.0)	24 (60.0)	40

*Includes *C. tropicalis*, *C. krusei*, *C. parapsilosis* and other non-albicans isolates. Pearson $\chi^2(2) = 0.25, p = 0.88$

Table 4: Minimum inhibitory concentration (MIC) and susceptibility profiles of Candida isolates (n = 16 tested)

Anti-fungal agent	Sensitive n (%)	Resistant n (%)	MIC range
Fluconazole	16 (100)	0	0.3125 – 0.625 mg L ⁻¹
Nystatin	7 (43.8)	9 (56.2)	50 – 500 IU mL ⁻¹
Ketoconazole	3 (18.8)	13 (81.2)	0.002 – 2 mg L ⁻¹

Discussion

Candida colonization is widely implicated in most urinary tract infections and there are reports in which non- albicans is fast gaining ground as potential pathogen. In this study which relied on culture- based methods for the identification and differentiation of Candida species, however, molecular techniques such as 16S rRNA sequencing could have provided a more comprehensive and improved species resolution.. Notwithstanding, the carriage rate of candida species in the population studied was 33%. This value is low when compared to reported prevalence from other Nigerian settings such as Port Harcourt, North central and Eastern Nigeria using similar population (Fajoyomi *et al.*, 2022; Aleruchi *et al.*, 2019; Chukwunwejim *et al.*, 2024), with prevalence of 64%, 44.37% and 88.76% respectively. The figure suggests that a sizeable reservoir of yeasts exists in apparently healthy residents, providing a potential source for future invasive disease should local or systemic immunity decline (Scorzoni *et al.*, 2016).

Age emerged as a key determinant of candida colonization. Individuals within the age group 21 to 24 years showed the highest prevalence at 44.2% and the odds of colonisation in this group were over four times that of adolescents aged 17 to 20 years. Such transitional changes in acquisition of candida species could be attributed to hormonal changes, rising sexual activity, and lifestyle factors (Faustino *et al.*, 2025; Gaziano *et al.*, 2023). Other factors such as oral contraceptive use and antibiotic misuse are common among students in Nigerian university communities, which further aggravate selective pressure for *Candida* overgrowth (Aminzadeh *et al.*, 2016).

Species-level data indicated that non-albicans Candida (NAC) species predominated, representing 60% of isolates. The dominance of NAC is now recognised across Africa and Asia and marks a shift from the historical pattern where

Candida albicans was the principal coloniser (Anh *et al.*, 2021). Several factors may account for this transition. NAC species often exhibit increased environmental robustness and greater capacity to form biofilms, which enhances persistence on mucosal surfaces (Gulati & Nobile, 2016). In addition, the expansive use of azole prophylaxis and over-the-counter antifungals can select for inherently less susceptible species such as *Candida krusei* and *Candida glabrata* (Akinosoglou *et al.*, 2024). In addition, the expansive use of azole prophylaxis and over-the-counter antifungals can select for inherently less susceptible species such as *Candida krusei* and *Candida glabrata*. Our finding that species distribution did not vary significantly across the three age bands suggests that once colonisation is established, host age plays a limited role in shaping the relative representation of *Candida* species.

Antifungal susceptibility testing revealed total sensitivity to fluconazole across the 16 isolates that underwent minimum inhibitory concentration testing. This result is encouraging because fluconazole is both affordable and widely stocked in Nigerian pharmacies. Comparable full sensitivity has been documented in community isolates from Rivers and Kumasi (Girah *et al.*, 2024; Aboagye *et al.*, 2025), although in a study conducted in Lagos, Fluconazole was the least effective (Samuel *et al.*, 2014). In contrast, resistance to nystatin and ketoconazole was high, measured at 56.2% and 81.2% respectively. Several regional studies have reported a similar pattern in resistance to some common antifungal drugs such as nystatin and ketoconazole, although variations in resistant trends were noted probably as a result of geographic locations of study and specie: In Nigeria, a similar study found 65% of candida isolates from high vagina specimen being resistant to nystatin, while ketoconazole and nystatin resistance of 61.9% and 44.3% respectively has been reported among women attending a clinic in Enugu state (Joachim *et al.*, 2020). Ketoconazole

has largely fallen out of favour because of hepatotoxicity, yet it remains available without prescription in rural settings, such tendencies may promote misuse and resistance. In a similar study conducted in Lagos university teaching Hospital, resistant rate obtained by *Candida albicans* was 86% and 71% respectively for Fluconazole and Ketoconazole respectively (Osinupebi *et al.*, 2021).

The predominance of NAC among resistant isolates mirrored reports from tertiary hospitals in Abuja and Pretoria, where NAC species were five times more likely than *C. albicans* to display azole resistance (Ezeadila *et al.*, 2020; Rabault *et al.*, 2025). NAC species possess a range of resistance mechanisms, including efflux pumps, altered sterol bio-synthesis pathways, and biofilm-associated tolerance, all of which can undermine standard dosing regimens (Li *et al.*, 2025). Consequently, clinicians should not assume that empiric therapy effective against *C. albicans* will cover NAC.

Several factors may help explain why fluconazole retained full activity as in relation to nystatin and ketoconazole. Fluconazole is often prescribed in short, single-dose regimens for vulvovaginal candidiasis, reducing exposure time and resistance selection compared with the prolonged topical courses typical for nystatin and ketoconazole (Donders *et al.*, 2022). Fluconazole's pharmacokinetic profile also allows for excellent mucosal penetration and long half-life, which may facilitate rapid clearance of yeast cells before resistance develops (Grant & Clissold, 1990).

Conclusion

Candida colonisation is common among symptom-free adults in Okada, affecting 33 percent of those sampled. Prevalence is highest in the 21 to 24 year age group, indicating that young adults represent a critical reservoir for potential transmission and infection. Non-*albicans* species are now the dominant colonisers, a change that has direct consequences for treatment because these species show greater resistance to several widely available antifungal drugs. Fluconazole remains fully active in vitro, but high resistance to nystatin and ketoconazole raises concern about the continued utility of these agents. The clear association between NAC species and resistance supports the routine use of species-level identification and susceptibility testing before therapy.

Recommendation

Public health authorities and clinicians in Okada should prioritise targeted education for young

adults on risk factors that support *Candida* overgrowth, enforce stricter regulation of over-the-counter topical antifungal, implement low-cost species identification in primary laboratories, and establish regular surveillance that couples antifungal susceptibility testing with molecular typing to detect emerging resistance and guide empiric therapy.

Limitations

This research highlights ongoing gaps. Only sixteen isolates were subjected to full MIC testing because of resource constraints. Future studies would embrace larger sample sizes and molecular identification to detect cryptic species such as *Candida auris*, which is emerging in West Africa. Antifungal resistance genes could not be sought using polymerase chain reaction to pinpoint mechanisms and monitor for clonal spread due to lack of funds.

Contribution to Knowledge

1. *Candida* infections correlates with age in asymptomatic individual, thus age is a determinant to acquisition of *Candida*. The early-adult peak in carriage should inform health education campaigns targeted at university students and young workers.
2. Fluconazole exhibit high susceptibility pattern and should be considered as drug of choice for vagina candidiasis

Declaration

The authors declared that this research could be used by other researchers with due citations of authors

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Conflict of interest

The authors have declared that there is no conflicts of interest.

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Author's Contributions

OM performed the write-up and mycological analysis of samples, ZS conceptualized the study and was a major contributor in writing the manuscript. FO recruited participants for the study. All authors read and approved the final manuscript.

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