



Research Article

Antifungal Susceptibility Pattern of *Candida* species Isolated from Non-Vaginal Infection Sites

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ABSTRACT

Candida species are opportunistic fungal pathogens whose management is increasingly complicated by a global shift toward non-albicans species and antifungal resistance. Although most *Candida* research in Nigeria has focused on vaginal candidiasis, non-vaginal sites such as blood, urine, sputum, swabs and cerebrospinal fluid remain under-characterised. This study determined the antifungal susceptibility pattern of *Candida* species isolated from non-vaginal clinical specimens at the University of Ilorin Teaching Hospital (UITH), Nigeria. In this cross-sectional laboratory-based study, 28 non-vaginal *Candida* isolates were identified to species level by internal transcribed spacer (ITS) region sequencing, and antifungal susceptibility was determined using the automated VITEK 2 system (AST-YST card), interpreted against CLSI M27 breakpoints. Data were analysed in SPSS version 26 using Fisher's exact test, with significance set at $p \leq 0.05$. *Candida tropicalis* (39.3%) and *Candida albicans* (32.1%) were the predominant species, alongside non-albicans and emerging species including *Pichia kudriavzevii*, *Candidozyma auris*, *Candida auris*, *Nakaseomyces glabrata* and *Clavispora lusitanae*. Isolates were completely susceptible to fluconazole, voriconazole and micafungin (100.0% each), with high susceptibility to caspofungin (95.8%) and flucytosine (88.0%); amphotericin B recorded the lowest susceptibility (80.0%), with 16.0% resistance. Susceptibility to fluconazole and flucytosine varied significantly with species ($p < 0.001$), while susceptibility to the other agents did not differ significantly between species. These findings show that non-vaginal *Candida* isolates at UITH remain broadly susceptible to first-line antifungals, but species-dependent variability in azole and flucytosine susceptibility, together with the recovery of *Candida auris*/*Candidozyma auris*, supports routine species-level identification and continued surveillance to guide rational antifungal use.

Keywords: Antifungal resistance; Antifungal susceptibility; *Candida* species; Nigeria; Non-vaginal infection; VITEK 2

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INTRODUCTION

Fungal infections are an increasing global health concern, particularly among immunocompromised populations, with *Candida* species remaining the most common opportunistic fungal pathogens (Camp *et al.*, 2020). *Candida* causes a spectrum of disease, candidiasis, ranging from superficial mucocutaneous

infection to invasive, life-threatening disease, and its pathogenicity derives from virulence factors such as tissue adherence, biofilm formation and phenotypic switching that enable colonisation of the bloodstream, urinary tract, respiratory tract and other deep-seated sites. Because different *Candida* species vary substantially in virulence and intrinsic

antifungal susceptibility, accurate species-level identification is a prerequisite for rational antifungal therapy.

Historically, research on candidiasis in Nigeria has focused predominantly on vulvovaginal infection. However, rising rates of invasive and non-genital candidiasis, particularly among critically ill and hospitalised patients, have shifted attention toward bloodstream, urinary tract, respiratory tract and other deep-seated infections, driven by the emergence of non-*albicans* *Candida* species and antifungal resistance (Camp *et al.*, 2020). The epidemiology of candidiasis has changed markedly worldwide, with increasing isolation of non-*albicans* species such as *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei* and the multidrug-resistant *C. auris*, many of which show reduced susceptibility to commonly used antifungal agents (Chowdhary *et al.*, 2020).

Antifungal susceptibility testing is therefore a critical component of *Candida* management. Automated platforms such as the VITEK 2 system (bioMérieux) provide standardised, reproducible susceptibility results with high categorical agreement relative to the CLSI broth microdilution reference method, reducing turnaround time relative to manual testing (Durand *et al.*, 2021; Wiederhold, 2021). In many Nigerian laboratories, however, antifungal susceptibility testing is not performed routinely alongside identification, and locally-generated non-vaginal *Candida* susceptibility data remain scarce, which limits antifungal stewardship and evidence-based empirical therapy (Girah *et al.*, 2024).

Despite the clinical importance of non-vaginal candidiasis, information on the antifungal susceptibility profile of *Candida* species isolated from non-vaginal anatomical sites at the University of Ilorin Teaching Hospital (UITH) remains limited. This study therefore determined the species distribution and antifungal susceptibility pattern of *Candida* isolates recovered from non-vaginal clinical specimens (blood, urine, sputum, swab and cerebrospinal fluid) at UITH, to generate baseline local data to support antifungal stewardship and empirical treatment decisions.

MATERIALS AND METHODS

Study Area and Population

This study was conducted at the Department of Medical Microbiology and Parasitology, University of Ilorin Teaching Hospital (UITH), Ilorin, Kwara State, Nigeria, a tertiary referral centre for Kwara State and neighbouring states. Molecular characterisation was

carried out at Celkha Biosciences Research Laboratory, Abuja, Nigeria. The study population comprised non-vaginal clinical specimens (urine, blood, sputum, swab and cerebrospinal fluid) obtained from patients of all age groups and both sexes, including inpatients and outpatients, attending UITH between January 2025 and July 2026, from which *Candida* was isolated on primary culture. A total of 28 viable *Candida* isolates were recovered and characterised.

Study Design

A cross-sectional, laboratory-based study design was employed. Recovered *Candida* isolates were subjected to presumptive phenotypic identification, definitive molecular identification, and antifungal susceptibility testing, and the resulting species-level data were analysed against susceptibility outcomes for all confirmed isolates.

Determination of Sample Size

The minimum sample size was calculated using Fisher's formula: $n = Z^2 P(1-P)/d^2$, where Z is the statistic corresponding to the 95% confidence level (1.96), P is the expected prevalence of *Candida* species isolated from different anatomical sites (10%, i.e., 0.1) (Oladele *et al.*, 2020), $(1-P)$ is the complement of the prevalence (0.9), and d is the desired precision (0.05). Substituting these values gave $n = (1.96)^2 \times 0.1 \times 0.9 / (0.05)^2 = 138$. A 10% attrition allowance (13.8) was added to compensate for possible loss of isolates during laboratory processing, giving an adjusted minimum sample size of 152 participants.

Inclusion and Exclusion Criteria

Isolates were included if they were: (i) recovered from non-vaginal clinical specimens; (ii) viable, with adequate growth on subculture; and (iii) obtained from inpatients or outpatients at UITH during the study period. Isolates were excluded if they: (i) originated from vaginal clinical specimens, which were excluded because of their high rate of colonisation and limited specificity for invasive disease; (ii) were non-viable and failed to grow after repeated subculture; or (iii) were linked to incomplete demographic or laboratory information. Contaminated cultures were also excluded.

Ethical Consideration and Informed Consent

Ethical approval was obtained from the Health Research Ethics Committee of UITH (Approval No. HREC PAN/2026/05/1639) prior to commencement of the study. An approved waiver of consent for laboratory analysis of routinely collected isolates. Patient confidentiality was maintained throughout the study; unique laboratory identification numbers

were used in place of patients' names during data collection and analysis, in accordance with the ethical principles governing biomedical research involving human participants.

Data Collection

A simple random sampling technique was employed in selecting eligible *Candida* isolates recovered from non-vaginal clinical specimens submitted to the Department of Medical Microbiology and Parasitology, UITH, with consecutive eligible isolates recruited until the required sample size was obtained. Data collection spanned eighteen months (January 2025 to July 2026), covering specimen collection, isolation and characterisation of *Candida* species, antifungal susceptibility testing, molecular analysis, and statistical analysis of the generated data. Presumptively identified isolates were preserved in 20% glycerol broth and stored at -20°C pending molecular characterisation and antifungal susceptibility testing. Demographic and specimen-source data (age, sex, specimen type) were extracted alongside laboratory results for each isolate.

Laboratory Analysis

Isolates were subcultured on Sabouraud Dextrose Agar (SDA) supplemented with gentamicin and chloramphenicol and incubated at 25°C for 24–48 hours. Pure colonies underwent presumptive phenotypic identification on CHROMagar *Candida* Plus, then definitive molecular identification by internal transcribed spacer (ITS) region sequencing, which served as the reference method. This comprised genomic DNA extraction, PCR amplification of the ITS region using the universal fungal primers ITS1 and ITS4, agarose gel electrophoresis, purification of PCR products, Sanger sequencing, and sequence analysis by BLAST search against the NCBI database.

Antifungal susceptibility testing was performed on all confirmed isolates using the automated VITEK 2 system (bioMérieux) with AST-YST cards. A standardised yeast suspension (McFarland 1.8–2.2) was prepared from a pure colony in 3.0 mL sterile 0.5% saline, and the AST-YST08 card was loaded and processed automatically, with incubation at 35.5°C and automated turbidimetric readings over 9–18 hours. Minimum inhibitory concentrations (MICs) generated by the instrument were interpreted as susceptible, susceptible dose-dependent, intermediate or resistant using CLSI M27 breakpoints

(Clinical and Laboratory Standards Institute, 2017). *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were used as quality-control strains.

Data were entered in Microsoft Excel and analysed in SPSS version 26. Descriptive statistics (frequencies and percentages) summarised specimen and species distribution and antifungal susceptibility profiles. Fisher's exact test was used to assess the association between *Candida* species and susceptibility to individual antifungal agents, with statistical significance set at $p \leq 0.05$.

RESULTS

Of the 152 participants recruited, 37 yielded *Candida* isolates on primary culture; nine of these became non-viable during subsequent molecular characterisation, leaving 28 viable *Candida* isolates that were confirmed by ITS sequencing and constituted the final analytic sample for species identification and antifungal susceptibility testing. Urine specimens accounted for the largest share of isolates (32.1%), followed by sputum (25.0%), blood (21.4%), swab (17.9%) and cerebrospinal fluid (3.6%). Molecular identification revealed *Candida tropicalis* as the predominant species (39.3%), followed by *Candida albicans* (32.1%), with the remainder comprising non-albicans and emerging species — *Pichia kudriavzevii* (10.7%), *Candidozyma auris* (7.1%), *Candida auris* (3.6%), *Nakaseomyces glabrata* (3.6%) and *Clavispora lusitaniae* (3.6%) (Table 1).

Among the antifungal agents tested, fluconazole, voriconazole and micafungin each showed 100.0% sensitivity. Caspofungin showed 95.8% sensitivity, with one resistant isolate (4.2%). Flucytosine showed 88.0% sensitivity, with 12.0% resistance. Amphotericin B recorded the lowest sensitivity (80.0%), with 4.0% intermediate susceptibility and 16.0% resistance (Table 2).

A statistically significant association was observed between *Candida* species and susceptibility to fluconazole ($p < 0.001$) and flucytosine ($p < 0.001$). Among fluconazole-sensitive isolates, *C. tropicalis* accounted for the highest proportion (55.0%), followed by *C. albicans* (45.0%); a similar pattern was observed for flucytosine (*C. tropicalis* 50.0%, *C. albicans* 40.9%). No statistically significant species-level association was found for voriconazole ($p = 1.000$), caspofungin ($p = 0.093$), micafungin ($p = 0.123$) or amphotericin B ($p = 0.933$) (Table 3).

Table 1: Species distribution of *Candida* isolates from non-vaginal specimens (n=138)

Species	Frequency	Percentage (%)
<i>Candida tropicalis</i>	11	39.3
<i>Candida albicans</i>	9	32.1
<i>Pichia kudriavzevii</i>	3	10.7
<i>Candidozyma auris</i>	2	7.1
<i>Candida auris</i>	1	3.6
<i>Nakaseomyces glabrata</i>	1	3.6
<i>Clavispora lusitaniae</i>	1	3.6
Total	28	100.0

Table 2: Antifungal susceptibility profile of *Candida* isolates from non-vaginal specimens

Antifungal Agent	Number Tested	Sensitive n(%)	Resistant n(%)
Fluconazole	20	20 (100.0)	0 (0.0)
Voriconazole	22	22 (100.0)	0 (0.0)
Caspofungin	24	23 (95.8)	1 (4.2)
Micafungin	22	22 (100.0)	0 (0.0)
Amphotericin B	25	20 (80.0)	4 (16.0)
Flucytosine	25	22 (88.0)	3 (12.0)

Table 3: Association between *Candida* species and antifungal susceptibility

Antifungal Agent	Predominant Sensitive Species (%)	P-value
Fluconazole	<i>C. tropicalis</i> 55.0; <i>C. albicans</i> 45.0	<0.001*
Voriconazole	<i>C. tropicalis</i> 40.9; <i>C. albicans</i> 36.4	1.000
Caspofungin	<i>C. tropicalis</i> 47.8; <i>C. albicans</i> 34.8	0.093
Micafungin	<i>C. tropicalis</i> 50.0; <i>C. albicans</i> 31.8	0.123
Amphotericin B	<i>C. tropicalis</i> 45.0; <i>C. albicans</i> 30.0	0.933
Flucytosine	<i>C. tropicalis</i> 50.0; <i>C. albicans</i> 40.9	<0.001*

*Statistically significant ($p \leq 0.05$)

DISCUSSION

This study characterised the antifungal susceptibility pattern of *Candida* species isolated from non-vaginal clinical specimens at UITH. Urine yielded the largest share of isolates, consistent with the well-documented burden of candiduria among catheterised, diabetic and critically ill inpatients (Gajdacs *et al.*, 2019), and reflects a case mix distinct from the predominantly gynaecological samples typical of most Nigerian *Candida* surveys.

Candida tropicalis emerged as the leading species (39.3%), ahead of *C. albicans* (32.1%). This finding adds to a growing body of evidence documenting a shift away from *C. albicans* dominance in non-genital *Candida* infections in tropical settings. *Candida tropicalis* is repeatedly identified as one of the most virulent non-albicans species, with an efficient biofilm-forming capacity favoured by warm, humid conditions, and is disproportionately represented in bloodstream and other deep-seated infections (Silva *et al.*, 2021). The recovery of *Candida auris* and *Candidozyma auris* together in three isolates (10.7%) is a particularly important finding, consistent with recent confirmation of local *C. auris* transmission and

bloodstream infection in Nigeria (Oladele *et al.*, 2022) and with reports of its adaptability and variable susceptibility profile (Semenya *et al.*, 2024).

Isolates in this study remained completely susceptible to fluconazole, voriconazole and micafungin, with high susceptibility to caspofungin and flucytosine. This favourable overall pattern contrasts with reports from other Nigerian tertiary centres, while Girah *et al.* (2024), studying isolates from Port Harcourt, recorded comparatively lower fluconazole susceptibility and itraconazole resistance approaching one-third of isolates tested, attributed to widespread unsupervised over-the-counter antifungal use. The higher susceptibility observed at UITH may reflect lower local antifungal selection pressure, more restricted prescribing practices within the teaching hospital, or the particular mix of species recovered, since azole susceptibility is intrinsically species-dependent.

Amphotericin B recorded the lowest susceptibility of the antifungals tested (80.0%), a finding that merits attention given its traditional role as a reliable broad-spectrum agent and its frequent use as salvage therapy when azole or echinocandin options fail. This

is broadly consistent with an increasing trend of polyene resistance reported in several tertiary hospital settings and reinforces the sentry surveillance observation that continuous monitoring of amphotericin B susceptibility remains necessary even where azole susceptibility appears preserved (Pfaller *et al.*, 2019).

The significant species-level association observed for fluconazole and flucytosine susceptibility (both $p < 0.001$), contrasted with the absence of a significant association for voriconazole, the echinocandins and amphotericin B, is consistent with the pharmacological principle that intrinsic susceptibility to azoles and to flucytosine varies considerably between *Candida* species, whereas echinocandins tend to retain broadly consistent activity across most species (Wiederhold, 2021; Durand *et al.*, 2021). Because *C. tropicalis* and *C. albicans* together accounted for the large majority of fluconazole- and flucytosine-sensitive isolates, while non-susceptible isolates clustered among rarer non-albicans species, this finding underscores the clinical importance of species-level identification before initiating fluconazole or flucytosine therapy for non-vaginal candidiasis, since empirical treatment based on morphology alone risks selecting an inappropriate agent for an intrinsically less susceptible species. This study is limited by its modest sample size ($n=138$), which constrains the precision of species-specific susceptibility estimates for less common taxa represented by only one or two isolates, and by its single-centre, cross-sectional design, which limits generalisability to other Nigerian tertiary hospitals.

CONCLUSION

Candida species isolated from non-vaginal clinical specimens at UITH were predominantly *C. tropicalis* and *C. albicans*, alongside a notable minority of non-albicans and emerging species, including the multidrug-resistant *Candida auris*/*Candidozyma auris*. Isolates remained highly susceptible to fluconazole, voriconazole and micafungin, with more variable susceptibility to flucytosine and amphotericin B, and clear species-dependent variability in fluconazole and flucytosine susceptibility. Routine species-level identification alongside antifungal susceptibility testing, and continued local surveillance for emerging resistant species, are recommended to support antifungal stewardship for non-vaginal candidiasis at UITH and comparable Nigerian tertiary hospitals.

Conflicts of Interest

The authors declare no conflict of interest.

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Data Availability

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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