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Antifungal resistance among Candida albicans isolates from vulvovaginal candidiasis patients at a referral hospital in Malawi

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Research Article

Keywords: antifungal resistance, Candida albicans, vulvovaginal candidiasis, minimum inhibitory concentration, Malawi

Posted Date: November 3rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11594324/v1

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Additional Declarations: No competing interests reported.

Abstract

Background

Antifungal resistance (AFR) is a growing global health concern, particularly among *Candida* species including *Candida albicans*, contributing to increased morbidity and treatment failure. In Malawi, despite antimicrobial resistance (AMR) surveillance strategies outlined in the National Action Plan on AMR, antifungal treatment remains largely empirical, with limited AFR interventions.

Methods

This study investigated phenotypic resistance in *Candida albicans* isolates from patients at Bwaila District Hospital in Lilongwe, Malawi. We employed a laboratory-based, cross-sectional, analytical design and analysed 123 high vaginal swab samples from patients with suspected vulvovaginal candidiasis. Isolation was performed using culture methods, and antifungal susceptibility testing was conducted by determining minimum inhibitory concentrations (MICs).

Results

Of the 123 samples analyzed, 28.5% (35/123) yielded *Candida albicans* isolates. Out of the 17 isolates tested for antifungal drug susceptibility, 41.2% (7/17) of isolates exhibited resistance to at least one antifungal agent, with the highest resistance observed against voriconazole 41.2% 7/17). Echinocandins (caspofungin and micafungin) showed the lowest resistance, 18% (3/17). Fluconazole and Flucytosine displayed dose-dependent susceptibility and had intermediate effects of 35.3% (6/17) and 29.4% (5/17), respectively. Minimum inhibitory concentration (MIC) profiles demonstrated significant variation across antifungal agents (ANOVA, $p < 0.001$; $\eta^2 = 0.217$).

Conclusions

The resistance and dose-dependent susceptibility observed among the antifungal drugs highlight the need for strengthened AFR surveillance and antifungal stewardship to guide treatment and clinical decision-making.

Background

Antifungal resistance (AFR) is a rising global threat interfering with the effective treatment of candidiasis patients. *Candida* species are the most common cause of fungal infections worldwide, and candidiasis is the most prevalent fungal infection affecting patients, causing over 995,000 deaths per year (Denning, 2024). The global rise in fungal infections over the past decade, accounting for approximately 1.5 million individuals annually, has been perpetuated by the increase in immunocompromised individuals due to

HIV/AIDS, cancer, organ transplantation, and the increasing elderly population (Zhang et al., 2020). Fungal infections have been reported as difficult to treat, largely because of AFR, which limits therapeutic options and contributes to treatment failure.

AFR is not a localized problem but a global phenomenon with geographically heterogeneous patterns. In some settings, *Candida* has emerged with resistance exceeding 93%; for example, fluconazole resistance in *Candidozyma auris* (formerly *Candida auris*) in Asia has reached levels above 93% (Lockhart et al., 2017). This is particularly critical considering that *Candida* species are a major cause of systemic fungal infections, which carry high mortality even with appropriate treatment (Lee, 2023). The emergence and spread of resistant *Candida* strains therefore represents a direct threat to clinical management of both mucosal and invasive candidiasis. In recent years, AFR has spread due to both innate and acquired factors. These include narrow-spectrum antifungal regimens, prolonged drug exposure, and most importantly, widespread use of azoles administered for both treatment and prophylaxis. Azole exposure has triggered various resistance mechanisms, including point mutations in drug target genes such as *ERG11*, *FKS*, and *CDR*, as well as overexpression of efflux pumps and drug-modifying enzymes (Lee et al., 2021). These mechanisms reduce the binding affinity of antifungals to their targets and increase efflux of the drug from fungal cells, thereby reducing antifungal efficacy. The result has been a steady erosion of the effectiveness of first-line antifungal agents, particularly the azoles, which remain the cornerstone of candidiasis management in most low-resource settings.

The burden of AFR is severe in low- and middle-income countries, such as Malawi, where diagnostic infrastructure for fungal pathogens is often inadequate or absent. Malawi lacks the diagnostic capacity to undertake routine *Candida* identification and antifungal susceptibility testing (AFST). As a result, antifungal drugs are administered empirically, a strategy that is inefficient for the treatment of high-risk and systemic candidiasis patients and contributes to both overtreatment and undertreatment. Despite the establishment of the Global Antimicrobial Resistance and Use Surveillance System and the National Action Plan on Antimicrobial Resistance, there remains limited antifungal resistance surveillance in Malawi (Malawi NAP, 2022). This has presented a persistent gap in health service delivery for the treatment of fungal infections. The consequences of this gap are multiple and interlinked. First, there is limited infrastructure and diagnostic capacity for fungal pathogens, meaning that even when AFR is suspected clinically, it cannot be confirmed. Second, there is a lack of prevention and control measures specifically targeting antifungal resistance, leaving clinicians without guidance on how to slow the emergence of resistance. Third, there is insufficient education on antifungal resistance among healthcare workers, which limits awareness of the problem and delays appropriate clinical responses. Fourth, there is a lack of policies on antifungal use and consumption, meaning that antifungal prescribing remains largely unregulated and driven by availability rather than by evidence. Together, these factors create an environment in which AFR can emerge and spread unnoticed.

Candida albicans is the most common *Candida* species causing human infection, including vulvovaginal candidiasis (VVC). VVC affects a substantial proportion of women of reproductive age and is a common reason for seeking primary care in Malawi and across sub-Saharan Africa. While VVC is rarely life-

threatening, it causes significant morbidity, reduces quality of life, and is associated with substantial healthcare costs. In settings where diagnostic capacity is limited, VVC is managed empirically, without species-level identification or AFST, further complicating the detection of resistance. Compounding this problem, VVC is frequently recurrent, and repeated exposure to azole antifungals is a recognised driver of resistance development in *C. albicans*.

The evidence base on AFR in *C. albicans* from Malawi is virtually absent. Studies from other sub-Saharan African countries have reported varying resistance profiles, but these cannot be extrapolated directly to Malawi, given differences in antifungal prescribing practices, healthcare infrastructure, and population characteristics. The absence of local data means that Malawian clinicians must rely on either empirical treatment or on guidelines developed for other settings, neither of which is optimal. Generating local evidence on AFR patterns is therefore essential for informing treatment guidelines, optimising antifungal use, and designing targeted surveillance strategies.

This research investigated the mechanisms of antifungal resistance in *C. albicans* isolates from patients with VVC at a referral hospital in Lilongwe, Malawi. The study integrates phenotypic approaches in understanding AFR in Malawi, focusing on resistance patterns across five antifungal agents representing different drug classes. The findings are intended to aid the administration of appropriate and targeted antifungal therapies to reduce morbidity, mortality rates, healthcare costs, and length of hospital stay. Additionally, the findings provide evidence to support the Malawi National Action Plan on AMR in strengthening antifungal resistance surveillance and informing antifungal drug policies to guide clinical decision-making in the treatment of fungal infections.

Methods

Study design

The study was a laboratory-based cross-sectional analytical study and was conducted to estimate the prevalence of *Candida albicans* among patients with clinically suspected vulvovaginal candidiasis and to characterise the phenotypic susceptibility of recovered *C. albicans* isolates to selected antifungal agents. Participants were enrolled once during the study period, and all microbiological procedures were performed under standardised laboratory conditions.

Study setting and population

The study was conducted from January to February 2026 at the Rush Clinic of the Sexually Transmitted Infections Unit, Bwaila District Hospital, Lilongwe, Malawi. Bwaila District Hospital is a referral facility serving Lilongwe District. The study population comprised female outpatients who presented with symptoms clinically compatible with vulvovaginal candidiasis.

Eligibility and recruitment

Patients were eligible if they presented with one or more symptoms suggestive of vulvovaginal candidiasis, including vulvar or vaginal pruritus, burning or irritation; thick, white, curd-like vaginal discharge; vulvar swelling, erythema or soreness; dyspareunia; or dysuria. Participants were also required to have received no antifungal treatment during the 10 days preceding specimen collection, to provide a specimen that met the laboratory acceptance criteria for culture and susceptibility testing, and to provide written informed consent. For participants below the legal age of consent, assent and permission from a parent or legal guardian were required. Patients were excluded if consent or assent was not provided, if they were attending solely for a pre-employment medical examination, or if they had used an antifungal agent within the preceding 10 days.

Sampling and sample-size determination

Eligible patients presenting during the recruitment period were enrolled consecutively. Because recruitment was facility-based and non-probabilistic, prevalence estimates describe the study population and should not be interpreted as population-representative estimates for all women in Lilongwe. The initial sample size for estimating a single population proportion was calculated using Cochran's formula presented in Eq. 1 (Cochran, 1977):

$$n_0 = \frac{Z^2 p(1-p)}{e^2}$$ [equation 1]

where n_0 is the initial sample size, Z is the standard-normal critical value for the selected confidence level, p is the anticipated prevalence, and e is the absolute precision. With Z = 1.96, p = 0.50 and e = 0.05, the initial estimate was n_0 = 384.16, rounded up to 385. The value p = 0.50 was selected because it maximises p (1 − p) and therefore yields the most conservative sample size when a reliable local prevalence estimate is unavailable. Because the number of eligible patients expected during the recruitment period was finite, the estimate was adjusted using the finite-population correction given in Eq. 2:

$$n = \frac{n_0}{1+\frac{n_0-1}{N}}$$ [equation 2]

where N is the projected number of eligible patients during the study period. After applying the finite-population correction and allowing 10% for specimen or isolate loss, the minimum target was approximately 120 participants. A total of 123 participants were recruited. Antifungal susceptibility testing was subsequently restricted to viable *C. albicans* isolates; analyses of these isolates were treated as exploratory, and uncertainty was quantified using confidence intervals and bootstrap resampling.

Specimen collection, transport and storage

One high vaginal swab was collected from each participant by trained clinical personnel using a sterile cotton-tipped swab supplied with semisolid Amies transport medium. Under aseptic conditions, the swab was inserted into the posterior vaginal fornix and rotated gently for approximately 10 seconds. Specimens were transported at 2–8°C and delivered to the laboratory within 2 hours of collection. If immediate processing was not possible, specimens were refrigerated at 2–8°C for no longer than 96 hours; longer-term storage was at − 20 to − 70°C until processing (Yassin et al., 2020).

Isolation and identification of Candida species

All 123 high vaginal swabs were inoculated onto Sabouraud dextrose agar and incubated at 35–37°C for 24–72 hours (Seyoum et al., 2020). Plates showing mixed growth were subcultured onto fresh Sabouraud dextrose agar and incubated for a further 24 hours to obtain pure colonies. Presumptive yeast colonies were examined microscopically after crystal violet staining. Species-level presumptive identification was performed on *CHROMagar Candida* medium (HiMedia Laboratories, India). Pure colonies were inoculated onto the chromogenic medium, incubated at 35–37°C for 72 hours, and identified from the manufacturer-specified colony colour and morphology (Seyoum et al., 2020; Yassin et al., 2020).

Quality assurance

Each batch of culture medium underwent sterility and performance checks before clinical specimens were processed. Reference strains were included to verify the performance of *CHROMagar Candida* and the VITEK 2 system. The quality-control panel comprised *C. albicans* and reference strains of *Nakaseomyces glabratus*, *Candida tropicalis*, *Candida parapsilosis*, *Candidozyma auris* and *Pichia kudriavzevii*. Quality-control results were required to fall within the applicable acceptance ranges before patient-isolate results were released.

Antifungal susceptibility testing

Antifungal susceptibility testing was performed at the National Microbiology Reference Laboratory, Public Health Institute of Malawi, using the VITEK 2 Compact system (bioMérieux, Marcy-l'Étoile, France) and AST-YS08 yeast susceptibility cards in accordance with the manufacturer's instructions (Partha et al., 2022). The panel comprised fluconazole, voriconazole, caspofungin, micafungin, and flucytosine. For each isolate, 3.0 mL of sterile 0.45–0.50% sodium chloride solution (pH 4.5–7.0) was dispensed aseptically into a 12 × 75 mm clear polystyrene tube. Pure colonies were transferred into the saline and mixed to produce a homogeneous suspension. Turbidity was adjusted to 2.0 McFarland (acceptable range, 1.8–2.2) using the VITEK 2 DensiCHEK Plus. The AST-YS08 card was scanned, associated with the corresponding suspension, and loaded into the instrument. Cards were incubated and read automatically over approximately 18 hours. Minimum inhibitory concentrations (MICs) and categorical

interpretations were recorded from the instrument output. Interpretations were based on the study-specified CLSI M27M44S criteria (CLSI, 2022), shown in Table 1.

Table 1
Study-specified MIC interpretive thresholds for Candida albicans

Antifungal agent	Susceptible (≤)	Intermediate/SDD	Resistant (≥)
Fluconazole	8	16–32	64
Voriconazole	1	2	4
Flucytosine	4	8–16	32
Micafungin	0.06	0.12	0.25
Caspofungin	0.25	0.5	1.0

MIC values are expressed in μg/mL; SDD, susceptible dose-dependent.

Of the 35 *C. albicans* isolates recovered, 17 (48.6%) remained viable for susceptibility testing after storage. The other 18 isolates were excluded from the susceptibility analysis because they could not be recovered on subculture.

Data management and statistical analysis

Data were entered in Microsoft Excel 2024, checked for completeness and internal consistency, and analysed in R version 4.3.3 (R Core Team, 2024). Data preparation included harmonisation of categorical variables, date validation, and parsing of MIC results reported with inequality signs. Continuous variables were summarised using the mean and standard deviation or the median and interquartile range, according to their distribution. Categorical variables were summarised as frequencies and percentages. Unless otherwise specified, tests were two-sided, and statistical significance was assessed at α = 0.05.

Interval-censored MIC analysis

MIC measurements were represented as intervals. A result reported as ≤ x was treated as left-censored on (0, x], a result reported as ≥ x as right-censored on [x, ∞), and an unqualified numeric result as point-observed. The primary comparison across antifungal agents used a Weibull accelerated failure-time model for interval-censored outcomes, fitted with the icenReg package (Anderson-Bergman, 2017; Turnbull, 1976):

$$log\,(T_i) = \mu\ + \beta^{\,T} Z_i + \sigma\,\epsilon_{\,i}$$ [equation 3]

where T_i denotes the unobserved true MIC for observation *i*, Z_i is a vector of indicator variables for antifungal agent, fluconazole is the reference category, β is the vector of log-acceleration coefficients, σ is the scale parameter, and ε_i follows the standard extreme-value distribution. For antifungal agent k, the acceleration factor was calculated as:

$$AF_k = exp\left(\beta_k\right)$$ [equation 4]

An acceleration factor below 1 indicates a leftward shift toward lower MICs relative to fluconazole, whereas a value above 1 indicates a shift toward higher MICs. Parameters were estimated by maximum likelihood. The overall contribution of antifungal agent was evaluated by a likelihood-ratio test comparing the full model with an intercept-only model. As a secondary descriptive analysis, numeric MIC values were compared across agents using one-way analysis of variance. Because this analysis does not fully accommodate censoring, it was interpreted as supportive rather than primary. The corresponding effect size was eta-squared:

$$\eta^2 = \frac{SS_{between}}{SS_{total}}$$ [equation 5]

where $SS_{between}$ is the between-agent sum of squares and SS_{total} is the total sum of squares. Agent-specific mean MICs were reported with 95% confidence intervals.

Bootstrap confidence intervals

Percentile bootstrap confidence intervals were used when small-sample or censoring assumptions made closed-form intervals unreliable (Efron & Tibshirani, 1993). Acceleration factors were estimated from 2,000 bootstrap resamples, and drug-specific resistance proportions from 5,000 resamples. For an estimator θ and significance level α, the percentile interval was computed as given in Eq. 6.

$$CI_{1-\alpha} = \left[\theta^*_{\alpha/2},\ \theta^*_{1-\alpha/2}\right]$$ [equation 6]

where θ^* denotes the ordered bootstrap estimates and the subscripts identify the corresponding empirical quantiles.

Pairwise co-resistance analysis

Pairwise co-resistance was examined using 2 × 2 contingency tables of isolate-level resistance classifications. Fisher's exact test was used because of the small cell counts (Fisher, 1935). An odds ratio of 1 indicates no association; values above 1 indicate positive co-occurrence of resistance. To control the false discovery rate across drug-pair comparisons, p-values were adjusted using the Benjamini-Hochberg step-up procedure (Benjamini & Hochberg, 1995):

$$q_{(i)} = min_{j=i,\ldots,m}\left(1,\ \frac{mp_{(j)}}{j}\right)$$ [equation 7]

where p(1) ≤ p(2) ≤ … ≤ p(m) are the ordered p-values and m is the number of pairwise tests. Drug pairs for which either agent had no resistant isolates were not tested because the resulting contingency tables were degenerate.

Sensitivity analyses

Robustness was assessed in three prespecified sensitivity analyses. First, results were compared with and without the ATCC quality-control strain. Second, isolates classified as intermediate were reclassified as non-susceptible to represent a conservative clinical scenario. Third, alternative substitutions were examined for left-censored MIC values in the secondary numeric analysis: x/100 (extreme), x/2 (conventional), and x (conservative), where x was the reported upper bound. Conclusions were considered robust when their direction and substantive interpretation remained unchanged across specifications.

Software and reproducibility

Analyses were performed in R version 4.3.3 (R Core Team, 2024). The tidyverse and readr packages were used for data management and import/export (Wickham et al., 2019, 2023), icenReg for interval-censored modelling (Anderson-Bergman, 2017), boot for bootstrap resampling (Canty & Ripley, 2021), and survival for supporting survival-analysis functions (Therneau, 2023). The analysis workflow included scripts for data cleaning, statistical analysis, table production and figure generation.

Results

Participant characteristics and recruitment

Of 127 assigned study IDs, 123 women were enrolled and analysed, with four IDs (116–119) excluded due to non-enrolment (Fig. 1). The mean age was 28.8 years (SD 8.5), median 27 years (interquartile range [IQR] 17–57), with a range of 17 to 57 years. Four participants (3.3%) did not provide age information.

A total of 75 participants (61.0%) were married, and 44 (35.8%) were single; four participants (3.3%) did not provide marital status. Pregnancy status was available for 120 participants: 11 (8.9%) were pregnant, and 109 (88.6%) were not pregnant; three participants (2.4%) had missing pregnancy data. All 123 participants (100%) presented with vaginal discharge and vulvar itching (Fig. 2). Additional symptoms were less common: pain or soreness was reported by 13/123 (10.6%) and swelling or erythema by 4/123 (3.3%) of participants. One participant did not provide symptom information. One participant (0.8%) reported being on pre-exposure prophylaxis (PrEP) at the time of enrolment; the remaining 121 participants (98.4%) were not on any medication, and one participant (0.8%) had missing medication data.

Culture Outcomes and Species Distribution

Of 123 high vaginal swabs cultured, 59 (48.0%) yielded yeast, 30 (24.4%) yielded bacterial growth (predominantly Gram-positive lactobacilli and cocci), and 34 (27.6%) showed no growth. Among the yeast isolates, four *Candida* species were identified; (i) *Candida albicans*: 35/123 (28.5% of cohort; 59.3% of yeast isolates); (ii) *Candida glabrata* (*Nakaseomyces glabratus*): 15/123 (12.2%; 25.4% of yeast isolates); (iii) *Candida tropicalis*: 8/123 (6.5%; 13.6% of yeast isolates); Mixed infection (*C. glabrata* + *C. tropicalis*): 1/123 (0.8%; 1.7% of yeast isolates) (Fig. 2).

Antifungal Susceptibility Testing Coverage

Of 35 *C. albicans* isolates, 17 (48.6%) were viable for AFST after storage; the remaining 18 (51.4%) were excluded due to viability loss (Fig. 1). All 17 tested isolates were assessed against five antifungal agents (fluconazole, voriconazole, caspofungin, micafungin, and flucytosine) using the VITEK 2 Compact AST-YS08 card. Each drug–isolate combination produced a quantitative MIC value classified per CLSI M27M44S as Sensitive (S), Intermediate (I), or Resistant (R) (Table 2; CLSI, 2022).

Antifungal Resistance Profile

Overall, 7/17 isolates (41.2%) were resistant to at least one antifungal agent. Resistance rates varied markedly by drug (Fig. 3, Table 2):

Table 2
Antifungal Susceptibility Frequencies of Candida albicans Isolates

Drug	Susceptible	Intermediate	Resistant	Resistance (95% CI)
Voriconazole	11/17 (64.7%)	0 (0%)	**7/17 (41.2%)**	17.6–66.7%
Caspofungin	15/17 (82.4%)	0 (0%)	3/17 (17.6%)	0.0–38.5%
Micafungin	15/17 (82.4%)	0 (0%)	3/17 (17.6%)	0.0–38.5%
Fluconazole	11/17 (64.7%)	6/17 (35.3%)	0 (0%)	—
Flucytosine	12/17 (70.6%)	5/17 (29.4%)	0 (0%)	—

Voriconazole exhibited the highest resistance rate (41.2%, 95% CI 17.6–66.7%). Echinocandins (caspofungin and micafungin) showed identical resistance rates of 17.6% (3/17 each). Fluconazole and flucytosine showed no resistant isolates, but both exhibited notable intermediate rates (35.3% and 29.4%, respectively), indicating dose-dependent susceptibility. When intermediate isolates were classified as non-susceptible (worst-case sensitivity analysis), non-susceptibility rates were:

Table 3
Non-susceptibility Frequencies of Candida albicans Isolates

Drug	Non-susceptible (I + R)	95% CI
Voriconazole	7/17 (41.2%)	16.7–66.7%
Fluconazole	6/17 (35.3%)	12.5–60.0%
Flucytosine	5/17 (29.4%)	7.7–52.9%
Caspofungin	3/17 (17.6%)	0.0–38.5%
Micafungin	3/17 (17.6%)	0.0–37.5%

MIC Distributions

MIC distributions varied markedly across drugs (Fig. 4). Summary statistics are presented in Table 3:

Table 4
MIC Distributions of Candida albicans Isolates

Drug	Median MIC (μg/mL)	IQR (μg/mL)	Range (μg/mL)	Censoring pattern
Fluconazole	1	1–16	≤ 0.05 to 16	3 left, 14 exact
Voriconazole	1	0.06–4	≤ 0.12 to ≥ 8	8 left, 8 exact, 1 right
Caspofungin	0.06	0.06–0.06	≤ 0.12 to ≥ 8	14 left, 3 right
Micafungin	0.03	0.03–0.03	≤ 0.06 to ≥ 8	14 left, 1 exact, 2 right
Flucytosine	2	0.5–8	≤ 1 to 16	4 left, 13 exact

Echinocandins (caspofungin and micafungin) exhibited the lowest MICs, with the majority of isolates clustering at or below the detection limit (14/17 at ≤ 0.12 μg/mL for caspofungin and ≤ 0.06 μg/mL for micafungin). Fluconazole displayed the widest distribution (right-skewed, ranging from ≤ 0.05 to 16 μg/mL), suggesting a subpopulation with elevated MICs. Voriconazole exhibited a bimodal distribution with a distinct high-MIC cluster (n = 7 at ≥ 4 μg/mL, all classified as resistant), consistent with the categorical resistance profile. Flucytosine demonstrated intermediate variability.

Interval-Censored Weibull Model

An interval-censored Weibull AFT model was fitted to all 85 MIC observations (17 isolates × 5 drugs), appropriately handling the left- and right-censored values. Drug identity was a significant predictor of MIC distribution (likelihood-ratio test, $\chi^2 = 62.31$, df = 4, $p < 0.001$).

Table 5
Acceleration factors (AFs) relative to fluconazole (reference)

Drug	AF (bootstrap point estimate)	95% CI (bootstrap)	Interpretation
Fluconazole	1.00 (reference)	—	—
Voriconazole	0.517	0.022–3.812	48% lower MIC
Caspofungin	0.091	0.0001–5.281	91% lower MIC
Micafungin	0.039	0.0000–1.348	96% lower MIC
Flucytosine	1.473	0.119–5.999	47% higher MIC

The echinocandins (caspofungin, micafungin) shifted the MIC distribution most dramatically downward (AFs of 0.091 and 0.039, respectively), confirming their potent in vitro activity. Voriconazole also shifted MIC downward (AF = 0.517) despite having the highest categorical resistance rate, this reflects the bimodal MIC distribution where 10 isolates had very low MICs while 7 had MICs above the CLSI breakpoint. Flucytosine shifted the MIC distribution upward (AF = 1.473), consistent with its intermediate

activity profile. Bootstrap CIs were wide due to the small sample size (n = 17), but the point estimates clearly separated echinocandins from the azoles and flucytosine.

Per-Isolate Susceptibility Patterns and Multi-Drug Resistance

Per-isolate susceptibility profiles revealed substantial heterogeneity across isolates (Fig. 5). Isolates were ordered by resistance burden (most resistant at top) in the dot plot visualisation. 6 isolates (35.3%) were pan-susceptible to all five drugs. 4 isolates (23.5%) were resistant to voriconazole only (S11, S17, S18, S56). 2 isolates (11.8%) displayed multi-drug resistance (MDR) involving voriconazole, caspofungin, and micafungin (S20, S57). 1 additional isolate (S59) was intermediate for fluconazole and flucytosine, and resistant to voriconazole, caspofungin, and micafungin. 3 isolates demonstrated isolated intermediate phenotypes (S4, S45, S46). The two MDR isolates (S20, S57) were characterised by concurrent resistance to three drug classes: second-generation azole (voriconazole), and both echinocandins (caspofungin, micafungin). Neither showed resistance to fluconazole or flucytosine, but both showed intermediate susceptibility to those agents.

Co-resistance analysis

Pairwise co-resistance was assessed using Fisher's exact tests with Benjamini-Hochberg correction for multiple comparisons (Table 6). Only drug pairs with at least one resistant isolate in both drugs could be tested; fluconazole and flucytosine produced no resistant isolates and were excluded from pairwise testing.

Table 6
Co-resistance analysis of Antifungal Drugs

Drug Pair	Odds Ratio	95% CI	p-value	Adjusted p (BH)
Caspofungin × Micafungin	∞	3.674 – ∞	**0.0015**	**0.0045**
Voriconazole × Caspofungin	∞	0.682 – ∞	0.0515	0.0515
Voriconazole × Micafungin	∞	0.682 – ∞	0.0515	0.0515

Caspofungin–micafungin co-resistance was highly statistically significant (p = 0.0015; adjusted p = 0.0045), demonstrating complete cross-resistance within the echinocandin class in this cohort. All three isolates resistant to caspofungin were also resistant to micafungin, and vice versa (odds ratio = ∞). Voriconazole showed borderline significant co-resistance with each echinocandin (p = 0.0515 for each pair), reflecting the MDR triad observed in the two MDR isolates. The co-resistance network visualisation (Fig. 6) illustrates these relationships, with caspofungin and micafungin forming the tightest cluster, and voriconazole linked to both. Fluconazole and flucytosine appear as isolated nodes (no resistance events), reflecting their favourable resistance profiles in this cohort despite elevated intermediate rates.

Sensitivity Analyses

Three sensitivity analyses confirmed the robustness of our findings (Table 6). The first is ATCC control inclusion. When the *C. albicans* ATCC control strain was included in the analysis, resistance proportions changed by less than 2 percentage points for all drugs, confirming that the control behaved as expected and did not bias results. Second was the worst-case classification. When intermediate isolates were reclassified as non-susceptible, non-susceptibility rates rose to 41.2% for voriconazole, 35.3% for fluconazole, and 29.4% for flucytosine, while echinocandins remained at 17.6%. This did not alter the overall conclusion that voriconazole was the least effective agent. Last sensistivity analysis was censoring assumptions. For fluconazole, voriconazole, and flucytosine, median MIC values were identical under extreme, standard, and conservative censoring treatments (median = 1, 1, and 2 µg/mL, respectively). For echinocandins, medians remained at or below detection limits regardless of assumption (0.0006–0.06 µg/mL for caspofungin; 0.0003–0.03 µg/mL for micafungin), confirming robust in vitro potency.

Discussion

This study found a high prevalence of *Candida albicans* among the VVC patients as compared to the non-albicans species recovered, *Candida glabrata* (*Nakaseomyces glabratus*) and *Candida tropicalis*. As *Candida* is a normal flora and therefore an opportunistic pathogen, the virulence factors that enable its ability to cause infection include germ-tube formation, adhesion, phenotypic switching, biofilm formation, and production of hydrolytic enzymes. A majority of candidiasis infections have been reported as caused by biofilm formation, which is the formation of a three-dimensional structure on biotic and antibiotic surfaces such as mucosal surfaces caused by the embedding of the fungus in an extracellular matrix. Among the *Candida* species, *C.albicans* is the most virulent *Candida* species due to its distinct ability to switch morphology, and it can exist as yeast, pseudohyphal, and hyphal forms influenced by its environment. Furthermore, it also forms a biofilm which enhances drug resistance and the ability to adhere to surfaces in the hospital also causing nosocomial infections (Pristov & Ghannoum, 2019). The findings are supported by a study conducted in Ethiopia, which found the highest prevalence in *C.albicans* of 49.8% (104/209) in candidiasis clinical samples (Seyoum et al., 2020). These findings call for monitoring of *C. albicans* in hospitals to assess threats of development of antifungal resistance and effectively mitigate AMR.

The highest resistance was observed against voriconazole, which is from the azole group of antifungals. Resistance in *Candida albicans* is explained to be either clinical or mycological. Clinical resistance occurs when an antifungal drug with in vitro activity against the fungus fails to eradicate the fungal infection. In contrast, mycological resistance is when the fungus is able to grow in the presence of antifungal drugs that would otherwise kill or limit its growth in vitro (Pristov & Ghannoum, 2019). Particularly, for *Candida albicans*, the resistance observed in this study may be explained using three main mechanisms, and this includes, multi-drug pumps in the fungal cell wall that are the result of up-regulation genes through point mutations in CDR1/CDR2, and MDR1; as well as transcription factors,

TAC1 and MDR1 encoding for efflux pumps. The second suggestive mechanism of resistance is the alteration or up-regulation for genes encoding target enzymes, ERG11. This alters the binding site of the enzymes and prevents binding of the antifungal drug. The last suggestive mechanism is the bypass pathway development caused by mutations and it blocks the alteration of the cell membrane and accumulation of drugs (Ford et al., 2015).

Azoles are the largest group of antifungals used to treat candidiasis as compared to the other groups, polyenes, echinocandins, and fluoropyrimidines. Azoles inhibit 14-alpha-sterol demethylase, which is encoded by the ERG11 gene, an enzyme responsible for the biosynthesis of the sterol ergosterol, a fungal-specific membrane component (Whaley et al., 2017). AFR patterns differ geographically and require localised surveys to localise antifungal therapy; however, there has been slow advancement in routine antifungal susceptibility testing, which has led to scanty data availability in other regions (Subira, 2019). The most common mechanism of azole resistance in *C.albicans* recorded is increasing the number of efflux pumps in the cell. Efflux pumps are membrane transporters that function by reducing the intracellular accumulation of drugs, avoiding toxic levels that can kill the fungal cell. Thereby, the overexpression may lead to cross-resistance in vitro and clinically. A systematic review and meta-analysis conducted also showed the highest resistance in azole antifungals. The mechanisms suggested were the ability of the microorganism to survive environmental stresses, including thermal, oxidative, osmotic, changes to pH, and nutrient limitations (Kessler et al., 2022). Azoles have been the commonly administered drugs to treat candidiasis for many years. However, the emergence of resistance, as displayed in this study and several studies, has posed a threat to patient management, and this has increased patient morbidity and mortality, especially in immunocompromised individuals. In this study, fluconazole displayed the highest dose-dependent effects, which is a threat to public health and displays the emergence of resistance. Fluconazole is the most commonly used azole drug and the primary therapeutic option for treatment of candidiasis. It belongs to the triazole antifungal group, and it is fungistatic rather than fungicidal; as such, there is a higher risk of development of acquired resistance in the presence of the antifungal. The USA has reported incidences of resistance 0.5–2% in contrast to this study which only found intermediate effects (Berkow & Lockhart, 2017). Other studies in Africa have also reported incidences of resistance to fluconazole, such as in Ethiopia and Kenya, which reported 10% and 9.09%, respectively (Seyoum et al., 2020; Subira, 2019). Voriconazole and fluconazole belong to the same antifungal group, the azoles; however, only voriconazole displayed resistance. This may be attributed to different generations of these drugs as voriconazole is in the second generation and fluconazole is in the first generation. Voriconazole has a lower binding affinity as compared to fluconazole, and this may explain the observed difference in susceptibility between these two drugs (Partha et al., 2022). These results contrast with the Malawi Standard Treatment Guidelines, which recommend voriconazole as a drug for candidiasis (Malawi Standard Treatment Guidelines). This may be explained by the cross-resistance nature of *Candida albicans*. The wide use of azoles in the environment for agriculture, as fungicides, such as tebuconazole, and epoxiconazole, has led to the development of resistance mechanisms in *Candida albicans* species affecting the effectiveness of azoles administered to humans. Therefore, this highlights the need to conduct one health antifungal

resistance surveillance in order to mitigate cross-resistance. Furthermore, the problem is perpetuated due to limited financial capacity (Wesangula et al., 2025). Malawi procures cost-effective drugs on the market, and the commonly administered drugs in government hospitals are the imidazole group of antifungals, encompassing drugs such as Clotrimazole, which are mainly supplied in government hospitals. Therefore, there is limited choice of antifungal drugs for patients.

Echinocandins displayed lower resistance as compared to azoles. Over the years, there has been a shift from the use of azoles to the use of echinocandins due to the development of resistance to azoles. Echinocandins are lipopeptide antifungals and are secondary metabolites of fungi which inhibit the biosynthesis of D-glucan, and they can be fungistatic or fungicidal. They work through inhibiting the synthesis of D-glucan, which is a critical cell wall polysaccharide and also a target for broad-spectrum antifungal drugs. The genes responsible for encoding D-glucan synthase are FKS1 and FKS2 (Kessler et al., 2022). Echinocandin resistance is rare (Mroczyńska & Brillowska-Dąbrowska, 2020). However, resistance was detected in this study to micafungin and caspofungin. This may be explained by mutations in the encoding genes FKS1 and FKS2, which alter the drug target and result in resistance (Lee et al., 2021). The detected resistance is therefore a threat to patients with invasive fungal infections, and this calls for the need to conduct surveillance of antifungal resistance to reduce patient morbidity and mortality, as well as healthcare costs from prolonged hospital stays.

Flucytosine displayed dose-dependent susceptibility in this study. Flucytosine belongs to the group of fluoropyrimidines and it is an overlooked drug as well as has limited availability and used in combination with Amphotericin B for invasive candidiasis. It has good solubility in *Candida* biofilms, which makes it a good drug. The intermediate results displayed in this study reflect that it is not effective for use as monotherapy and requires combination use as supported by other studies which indicated Amphotericin B as the combination drug of choice (Sigera & Denning, 2023).

Two isolates displayed multi-drug resistance to voriconazole, caspofungin, and micafungin, and intermediate effects to Fluconazole and Flucytosine. This reflects drug failure in these patients and the importance of conducting AFST routinely. This may be explained by a study in 2018 in Iran that indicated that the increased use of azole antifungal agents has led to the emergence of resistant isolates, and mechanisms suggested include mutations in the encoding gene ERG11, which have reduced the affinity between the protein and azole (Sardari et al., 2019). However, the present study did not test for the Imidazole group of azoles, in contrast to the study conducted in Kenya, which tested clotrimazole. This was due to the Vitek 2 Compact drug spectrum, which targeted the Triazole group of Azoles, Polyenes, Fluoropyrimidines, and Echinocandins (Miruka et al., 2019) (Dovo et al., 2022).

Conclusions

This study demonstrated significant differences in antifungal susceptibility among *C. albicans* isolates, with the drugs of choice being fluconazole and flucytosine, which exhibited dose-dependent susceptibility. Echinocandins and voriconazole showed less reliable effectiveness due to the resistance

observed to these agents. These findings raise a need for action regarding the preservation of fluconazole from developing resistance due to its overuse for treatment and prophylaxis. Furthermore, there is a need for routine antifungal susceptibility testing and consideration of alternative agents in clinical management. Ongoing surveillance is essential to monitor emerging resistance patterns and guide treatment strategies.

Abbreviations

AFR
Antifungal Resistance
AFST
Antifungal Susceptibility Testing
AMR
Antimicrobial Resistance
AMRCC
Antimicrobial Resistance Coordinating Centre
MDR
Multidrug Resistance
MIC
Minimum Inhibitory Concentration
NMRL
National Microbiology Reference Laboratory
PHIM
Public Health Institute of Malawi
VVC
Vulvovaginal Candidiasis

Declarations

Ethical approval and consent to participate

Ethical approval was obtained from the Malawi University of Science and Technology Research Committee and the Bwaila District Hospital Research Committee. Institutional approval was provided by the Public Health Institute of Malawi and the National Public Health Reference Laboratories. Written informed consent was obtained from all participants, and participants below 18 years of age provided assent with parental or guardian consent. All samples were anonymised, and data were encrypted with restricted access to authorised personnel only.

Competing interests

The authors declare that they have no competing interests.

Funding

The authors declare that this research did not receive funding.

Author Contribution

DK was a major contributor to writing the manuscript and performed all the laboratory experiments and produced the data sets used for this study including the culture experiments and antifungal susceptibility tests and also analyzed the antifungal susceptibility data frequencies and distribution of minimum inhibitory concentrations and prepared tables 1-6.WM prepared figures 1-6, and conducted the statistical analysis of the bootstrap confidence intervals and pairwise co-resistance analysis. CM and PC contributed to editing the manuscript, including citation and referencing formatting as well as the methodology. All authors read and approved the final manuscript.

Acknowledgement

We thank the Antimicrobial Resistance Coordinating Centre (AMRCC) for the support and resources that enabled this study. In a special way, we thank all the nurses from Bwaila District Hospital who collected the high vaginal swabs and ensured quality data was obtained. Furthermore, we thank Ronald Chaduka, a laboratory technologist at the National Microbiology Reference Laboratory, who verified the machines for the experiments. We acknowledge Harry Milala and Lusayo Mwakilama for the mobilisation of nurses who collected the samples in this study. We thank Moses Chitenje for the installation of the data analysis software.

Data Availability

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

References

1. Berkow E, Lockhart S. Fluconazole resistance in Candida species: A current perspective. Infect Drug Resist. 2017;10:237–45. https://doi.org/10.2147/IDR.S118892.
2. Dovo EE, Zohoncon TM, Tovo SF, Soubeiga ST, Kiendrebeogo IT, Yonli AT, Ouedraogo RA, Dabire AM, Djigma FW, Nadembega CW, Belemgnegre M, Ouedraogo P, Obiri-Yeboah D, Simpore J. First detection of mutated ERG11 gene in vulvovaginal Candida albicans isolates at Ouagadougou/Burkina Faso. BMC Infect Dis. 2022;22(1). https://doi.org/10.1186/s12879-022-07619-5.

3. Ford CB, Funt JM, Abbey D, Issi L, Guiducci C, Martinez DA, Delorey T, Li BY, White TC, Cuomo C, Rao RP, Berman J, Thompson DA, Regev A. The evolution of drug resistance in clinical isolates of Candida albicans. eLife. 2015;4:e00662. https://doi.org/10.7554/eLife.00662.
4. Kessler SQS, Lang PM, Dal-Pizzol TS, Montagner F. Resistance profiles to antifungal agents in Candida albicans isolated from human oral cavities: Systematic review and meta-analysis. Clin Oral Invest. 2022;26(11):6479–89. https://doi.org/10.1007/s00784-022-04716-2.
5. Lee Y, Puumala E, Robbins N, Cowen LE. Antifungal Drug Resistance: Molecular Mechanisms in *Candida albicans* and Beyond. Chem Rev. 2021;121(6):3390–411. https://doi.org/10.1021/acs.chemrev.0c00199.
6. Miruka H, Omwenga EO, Musyoki S, Awuor SO. (n.d.). *Incidence, antifungal resistance properties, and virulence traits of candida species isolated from HIV/AIDS patients from county hospitals in Kenya*.
7. Mroczyńska M, Brillowska-Dąbrowska A. Review on Current Status of Echinocandins Use. Antibiotics. 2020;9(5):227. https://doi.org/10.3390/antibiotics9050227.
8. Partha ADSL, Widodo ADW, Endraswari PD. (2022). Evaluation of fluconazole, itraconazole, and voriconazole activity on Candida albicans: A case control study. *Annals of Medicine & Surgery*, *84*. https://doi.org/10.1016/j.amsu.2022.104882
9. Pristov KE, Ghannoum MA. Resistance of Candida to azoles and echinocandins worldwide. Clin Microbiol Infect. 2019;25(7):792–8. https://doi.org/10.1016/j.cmi.2019.03.028.
10. Sardari A, Zarrinfar H, Mohammadi R. Detection of ERG11 point mutations in Iranian fluconazoleresistant Candida albicans isolates. Curr Med Mycol. 2019. https://doi.org/10.18502/cmm.5.1.531.
11. Seyoum E, Bitew A, Mihret A. Distribution of Candida albicans and non-albicans Candida species isolated in different clinical samples and their in vitro antifungal suscetibity profile in Ethiopia. BMC Infect Dis. 2020;20(1):231. https://doi.org/10.1186/s12879-020-4883-5.
12. Sigera LSM, Denning DW. Flucytosine and its clinical usage. Therapeutic Adv Infect Disease. 2023;10:20499361231161387. https://doi.org/10.1177/20499361231161387.
13. Subira A. (n.d.). *PREVALENCE, SPECIES DISTRIBUTION, ANTIFUNGAL SUSCEPTIBILITY PROFILES OF CANDIDA AND RISK FACTORS ASSOCIATED WITH ISOLATION OF CANDIDA IN PATIENT SAMPLES SUBMITTED FOR ANALYSIS AT THE MOMBASA HOSPITAL*.
14. Wesangula E, Chizimu JY, Mapunjo S, Mudenda S, Seni J, Mitambo C, Yamba K, Gashegu M, Nhantumbo A, Francis E, Moremi N, Athiany H, Matu M. (2025). *A Regional Approach to Strengthening Implementation of Sustainable Antimicrobial Stewardship Programs in Five countries in East, Central, and Southern Africa*. Public Health and Healthcare. https://doi.org/10.20944/preprints202502.0295.v1
15. Whaley SG, Berkow EL, Rybak JM, Nishimoto AT, Barker KS, Rogers PD. (2017). Azole Antifungal Resistance in Candida albicans and Emerging Non-albicans Candida Species. *Frontiers in Microbiology*, *7*. https://doi.org/10.3389/fmicb.2016.02173

Figures

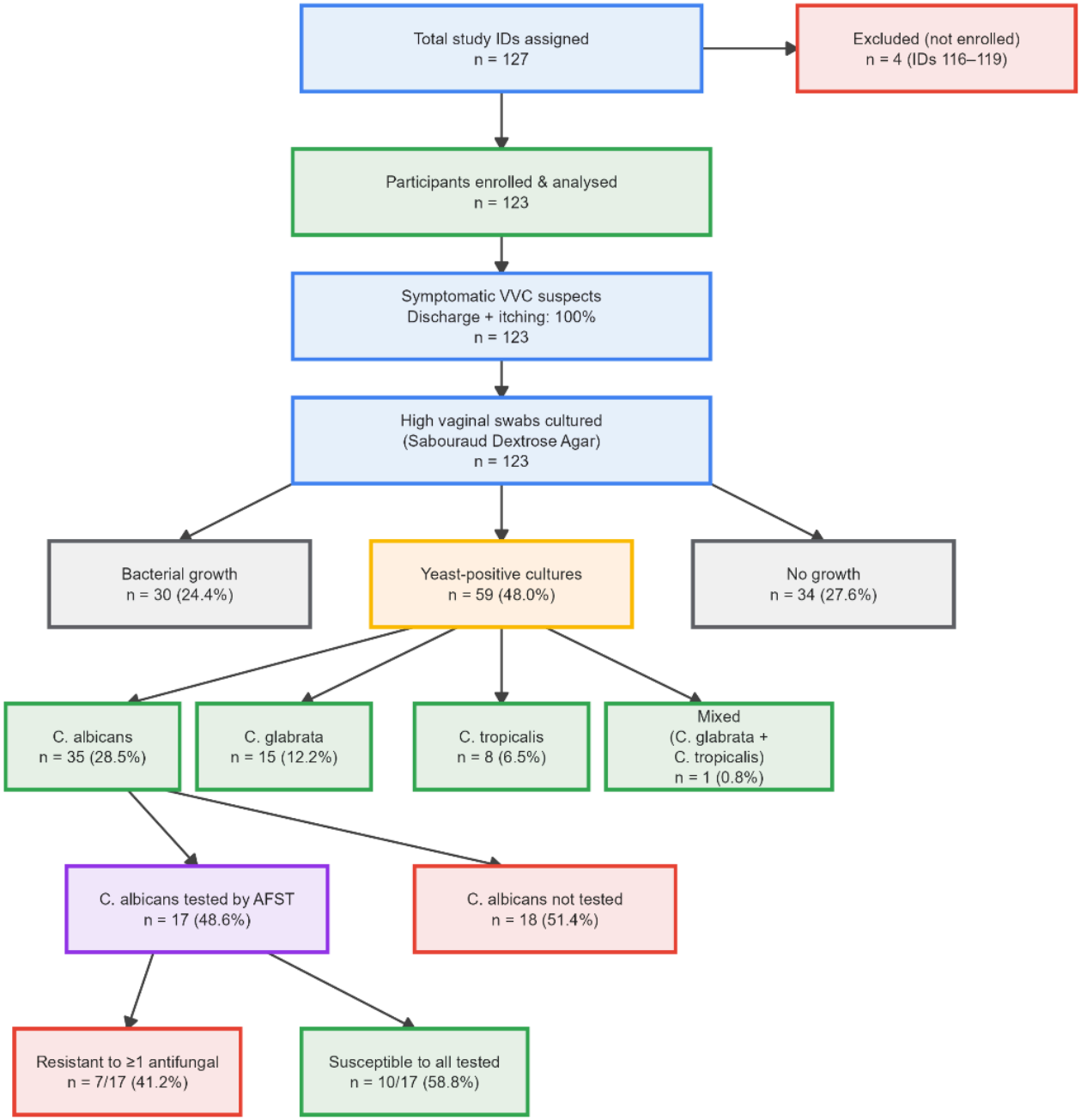


Figure 1

Study cohort flow diagram showing recruitment, culture outcomes, species distribution, and AFST coverage of 123 VVC suspects at Bwaila District Hospital, Malawi.

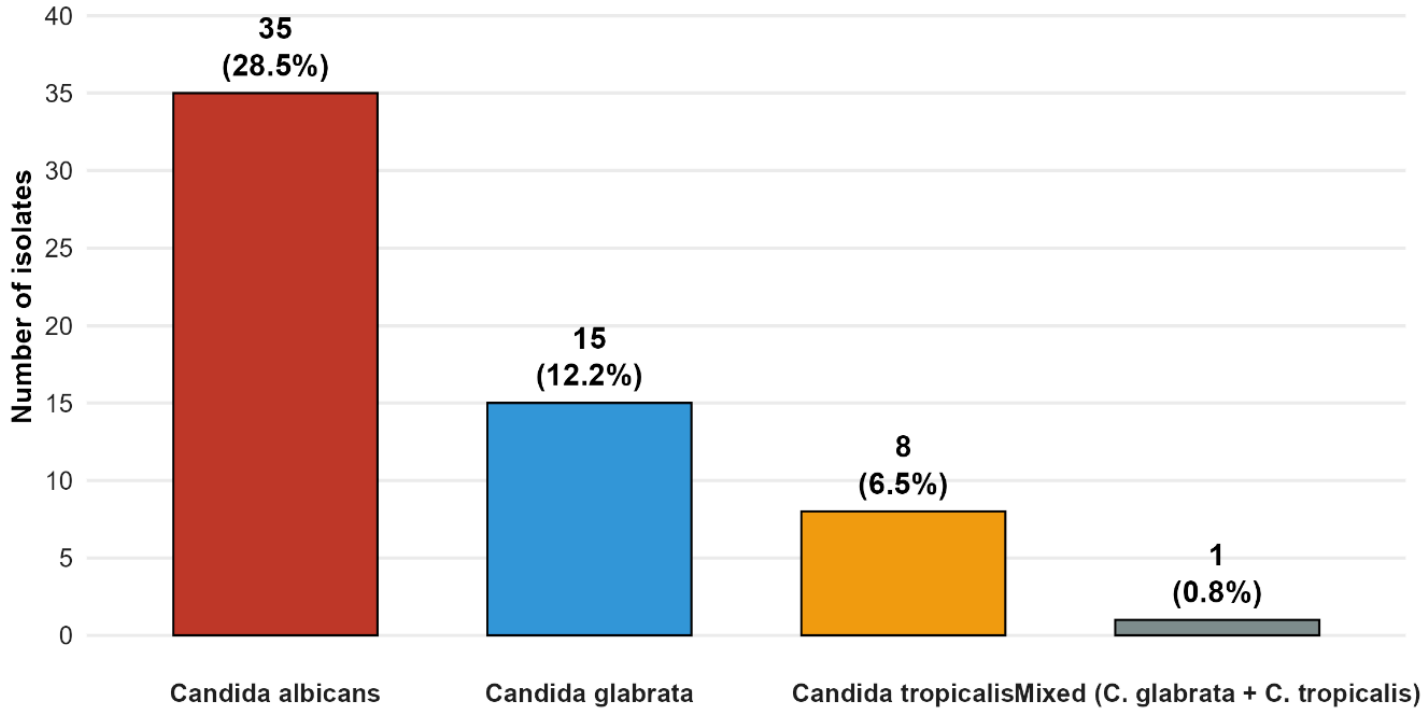


Figure 2

Distribution of *Candida*species isolated from VVC patients (n = 123). Percentages are relative to the total cohort.

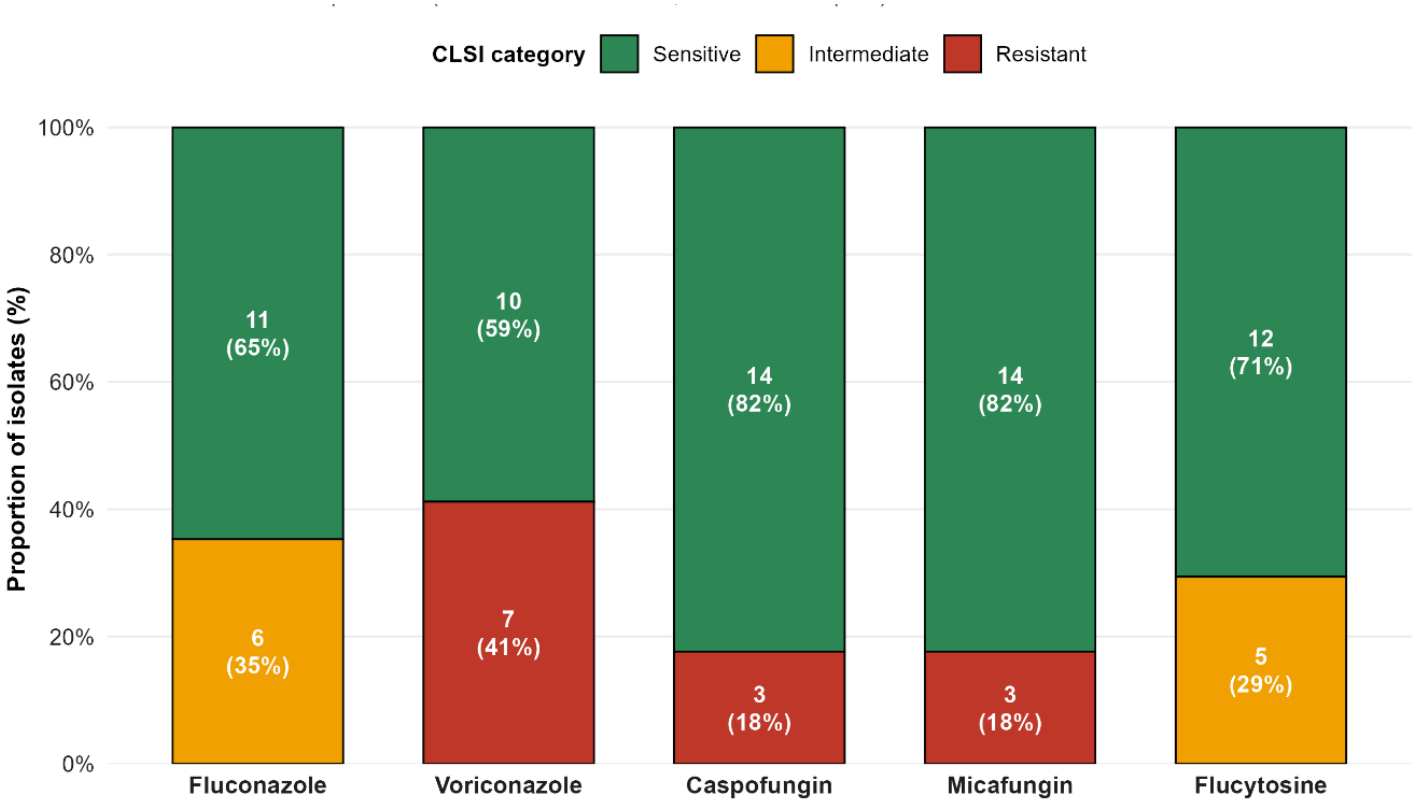


Figure 3

Antifungal susceptibility profile of *Candida albicans* isolates (n = 17) by CLSI M27M44S category. Percentages shown within each drug bar. Resistance was highest for voriconazole (41.2%); echinocandins (caspofungin and micafungin) showed 17.6% resistance; fluconazole and flucytosine showed no resistance but elevated intermediate rates (35.3% and 29.4%, respectively).

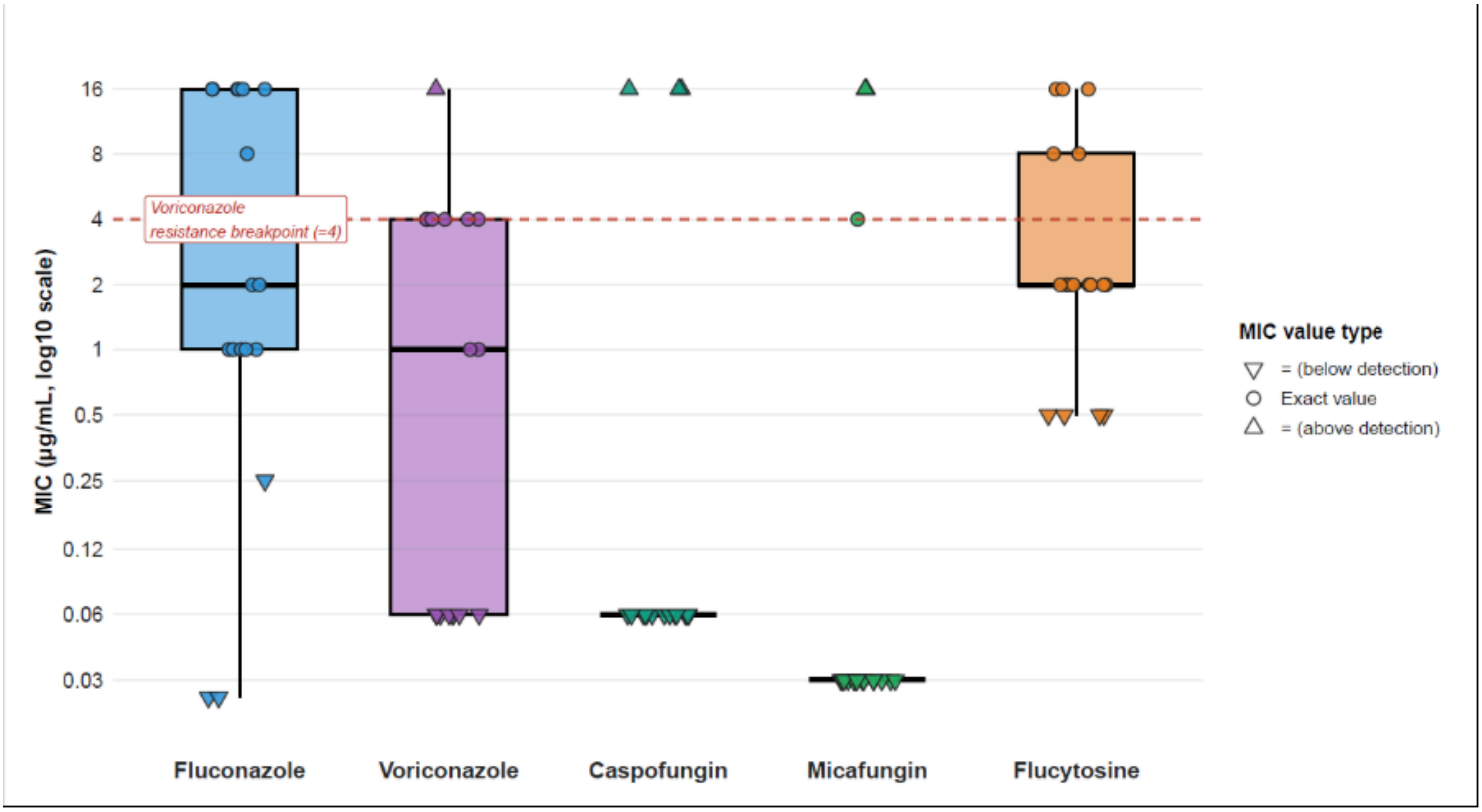


Figure 4

Distribution of minimum inhibitory concentrations across five antifungal agents (n = 17 *C. albicans*isolates). Boxes show median and interquartile range; individual points are jittered to avoid overlap. Point shape indicates MIC censoring. The dashed red line marks the voriconazole resistance breakpoint (≥4 µg/mL). Echinocandins cluster at the lowest detectable MICs; fluconazole shows the widest distribution.

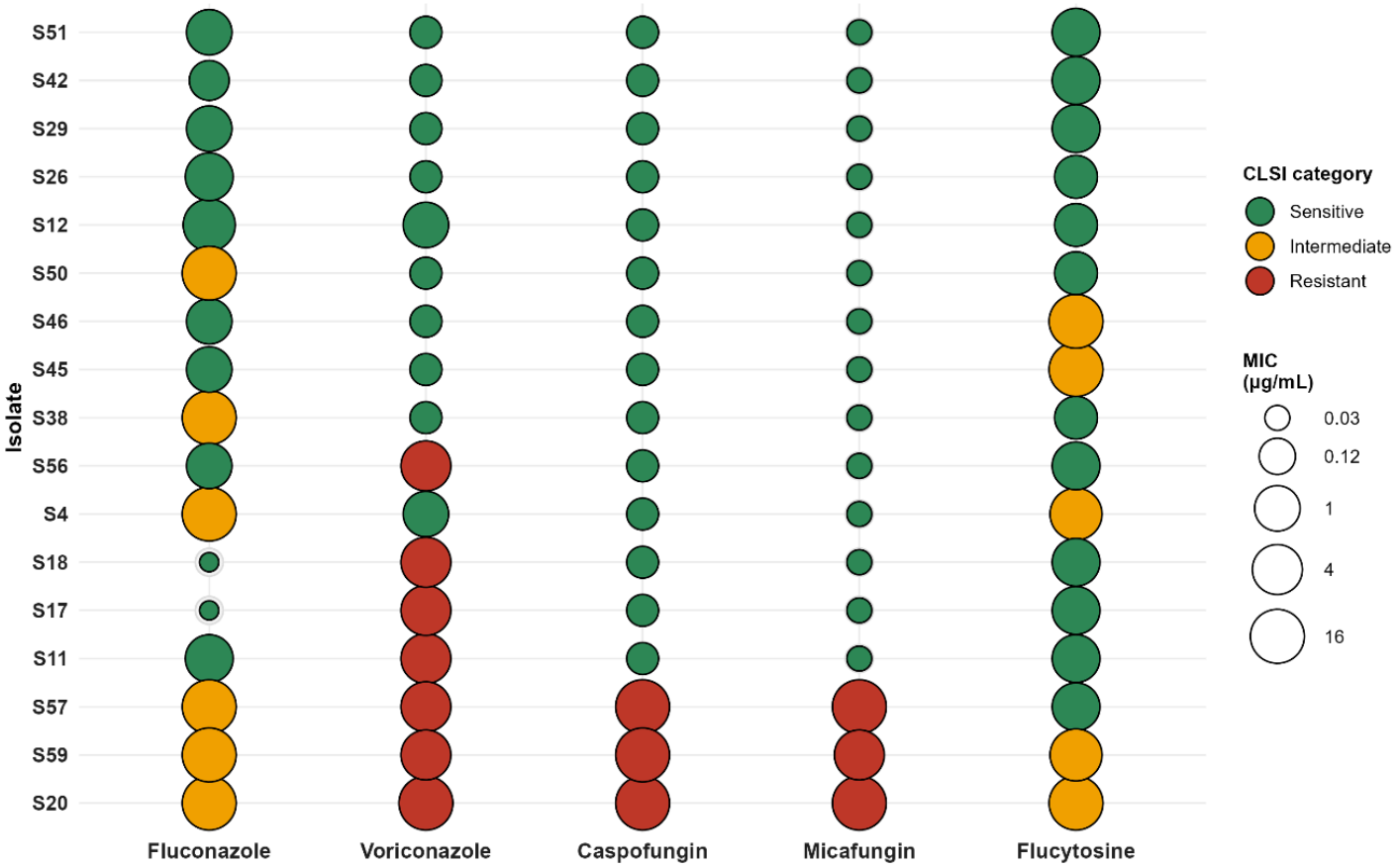


Figure 5

Per-isolate antifungal susceptibility profiles of *C. albicans* (n = 17). Dot size reflects MIC magnitude (log scale); colour reflects CLSI interpretation (green = Sensitive, amber = Intermediate, red = Resistant). Isolates are ordered by resistance burden, with the most resistant isolate at top. Two MDR isolates (S20, S57) are resistant to voriconazole, caspofungin, and micafungin.

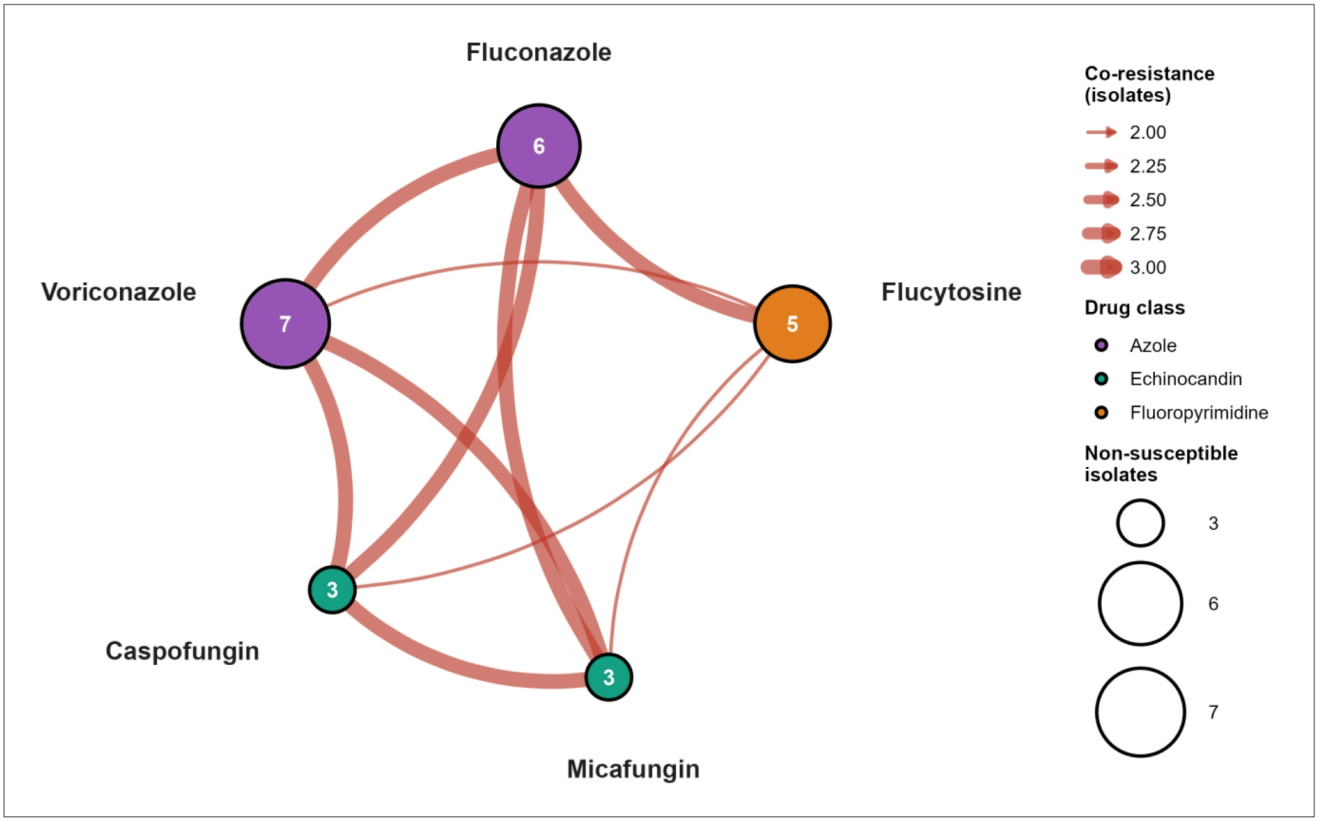


Figure 6

Co-resistance network of antifungal agents. Nodes represent antifungal drugs; node size reflects the number of non-susceptible isolates; node colour indicates drug class (purple = azole, teal = echinocandin, orange = fluoropyrimidine); edge thickness reflects the number of isolates co-resistant to both drugs. Voriconazole, caspofungin, and micafungin form a tight co-resistance cluster (MDR).