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Symptomatic vulvovaginal candidiasis and antifungal resistance in HIV-1 positive women at Mbarara city health centre IV

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Vulvovaginal candidiasis (VVC) represents a universal health hazard that contributes to significant morbidity in HIV-positive women. Antifungal resistance is a worldwide increasing health problem that reduces treatment options while increasing treatment costs. The purpose of this study was to determine the prevalence of VVC, identify associated risk factors, characterize the distribution of *Candida* species, and assess their antifungal susceptibility patterns among HIV-positive women attending Mbarara City Health Centre IV. A laboratory-based, cross-sectional study design was conducted on 146 high vaginal swabs collected from HIV- positive women aged 18 years and above attending routine HIV care that presented with signs and symptoms of vulvovaginal candidiasis. These were subjected to microscopy and culture on Sabouraud Dextrose Agar. *Candida* isolates were identified by gram stain, germ tube, CHROM agar™, and Analytical profile index (API® *Candida*) tests. Susceptibility to fluconazole, clotrimazole, voriconazole, amphotericin B, miconazole, and nystatin by the disc diffusion method on Mueller Hinton agar supplemented with 2%w/v glucose and 0.5 µg/ml methylene blue dye. Out of the 146 high vaginal swabs collected, 71(49%) were colonized with *Candida* species with 43(62%) being *Candida albicans* while 26 (37.6%) were non-*Candida albicans*. *Candida* species were susceptible to amphotericin B (68/71, 95.7%) and resistant to fluconazole and clotrimazole (33/71, 45%). Elevated blood glucose levels were significantly associated with vulvovaginal candidiasis ($p = 0.018$; odds ratio = 2.93). *Candida albicans* is the leading cause of VVC, with a higher prevalence than non-*Candida albicans*. It also demonstrates that amphotericin B and nystatin are the most effective antifungal medications. Furthermore, diabetes is associated with VVC compared to other studied factors. We recommend the use of nystatin for the management of vulvovaginal candidiasis among HIV adult women.

Keywords Antifungal susceptibility, Human immunodeficiency virus, Vulvovaginal candidiasis

Vulvovaginal candidiasis (VVC) is a symptomatic vaginitis caused by infection with *Candida* yeast[1]. It is the most prevalent and common fungal infection affecting women worldwide; it is estimated to afflict approximately 75% of all women at least once in their lifetime[2]. Nearly 8% of women globally suffer from recurrent VVC[3] yet cases of reoccurrences are sporadic and have been reported in HIV-positive women[4].

Vulvovaginal candidiasis presentation among women can occur in various forms irrespective of their HIV serostatus[2]. It usually first presents in a mild form as a thick white discharge, itching, burning, irritation, pain during sex, pain, or discomfort when urinating and can go on to a severe form including redness, swelling, and cracks in the wall of the vagina[5]. However, HIV-positive women are more susceptible to vulvovaginal candidiasis than HIV-negative women due to immunosuppression associated with HIV infection[4].

Recurrent VVC is linked to pain, loss of self-esteem, reduced work performance, discomfort, interference with sexual activity, mental anguish, and significant direct and indirect financial burdens[6]. About 138 million women worldwide suffer from recurrent vvc each year. The economic impact of decreased productivity due to vvc in high-income nations could reach around US$14.39 billion annually by 2030. The financial effect in low- to middle-income countries is projected to be twice as high as that in high-income countries such as the United States of America[3].

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The common antifungal regimens employed in the Ugandan setting include the azoles like fluconazole and itraconazole. Although there are many effective antifungal drugs both topical and oral that are used for treating vulvovaginal candidiasis, reduced susceptibility of some vulvovaginal candida isolates to some antifungal agents has been reported in some countries in the world involving yeasts isolated from high vaginal swabs[7–9].

Understanding the prevalence of vulvovaginal candidiasis, associated factors, and antifungal susceptibility patterns of Candida species in HIV-positive women is key in guiding appropriate antifungal therapy selection.

Methods

Study area

This study employed a cross-sectional design that included HIV-positive women aged 18 years and above who received regular HIV care at the Mbarara City Health Centre IV and consented to participate in the study.

Ethical consideration

Ethical approval was granted by the Research Ethics Committee of Mbarara University of Science and Technology (MUST-2023-1284), and administrative clearance was obtained from the Mbarara City Health Officer. Prior to enrollment, written informed consent was obtained from all participants. Confidentiality and privacy were strictly maintained in accordance with standard clinical protocols. Participants retained the right to withdraw at any time without compromising their medical care. Study supervisors ensured compliance with ethical guidelines, safeguarding patient rights and dignity throughout all procedures.

Sample size

According to Mbarara City Council HCIV's Open Medical Records System (OpenMRS), which was used to determine the sample size of participants, 10 HIV-positive women on average report VVC signs and symptoms each week (MCC Weekly Reports, 2023). Therefore, the sample size to a finite population using the formula $n_1 = N \times n/ (n + N\text{-}1)$ where N is the finite population and n_1 is the corrected sampled size. We adjusted for a 10% attrition rate (131/0.1). Therefore, we enrolled 144 participants in this study.

Data collection

Quantitative data were collected through structured questionnaires and medical record reviews. A systematic questionnaire conducted by an interviewer was utilized to obtain data about the participants' socio-demographics of participants and the variables associated with vulvovaginal candidiasis.

Sample collection, culture and identification

Following a clinical examination by the midwife, vulvovaginal samples were collected with the patient in lithotomy position. The vulva was swabbed front-to-back using sterile saline-soaked gauze swabs. A sterile cotton swab was then inserted 20–30 mm into the vaginal canal and rotated gently for sample collection. Two vaginal swabs were taken; one for gram staining and the other for culture and sensitivity testing.

The labelled samples were transported to the Microbiology laboratory of Mbarara university of science and technology immediately after collection. In the laboratory, the swabs were inoculated on Sabouraud Dextrose Agar (SDA) at 37 °C for 48 h[10]. A wet mount preparation using saline and gram staining was performed to examine microscopically for budding yeast cells. Species identification was achieved through germ tube test, using Candida API strips, and subculturing on CHROMagar[10,11].

Antifungal susceptibility testing was performed using the disc diffusion method on Mueller Hinton Agar supplemented with 2% glucose and methylene blue[12]. The antifungal agents tested included Fluconazole (25 µg), Voriconazole (1 µg), Nystatin (100 IU), Clotrimazole (50 µg), Miconazole (10 µg), and Amphotericin B (2.5 µg/mL). Plates were incubated at 37 °C for 24–48 h, allowing detection of slower-growing species like *Candida glabrata* and *Candida krusei*[10]. *C. albicans* ATCC 90,028, *C. parapsilosis* ATCC 22,019 and, *C. krusei* ATCC 6258 were used as control strains.

Every tenth sample was evaluated at the Epi-Centre laboratory Mbarara), a level three authorized laboratory in Mbarara, for external quality control.

Data analysis

Questionnaires were checked for completeness on the same day of the data collection. Data was entered in Excel, cleaned, coded, and backed up. Data were exported to STATA V17 for analysis.

The baseline characteristics of participants were summarized using mean with SD, median with IQR, or proportions as deemed appropriate. Descriptive statistics were used to summarize the demographic, behavioral, and clinical characteristics of the study population. The prevalence of VVC was estimated with confidence intervals. Logistic regression analysis was performed to identify factors associated with VVC adjusting for potential confounders.

Results

Social demographic characteristics of study participants

In this study, 420 HIV positive women at least 18 years old were screened of which 150 were eligible. Of the eligible participants, 146 participated in the study and 4 declined to participate. Growth was observed in 86 participants of which 71 were *Candida* species and 15 were other organisms and not *Candida* species therefore excluded from the study (Table 1).

Variable	Category	Frequency	Percentage (%)
Age (Years)	< 20	1	0.68
	20–29	27	18.49
	30–39	60	41.10
	40–49	49	33.56
	>50	9	6.16
Blood glucose levels (random blood sugar)	Abnormal	112	76.71
	Normal (less than 7.8 mmol/L)	34	23.29
Pregnancy	Yes	29	18.18
	No	117	81.82
TB disease	Detected	12	8.39
	Not Detected	134	91.61
Occupation	Business	66	45.21
	Crop farming	61	41.78
	Formal	8	5.48
	Employment	10	6.85
	Housewife	1	0.68
Marital status	Married	76	52.05
	Divorced	48	32.88
	Single	20	13.70
	Cohabiting	1	0.68
	Widowed	1	0.68
Educational level	No Education	14	9.59
	Primary	75	51.37
	Secondary	45	30.82
	Tertiary	12	8.22

Table 1. Distribution of sociodemographic characteristics among study participants ($N = 420$).

Organism	Frequency	percentage
Candida albicans	43	60.5
Candida krusei	8	11.2
Candida ciferrii	6	8.5
Candida guilliermondii	6	8.5
Candida glabrata	4	5.6
Candida famata	3	4.2
Candida parapsilosis	1	1.4

Table 2. Candida species of isolated organisms

Prevalence of VVC among HIV-positive women at Mbarara city health centre IV

Of the 71 culture-positive cases, Candida species were isolated from 49% of HIV-positive women presenting with vulvovaginal candidiasis symptoms at Mbarara City Council health centre IV ART clinic. Candida albicans accounted for the majority of isolates (60.5%, $n = 43$), while non-albicans species constituted 39.4% ($n = 28$) of cases (Table 2, Fig. 1).

Antifungal susceptibility patterns

Antifungal susceptibility patterns of the isolated candida species among HIV-positive women at Mbarara City Health Centre IV. *Candida* species showed the highest susceptibility to polyenes (amphotericin B and nystatin) than to selected azoles as shown in Table 3.

Factors associated with symptomatic VVC among HIV-positive women at Mbarara City health centre IV

The factors that were assessed in this study were age, random blood glucose levels, education level, pregnancy status, TB disease, occupation, and marital status. The blood glucose levels of the participants showed a significant association ($p = 0.018$ and OR = 2.93) with infection with vulvovaginal candidiasis (see Table 4).

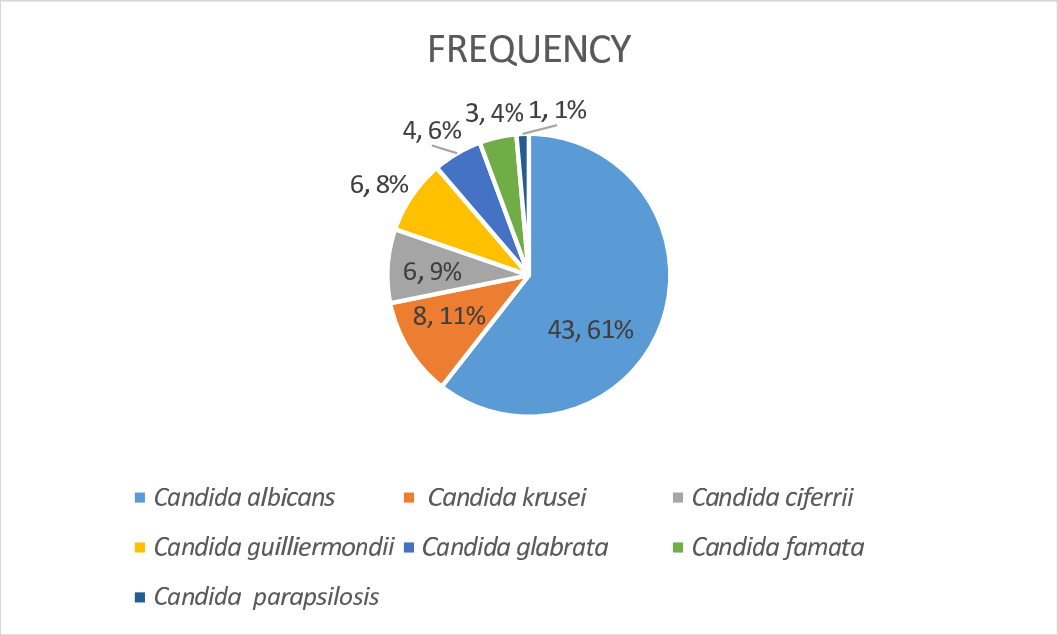

Fig. 1. Distribution of *Candida* species isolated from HIV positive women with vulvovaginal candidiasis at Mbarara City Health Centre IV. Percentages represent the proportion of each species among the 71 isolates.

Antifungal drug	Candida species, *n*(%)						
	C.albicans	*C.krusei*	*C.guilliemondi*	*C.glabrata*	*C.famata*	*C.parapsilosis*	*C.ferrii*
Fluconazole							
S	23 (53.5%)	4 (50.0%)	2 (33.3%)	2 (50.0%)	2 (66.7%)	1 (100%)	2(33.3%)
R	12 (27.9%)	2 (25.0%)	2 (33.3%)	0	1 (33.3%)	0	1(16.7%)
I	8 (18.6%)	2 (25.0%)	2 (33.3%)	2 (50.0%)	0	0	3(50%)
Clotrimazole							
S	19 (44.2%)	3(37.5%)	4 (66.6%)	3 (75.0%)	2 (66.7%)	0	3 (50.0%)
R	15 (34.9%)	3 (37.5%)	1 (16.7%)	1 (25.0%)	1 (33.3%)	1 (100%)	1(16.7%)
I	9 (20.9%)	2 (25%)	1 (16.7%)	0	0	0	2(33.3%)
Nystatin							
S	35 (81.4%)	7 (87.5%)	6 (100%)	4 (100%)	3 (100%)	1 (100%)	5 (83.3%)
R	3(7.0%)	1 (12.5%)	0	0	0	0	1 (16.7%)
I	5 (11.6%)	0	0	0	0	0	0
Miconazole							
S	37 (86.0%)	5 (62.5%)	6 (100%)	4 (100%)	3 (100%)	1 (100%)	4 (66.7%)
R	0	0	0	0	0	0	0
I	6 (14.0%)	3 (37.5%)	0	0	0	0	2 (33.3%)
Voriconazole							
S	28 (65.1%)	5 (62.5%)	6(100%)	4(100%)	2(66.7%)	1(100%)	4(66.7%)
R	7 (16.3%)	1(12.5%)	0	0	0	0	2(33.3%)
I	8 (18.6%)	2(25%)	0	0	1(33.3%)	0	0

Table 3. In vitro antifungal susceptibility patterns of *Candida* species isolated from HIV-positive women with vulvovaginal candidiasis. Key; S (susceptible), I(Intermediate), R (Resistant).

Discussion

Candida species can cause VVC in both HIV-positive and HIV-negative women. However, compared to HIV Negative women, HIV + women (with lower CD4 + T-cell counts) had a higher chance of acquiring VVC[13]. Effective management of vulvovaginal candidiasis (VVC) in HIV-positive women requires identification of causative Candida species, determination of their antifungal susceptibility patterns, and assessment of risk factors associated with VVC.

Variables				
Variable	**Frequency**	**OR**	**P-value**	**CI**
Age (years)				
20–29	27 (18.49)			
30–39	60 (41.10)	0.50	0.23	0.16–1.55
40–49	49 (33.56)	0.99	1.00	0.29–3.39
˃50	9 (6.16)	1.06	0.95	0.16–6.81
Glucose level				
Abnormal	112 (76.71)	***2.93	***0.018	1.21–7.14***
Normal				
Pregnancy				
Yes	29 (18.18)	0.51	0.204	0.18–1.44
No	117 (81.82)			
TB disease				
Detected	12 (8.39)	1.10	0.90	0.27–4.53
Not detected	134 (91.61)	2.41	0.42	0.29–20.7
Occupation				
Crop farming	61 (41.78)	1.12	0.80	0.48–2.59
Formal Employment	8 (5.48)	0.69	0.63	0.15–3.15
Housewife	10 (6.85)	0.85	0.87	0.12–6.16
Marital status				
Divorced	48 (32.88)	0.71	0.44	0.29–1.70
Married	76 (52.05)		Ref. value	
Single	20 (13.70)	0.43	0.19	0.12–1.50
Education level				
No formal education	14 (9.59)	1.09	0.90	0.30–3.93
Primary	75 (51.37)		Ref. value	
Secondary	45 (30.82)	1.05	0.91	0.43–2.58
Tertiary	12 (8.22)	0.61	0.59	0.10–3.68

Table 4. Factors associated with vulvo vaginal candidiasis for multivariate analysis.

The observed 49% prevalence of vulvovaginal candidiasis (VVC) among HIV-positive women in our study aligns closely with the 50% reported by Merenstein et al.,[14] in the US suggesting similar ART efficacy and diagnostic approaches between the populations. However, our prevalence was substantially higher than the 20% documented in Namibia[15] reflecting differences in ART accessibility, regional *Candida* species distribution, or climatic conditions favoring fungal growth. Conversely, the markedly lower prevalence compared to Brazil's 100%[16] may indicate divergent study methodologies (e.g., inclusion of asymptomatic cases) or variations in HIV disease progression among cohorts. Notably, reduced VVC risk observed in women on combination ART underscores the critical role of sustained antiretroviral therapy in mitigating opportunistic infections. These disparities highlight the interplay of immunological (e.g., CD4 + counts), therapeutic (e.g., ART adherence), and environmental (e.g., humidity) factors in shaping VVC epidemiology across global settings[17–19].

Proper identification of *Candida* species is important to aid in proper management of vaginal candidiasis. *C. albicans* is still the predominant species and this finding is consistent with many other studies[7,13,20,21]. However, some studies have reported a high prevalence of non-Candida albicans in vulvovaginal candidiasis[22,23]. The shifting epidemiology of vulvovaginal candidiasis, characterized by increasing non-albicans *Candida* (NAC) species prevalence alongside decreasing but still dominant *C. albicans* cases, likely results from multiple factors such as widespread use of broad-spectrum antibiotics that disrupt vaginal microbiota, increased availability and often indiscriminate use of antifungal agents (both over-the-counter and prescribed), and host immune suppression. While *C. albicans* remains the most prevalent species[23].

In our study, factors such as pregnancy, occupation, education levels, and marital status were not associated with VVC among HIV positive women. however, the diabetic status of HIV-positive women in our study was found to be associated with VVC ($p = 0.018$) which agrees with the study of Keran et al.,[24] who concluded that 8.8% had positive culture of *Candida* species. Hyperglycemia increases glucose concentration in vaginal secretions and tissues, thereby enhancing yeast adhesion and growth.This is because high blood sugar levels decrease neutrophil migration thereby weakening their chemotactic and phagocytic activity[25].

In this study, *Candida* species showed the highest susceptibility to Amphotericin B except *C.guilliermondii* which showed intermediate susceptibility. The results are similar to the results of Kan et al.,[26] that reported susceptibility of 98% to Amphotericin B by *Candida* species. Susceptibility of NAC to nystatin and resistance among *C.albicans* was observed which is in consistence with other studies[27,28]. This may because these drugs are both fungicidal and fungistatic reducing the likelihood for *Candida* species to develop resistance. Similar investigations have found similar susceptibility patterns for Amphotericin B, which is less widely used to treat

VVC. However, the findings from this present study differ from a study by Khan, 2018[29] that reported a resistance of 41.6% in *Candida albicans*.

The high resistance of *candida* species to azoles in our current study is consistent with the outcomes of clotrimazole and fluconazole susceptibility patterns conducted by Sathi et al.[30] and Mukasa et al.[20] respectively. This may be attributed to the 'over the counter' antifungal vaginal pessaries and creams containing clotrimazole and fluconazole thus there is higher exposure of the organism and likely development of resistance.

Conclusions

This study demonstrates that vulvovaginal candidiasis (VVC) is a prevalent opportunistic infection among HIV-positive women at Mbarara City Health Centre IV, with *Candida* species identified in 49% of symptomatic cases. *Candida albicans* was the predominant isolate (60.5%), reinforcing its central role in VVC etiology in this population. Notably, polyenes specifically amphotericin B and nystatin exhibited the highest antifungal activity, suggesting their potential as first-line treatments. Furthermore, elevated blood glucose levels were significantly associated with VVC ($p = 0.018$), implicating hyperglycemia as a key modifiable risk factor. These findings underscore the need for targeted antifungal stewardship and glycemic control interventions to mitigate the burden of VVC in immunocompromised women.

Data availability

The data that support the findings of this study are available on request from the corresponding author. The data set is not publicly available due to privacy or ethical restrictions.

Received: 7 April 2025; Accepted: 25 June 2025
Published online: 06 July 2025

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Acknowledgements
We acknowledge the district health office for their cooperation during the data collection. We also appreciate the data collectors, supervisors, and study participants for their invaluable input to the success of this study.

Author contributions
All authors contributed equally and reviewed the manuscript.

Funding
The authors received no funding for this study.

Declarations

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-09215-4.

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