International Journal of Health Sciences and Research
[Indexed, Peer-Reviewed Journal]
Volume 15; Issue: 8; August 2025
Website: www.ijhsr.org
Original Research Article *ISSN: 2249-9571*

Vulvovaginal Candidiasis: Characterization of Etiologic Agent with Their Antifungal Susceptibility Pattern

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DOI: https://doi.org/10.52403/ijhsr.20250829

ABSTRACT

Introduction: Vulvovaginal candidiasis (VVC) remains one of the most common causes of gynecological consultations worldwide, with *Candida albicans* being the predominant etiological agent. In recent years, however, there has been a notable rise in cases caused by non-*albicans Candida* (NAC) species, many of which exhibit resistance to commonly used antifungal agents. This study aimed to investigate the spectrum of *Candida* species responsible for vulvovaginitis and to assess their antifungal susceptibility patterns at a tertiary care center in North India.

Material And Methods: A pair of High vaginal swabs (HVS) were collected from female patients exhibiting clinical features indicative of VVC. For each patient, one swab was processed for direct microscopic examination, while the second was cultured on Sabouraud Dextrose Agar (SDA) for the isolation of *Candida* species. Identification was performed using established mycological techniques, including the germ tube test, carbohydrate fermentation and assimilation tests, morphological assessment on cornmeal agar, and differentiation of colony coloration on HiCHROMagar. Antifungal susceptibility testing to fluconazole, voriconazole, amphotericin B, and caspofungin was conducted using the broth microdilution method, following Clinical and Laboratory Standards Institute (CLSI) M27-A3 guidelines. Isolates were categorized as susceptible, intermediate, or resistant according to interpretive breakpoints defined by CLSI document M60.

Results: A total of 101 non-duplicate Candida isolates were obtained, including 49 strains of *C. albicans*. Among the NAC species, *C. tropicalis* was the most prevalent (n = 20), followed by *C. glabrata* (n = 18) and *C. krusei* (n = 14). *C. albicans* (n=49) showed high susceptibility to all tested antifungals. *C. tropicalis* (n=20) was generally susceptible to all agents, though fluconazole MICs were slightly higher than those for *C. albicans*. *C. glabrata* (n=18) exhibited reduced susceptibility and *C. krusei* (n=14) displayed intrinsic resistance to fluconazole respectively but both remained sensitive to voriconazole, amphotericin B, and caspofungin.

Conclusion: This study provides valuable insights into the diverse etiological spectrum of vulvovaginal candidiasis and their respective antifungal susceptibility profiles. The findings highlight the importance of targeted antifungal stewardship and species-specific therapy to optimize treatment outcomes and reduce the burden of VVC among women.

Keywords: Caspofungin, *Non-albicans Candida*, Broth microdilution method, fluconazole, *C. albicans*

INTRODUCTION

Vaginal discharge is one of the most common reasons for healthcare visits among young females worldwide (1). It is most frequently associated with four pathological conditions: candidiasis, bacterial vaginosis, aerobic vaginitis, and trichomoniasis (2). Candida vaginitis is the second most common cause, characterized by inflammation of the vulva and/or vagina in the presence of *Candida* species, with no other identifiable etiology (2). Epidemiological studies suggest that approximately 75% of women experience vulvovaginitis at least once in their lifetime, with nearly half of them having a second episode and 5–10% developing recurrent vulvovaginitis (>4 episodes per year) (3). Clinically, the condition is associated with leucorrhea, lower abdominal pain, itching, and burning sensations.

Candida vulvovaginitis occurs more frequently in certain risk groups, including women with diabetes, pregnant women, and those using oral contraceptives or antibiotics (4). Although *Candida albicans* was historically considered the predominant pathogen, recent studies have reported a rising incidence of non-*albicans Candida* (NAC) species worldwide (5). These NAC species exhibit variable susceptibility to different classes of antifungal agents, posing potential therapeutic challenges.

In this context, we conducted a prospective study to identify the causative agents of Candida vulvovaginitis and to determine their antifungal susceptibility patterns to four major classes of antifungal drugs and compare the antifungal susceptibility pattern amongst candida and NAC spp isolated at our tertiary care centre.

MATERIALS & METHODS

Sample Collection and Processing Two high vaginal swabs were collected from young female patients (20-45 years) presenting with complaints of vaginal discharge. The swabs were immediately transported to the Mycology Section, Department of Microbiology, and processed without delay. One swab was used to prepare a Gram-stained smear for microscopic examination, while the other was inoculated onto blood agar and Sabouraud Dextrose Agar (SDA) tubes. Cultures were incubated at 37 °C for up to seven days, with daily observation for fungal growth.

Identification of Candida spp. Fungal growth on blood agar and SDA was processed using standard mycological procedures. Candida species were identified based on colony morphology, Gram staining, germ tube test, morphological features on cornmeal agar, carbohydrate fermentation and assimilation tests, and colony colour differentiation on HiCHROM agar. Confirmed isolates were stored in glycerol broth at −20 °C for further analysis.

Antifungal Susceptibility Testing - Antifungal susceptibility testing for fluconazole, voriconazole, amphotericin B, and caspofungin was performed according to Clinical and Laboratory Standards Institute (CLSI) M27-A3 guidelines, using *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 as quality control strains. Antifungal powders (Sigma Pharmaceuticals) were dissolved in dimethyl sulfoxide (DMSO) to prepare 100× stock solutions (fluconazole 12.8 mg/ml; voriconazole 3.2 mg/ml; amphotericin B 3.2 mg/ml; caspofungin 0.8 mg/ml). These were further diluted in RPMI 1640 medium (without bicarbonate, buffered with 0.165 M MOPS, pH 7.0) to obtain working concentrations of 128 µg/ml (fluconazole), 32 µg/ml (voriconazole), 32 µg/ml (amphotericin B), and 8 µg/ml (caspofungin).

Inoculum Preparation - Stored yeast isolates were subcultured on potato dextrose agar (PDA) tubes. After incubation, 1–2 colonies were suspended in 5 ml sterile normal saline (0.145 M) and vortexed for 15 s. The turbidity was adjusted to a 0.5 McFarland standard at 530 nm, yielding a yeast suspension of 1–5 × 10^6 cells/ml. The working inoculum was prepared by sequential 1:100 and 1:20 dilutions in RPMI 1640 to achieve a final concentration of 5 × 10^2 to 2.5 × 10^3 cells/ml.

MIC Determination - Minimum inhibitory concentrations (MICs) were determined using the broth microdilution method in U-bottom 96-well microtiter plates, with a total volume of 200 µl per well (100 µl drug solution + 100 µl inoculum). Plates were incubated at 35 °C and read at 48 h for fluconazole, voriconazole, and amphotericin B, and at 24 h for caspofungin. For amphotericin B, MIC was defined as the lowest concentration showing complete growth inhibition; for the other agents, MIC was defined as the lowest concentration producing ≥50% growth reduction compared with the drug-free control (6).

STATISTICAL ANALYSIS

For statistical analysis, the data collected were expressed as mean and standard deviation for numerical variables. We used the Kruskal–Wallis (KW; nonparametric ANOVA) test to analyze the mean rank difference among the groups. The significance level of $p < 0.05$ was adopted.

RESULT

A total of 101 strains of the *Candida* spp were isolated from the vaginal swabs, collected from females presenting with the complaint of vaginal discharge. The species distribution of the isolated 101 strains is shown in Fig. 1.

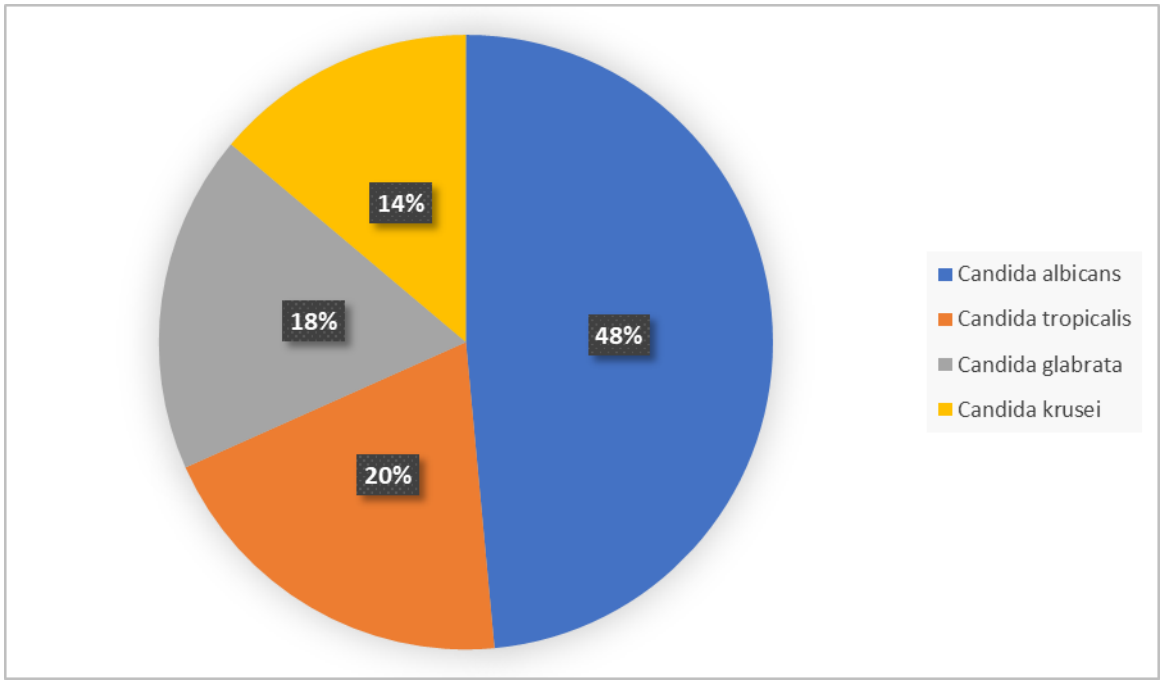

Fig 1. Species distribution of various Candida spp. in our study

Antifungal susceptibility testing of these isolated *Candida* spp was determined by broth microdilution method. Table 1 shows the MIC profiles of four antifungal agents—Voriconazole, Fluconazole, Amphotericin B, and Caspofungin—against different Candida species isolated from high vaginal swabs (n=101 isolates).

***Candida* species**	**Antifungal agent**	**No. of isolates with MIC (µg/ml)**											**MIC_{50}**	**MIC_{90}**	**Range**
		≤0.06	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**≥64**			
C. albicans (49)	V	44	3	0	2	0	0	0	0	0	0	0	0.12	0.5	0.005-0.5
	F	0	17	13	13	4	2	0	0	0	0	0	0.25	1	0.12-2
	A	7	4	8	24	6	0	0	0	0	0	0	0.5	1	0.06-1
	C	41	5	2	0	0	1	0	0	0	0	0	0.03	0.12	0.008-0.25
*C. tropicalis (*20)	V	17	2	1	0	0	0	0	0	0	0	0	0.03	0.125	0.01-0.5
	F	0	4	5	6	2	2	1	0	0	0	0	0.5	2	0.12-4
	A	3	1	4	11	1	0	0	0	0	0	0	0.5	0.5	0.12-1
	C	17	3	0	0	0	0	0	0	0	0	0	0.01	0.125	0.01-0.5
*C. glabrata (*18)	V	9	3	2	4	0	0	0	0	0	0	0	0.12	0.5	0.01-0.5
	F	0	2	3	4	4	4	1	0	0	0	0	1	2	0.125-4
	A	0	1	3	6	8	0	0	0	0	0	0	0.5	1	0.125-1
	C	12	0	2	4	0	0	0	0	0	0	0	0.06	0.5	0.008-0.5
C. krusei (14)	V	9	3	1	1	0	0	0	0	0	0	0	0.06	0.25	0.01-1
	F	0	1	6	0	3	1	3	0	0	0	0	1	4	0.25-4
	A	1	2	2	3	6	0	0	0	0	0	0	1	1	0.06-1
	C	13	1	0	0	0	0	0	0	0	0	0	0.01	0.25	0.0008-0.125

Table 1. Minimum Inhibitory Concentration (MIC) profiles of different *Candida* species (n=101) isolated from high vaginal swab against four antifungal agents. V: Voriconazole, F: Fluconazole, A: Amphotericin B, C: Caspofungin.

We further compared the MICs of four antifungal agents against *Candida albicans* (n=49 isolates) and NAC isolates (n=52 isolates) as shown in Table 2.

Drug	**Isolated *Candida* sp**	**MIC range (mcg/ml)**	**Mean MIC ± SD (mcg/ml)**	**MIC_{50} (mcg/ml)**	**MIC_{90} (mcg/ml)**	**p-value**
Fluconazole	NAC (52)	0.06-4	1.006±1.141	0.5	4	p-value < 0.05
	C. albicans (49)	0.03-2	0.403±0.411	0.25	1	
Voriconazole	NAC (52)	0.005-1	0.1475±0.298	0.06	0.5	p-value >0.05
	C. albicans (49)	0.005-1	0.060±0.153	0.03	0.12	
Amphotericin B	NAC (52)	0.06-1	0.548±0.320	0.5	1	p-value <0.05
	C. albicans (49)	0.03-1	0.426±0.274	0.5	1	
Caspofungin	NAC (52)	0.0008-0.5	0.076±0.133	0.03	0.25	p-value >0.05
	C. albicans (49)	0.0008-0.25	0.044±0.054	0.03	0.12	

Table 2 Comparative Antifungal Susceptibility Profiles of Candida albicans and Non-albicans Candida Species (NAC spp) to fluconazole, voriconazole, amphotericin B and caspofungin

DISCUSSION

Vaginal discharge is a common health concern among sexually active females and can be caused by bacterial, fungal, or parasitic infections. Among these, *Candida* species are responsible for a significant number of cases. Historically, *Candida albicans* was regarded as the predominant cause of vulvovaginal candidiasis. However, in recent years, there has been a global increase in the incidence NAC species in these infections (7, 8). This shift in the etiological landscape may be attributed to factors such as the widespread use of fluconazole prophylaxis and enhanced virulence traits of NAC species (9). In the present study, NAC strains slightly outnumbered *Candida albicans* among vulvovaginitis cases. Similar findings of NAC predominance over *C. albicans* have been reported by Kumari et al. and Hedayati et al. (7, 8).

In our study, *C. albicans* accounted for 48.5% of isolates, followed by *C. tropicalis* (19.8%), *C. glabrata* (17.8%), and *C. krusei* (13.8%). Notably, no isolates of *C. parapsilosis* were recovered. This variation in NAC species distribution may reflect regional differences in fungal epidemiology influenced by factors such as azole antifungal usage patterns. DS Elfeky et al. reported recovery rates of *C. glabrata* (12.7%), *C. krusei* and *C. parapsilosis* (7.9%), and *C. tropicalis* (6.3%) among NAC isolates from vulvovaginitis cases (10). In contrast, Sobel et al., Bauters et al., and Babić M et al. identified *C. glabrata* as the most common NAC species (11-13), while Bitew A et al. documented *C. krusei* as predominant among NAC strains (14).

Among the 101 *Candida* isolates from VVC cases, *C. albicans* (n=49) showed high susceptibility to all tested antifungals, with the lowest MICs for caspofungin. *C. tropicalis* (n=20) was generally susceptible to all agents, though fluconazole MICs were slightly higher than those for *C. albicans*. *C. glabrata* (n=18) exhibited reduced susceptibility to fluconazole but remained sensitive to voriconazole, amphotericin B, and caspofungin. Although, one strain of *C. glabrata* showed higher voriconazole (1mcg/ml) and fluconazole MIC (4mcg/ml). Similar results of fluconazole sensitive *C. glabrata* strains have been reported by Bitew A *et al* & Richter *et al* (14,15). *C. krusei* (n=14) displayed intrinsic resistance to fluconazole while showing good susceptibility to voriconazole, amphotericin B, and caspofungin. All the isolated strains were sensitive to caspofungin, member of echinocandin, another class of antifungal agents which inhibit the synthesis of β-glucan. Our results were in agreement with another study, executed by Pappas *et al* (16). Echinocandin resistance is still relatively rare in Candida spp. but increasingly notable in *C. glabrata*, with reported rates reaching 13% (17).

The comparative antifungal susceptibility analysis revealed that NAC isolates generally exhibited higher MIC values than *C. albicans*. For fluconazole, NAC isolates demonstrated a higher mean MIC (1.006 ± 1.141 µg/ml) and a broader range (0.06–4 µg/ml) compared to *C. albicans*, with the difference being statistically significant ($p < 0.05$). Similar patterns have been reported in earlier studies, particularly for *C. glabrata* and *C. krusei*, which often display reduced fluconazole susceptibility relative to *C. albicans* (15). In contrast, voriconazole and caspofungin exhibited comparable MIC ranges in both groups, with no statistically significant differences ($p>0.05$); consistently low MICs for these agents across *C. albicans* and NAC species have also been documented previously (18). For amphotericin B, NAC isolates showed a slightly higher mean MIC (0.548 µg/ml) than *C. albicans* (0.426 µg/ml), a difference that was statistically significant ($p < 0.05$). Similar trends of elevated amphotericin B MICs in NAC species have been observed in multicenter surveillance studies from Asia and other regions (19). Collectively, these findings highlight the clinical importance of routine antifungal susceptibility testing and support the

adoption of species-specific therapeutic strategies to optimize management of VVC and mitigate antifungal resistance development.

CONCLUSION

Vulvovaginal candidiasis is caused by a diverse group of *Candida* species, each exhibiting variable susceptibility to commonly used antifungal agents. This variability necessitates prompt and accurate species-level identification accompanied by comprehensive antifungal susceptibility testing. Tailored antifungal therapy is crucial to ensure effective treatment, prevent therapeutic failures, and reduce the incidence of recurrent infections. Given the rising prevalence of non-*albicans* *Candida* species with differing antifungal resistance patterns, routine susceptibility testing supports evidence-based clinical decision-making and helps curb the emergence of drug-resistant strains. Ultimately, these measures improve patient outcomes, minimize complications, and contribute to antimicrobial stewardship in the management of vulvovaginal candidiasis.

Declaration by Authors

Ethical Approval: The study was approved by the Ethical Committee of the Institute (ECR/526/Inst/UP/2014).

Acknowledgement: We acknowledge Department of Science and Technology. We are also thankful to the Department of Obstetrics & Gynaecology, for the clinical samples.

Source of Funding: None

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

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How to cite this article: Pradeep Paul, Parijat Das, Nidhi Pandey. Vulvovaginal candidiasis: characterization of etiologic agent with their antifungal susceptibility pattern. *Int J Health Sci Res.* 2025; 15(8):248-254. DOI: *https://doi.org/10.52403/ijhsr.20250829*

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