

# Phenotypic characterization and antifungal susceptibility profile of *Candida* isolates from women with vulvovaginal candidiasis at a community health center linked to a teaching institution in the Sub-Himalayan region of North India

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## ABSTRACT

**Introduction:** Vulvovaginal candidiasis (VVC) is considered a common gynecological problem among females of reproductive age group. 70–75% of women report having had candidal vulvovaginitis at some point in their lifetimes and 40–50% suffer recurrent candidal vulvovaginitis. **Objectives:** This study aims to identify the *Candida* species involved in VVC and to determine their antifungal susceptibility pattern. **Materials and Methods:** The present study was a cross-sectional study conducted on 257 females (18–55 yr) with complaints of abnormal vaginal discharge. For detection of *Candida*, the swab samples were subjected to Gram stain, 10% KOH mount, and culture on Sabouraud dextrose agar (SDA). *Candida* species identification was done by subculturing *Candida* isolates onto CHROMagar, corn meal agar (Himedia), and further confirmation was done by MALDI-TOF MS. Antifungal testing was done using the disk diffusion method. **Results:** A total of 257 females with complaints of abnormal discharge were enrolled in this study. Out of 257, *C. albicans* 37 (58.7%) and 26 (41.3%) isolates were identified as non-*albicans Candida*. Out of 63 positive cases, a maximum number of study subject belongs to the age group 26–35 years (50.8%). Along with vaginal discharge, itching (65.37%) is the most common complaint. VVC was found to be most predominant in patients with prolonged antibiotic therapy (38.1%), and in pregnant females (15.9%). **Conclusion:** Understanding the emerging fungal pathogens and their drug susceptibility patterns is essential for the effective management of infections. Drug resistance can lead to treatment failure and highlights the need for alternative treatment options or strategies.

**Keywords:** Intrauterine devices, vaginal discharge, vulvovaginal candidiasis

## Introduction

Vulvovaginal candidiasis (VVC) is an opportunistic fungal infection that is observed commonly in females of the reproductive age group, and it is caused by an overgrowth of *Candida* species in the vagina. Predominant symptoms of VVC are curd-like vaginal discharge, vulvar itching, dyspareunia, dysuria,

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edema, and vulvovaginal erythema. It has been observed that about 70–75% of sexually active females may experience at least one episode of vulvovaginal candidiasis in their lifetime.<sup>[1]</sup> 50% of initially infected females may experience a second episode and only 5 to 10% of individuals may develop recurrent infection.<sup>[2,3]</sup> In 85%–90% of cases of VVC, the most commonly identified species is *Candida albicans*.<sup>[4]</sup> Whereas, in non-*albicans Candida* (NAC), *Candida glabrata* appeared as the most common pathogen followed by *Candida tropicalis*, *Candida krusei*, and *Candida parapsilosis*.<sup>[5]</sup> Some important predisposing factors which increase the risk of VVC, include pregnancy (55%) and broad-spectrum antibiotics (8%), other risk factors are uncontrolled diabetes mellitus, use of contraceptives, immunosuppression, and HIV.<sup>[6-8]</sup> *Candida* isolates obtained from VVC could not be entirely recognized at the species level by conventional methods, alternatively, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) can be used for the reliable identification of *Candida* species. The MALDI-TOF MS facilitates the identification of a large spectrum of proteins directly from intact microorganisms.<sup>[9]</sup> Until now, a single dose of fluconazole is recommended for treating VVC, but some NAC, *C. krusei* is intrinsically resistant to fluconazole, However, *C. glabrata* is reported to be less susceptible to fluconazole or related azole antifungals, but few recent studies show increasing susceptibility of *C. glabrata* toward fluconazole.<sup>[10,11]</sup> Further studies are necessary to validate any inconsistencies observed in the fluconazole susceptibility pattern across different research endeavors.

The inappropriate and over usage of antimycotics leads to the development of antifungal resistance. Due to the emergence of drug resistance, there should be identification and antifungal susceptibility testing of *Candida* species in the clinical setting. This study was carried out to identify *Candida* species involved in VVC and determine their antifungal susceptibility pattern.

## Materials and Methods

This is a cross-sectional study conducted from October 2019 to December 2022, vaginal swabs were collected from 257 women (18–55yr) with complaints of abnormal vaginal discharge. Ethical approval for the study was obtained from the ethical committee (EC registration No.: EC/NEW/INST/2022/UA/0152 dated 28/5/22) of Swami Rama Himalayan University (SRHU), Dehradun, and participants informed written consent was taken. Patients who are unmarried and menstruating were excluded from this study.

Two vaginal swabs samples were collected from 257 women who had a history of abnormal vaginal discharge. One swab sample was subjected to gram stain and 10% KOH mount. Another swab sample was inoculated on Sabouraud dextrose agar (SDA) without antibiotic (Himedia) and incubated at 37°C for 24–48 hours. Isolates on SDA were identified as *Candida* by colony morphology, gram stain, and germ tube test. *Candida* species identification was done by subculturing *Candida* isolates onto CHROMagar, corn meal agar (Himedia),

and further confirmation was done by MALDI-TOF MS [Vitek-MS™ (BioMérieux)].

## Antifungal susceptibility testing

Antifungal susceptibility testing was performed according to CLSI-M44-A2 guidelines 3<sup>rd</sup> edition.<sup>[12]</sup> The recommended antifungal disk diffusion testing medium is Muller Hinton agar with 2% glucose and 0.5 µg Methylene blue. The suspension was prepared by picking 5–6 colonies of SDA plate from a 24-hrs old culture of *Candida* species. Colonies were then inoculated in 5 ml of sterile saline, and their turbidity was adjusted to 0.5 McFarland standard. A sterile cotton swab was moistened with the adjusted inoculum suspension, and then excess fluid was removed by pressing the swab on the inside wall of the tube. Then the inoculation was done on the entire agar surface. Antifungal disks (Biogram) of fluconazole (25 µg), itraconazole (10 µg), ketoconazole (10 µg), nystatin (100 U) and amphotericin B (100U) were used.

Due to the lack of defined breakpoints for itraconazole, ketoconazole, amphotericin B, and nystatin arbitrary values based on the other studies and manufacturer guidelines were employed.<sup>[12-16]</sup>

## Statistical analysis

The data were collected and entered in MS Excel 2010 and statistical analysis was done using the SPSS software version 20. Any association between categorical variables has been calculated via a non-parametric test, viz., Chi-square and Fisher exact test. *P* value <0.05 was considered significant.

## Results

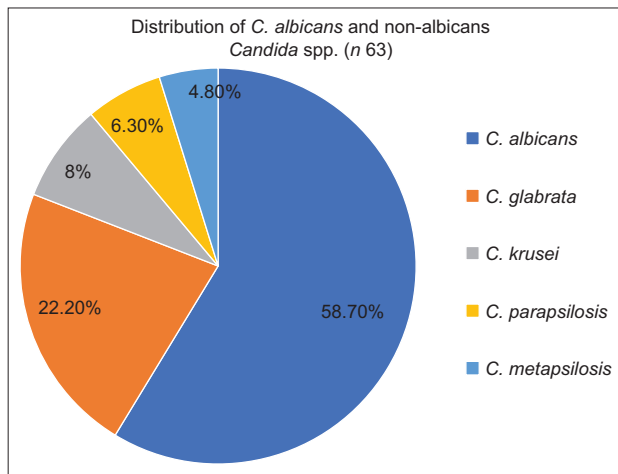
A total of 257 females with complaints of abnormal discharge were enrolled in this study. Out of 257, 63 (24.5%) females were found positive for vulvovaginal candidiasis, 59 (23%) for Bacterial vaginosis, 6 (2.3%) for Trichomonas vaginalis, and 10 (3.9%) for Mixed infection. Out of 63 positive cases, a maximum number of study subject belongs to the age group 26–35 years (50.8%) followed by the 16–25 years (31.7%) age group [Table 1]. However, no statistically significant association exists between age group and infection. Out of 63, the predominant isolates identified were *C. albicans* 37 (58.7%) and 26 (41.3%) isolates were identified as NAC [*C. glabrata* (22.2%), *C. krusei* (8%), *C. parapsilosis* (6.3%), and *C. matapsilosis* (4.8%)] [Figure 1]. Along with vaginal discharge, itching (65.37%) is the most common complaint

**Table 1: Distribution of study subjects (n=257) and patients with VVC (n=63) according to age group**

Age group	Total number of subjects (n=257)	Patients with VVC (n=63)
16–25	70 (27.2%)	20 (31.7%)
26–35	120 (46.7%)	32 (50.8%)
36–45	51 (19.8%)	9 (14.3%)
46–55	16 (6.3%)	2 (3.2%)

observed in the study group followed by lower abdominal pain (55.4%) and burning micturition (44.5%) [Figure 2]. There is a statistically significant correlation between VVC with burning micturition and itching ( $P = 0.0001$ ) [Figure 2]. VVC was found to be most predominant in patients with prolonged antibiotic therapy (38.1%), and in pregnant females (15.9%). There is a statistically significant association between infection and prolonged antibiotic therapy ( $P = 0.0001$ ) [Table 2].

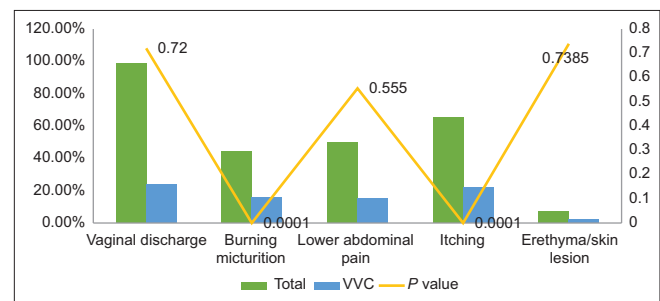
The antifungal susceptibility test shows that the most effective antifungal agent used in this study was nystatin, where 97% of isolates were sensitive followed by amphotericin B (81%). 52.3% of *Candida* isolates were sensitive to fluconazole, whereas only 14.3% of *Candida* isolates were sensitive to ketoconazole and 6.3% to itraconazole. No susceptible dose-dependent (SDD) isolates were detected for nystatin, whereas SDD isolates detected for fluconazole (9.5%), ketoconazole (9.5%), itraconazole (23.8%) and amphotericin B (12.7%). *Candida* isolates showed high resistance to ketoconazole (76.2%) followed by itraconazole (69.8%), fluconazole (38.1%), and only 6.3% of isolates showed resistance to amphotericin B and 3.2% to nystatin. In the present study, ketoconazole resistance was maximum among all antifungal drugs to all *Candida* species as shown in [Table 3]. Chi-squares ( $\chi^2$ ) of fluconazole, ketoconazole, itraconazole, nystatin, and amphotericin B are 15.724, 13.557, 23.258, 2.528, and 22.617, respectively, with degrees of freedom of 8 in all the antifungal agents. The findings were significant for fluconazole ( $P$  value  $< 0.015$ ), ketoconazole ( $P$  value  $< 0.037$ ) itraconazole ( $P$  value = 0.000) and amphotericin B ( $P$  value = 0.001), and insignificant for nystatin ( $P$  value = 1).



**Figure 1:** Distribution profile of *Candida* species among study participants causing Vulvovaginal candidiasis

## Discussion

VVC most commonly occurs in females of the reproductive age group and in this condition is characterized as inflammation, intense itchiness, and a thick, white discharge from the vagina. The present study is a cross-sectional study conducted on females with complaints of abnormal vaginal discharge. In the present study, the prevalence of vulvovaginal candidiasis is 24.5%, similar results were found in the study conducted by Bignoumba *et al.* and Krishnapriya Kalaiarasan *et al.* 28.5% and 23.7%, respectively.<sup>[5,17]</sup> In contrast to this study, some studies that showed a higher prevalence of VVC were Shrestha *et al.* (Nepal, 42.1%), and Waikhom *et al.* (Ghana, 30.7%).<sup>[16,18]</sup> In this study, *C. albicans* (58.7%) was the most predominant among the isolated species which is similar to the study carried out by Swaminathan *et al.*, Gandhi *et al.*, and Arastehfar *et al.* where the prevalence of *C. albicans* was 60%, 66.3%, and 78.6%, respectively.<sup>[19-21]</sup> The second most common *Candida* species was *C. glabrata* (22.2%) which was found similar to the study conducted by Gandhi *et al.* (9.8%), Arastehfar *et al.* (11.7%), and Swaminathan KR *et al.* (40%).<sup>[19-21]</sup> In contrast to this study, Deepak Kumar *et al.* study showed a prevalence of *C. tropicalis* (25%), and Mohanty *et al.* (57%) showed a higher prevalence of *C. glabrata* than *C. albicans*.<sup>[13,22]</sup> In the present study, 96.8% of study subjects were between the age group 16–45 years; these findings were similar to the study conducted by Latha Ragunathan *et al.*, where 88.9% of women belonged to this age group.<sup>[6]</sup> The higher incidence of infection in this age group due to high exposure to sexual activity and the presence of high levels of reproductive hormones present during this stage might be a reason behind to increase in the susceptibility to reproductive tract infections. In the present study, there is a significant association between itching and burning micturition. Waikhom *et al.* conducted a study on pregnant women in Ghana where they found a significant association between VVC and burning sensation and irritation.<sup>[16]</sup>



**Figure 2:** Symptoms presented in the study group ( $n=257$ ) and VVC ( $n=63$ )

**Table 2:** Risk factors associated with total no. of study participants ( $n=257$ ) and with vulvovaginal candidiasis ( $n=63$ )

Risk factors	Total number of subjects ( $n=257$ )	Patients with VVC ( $n=63$ )	$P$
Pregnancy	27 (10.5%)	10 (15.9%)	0.84
Intrauterine devices	13 (5.1%)	1 (1.6%)	0.11
Prolonged antibiotic therapy	30 (11.7%)	24 (38.1%)	0.0001
Diabetes	15 (5.8%)	4 (6.3%)	0.12
No risk factors	172 (66.9%)	24 (38.1%)	

**Table 3: Antifungal susceptibility profile of different *Candida* species (n=63)**

Antifungal agents	C. albicans (n=37)			C. glabrata (n=14)			C. matapsilosis (n=3)			C. krusei (n=5)			C. parapsilosis (n=4)			Total		
	S (%)	SDD (%)	R (%)	S (%)	SDD (%)	R (%)	S (%)	SDD (%)	R (%)	S (%)	SDD (%)	R (%)	S (%)	SDD (%)	R (%)	S (%)	SDD (%)	R (%)
Fluconazole	19 (51.4)	4 (10.8)	14 (37.8)	11 (78.6)	0	3 (21.4)	2 (67)	1 (33.3)	0	0	0	5 (100)	1 (25)	1 (25)	2 (50)	33 (52.3)	6 (9.5)	24 (38.1)
Ketoconazole	5 (13.5)	5 (13.1)	27 (73)	1 (7.1)	0	13 (93)	3 (100)	0	0	0	0	5 (100)	0	1 (25)	3 (75)	9 (14.3)	6 (9.5)	48 (76.2)
Itraconazole	5 (13.5)	8 (21.6)	27 (73)	1 (7.1)	3 (21.4)	10 (71.4)	1 (33.3)	2 (67)	0	0	0	5 (100)	0	2 (50)	2 (50)	4 (6.3)	15 (23.8)	44 (69.8)
Nystatin	35 (94.6)	0	2 (5.4)	14 (100)	0	0	3 (100)	0	0	5 (100)	0	0	4 (100)	0	0	61 (97)	0	2 (3.2)
Amphotericin B	36 (97.3)	1 (2.7)	0	6 (43)	6 (43)	2 (14.3)	3 (100)	0	0	2 (40)	1 (20)	2 (40)	4 (100)	0	0	51 (81)	8 (12.7)	4 (6.3)

S=Sensitive, SDD=Susceptible dose-dependent, R=Resistant

In this study, the prevalence of VVC was found to be higher in patients who had previously been exposed to antibiotics or prolonged treatment in the past (38.1%), and also in pregnant females (15.9%). Swaminathan *et al.* reported that 13.3% of females with overused antibiotics and 29.1% of pregnant females had VVC.<sup>[19]</sup> The use of prolonged antibiotic agents increases vaginal yeast colonization by eliminating lactobacilli. Khan *et al.* detected a high prevalence of VVC in the pregnant female when they were in their second trimester of pregnancy, and concluded that this was likely due to raised hormone levels in pregnancy.<sup>[23]</sup>

The *in-vitro* antifungal susceptibility test showed that the most effective antifungal agent was nystatin, where 97% of isolates were sensitive followed by amphotericin B (81%). No susceptible dose-dependent (SDD) isolates were detected for nystatin except *C. albicans* (5.4%) isolates that show resistance to nystatin. In the study conducted by Khan *et al.*, 35% of *Candida* isolates showed sensitivity to nystatin, 16.7% SDD, and 58.3% were resistant,<sup>[23]</sup> and in a study conducted by Shrestha *et al.* where 100% of *Candida* isolates were sensitive to nystatin.<sup>[18]</sup> In the current study, 52.3% of *Candida* isolates were sensitive to fluconazole. The study by Deepak Kumar *et al.* reported that 72% of *Candida* isolates were sensitive to fluconazole.<sup>[13]</sup> In the present study, 38.1% of *Candida* isolates showed resistance to fluconazole, which was similar to the result observed by Kombade *et al.* (34.6%).<sup>[24]</sup> In contrast to this study, Latha Raghunathan *et al.* study showed that only 5% of *C. albicans* isolates were resistant to fluconazole by E-test.<sup>[6]</sup> Dota *et al.* reported a higher resistance rate for fluconazole (32%) by the disk diffusion method whereas no resistance was detected by the micro-dilution method.<sup>[25]</sup> In the present study, a maximum (76.2%) of *Candida* isolates showed resistance to ketoconazole, whereas Urvasi Chongtham *et al.* observed that 39% of *Candida* isolates were resistant to ketoconazole.<sup>[15]</sup> In this study, only 14.3% of *Candida* isolates were sensitive to ketoconazole whereas DEIFeky *et al.* observed 85.7% of *Candida* isolates were sensitive.<sup>[14]</sup> In this study, 6.3% of *Candida* isolates were sensitive to itraconazole, and 69.8% were resistant. Kombade *et al.* study showed 81% of *Candida* isolates were resistant to itraconazole.<sup>[24]</sup>

Amphotericin B was sensitive in 81% of *Candida* isolates and resistant in 6.3% of isolates, similar results were found in a study conducted by Urvasi Chongtham *et al.*, where 81% of *Candida* isolates were sensitive, and 8% were resistant.<sup>[15]</sup> In contrast to this study, Kombade *et al.* that observed 34.6% of *Candida* isolates were resistant to amphotericin B.<sup>[24]</sup> It has also been observed that all of the *C. krusei* were resistant to fluconazole as they were intrinsically resistant to fluconazole but also showed 100% resistance to itraconazole and ketoconazole, which was similar to the result of a study conducted by Kombade *et al.* where *C. krusei* shows 100% resistance to itraconazole.<sup>[24]</sup> In contrast to this Urvasi Chongtham *et al.* reported 60% resistance to ketoconazole and none of the isolates was resistant to itraconazole.<sup>[15]</sup>



## Conclusion

Firstly, the study's findings regarding the prevalence of VVC, along with the identification of the most common *Candida* species, provide valuable insights into the epidemiology of this condition. Understanding the prevalence rates and the predominant species can aid healthcare providers and public health officials in targeting preventive and treatment strategies effectively. Moreover, the study's emphasis on the association between VVC and factors such as antibiotic exposure and pregnancy underscores the need for tailored approaches for specific subgroups of women, enhancing the quality of reproductive healthcare.

The analysis of antifungal susceptibility patterns is particularly significant. The observed resistance to commonly used antifungal drugs, such as fluconazole and itraconazole, has important clinical implications. It underscores the urgency of monitoring drug resistance trends and emphasizes the necessity for diversifying treatment options. This aspect is crucial not only for individual patient care but also for shaping treatment guidelines and public health policies. Addressing drug resistance can prevent treatment failures, reduce the risk of recurrent infections, and contribute to the overall well-being of women in the community.

Furthermore, the study's acknowledgement of limitations, such as sample size and methodology, highlights the ongoing need for rigorous research in the field of female reproductive health. As women's health concerns evolve, comprehensive studies with larger and more diverse samples, as well as advanced analytical methods, become imperative to provide a comprehensive understanding of the challenges and opportunities in managing conditions like VVC.

In conclusion, the data analysis presented in this study is of great significance to the broader perspective of female reproductive health in the community. It informs healthcare professionals, researchers, policymakers, and advocates about the prevalence, characteristics, and treatment patterns of VVC. The study's findings contribute to a more nuanced understanding of the challenges women face in their reproductive health journey and emphasize the importance of tailored interventions, effective antimicrobial stewardship, and ongoing research to ensure the best possible care for women in the community.

The limitations of this study are firstly, the small sample size may not accurately reflect the actual burden of the disease in the community. Larger studies with a more representative sample size would provide more reliable information. Additionally, the use of the disk diffusion method has limitations in detecting patient-to-patient variation in drug susceptibility. Other more sensitive and specific methods, such as broth micro-dilution, may be needed to comprehensively assess antifungal susceptibility.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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