

Vulvovaginal Candidiasis in Pregnant Women and Susceptibility Profile to Fluconazole and Voriconazole

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Abstract

Objective: To evaluate the prevalence of vulvovaginal candidiasis (VVC) among pregnant women, identify the species of candida responsible for infections, and evaluate their susceptibility to antifungal agents, specifically fluconazole and voriconazole.

Methodology: The study was conducted at the Department of Microbiology at Basic Medical Sciences Institute (BMSI), in collaboration with the Gynaecology Outpatient Department at Jinnah Postgraduate Medical Centre (JPMC), Karachi from Feb 2021 to Oct 2022. All pregnant patients suspected of vulvovaginal candidiasis irrespective of age have been included. After taking informed vaginal swabs were collected and analyzed for Candida species using Gram staining and antifungal susceptibility testing via disk diffusion on Mueller-Hinton agar. Statistical analysis was done using SPSS version 26

Results: Overall mean age was 27 years. Among 323 patients, 88% belonged to lower socioeconomic classes, and 38% were in their first trimester. *Candida albicans* was the most common species (88%), followed by *C. glabrata* (8%) and *C. krusei* (4%). Antifungal susceptibility showed resistance to fluconazole in 57% of *C. albicans*, 81% in *C. glabrata*, and 100% in *C. krusei*. *C. albicans* and *C. glabrata* were more susceptible to voriconazole (85% and 69%, respectively)

Conclusion: A high prevalence of VVC observed among pregnant women, with *Candida albicans* being the most frequently isolated species, followed by *C. glabrata* and *C. krusei*. There was a significant antifungal resistance, especially to fluconazole, in *C. albicans* and *C. glabrata*, with *C. krusei* exhibiting complete resistance to fluconazole. Voriconazole found to be the better effective across all *Candida* species.

Keywords: Vulvovaginal candidiasis, Species, Resistance, Fluconazole, Voriconazole.

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Introduction

Vulvovaginitis, characterized by inflammation of the vulva and vagina, is a frequent concern among adolescents and young women seeking gynecologic care. Common causes of vaginal inflammation include bacterial vaginosis, VVC, and trichomonal vaginitis. Among these, VVC stands out as a major public health issue due to its high prevalence and the morbidity it causes, which has increased over recent decades.¹

Candida infections range from superficial or mucosal to life-threatening disseminated forms, such as candidemia and hepatosplenic candidiasis.² The most

common fungal pathogen causing these infections is *Candida albicans*, which inhabits the skin, reproductive, and gastrointestinal tracts. This shift and the increasing antifungal resistance among both *C. albicans* and NAC present significant challenges to effective treatment.²

Given the growing diversity and antifungal resistance among *Candida* species, timely diagnosis and effective management of VVC are essential.³ About 10–20% of women experience complicated VVC, characterized by severe or recurrent infections, often caused by NAC species. These cases have diagnostic and therapeutic

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implications, particularly for individuals with conditions like pregnancy, immunosuppression, or diabetes.⁴

Azole antifungals, including fluconazole and clotrimazole, are frequently used to treat VVC. Fluconazole, often administered as a single oral dose, is effective against *C. albicans*, the primary cause of VVC.^{5,6} However, its use during pregnancy is subject to scrutiny due to potential risks to the fetus. Oral antifungal medications like fluconazole require careful risk-benefit evaluation during pregnancy, as some studies suggest a possible association with birth defects when used in high doses or for prolonged periods during the first trimester.^{7,8}

Voriconazole, another azole antifungal, offers a broader range of activity compared to fluconazole. While it is highly effective against systemic fungal infections, including those caused by *Aspergillus* species, its use for localized infections like VVC is limited due to its broader systemic impact and potential risks during pregnancy.^{9,10} The administration of voriconazole in pregnant patients remains controversial due to insufficient safety data and is typically reserved for severe cases when the benefits outweigh the risks.^{11,12}

Healthcare practitioners play a crucial role in assessing individual patient needs and tailoring treatment strategies. For pregnant women, this involves a careful evaluation of infection severity, potential drug risks, and overall maternal-fetal health.¹³ As fluconazole is often the first-line treatment for VVC, its administration during pregnancy should be based on a meticulous assessment of risks and benefits.^{14,15} Voriconazole, on the other hand, is primarily reserved for serious systemic fungal infections due to its wide spectrum of activity and pharmacokinetics.^{16,17} Despite its prevalence, there is limited data on *Candida* species' distribution and antifungal susceptibility in VVC in Pakistan. This study aims to address this gap by determining the frequency of *Candida* species in VVC and their susceptibility to fluconazole and voriconazole.² Such findings could guide clinicians in optimizing antifungal treatment and improving patient outcomes.

Methodology

The descriptive cross-sectional study was conducted at Department of Microbiology at the Basic Medical Sciences Institute (BMSI), with involved collaboration with the department of Gynaecology Outpatient Department (OPD) at Jinnah Postgraduate Medical

Centre (JPMC) in Karachi. A 354 sample size was performed using OpenEpi, with 95% CL and 5% margin of error. Study was conducting from Feb-2021 to Oct-2022. All pregnant patients suspected of vulvovaginal candidiasis irrespective of age have been included.

Women on antifungal drugs and the patients who refused to give consent were excluded. After taking informed consent two vaginal swabs has been obtained from the posterior fornix of the vagina by gynecologists having minimum experience of 5 years and samples were transported to the microbiology department at BMSI. Gram staining was conducted to assess the presence of Gram-positive and Gram-negative bacteria, as well as *Candida* and clue cells. The preparation of a smear involves the application of a drop of normal saline onto a glass slide, followed by the mixing of this saline with a vaginal swab. The samples were allowed to air dry and subsequently treated with absolute methanol for fixation. To perform the staining procedure, the slide was placed on a staining rack and covered with crystal violet solution for 30 to 60 seconds before being rinsed with fresh water. Next, Lugol's iodine was applied for 30 to 60 seconds, followed by another rinse with distilled water. The slide was then decolorized briefly with an acetone-alcohol mixture and rinsed with tap water. Safranin or a neutral red stain was applied for 2 minutes, after which the stain was removed by rinsing with clean water. The back of the slide was wiped, and it was placed on a draining rack to air-dry. The smear was subsequently examined microscopically to detect bacteria, *Candida*, and clue cells. The antifungal resistance testing followed a multi-step approach to identify and test fungal pathogens.

Vaginal discharge samples were characterized based on pH and appearance to indicate possible pathogens, such as *Trichomonas vaginalis* or *Candida albicans*. Rapid Yeast Plus panels were used for further species identification based on substrate breakdown. Antifungal susceptibility testing was performed using the disk diffusion method on Mueller-Hinton agar to evaluate resistance, measuring zones of inhibition around antifungal-impregnated disks to determine sensitivity profiles. Data was entered and analyzed using SPSS version 26.

Results

The patients' ages ranged from 17 to 38 years, with a mean age of 27 years. Of the 323 patients, 88% were from the poor social class and 12% from middle-class families. Pregnancy trimester distribution showed that

38% were in the first trimester, 43% in the second trimester, and 19% in the third. Among the symptoms, 45.8% reported itching, 51.1% had discharge, 30% noticed a smell, 39.3% experienced pain, 43.3% had dyspareunia (painful intercourse), and 57.6% experienced dysuria (painful urination). Diabetes was present in 21.4% of cases. Regarding the color of vaginal discharge, 3.1% reported yellow discharge, while 57% had white discharge. Most (98%) of the women had a vaginal pH level between 1 and 3. Previously antifungal treatment used history was in 77% of patients, while 19.5% had repeated antibiotic treatments history. Table I

Table I. Clinical characteristics of pregnant women. (n=323)

Symptoms of infected pregnant women	
Itching	148(45.8%)
Discharge	165(51.1%)
Smell	97(30.0%)
Pain	127(39.3%)
Dyspareunia	140(43.3%)
Dysuria	186(57.6%)
Diabetes	69(21.4%)
Color of Vaginal discharge	
Yellow	10 (3.1%)
Grayish	--
White	184 (57.0%)
pH of vaginal discharge	
pH 7 and more	--
pH 4-6	2.0%
pH 1-3	98.0%
Antifungal	
Yes	249 (77.0%)
No	74 (23.0%)
Repeated Antibiotic	
Yes	63 (19.5%)
No	260 (80.5%)

Table II: Frequency of Candida species among women. (n=323)

Candida species	Frequency	Percentage
<i>Candida glabrata</i>	16	8%
<i>Candida krusei</i>	7	4%
<i>Candida albicans</i>	171	88%

On the distribution of *Candida* species among patients with VVC was analyzed as follows: 171 patients (88%) had isolated *C. albicans*, 16 patients (8%) had *C. glabrata*, and 7 patients (4%) had isolated *C. krusei*. Table II.

According to antifungal susceptibility of 171 *Candida albicans* isolates, the Nystatin showed sensitivity in 79% of isolates and resistance in 21%. Clotrimazole was effective in 74% of isolates, with 26% showing resistance. Fluconazole exhibited sensitivity in 43% and resistance in 57% of cases. Voriconazole had the highest sensitivity at 85%, with 15% of isolates being resistant. Itraconazole was sensitive in 71% and resistant in 29% of isolates, while Amphotericin showed sensitivity in 68% and resistance in 32% of the isolates.

On the antifungal susceptibility for *Candida glabrata* isolates, the Nystatin was effective in 69% of isolates, while 31% showed resistance. Clotrimazole showed 81% sensitivity and 19% resistance. Fluconazole was effective in only 19% of isolates, with 81% resistant. Voriconazole demonstrated 69% sensitivity and 31% resistance. Itraconazole was sensitive in 56% of isolates and resistant in 44%. Amphotericin had 44% sensitivity, with 56% of isolates showing resistance. Furthermore, the antifungal susceptibility of 7 *Candida krusei* isolates showed that Nystatin was effective in 57% of isolates and resistant in 43%. Clotrimazole and Voriconazole each showed 71% sensitivity and 29% resistance. Fluconazole was ineffective, with 100% resistance. Itraconazole had 29% sensitivity and 71% resistance, while Amphotericin was sensitive in 43% of isolates and resistant in 57%. Table III

Discussion

Candida species are regarded as the most significant opportunistic fungi. Vulvovaginal candidiasis is a prevalent infection that affects females within the reproductive age range. Research suggests that approximately 75% of females in the childbearing age group experience at least one episode of VVC

Table III: Antifungals susceptibility pattern against candida species.

Antifungals	<i>Candida albicans</i>		<i>Candida glabrata</i>		<i>Candida krusei</i>	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Nystatin	135(79%)	36(21%)	11(69%)	5(31%)	4(57%)	3 (43%)
Clotrimazole	127(74%)	44(26%)	13(81%)	3(19%)	5 (71%)	2 (29%)
Fluconazole	74(43%)	97(57%)	3(19%)	13(81%)	0(0%)	7(100%)
Voriconazole	146(85%)	25(15%)	11(69%)	5(31%)	5(71%)	2(29%)
Itraconazole	120(71%)	51(29%)	9(56%)	7(44%)	2(29%)	5(71%)
Amphotericin B	116(68%)	55(32%)	7(44%)	9(56%)	3 (43%)	4 (57%)

throughout their lifetime. The study observed individuals aged 18 to 46, which aligns with previous research that shows a higher incidence of VVC in those aged 15-45, likely due to elevated estrogen levels and the use of contraceptive methods.¹⁸ However, this finding contrasts with a study conducted in Nigeria, where a higher prevalence of *Candida albicans* was noted in individuals aged 36-40.¹⁸ The study also found that 43% of pregnant participants were in their second trimester, which aligns with research conducted in Nepal¹⁹ and India, both of which reported the second trimester as the period with the highest prevalence of VVC.

The present study revealed varied susceptibility patterns of *Candida albicans* to antifungal agents in pregnant women. Nystatin was effective in 79% of cases, with resistance observed in 21%. Clotrimazole showed sensitivity in 74% of cases, while 26% exhibited resistance. Fluconazole demonstrated sensitivity in 43% of isolates, with 57% resistant. Voriconazole had the highest sensitivity at 85%, with 15% showing resistance. Itraconazole was effective in 71% of cases, with resistance in 29%, while Amphotericin showed sensitivity in 68% and resistance in 32%. These findings are consistent with a study in Kenya where *C. albicans* isolates showed 88.33% sensitivity to Itraconazole. Resistance to fluconazole, voriconazole, and itraconazole was also observed in a Turkish study by Yenisehirli et al²⁰ Furthermore, studies from Pakistan and China indicate that *C. albicans* isolates were more sensitive to voriconazole, with *C. albicans* showing 85% sensitivity in this study. In contrast, Zaman et al¹⁸ reported higher sensitivity to itraconazole, while research from Iran²¹ and China found varying resistance levels to fluconazole and itraconazole. For example, 25.8% of *C. glabrata* isolates showed fluconazole resistance, while 7.8% of *C. albicans* isolates exhibited resistance. Studies from both Iran and China emphasize the evolving patterns of antifungal resistance across different regions.

This study identified *C. albicans* as the prevailing species responsible for *Candida* vaginitis. The observed results may be attributed to the heightened virulence exhibited by *Candida albicans* relative to other species within the *Candida* genus, as suggested by Mushi et al.²² A study in Kenya found *C. albicans* in 73.7% of cases, and a study in India noted a lower prevalence of 35.5%, with a higher occurrence of non-*albicans* species like *C. tropicalis* and *C. glabrata*. Resistance to antifungals, particularly among *C.*

glabrata, is a growing concern. For example, the present study found a resistance rate of 31% to Nystatin and Voriconazole, and 44% to Itraconazole in *C. glabrata* isolates. This trend is consistent with other studies, such as in Uganda, where *C. krusei* showed significant resistance to fluconazole and itraconazole. The increasing resistance to antifungals, especially among non-*albicans* species, highlights the need for alternative treatments and careful monitoring of resistance patterns in VVC cases. In India, a study by Dharmik et al²³ found high sensitivity of *Candida* isolates to fluconazole (97.2%), clotrimazole (80%), and itraconazole (57%), with lower sensitivity to miconazole (37%). However, in the current study, *Candida krusei* isolates showed significant resistance to several antifungals. Specifically, 100% of *C. krusei* isolates were resistant to fluconazole, while 71% were resistant to itraconazole and amphotericin. Sensitivity to other antifungals was 57% for Nystatin, 71% for clotrimazole, and 71% for voriconazole. These findings contrast with reports from Khan et al²⁴, who observed 100% fluconazole resistance and 66.6% itraconazole resistance in *C. krusei* isolates. Additionally, in studies by Mishra et al²⁵, *C. glabrata* isolates showed resistance to fluconazole, and *C. tropicalis* demonstrated high resistance (37.7%) to fluconazole. The current study supports these trends, as fluconazole resistance was found in 38.8% of *C. tropicalis* isolates, with voriconazole showing the least resistance (10.2%).

Conclusion

The study revealed a high prevalence of VVC among pregnant women, with *Candida albicans* being the most frequently isolated species, followed by *C. glabrata* and *C. krusei*. The results highlighted significant antifungal resistance, especially to fluconazole, in *C. albicans* and *C. glabrata*, with *C. krusei* exhibiting complete resistance to fluconazole. Voriconazole observed better effective across all *Candida* species, emphasizing the need for careful antifungal management in VVC cases. Additionally, socioeconomic factors and prior antifungal use were associated with increased prevalence of infections, suggesting the importance of targeted treatment strategies and awareness

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