ORIGINAL RESEARCH

Prevalence Of Vulvovaginal Candidiasis And Associated Risk Factors: An Investigation Of Candida Species And Antifungal Susceptibility Testing Profile

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ABSTRACT

Background: Vulvovaginal candidiasis (VVC) is the most common vaginal infection affecting women of reproductive age. The present study aimed to assess the prevalence of VVC and associated risk factor for VVC, and antifungal susceptibility testing profile of Candida species causing VVC. **Methods:** This hospital-based prospective observational study included suspected cases of VVC, in married and sexually active women. The clinical and demographic data such as age, parity, oral contraceptive pills (OCPs) uses, antibiotic profile and diabetic history were recorded. **Results:** A total of 130 patients were included in the study, out of which the majority (54.6%) of women were in the 18-30 years of age group. A totalof 81.5% of women had vaginal discharge, whereas 14.6% of women had recurrent discharge. The patients from urban areas had a significantly higher proportion of Candida positive isolates than negative isolates [96.0% vs 69.9%; P=0.0001]. The patients who had OCPs had higher chances of development of Candida-positive isolates as compared to negative isolates [12.0% vs 3.0%; P=0.06]. Antibiotic use was associated with an increased risk of colonization by Candida species (44.0%). Biofilm production was majorly observed among patients who were positive for Candida glabrata and Candida albicans isolates. Fluconazole showed 24.0% resistance and 76.0% sensitivity, itraconazole showed 76.0% resistance and 24.0% sensitivityandvoriconazoleexhibited no resistance with 100.0% sensitivity. **Conclusion:** The VVC diagnosis was associated with antibiotic prescriptions and diabetes and pregnancy. The antifungal susceptibility result showed that all candida species showed higher sensitivity towards voriconazole treatment.

Keywords: Candida glabrata, Candida albicans, voriconazole, vaginal infection.

INTRODUCTION

Vulvovaginal candidiasis (VVC) is the second most common vaginal infection affecting women of reproductive age.[1] It is a common condition caused by yeast infection in the vulva and/or intravaginal area. The candida species are the most common cause of VVC, affecting approximately 75% of women, with 40%-45% experiencing recurrent episodes and 10%-20% developing complicated VVC.[2] Several risk factors contribute to the occurrence of VVC, including pregnancy, contraceptive use, diabetes mellitus, antibiotic usage, and certain behavioral factors.[1] Additionally, the use of immunosuppressants and glucocorticoids may lead to Candida infections.[3,4] Candida albicans is responsible for approximately 80-90% of VVC cases, while non-albicans species like Candida glabrata, Candida tropicalis, Candida krusei, and Candida parapsilosis account for only a minority of cases (10-20%). Non-albicans species typically presents as a mild infection.[5]

Most commonly used antifungal agents for yeast infections include flucytosine, fluconazole, amphotericin B, voriconazole, clotrimazole, nystatin, caspofungin, and ketoconazole. However, the use of

antifungal agents presents issues such as safety concerns, cost, and the emergence of drug-resistant strains. Azoles, which can be taken orally, have the advantage of increased potency.[6] However, the use of fluconazole in the syndromic management of Reproductive Tract/Sexually Transmitted Infections (RTI/STI) programs, including vaginal discharge kits, may contribute to recurrent infections and treatment failure by non-albicans Candida species. Therefore, prevention, early diagnosis, and prompt treatment of VVC are crucial to prevent complications such as pelvic inflammatory disease, infertility, ectopic pregnancy, pelvic abscess, spontaneous abortion, and menstrual disorders.[7]

Moreover, biofilms of Candida exhibit resistance to antifungal agents. This poses a significant challenge for both in vitro susceptibility testing and therapeutic purposes.[8,9]

The present study aimed to assess the prevalence of VVC and associated risk factors for VVC and antifungal susceptibility testing profile of Candida species causing VVC.

METHODS

Study design

This hospital-based prospective observational study was conducted at the Department of Microbiology, King George's Medical University, Lucknow between July 2014 and June 2015. The study was approved by the Institutional Ethical Committee of King George's Medical University, U.P. (approval no: ECR/262/Inst/UP/2013).

Inclusion and exclusion criteria

Suspected cases of VVC, married and sexually active women (age between 16 to 45 years) were included in the study. The unmarried and postmenopausal women were excluded from the study.

Data collection

Patient's detailed history and local examination were done. The clinical and demographic data such as age, parity, oral contraceptive pills (OCPs) uses, antibiotic profile and diabetic history were recorded.

Sample collection and processing

Testing for samples was done at the Mycology Laboratory of Microbiology KGMU, Lucknow. Two vaginal swabs were collected from each patient. The first swab was cultured on blood agar, MacConkey agar, and Sabouraud's dextrose agar (SDA), and incubated at 37^0 C and 25^0C for candida spices isolation. The second swab was used for 10% potassium hydroxide (KOH) mount, wet mount, gram stain, and microscopic examination for budding yeast-like cells with pseudohyphae and to distinguish from other sexually transmitted infections (STIs).

Identifications of isolates

The identification of isolates was made by gram staining and lacto phenol cotton blue staining.

Identification procedure for yeast

Germ tube formation test, sugar fermentation, sugar assimilation, and chromID agar tests were used for identification of Candida and its species.

Germ tube formation test

The three drops of fresh pooled human serum were dispensed into tubes using a Pasteur pipette. The yeast was suspended in the serum and the tube was incubated at 35°C. The suspension was examined using low power objectives, and the high power objective was used to confirm the presence or absence of germ tubes. This test was validated with the corn meal agar (CMA) test. CMA plate was prepared with 1% Tween 80 and divided into four quadrants. Four streaks of yeast colony were made on the agar plate. A flame sterilized and cooled cover glass was placed over the control part of the streak and incubated at 25°C. The morphological features were examined.

Sugar fermentation

The liquid fermentation media was prepared, and sterilized by autoclave at 120^0C for 15 min. About 2% of sterilized sugar was added in to the sterilized tube. The inoculum preparation was done by suspending heavy inoculum of yeast grown on sugar free medium and incubated at 25°C for 1 week. The tubes were examined at every 48 to 72 h of interval for the production of acid and gas. The production of gas in the tube indicates fermentation positive.

Sugar assimilation test

A yeast suspension was prepared by combining a 24-48 h old culture with 2ml of yeast nitrogen base (YNB). The suspension was added to 18mL of molted agar and poured into a petri plate. Kept the petri plate at room temperature and carbohydrate-impregnated discs were placed on the agar surface. The plates were then incubated at 37°C for 3-4 days. Presence of growth around each disc indicates yeast assimilation of sugar.

chromID agar candida medium

The yeast colony was streak inoculated on the surface of CHROM agar plate in a fish-tailing manner. The plate was then incubated at 37°C in ambient air for 48 hours followed by observation of colony colour.

Antifungal testing methods

Disk diffusion test

This test for fluconazole and voriconazole was performed in accordance with the Clinical & Laboratory Standards Institute (CLSI) document MAA-A3. Agar plates (90 mm) containing Muller-Hilton agar were used. Inoculum was prepared by picking five distinct colonies from 24-h old growth on sabouraud dextrose agar in saline solution. The

resulting suspension was vortex and turbidity adjusted to yield 1 x 10^6 - 5 x 10^6cells/mL (0.5 McFarland standard). The agar surface was then inoculated with the suspension using a swab. Fluconazole and voriconazole discs were placed on the plates. The plates were incubated at 37°C for 18-24 h, and the zone diameter of growth reduction was measured. Pinpoint micro colonies and large colonies within the zone were ignored.

Micro broth dilution method for yeast

The antifungal susceptibility testing for all isolates was performed in accordance with CLSI M27-A3. This test was performed by using sterile disposable multiwell micro dilution plates (96 U-shaped well). The stock solutions of voriconazole were prepared in distilled water whereas fluconazole, itraconazole, and amphotericin B were prepared in dimethylsulfoxide (DMSO) and stored at -70°C. RPMI 1640 medium was used as the broth medium. Inoculum was prepared from Candida species colonies grown on SDA. Voltex was suspended and the resulting suspension's turbidity was adjusted to yield 1 x 10^6 -5 x 10^6 cells/mL (0.5 McFarland standard). Minimal inhibitory concentration (MIC) was determined at 48 h.

Biofilm production methods

Yeast isolates were cultured in Yeast Extract-Peptone-Dextrose (YPD) broth and adjusted to 10^6 cells/L. Suspensions were dispensed in wells of flat-bottomed 96-well microtiter plate with normal saline as negative control and incubated at 37°C. Wells were washed, reloaded with 100 µL of respective liquid media, and observed under an inverted microscope. Optical density was measured using an enzyme-linked immunosorbent assay (ELISA) reader with incubation.

STATISTICAL ANALYSIS

Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 23.0. Descriptive statistics were used to describe categorical variables (frequency and percentages). Comparison of quantitative between the groups was done using chi-square test.

RESULTS

A total of 130 patients were included in the study. The majority (54.6%) of women were in the 18-30 years of age group. About 75.4% of women belonged to an urban area. Out of 130 women, 14.6% were pregnant. Overall, 92.3% of women were in moderate socioeconomic status. Approximately 81.5% of women had vaginal discharge, whereas 14.6% of women had recurrent discharge. The majority of women (82.3%) had a moderate hygiene status (Table 1).

Table 1: Demographic profile

Parameters	Number of patients (N=130)
Age groups (years)	
18-30	71 (54.6)
31-40	49 (37.7)
>40	10 (7.7)
Place of residence	
Rural	32 (24.6)
Urban	98 (75.4)
Pregnancy status	
Pregnant	19 (14.6)
Non- pregnant	111 (85.4)
Socioeconomic status	
Low	10 (5.4)
Moderate	120 (92.3)
Vaginal discharge	106 (81.5)
Hygiene status	
Moderate	107 (82.3)
Poor	23 (17.7)
Recurrent discharge	19 (14.6)
Candida isolates	25 (19.2)
Data presented as n (%).	

The incidence of Candida-positive isolates was significantly higher in 20-30 years of age group (64.0%) of patients compared to the 31-40 and >40 years of age group (32.0% and 4.0%, respectively). The patients from urban areas had a significantly higher proportion of Candida-positive isolates thannegative isolates [96.0% vs 69.9%; P=0.0001]. The patients with vaginal discharge had a significantly higher proportion of Candida-positive isolates than negative isolates [96.0% vs 77.1%; P=0.001]. Patients

with recurrent discharge had significantly higher Candida-positive isolates compared to negative isolates(44.0% vs 7.6%; P=0.0001) (Table 2).

Table 2: Association of Candida status with demographic and associated risk factor

Parameter	Candida		P value
	Positive isolates [n = 25]	Negative isolates [n = 105]	
Age in years, (n=130)			
20-30	16 (64.0)	55 (52.4)	0.0001
31-40	8 (32.0)	41 (39.0)	
>40	1 (4.0)	9 (8.6)	
Place of residence		[n = 103]	
Rural	1 (4.0)	31 (30.1)	0.0001
Urban	24 (96.0)	72 (69,9)	
Socioeconomic status			
Middle	19 (16.1)	99 (94.3)	-
Poor	4 (40.0)	6 (5.7)	
Upper	2 (100.0)	-	
Personal hygiene			
Moderate	9 (8.4)	98 (93.3)	-
Poor	16 (69.7)	7 (6.7)	
Vaginal discharge	24 (96.0)	81 (77.1)	0.001
Recurrent discharge	11 (44.0)	8 (7.6)	0.0001
Risk factors			
Oral contraceptives pills	3 (12.0)	3 (3.0)	0.06
Antibiotics	11 (44.0)	8 (8.0)	0.0001
Parity ≥3	3 (12.0)	47 (47.0)	0.0001
Diabetes	5 (20.0)	1 (1.0)	0.0001
Pregnancy	12 (48.0)	7 (7.0)	0.0001
Data presented as n (%).			

The patients who had OCPs had higher chances of development of Candida-positive isolates as compared to negative isolates [12.0% vs 3.0%; P=0.06]. Antibiotic use was associated with an increased risk of colonization by Candidaspecies(44.0%). Candida-positive isolates were significantly higher in pregnant patients (Table 2).

The majority of patients (36.0%) tested positive for Candida glabrata according to the cornmeal agar test. The Candida glabrata was most common isolates (28.0%) according to sugar fermentation test. The CHROM agar test showed that the Candida glabrata was observed in majority (40.0%) of patients. The Candida glabrata was found in majority of patients (32.0%) according to sugar assimilation test [Table 3].

Table 3: Candida species according to the yeast identification test

Parameter	Number of isolates (n=25)
Corn meal agar morphology	
Candida glabrata	9 (36.0)
Candida albicans	5 (20.0)
Candida lusitaniae	6 (24.0)
Candida tropicalis	4 (16.0)
Candida guilliermondii	1 (4.0)
Sugar fermentation test	
Candida glabrata	7 (28.0)
Candida albicans	6 (24.0)
Candida lusitaniae	5 (20.0)
Candida tropicalis	5 (20.0)
Candida krusei	2 (8.0)
CHROM agar morphology	
Candida glabrata	10 (40.0)
Candida lusitaniae	6 (24.0)
Candida albicans	5 (20.0)
Candida tropicalis	4 (16.0)
Sugar assimilations	

Candida glabrata	8 (32.0)
Candida lusitaniae	7 (28.0)
Candida tropicalis	5 (20.0)
Candida albicans	4 (16.0)
Candida guilliermondii	1 (4.0)
Data presented as n (%).	

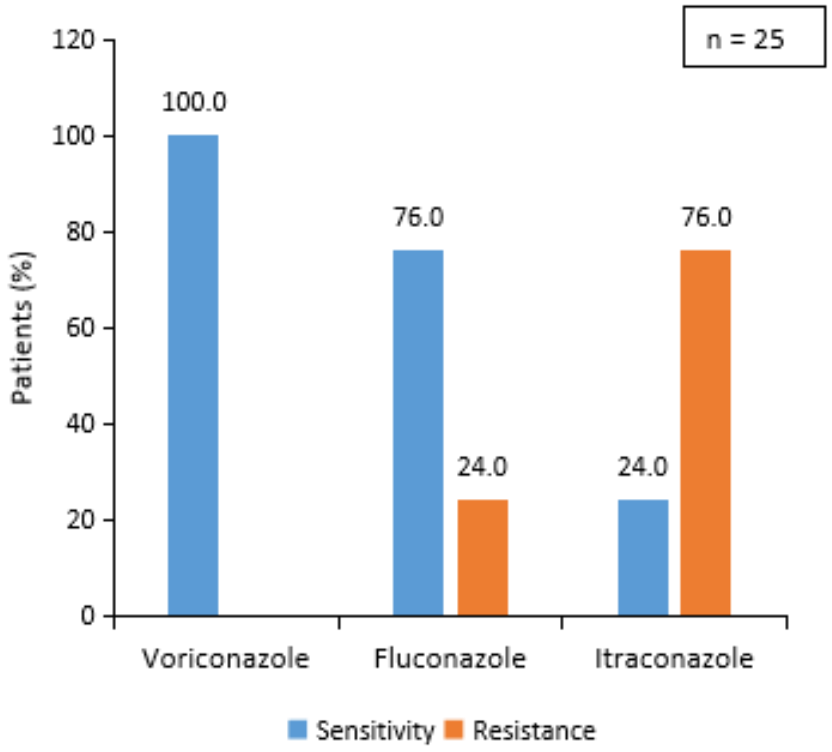
Out of 25 Candida-positive patients, the biofilm production was observed in 12 patients. Biofilm production was positive in four patients each from Candida glabrata and Candida albicans group (Table 4).

Table 4: Association of biofilm with various Candida species

Parameter	No. of patients (n=25)	Candida species				
		Candida glabrata	Candida albicans	Candida lusitaine	Candida tropicalis	Candida guilliermondii
Biofilm						
Present	12	4	4	1	2	1
Absent	13	5	1	5	2	0

The fluconazole showed 24.0% resistance and 76.0% sensitivity, itraconazole showed 76% resistance and 24% sensitivitywhile, voriconazole showed 100% sensitivity with no resistance (Figure 1).

Figure 1: Drug sensitivity pattern according disc diffusion test by M44A CLSI guidelines



DISCUSSION

The present study retrospectively determined the prevalence of VVC and associated risk factors. The salient observations were; i) The majority (54.6%) of women were in the 18-30 years of age group; ii) The patients from urban areas had a significantly higher proportion of Candida-positive isolates; iii) VVC diagnosis was associated with antibiotic prescriptions. iv) Patients with diabetes and pregnant women had significantly higher chances of development of Candida-positive isolates. iv) voriconazole showed no resistance with 100% sensitivity.

A high rate of VVC was reported in women inthe age range of 20-29 years in many studies,[10,11] which wasconsistent with the findings of the present study (18-30 years), probably due todrug usage and or contraceptives. Among the previousstudies that evaluated VVC prevalence, previous retrospective studies from Germany involved a large population (n=954,186), out of which 50,279 of these women were diagnosed with VVC. The highest prevalence rates were found in the age groups of 18-25 years (n = 10,063), 26-30 years (n = 7,631), and 31-35 years (n = 7,384) as compared to the older adult population (>60 years; n = 4,877).[12] The present study findings align with previous research conducted by Samal et al., demonstrating a decreased occurrence of VVC in women over the age of 40. This is consistent with the

results reported in their study.[13] Similarly, Sen's research also revealed that the age range of 21-30 years had the highest prevalence of VVC, which supports the present study observations. This suggests that women of reproductive age are more susceptible to developing VVC.[14]

The likelihood of developing VVC is higher in women who take oral contraceptives or undergo hormone replacement therapy after menopause than other women.[15] Using contraceptives elevates vaginal glycogen levels, creating a more favorable environment for Candida growth. Several studies indicate a higher incidence of Candida colonization and VVC among women who use OCPs.[1] Some studies suggest that estrogens directly influence Candida growth and its adherence to the vaginal epithelium, providing an environmentfor the increased incidence of VVC in women using OCPs.[16] In contrast to previous findings, the present study has shown a smaller number of (12.0%) patients experiencing VVC in patients taking OCPs.

The predisposing factors observed in this study, such as the use of multiple antibiotics (44.0%) and pregnancy (48.0%), were consistent with those previously reported.[17] Interestingly, multiple studies have established a connection between the trimester of pregnancy and the susceptibility of pregnant women to VVC infection. The vulnerability to infection among pregnant mothers increases as pregnancy progresses, resulting in the highest prevalence of VVC in the third trimester.[18] A previous prospective study evaluating the identification of Candida Species in women with VVC noted that out of 56 women with positive cultures, 20 were pregnant, six had diabetes, four had a history of antibiotic use, and 4 were using OCPs. Notably, there was a significant association between positive cultures and pregnancy, which may be attributed to elevated levels of reproductive hormones inducing a higher glycogen content in vaginal epithelial cells, creating a favorable environment for Candida growth.[19]However, Yadav and Prakash found no correlation between VVC and the gestational period.[20] Therefore, the role of the gestational period as a risk factor for VVC during pregnancy remains controversial.

Each Candida species exhibits differences in terms of biofilm formation. In this study, Candida glabrataand Candida albicansmainly contributed to the strong biofilm producers,which was similar to an earlier report.[21] However, another retrospective cohort noted that biofilm formation capacity was most commonly observed for isolates of Candia tropicalis (20 of 28 patients [71.4%]), followed by Candida glabrata (6 of 26 [23.1%]), Candidaalbicans (38 of 168 [22.6%]), and Candidaparapsilosis (14 of 64 [21.8%]).[22] In contrast, another author also reported Candida krusei and Candida tropicalis as strong biofilm producers than Candida albicans.[23]

In this study, fluconazole showed 24% resistance and 76% sensitivity, itraconazole showed 76% resistance and 24% sensitivity,while voriconazole showed no resistance with 100% sensitivity. Similarly previous noteworthy study reported that the majority of Candida species showed resistance to fluconazole (62%), followed by clotrimazole (59.3%), nystatin (58.3%), and itraconazole (40.7%). On the other hand, the lowest resistance rate was found for voriconazole (10.2%).[24] These findings are in concordance with cross sectional study by Bitew, et al. wherein voriconazole exhibited favorable effectiveness against all Candida among the azole drugs while only 10.6% of isolates of Candia albicans demonstrated resistance.[25]

CONCLUSION

The current research demonstrated that most of the patients withVVC belonged to the reproductive age category. The diagnosis of VVC was linked to the use of antibiotics as well as the presence of diabetes and pregnancy. Candida glabrata and Candida albicans were primarily responsible for producing robust biofilms. The findings of the antifungal susceptibility test revealed that all types of Candidaspecies exhibited greater sensitivity to voriconazole treatment.

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