Biofilm Production And Antifungal Resistance Among Candida Species In Patients With Vulvo Vaginitis: A Cross Sectional Study

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Abstracts: Background: Vulvovaginal candidiasis (VVC), one of the commonest fungal infections in women of reproductive age group, can become recurrent if not managed appropriately. Recently, production of biofilm, which reduces the susceptibility to anti-fungal agents, has also been implicated in its pathogenesis. Hence, the study was planned to determine the prevalence of VVC in cases of vulvovaginitis, identify the pre-dominant candida species, find out their antifungal susceptibility pattern and to detect biofilm production in the candida isolates. Methods: In this cross-sectional study, all isolates were identified and confirmed by various basic microbiological methods followed by the VITEK-2 system. Biofilm formation was detected by Congo red agar method and Tube method. Descriptive statistical methods were used to evaluate the data collected. Results: Out of total 25 patients, 7 were positive with VVC out of which 2 cases were caused by C.albicans and 5 by Candida Non Albicans (3 by C.glabrata and 2 by C.tropicalis). As compared to NAC, C. albicans is still partially sensitive to fluconazole. Fortunately, both C. albicans and C. tropicalis are still 100% sensitive to voriconazole although resistance has developed partially in C. glabrata. Biofilm production was observed in 3 cases. Conclusion: Majority of cases of VVC were due to NAC predominantly C. glabrata which has grave therapeutic implications as they show decreased susceptibility to all antifungals. Voriconazole is still effective but needs to be used rationally to avoid developing resistance to it also. The evidence of biofilm production could be an important contributor towards increased incidence of antifungal drug resistance [Lele G Natl J Integr Res Med, 2023; 14(6):35-39, Published on Dated: 28/12/2023]

Key Words: antifungal susceptibility pattern, biofilm, candida species, vulvovaginal candidiasis

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Introduction: Over the last few years, mycotic infections have posed a surprisingly substantial, yet unrecognized, health burden on the global population. Candida species, though commensals, can be opportunistic and initiate infections in both immunocompetent and immunocompromised individuals but higher incidence in the latter.[1]

Vulvovaginal candidiasis (VVC) is one of the commonest fungal reproductive tract infections especially in women of childbearing age. Around 75% of women experience at least one episode of VVC during their reproductive years.[2] Recurrent VVC [>3 episodes per year due to treatment failure] hampers the quality of life of women and can cause serious health complications. Earlier, Candida albicans was the causative agent in majority of cases; however, episodes due to nonalbicans candida species appear to be increasing.[3] The patient usually presents with symptoms such as intense vulval itching/irritation often accompanied by curdy-white discharge.

The transformation of Candida spp. from commensal to pathogen is enabled by expression of various virulence factors such as germ tube formation, adhesions, hydrolytic enzymes and formation of a biofilm.[4] Biofilms are found to be involved in an estimate of 80% infections of the human body. They are ubiquitous in nature and are embedded in the extracellular matrix. The presence of biofilms is of foremost medical significance as they provide the properties of better adhesion, resistance to clearance by the host immune system and decreased susceptibility to therapy.[5]

Antifungal agents commonly used to treat VVC include azoles (clotrimazole, ketoconazole, fluconazole, voriconazole) amphotericin B, nystatin, capsofungin and micafungin.[6,7] Apart from adverse drug reactions and cost factors, a major limitation with long term use of antifungals is the development of drug resistant strains especially to azoles.[8,9] The ability of Candida to develop drug-resistant biofilms plays a crucial role in their pathogenicity.

Identification of Candida species and their antifungal susceptibility testing are necessary for optimal treatment of these infections, especially in settings where diagnosis is based on syndromic approach. Hence, the study was planned to determine the prevalence of VVC in clinically suspected cases of vulvovaginitis and to find out the associated risk factors. It was also planned to identify the pre-dominant candida species, find out their antifungal susceptibility pattern and to detect biofilm production in the candida isolates. This study aims to raise awareness amongst various stakeholders namely clinicians, patients and microbiologists regarding the emerging local resistance pattern of antifungal drugs prescribed for VVC and the role of biofilm in its pathogenesis.

Material and Methods : This cross sectional, analytical study was performed on clinically suspected cases of Vulvovaginitis consulting the gynaecology department of a tertiary care hospital.

Study site: The study was conducted in the Department of Microbiology of a tertiary care Medical College and Hospital in the rural area of North India. Ethical clearance was obtained from the Institutional Ethics Committee before starting the study. Written informed consent was taken from each participant of the study.

Study period: The study was performed over a period of two months (July and August, 2021)

Sample size: It was conducted in clinically suspected cases of VVC attending the gynaecology department over the span of two months. The detailed history of each patient was taken in a semi structured proforma.

Inclusion criteria: Clinically suspected cases of Vulvovaginitis in females of all age groups.

Exclusion criteria: Cases other than Vulvovaginitis.

Study tool: Samples of high vaginal swabs were collected with the help of a gynaecologist under aseptic conditions. The specimen was immediately transported in a sterile tube containing normal saline to the microbiology lab for analysis.

Data collection: The high vaginal swab was subjected to KOH examination, Gram staining and culture on Sabouraud's dextrose agar (SDA) with Chloramphenicol. SDA slants were incubated at 37 °C for 7 days.

The colonies suggestive of Candida species were further identified and confirmed by Gram staining, Germ tube test, Chlamydospore formation and colour production on CHROM agar. Antifungal susceptibility was tested by VITEK automated system-2 using AST YSOR cards. Biofilm formation was detected by Congo red agar method and Tube method

Data analysis: Descriptive statistical methods were used to evaluate the data collected.

Result: Out of total 25 patients, who were included in the study, 7 (28%) reported positive with vulvovaginal candidiasis. Out of the total 7 positive cases, 5 cases (71.4%) were caused by Candida Non Albicans (3 by C.glabrata and 2 by C.tropicalis). C.albicans was responsible for infection in 2 (28.6%) cases.

Majority of cases (71.4%) belonged to the age group of 21-30 years and the remaining 28.6% were in the age group of 31-40 years. Mean age was 25 years with standard deviation (SD) ± 5.41. Out of the total cases, 6 (85.7%) were homemakers whereas only one case (14.3%) was employed as a clerk. All the cases presented with symptom of vaginal discharge which was thick curdy white in 5 cases and homogenous grey white in the remaining two cases. The discharge was odourless in majority (6) cases while it was foul smelling in 1 case. Total 6 cases (85.7%) cases also complained of pruritis and dysuria. Table 1 depicts various risk factors which were found in positive cases of VVC.

Table 1: Predisposing risk factors in positive VVC cases (n =7)

S.No.	Condition	Number of cases	Percentage cases
1	Pregnancy	2	28.6
2	Diabetes Mellitus	1	14.3
3	History of antibiotic intake	1	14.3
4	Poor personal hygiene	5	71.4

All the VVC cases showed budding yeast cells with pseudohyphae when tested with KOH mount followed by culture on SDA agar which demonstrated creamy white pasty colonies. Gram staining showed the presence of gram-positive budding yeast cells. Out of total 7 cases, only 2 cases showed germ tube projections (C. albicans) whereas the remaining 5 cases did not show any type of projections in their germ tube

(C. nonalbicans). Candida species identification was done by chrom agar.

Out of 7 cases, 3 cases showed pink smooth colour confirming them as C. glabrata, 2 cases bluish colour which confirmed as C. tropicalis and 2 cases light green colour identifying as C. Albicans.

The antifungal susceptibility testing was performed for three antifungals namely fluconazole, voriconazole and amphotericin B and the results are shown in Table 2. Figure 1, 2 and 3 depict the sensitivity profile of various candida species i.e., C. albicans, C. tropicalis and C. glabrata to various antifungals respectively. Biofilm production was observed in 3 cases (43%) and the results are depicted in figure 4.

Table 2: Antifungal susceptibility testing pattern of various candida species

Drug		FCA		VCA		Amp B	
Species	No.	S	R	S	R	S	R
C.albicans	2	1	1	2	0	2	0
C.glabrata	3	0	3	2	1	2	1
C.tropicalis	2	0	2	2	0	1	1

FCA = Fluconazole, VCA = Voriconazole, Amp B = Amphotericin B. S = Susceptible, R = Resistant

Figure 1: Antifungal susceptibility pattern in Candida albicans

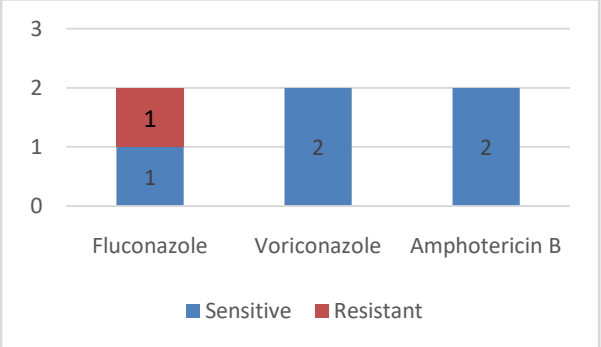
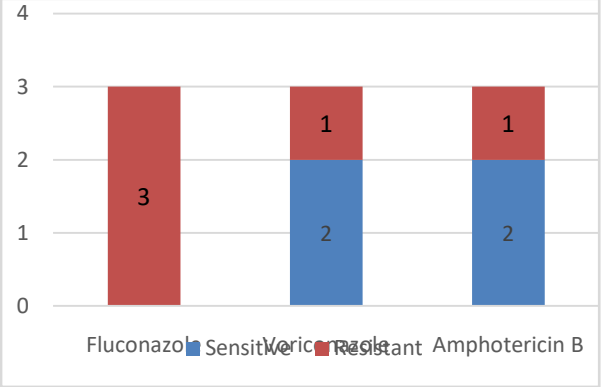


Figure 2: Antifungal susceptibility pattern in Candida glabrata



Discussion: The prevalence of VVC in our study is 28% and a similar prevalence rate has been observed in many recent studies. The prevalence rate of 24% was reported by Khan et al[10], 20% by Ahmed et al[11], 23.7% by Kalaiarasan et al[12] and 18.5% by Mohanty et al.[13] Much higher rates of around 40-50 % have also been reported in a number of studies from India.[14,15,16] Since VVC is more common in reproductive age group, majority of cases were in the age group of 21-30 years similar to other studies.[14] The findings of the present study reinforce the fact that the incidence of fungal infections are higher in the presence of various risk factors like diabetes, pregnancy, history of antibiotic intake etc.

Figure 3: Antifungal susceptibility pattern in Candida tropicalis

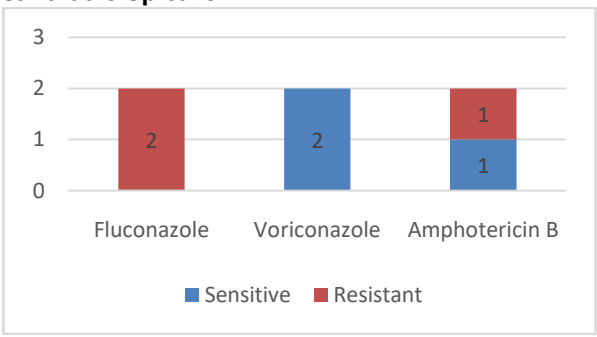
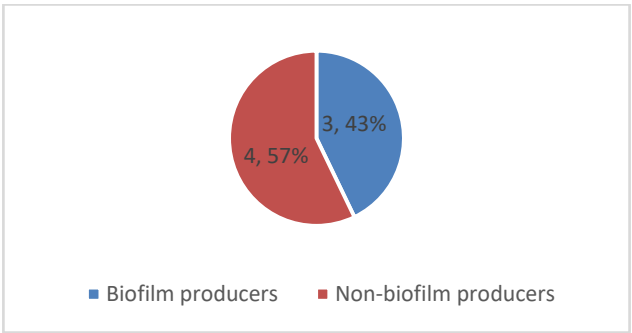


Figure 4: Biofilm production by Candida species in VVC



For many years, the predominant species isolated in candidiasis was C. albicans. However, recently there appears to have been a trend toward a greater prevalence of non-albicans species (NAC) such as C. glabrata, C. krusei, C. parapsilosis, and C. tropicalis in fungal cultures. In our study, majority of cases (71.4%) were due to NAC predominantly C. glabrata.

A study conducted by Kombade et al at AIIMS, Jodhpur reported that more than 80% of isolates were NAC with C. glabrata responsible for 58% of infections followed by C. albicans and C. tropicalis.[14]. A study at JIPMER, Puducherry by

Kalaiarasan et al reported C. glabrata (45.1%) to be the most common isolate followed by C. tropicalis (23.5% and C. albicans (17.6%).[12]Similarly, Mohanty et al at AIIMS, New Delhi also observed C. Glabrata (50.4%) to be the commonest isolate followed by C. albicans and C. tropicalis.[13] A number of other recent studies both in India (Deorukhkar et al[3],Tulasidas et al[17]) and worldwide (Khan et al[10]) also share similar findings . The emergence of NAC as a major contributor to VVC has grave therapeutic implications as these species show decreased susceptibility to all antifungals, particularly the azoles, which are generally used for the management of vaginal candidiasis. Tulasidas et al reported high resistance to fluconazole especially by C. glabrata (100%).[17] A number of other studies by Deorukhar etal,[3]Kalaiarasan et al,[12]Kombade et al[14] ,Patel et al[18] also echo similar views.

In our study also, a trend towards increased resistance to antifungals is observed especially in NAC species. As compared to NAC, C. albicans is still partially sensitive to fluconazole whereas both C. tropicalis and C. glabrata have developed complete resistance to fluconazole which may be due to their overuse during recent times. Fortunately, both C. albicans and C. tropicalis are still 100% sensitive to voriconazole although resistance has developed partially in C. glabrata. This implies that voriconazole is still effective but needs to be used rationally in VVC cases to avoid developing resistance to it also. Similarly, C. albicans is still sensitive to amphotericin B whereas NAC shows resistance partially. The fact that ours is a tertiary care hospital in a rural area, where chronic and complicated cases are treated, could explain the high incidence of resistance to antifungals. The patients would already have received these medicines intermittently before being referred to our hospital. This could also explain the higher prevalence of NAC in diagnosed cases.

In our study, 43% of candida species showed production of biofilm similar to the findings reported by tulasidaset al[17] (44%) and Thamke et al[19] (40%). The ability to form biofilms is regarded as one of the most powerful pathogenic characteristics, leading to treatment failure and the recurrence of infections.[20]

The major limitation of our study is relatively less sample size probably due to the limited span of data collection. Hence, further studies are needed with a much larger sample size to get more robust results in Indian scenario.

Conclusion: The prevalence of Vulvovaginal candidiasis in our study is 28% with highest incidence in the age group of 21-30 years. Risk factors reported in our study for VVC includes pregnancy, diabetes, history of antibiotic intake and poor personal hygiene. A pre-dominance of NAC was observed over C. albicans as causative species (C. glabrata-42.9%, C. tropicalis-28.6% and C. tropicalis-28.6%).

High incidence of resistance towards antifungals was observed especially towards fluconazole by NAC. Hence, voriconazole should be preferred as first line drug for the management of VVC instead of fluconazole in our setup but needs to be used judiciously. Biofilm production was observed in 43% of candida species which could be an important contributor towards increased incidence of antifungal drug resistance.

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