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Susceptibility Patterns of Vulvovaginal *Candida* isolates against Antifungal Drugs in a Tertiary Care Teaching Hospital, West Bengal, India

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Abstract: *Vulvovaginal candidiasis (VVC) is a common fungal infection among adult women during reproductive ages. This study was carried out to isolate Candida and to determine the species prevalence and the antifungal susceptibility of Candida species isolated from patients with VVC. A total of 239 women of reproductive age group (18-50years) with complaints of vaginal discharge and clinically diagnosed as vulvovaginal candidiasis were included in the study group. The study was conducted at Obstetrics and Gynaecology OPD (out patients department) of Malda Medical College, Malda and in the department of Microbiology of Malda Medical College, Malda. Two high vaginal swabs were collected from each patient, one for direct microscopy, another for culture on Sabouraud dextrose agar (SDA) with chloramphenicol. Isolates on SDA were identified and speciated using standard mycological tests – microscopic examination of Gram stained smear, germ tube test, sugar assimilation test and morphology on corn meal agar. Antifungal susceptibility testing was performed by modified Kirby-Bauer disc diffusion method. A total of 91grew Candida isolates giving a prevalence of 38.08% and highest numbers of isolates (53, 58.24%) were C. albicans followed by C. glabrata (17, 18.68%). Antifungal susceptibility testing showed that Candida isolates were more sensitive to Voriconazole, Itraconazole and Ketoconazole, compared to that of Fluconazole, Clotrimazole, Miconazole and Nystatin. Although Fluconazole still appeared to be active against C. albicans, high resistance rate of Non-albicans Candida (NAC) spp. against the drug may support a search for alternative antifungal drugs when treating VVC caused by NAC spp.*

Keywords: *Vulvovaginal candidiasis (VVC), women of reproductive age, antifungal susceptibility testing, disc diffusion method*

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I. Introduction

Vulvovaginal candidiasis (VVC) is a common fungal infection among adult women during reproductive ages. It has been estimated that 75% of all adult women experience at least one period of vulvovaginal candidiasis in their lifetime.[1]The risk of VVC is high in women with diabetes mellitus, Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS), oral contraceptives, using broad spectrum antibiotics therapy, pregnant women and women involved in receptive oral sex.[2] Pregnant women are the most predisposed to severe vulvovaginal candidiasis as a result of lowered immunity and this severity may involve among others chorioamnionitis which eventually leads to preterm labour.[3] According to available data, preterm birth is the leading cause of neonatal mortality worldwide.[4] The principal agent of VVC is *Candida albicans*, but other species known generally as C. non-albicans (*C. glabrata*, *C. tropicalis, C. krusei, C. parapsilisis, C. gullermondii*) are also isolated.[5] The inappropriate use of antifungal drugs and introduction of over-the-counter antimycotics in countries worldwide predispose development of antifungal resistance.[6] Understanding of the antifungal susceptibility patterns of pathogenic fungi is key in guiding appropriate therapy selection for mycoses.[3] A few studies have focused on the correlation of antifungal susceptibility with clinical results in VVC.[7] Therefore, the aim of this study is to isolate *Candida* from VVC patients, speciation of *Candida* isolates and to determine their susceptibility patterns to the commonly used antifungal drugs in a tertiary care hospital, West Bengal, India.

II. Aims And Objectives

1) To determine the frequency of *Candida* species isolation from VVC and speciation of *Candida* isolates
2) To assess their antifungal susceptibility patterns to commonly used antifungals, including Fluconazole, Ketoconazole, Voriconazole, Itraconazole, Clotrimazole, Miconazole and Nystatin.

III. Materials And Methods

The study was planned as a cross-sectional observational study. A total of 239 women of reproductive age group (18-50years) with complaints of vaginal discharge, itching, dyspareunia, low backache and pain in the lower abdomen and clinically diagnosed as vulvovaginal candidiasis and willing to provide written informed consent, were included in the study group. Women of reproductive age group (18-50years) with clinical symptoms of vulvovaginal candidiasis, unwilling to provide written informed consent, with history of vaginal bleeding and pregnant, were excluded from the study. The study was conducted at Obstetrics and Gynaecology OPD (out patients department) of Malda Medical College, Malda and in the department of Microbiology of Malda Medical College, Malda, from September 2017 to August 2018. Ethical clearance was obtained from the institution. An informed consent form was obtained from the participants after the contents of the form were clearly explained.

Two high vaginal swabs were collected from each patient, one for direct microscopy, another for culture. Direct microscopic examination of KOH (10%) mount and Gram stained smear were done and examined for the presence of budding yeast cells and pseudohyphae. The other one was inoculated on Sabouraud dextrose agar (SDA) with chloramphenicol (Hi Media Mumbai, India) and was incubated at 25^{o}C and at 37^{o}C for 48 to 72 hours aerobically. Colonies appeared within 1-3 days as creamy white, smooth and pasty. Isolates on SDA were identified and speciated using standard mycological tests – microscopic examination of Gram stained smear, germ tube test, sugar assimilation test and morphology on corn meal agar. The isolates on *Candida* CHROM agar were identified by noting the color of the colonies. Antifungal susceptibility testing was performed by modified Kirby-Bauer disc diffusion method using Fluconazole (25mcg), Ketoconazole (10mcg), Voriconazole (1mcg), Itraconazole (10mcg), Clotrimazole (10mcg), Miconazole (10mcg) and Nystatin (50mcg) [Hi-Media Mumbai, India] on Muller-Hinton Agar (MHA) (Hi-Media), supplemented with 2% Glucose and Methylene Blue dye 0.5 mcg/ml (GMB) as per the CLSI-M44-A2 guidelines.[8] After overnight incubation, zone of inhibition were noted and categorized in susceptible and resistance compared with the standard zones interpretive breakpoints published by CLSI M44-A2 and Rosco Diagnostica.[8,9]

Antifungal disc	Susceptible Zone(mm)	SDD Zone(mm)	Resistant Zone(mm)
Fluconazole	≥19	15-18	≤14
Ketoconazole	≥28	21-27	≤20
Voriconazole	≥17	14-16	≤13
Itraconazole	≥23	14-22	≤13
Clotrimazole	≥20	12-19	≤11
Miconazole	≥20	12-19	≤11
Nystatin	≥15	10-14	≤10, no zone

*SDD - Susceptible dose dependent

IV. Results

A total of 239 clinically suspected cases of VVC were presented during the study period. Out of 239 samples, 91 (38.08%) were culture positive for *Candida* and 148 (61.92%) were negative for *Candida* spp. As shown in Table1, highest frequency of culture confirmed cases of VVC were found in the age group of 26-35 years (43, 47.25%) followed by 36-45 years (27, 29.67%), 18-25 years (17, 18.68%) and 46-50 years (4, 4.40%).

In our study, diabetes mellitus (13, 14.29%) was the most common risk factor for VVC followed by broad spectrum antibiotic usage (11, 12.09%). Other risk factors were IUCDS (8, 8.79%) and usages of oral contraceptive pills (5, 5.49%). (Table 2)

Out of 239 high vaginal swabs cultured, 91grew *Candida* giving a prevalence of 38.08%. In our present study, highest numbers of isolates (53, 58.24%) were *C. albicans* followed by *C. glabrata* (17, 18.68%), *C. tropicalis* (14, 15.38%), *C. krusei* (4, 4.40%) and *C. parapsilosis* (3, 3.30%) as shown in Table 4. The overall rate of non-albicans *Candida* (NAC) was 41.76% (38/91) (Table 3) and among the NAC spp., *C. glabrata* (17/38, 44.74%) was the commonest isolate followed by *C. tropicalis* (14/38, 36.84%). Though C. albicans remained the predominant species isolated in the present study, NAC were isolated in considerable proportions.

In this study, antifungal susceptibility testing was done using Fluconazole (25mcg), Ketoconazole (10mcg), Voriconazole (1mcg), Itraconazole (10mcg), Clotrimazole (10mcg), Miconazole (10mcg) and

Nystatin (50mcg). Antifungal sensitivity pattern of C. albicans and non-albicans *Candida* (NAC) are shown in Table 5 and 6.

V. Discussion

Vulvovaginal candidiasis (VVC) remains one of the most common infections of the female genital tract. In the present study, out of 239 high vaginal swabs, 91 (38.08%) *Candida* isolates were found. This finding is in concordance with the study by Muthusamy S. et al (39.23%).[10] The highest frequency of culture confirmed cases of VVC were found in the age group of 26-35 years (47.25%), followed by the age group of 36-45 years (29.67%). Similar findings were observed by Dharmik P.G et al[11] and Laksmi N et al.[12]

In our study, major risk factors for VVC were Diabetes mellitus (13, 14.29%), broad spectrum antibiotic usage (11, 12.09%), use of IUCDS (8, 8.79%) and oral contraceptive pills (5, 5.49%). Our result coincides with studies of Lakshmi N et al[12] and Matehkolaei AR et al.[13]

In our present study, highest numbers of isolates (53, 58.24%) were *C. albicans* followed by *C. glabrata* (17, 18.68%). Our findings were similar to other studies publishing the highest occurrence of C. albicans followed by C. glabrata.[3,10,13] But this finding has been contraindicated by the studies done by Lakshmi N et al[12] and Mohanty S et al[14] which showed that C. parapsilosis (33.3%) and C. glabrata (50.4%) were the predominant isolates respectively followed by C. albicans.

In our study Fluconazole had shown 79.25% (42/53) sensitivity against *C. albicans*, which is quite comparable with study of Gandhi et al[5] (78%) and Babin et al[15] (76%), while study done by Ajitha et al[16] and Emam et al[17] showed higher sensitivity rate of 93.3% and 96.7% respectively. Fluconazole was 75% resistant against *C. krusei* in this study which was comparable to study of Muthusamy et al [10] (75%) while in study of Deorukhkar et al[18] Fluconazole showed 18.8% resistance, in Gandhi et al[5] 33% resistance and in Adesiji et al [19]100% resistance. Overall, Fluconazole was 72.53% (66/91) sensitive, while in study of Adesiji et al [19] Fluconazole showed 7.8% sensitivity , in Kelen Dota et al[20] 35.5% and in Dharmik et al[11] 97.2% sensitivity.

In our study, overall ketoconazole resistance was noted in 17.58% (16/91) isolates while it was 32.9% in study by Deorukhkar et al.[18] Ketoconazole had shown maximum resistance against *C. krusei* in our study as in other study .[5]

Voriconazole had shown 92.45% sensitivity against *C. albicans* which was comparable to Doddaiah et al (91.4%) [21] and Luo X et al (93.2%).[22] In the present study, *C.albicans* and majority of *NAC* species were found susceptible to voriconazole and overall sensitivity was 92.31% (84/91).

In our study, Itraconazole was 88.68% (47/53) sensitive among *C. albicans* which was comparable to study of Luo X et al[22] (85.8%), but in study of Gandhi et al[5] Itraconazole showed 52% and in Lakshmi et al[12] 56% sensitivity. In the study the overall Itraconazole sensitivity was 85.71% (78/91) which was comparable to study of Muthusamy et al[10] (83.34%).In this study Clotrimazole was 79.12% (72/91) sensitive in all *Candida* isolates which was quite comparable with Gandhi et al and Dharmik et al.[5,11]

In our study, Miconazole sensitivity was 56.04% (51/91) among all *Candida* spp. This finding was higher than Dharmik et al[11] (37%) but lower than Zahra et al[1] (89.6%).

Nystatin was 64.15% (34/53) sensitive among C. albicans in our study while the sensitivity was 100% in Emam et al[17]and only 26.6% in Khan et al.[23]

VI. Tables And Figures

Table 1: Age wise distribution of VVC cases

Age in years	Number	Percentage (%)
18-25 years	17	18.68
26-35 years	43	47.25
36-45 years	27	29.67
46-50years	4	4.40
Total	91	100

Table 2: Risk factors associated with VVC

Risk factors	Number (%)
Diabetes mellitus	13 (14.29)
Antibiotic usage	11 (12.09)
Pregnancy	0
IUCDS	8 (8.79)
Oral contraceptive pills	5 (5.49)

*****Pregnant women were excluded from the study

Table 3: Distribution of *C.albicans* and non albicans *Candida* (NAC)

C.albicans (%)	NAC (%)
53 (58.24)	38 (41.76)

Table 4: Distribution of different species of *Candida*

Candida isolates	Number n=91	Percent (%)
C.albicans	53	58.24
C.glabrata	17	18.68
C.tropicalis	14	15.38
C.krusei	4	4.40
C.parapsilosis	3	3.30

Table 5: Species wise antifungal susceptibility pattern

Antifungal agents	*Candida* species				
	C. albicans n=53	*C. glabrata* n=17	*C. tropicalis* n=14	*C. Krusei* n=4	*C. parapsilosis* n=3
Fluconazole					
S	42(79.25%)	11(64.71%)	9(64.29%)	1(25%)	2(66.67%)
R	11(20.75%)	06(35.29%)	5(35.71%)	3(75%)	1(33.33%)
Ketoconazole					
S	49(92.45%)	11(64.71%)	11(78.57%)	2(50%)	2(66.67%)
R	4(7.55%)	6(35.29%)	3(21.43%)	2(50%)	1(33.33%)
Voriconazole					
S	49(92.45%)	16(94.12%)	13(92.86%)	03(75%)	03(100%)
R	04(7.55%)	01(5.88%)	01(7.14%)	01(25%)	0
Itraconazole					
S	47(88.68%)	15(88.24%)	12(85.71%)	02(50%)	02(66.67%)
R	06(11.32%)	02(11.76%)	02(14.29%)	02(50%)	01(33.33%)
Clotrimazole					
S	41(77.36%)	16(94.12%)	11(78.57%)	01(25%)	03(100%)
R	12(22.64%)	01(5.88%)	03(21.43%)	03(75%)	0
Miconazole					
S	31(58.49%)	11(64.71%)	06(42.86%)	01(25%)	02(66.67%)
R	22(41.51%)	06(35.29%)	08(57.14%)	03(75%)	01(33.33%)
Nystatin					
S	34(64.15%)	15(88.24%)	11(78.57%)	02(50%)	03(100%)
R	19(35.85%)	02(11.76%)	03(21.43%)	02 (50%)	0

Table 6: Antifungal susceptibility pattern of *Candida* spp.

Antifungal drugs	Sensitivity (%)	Resistant (%)
Fluconazole	71.43	28.57
Ketoconazole	82.42	17.58
Voriconazole	92.31	7.69
Itraconazole	85.71	14.29
Clotrimazole	79.12	20.88
Miconazole	56.04	43.96
Nystatin	71.43	28.57

Figure 1: Gram stained smear from growth on SDA

Figure 2: Germ Tube Test in C. albicans

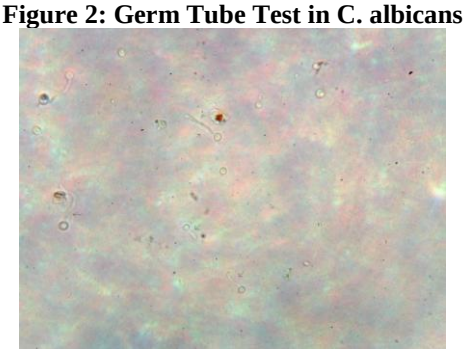


Figure 3: *Candida* species on CHROM agar

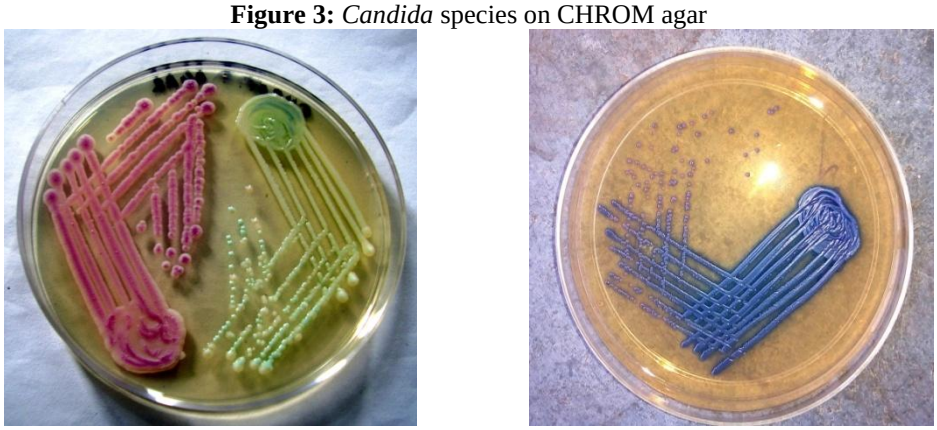
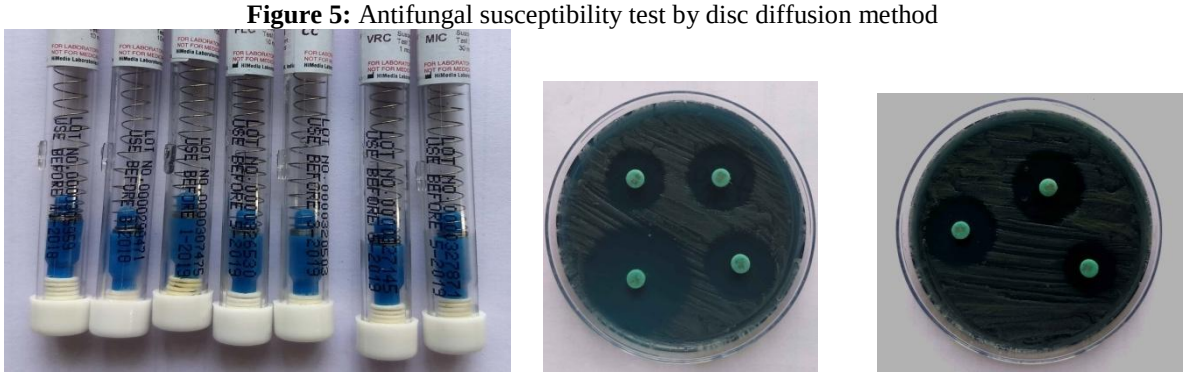


Figure 4: Speciation of *Candida* by carbohydrate assimilation

Figure 5: Antifungal susceptibility test by disc diffusion method



VII. Conclusion

High prevalence rate of vulvovaginal candidiasis and observation of a high prevalence rate of non-albicans *Candida* species recommends routine antifungal susceptibility testing of *Candida* isolates. Precise identification of *Candida* at the species level and judicious use of antifungal agents is very important for the prevention of emergence and spread of drug resistant *Candida* species and to avoid treatment failures.

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