

Isolation, Identification and Anti-Fungal Susceptibility of Candida Species from Clinically Suspected Cases of Vulvovaginitis in a Tertiary Care Hospital in Rural Area – A Cross-Sectional Study

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Abstract

Aim/Objectives: To determine the predominant candidal species in Vulvovaginal Candidiasis. To find out the antifungal susceptibility pattern of the candidal isolates and to assess the risk factor associated with Vulvo-Vaginal Candidiasis (VVC).

Methods: A total of 100 high vaginal swabs (HVS) were collected from women suspected of having Candidal Vulvovaginitis from May 2014 to February 2015. Wet mount preparations in potassium hydroxide (KOH) and Gram-stained smears were performed directly on specimens. Cultures for Candida species were done using Sabouraud's dextrose agar (SDA) at room temperature (25-28°C). Antifungal susceptibility of the Candida species to Amphotericin B, Fluconazole and Ketoconazole was assessed using the NCCLS guidelines on Mueller Hilton agar.

Results: The incidence of non albicans (60%) was significantly higher than the Candida albicans (40%) which was found to be sensitive to Fluconazole (95%) followed by Amphotericin B (85%). The common presenting symptoms of vulvovaginitis found to be thick white vaginal discharge followed by itching pruritus.

Conclusion: The predominant candidal species in vulvovaginitis was non-albicans (candida tropicalis). Candida was most sensitive to fluconazole.

Keywords: Vulvovaginal candidiasis; Candida tropicalis; Antifungal sensitivity.

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Introduction

The Reproductive tract infections, which may be communicable or non-communicable, are caused by a wide range of bacterial, protozoal, fungal and eco parasites.¹ They are a significant health problem as they cause wide spread mortality and morbidity in women especially during reproductive age, particularly in developing countries where Renal Tract Infections (RTIs) are endemic.^{2,3}

The reason for the high incidence in this age group includes low levels of protective cervical antibodies, increased sexual activity, and new influence of reproductive hormones which may lead to increased susceptibility to infections.⁴

Vaginal Candidiasis is very common yeast infection of the vagina and is caused by fungal micro-organisms. Fungi are eukaryotic organisms with approximately 300000 different species.⁵ Of these, about 200 are potential parasites, with only a few of these affecting humans.⁶ Of the Candida species

afflicting humans, *Candida albicans* is by far the most common.^{6,7}

Candida albicans is a normal commensal of the reproductive tract that is always present in small amounts.⁷ However, when an imbalance occurs such as the normal acidity of the vagina, changes or when hormonal balance changes, candida can multiply.⁸ When that happens, symptoms of Candidiasis appear.^{8,9}

Several studies have reported that among women with acute VV Candidiasis, *C. albicans* accounts for 80-90% of the isolated fungal species, whereas other species are less frequently isolated.^{9,10} In recent years, there appears to have been a trend toward a greater prevalence of non-albicans species such as *C. glabrata*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis* in fungal cultures.¹¹

Women who are diagnosed with recurrent Vulvovaginal Candidiasis usually respond favourably to antifungal therapies with very little resistance.¹²

Clinically, VVC is described as consisting of white, curdy, vaginal discharge which is probably associated with irritation and/or itching of the genital area, inflammation (redness and swelling caused by the presence of extra immune cells) of the labia majora, labia minora, or perineal area, pain and irritation with sexual intercourse.¹³ The patient should be asked about specific symptoms and their duration, any previous diagnosis and previous treatment and its effects. A general medical review, dermatologic review, social history and contraceptive history can also be helpful.¹⁴

There are very few studies from rural area of Northern India reporting prevalence and type of Candidal Vulvovaginitis. Hence, the present study was undertaken to identify the predominant candidal species in clinically suspected cases of Vulvovaginitis and to assess risk factors and the best possible treatment in this rural area of Haryana.

Material and Methods

A cross-sectional study was carried out in the Department of Microbiology, MMIMSR, Mullana on 100 clinically suspected cases of Vulvovaginitis attending gynaecology OPD of all age groups. Before the commencement of the study Ethical clearance was taken from institutional ethical committee. An informed written consent was obtained from the subjects taking part in the study after explaining the purpose of the study. Samples of high vaginal swabs (swab is inserted into upper part of vagina and rotated there before withdrawing it, so that exudate is collected from upper as well as lower vaginal wall) were collected with the help of gynaecologist under aseptic condition. The specimens were immediately transported in a sterile tube containing normal saline to the microbiology laboratory for analysis. The high vaginal swab was subjected to: KOH mounting, Gram staining, Culture (Sabouraud's dextrose agar (SDA) without antibiotics, Sabouraud's with antibiotics and Cycloheximide). SDA slants were incubated at 37°C for 7 days to 4 weeks as per standard guidelines. Colony Characteristics were also examined on Sabouraud's dextrose agar conformed by Gram staining and Species confirmation was done by morphology on CHROM agar and Germ tube test.

Antifungal susceptibility to Fluconazole (30mcg) Amphotericin B (50mcg) and Ketoconazole (30mcg) was tested by Disc diffusion method, as recommended by CLSI M-44A guidelines on Mueller Hilton agar.¹⁵

Results

Out of total 100 subjects, 20 (20%) were reported positive with Vulvovaginal Candidiasis. The most common (60%) cause of Vulvovaginal Candidiasis was Candida Non Albicans, which had bluish colour with pink halo on CHROM agar which confirmed it as Candida tropicalis.

The majority (95%) of the symptoms in the positive Vulvovaginal Candidiasis cases were itching pruritus. Computed chi-square value for the symptoms of Vulvovaginal Candidiasis i.e. Odourless discharge ($p=0.001$), itching pruritus ($p=0.000^*$) and dysuria ($p=0.000^*$) were found to be significant at 0.05 level of significance. The most common associated conditions among the positive VVC cases were Pregnancy (35%), followed by Diabetes (30%), Thyroid (25%) and History of antibiotic intake (10%).

Majority (70%) of cases were in the age group of 21-30 years and belonged to low socio-economic status. Majority (44.5%) were poorly educated (Primary education and below). These were found to be the common risk factors associated with VVC.

Both the species of Candida albicans (40%) and Candida tropicalis (60%) were found to be highly sensitive to Fluconazole followed by Ketoconazole. The calculated p value of 0.00 (<0.05) was statistically significant.

Table 1: Number of Positive samples of VVC with Different Diagnostic Methods (n = 100)

S. No.	Diagnostic Method	No. of Positive samples	% age of Cases
1.	KOH Mount (Budding yeast cells with pseudohyphae)	20	20%
2.	Culture on SDA	20	20%
3.	Gram stain (Gram positive budding yeast cells)	20	20%
	Total patients diagnosed with VVC	20	

The data presented in Table 1 shows that all of the positive cases of VVC have the fungal element when tested with KOH Mount (Budding yeast cells with pseudohyphae) followed by Culture on SDA (creamy, white, pasty colonies) and Gram Staining (Gram positive budding yeast cells). Out of 20 positive Vulvovaginal Candidiasis cases only 8 (40%) of the cases showed germ tube projections.

Table 2: Distribution of candidal species on chrom Agar

Test	Candida Albicans		Candida Non- Albicans	
	No.	(%)	No.	(%)
CHROM Agar	8	40	12	60
Total	20	100	20	100

Table 3: Symptoms of vulvovaginitis among all cases of suspected VVC

S. No	Symptoms		No. (%) of Cases		Chi Square	df	p-value
			Candia Positive (n=20)	Candida Negative (n=80)			
1.	Discharge	Foul Smelling	4(20)	48(60)	8.716	1	0.001*
		Odourless	16(80)	32(40)			
2	Itching Pruritis		19(95)	35(44)	14.918	1	0.000*
3	Dysuria		19(95)	34(43)	15.659	1	0.000*

df=1 *Significant ($p \leq 0.05$) ^{NS}= Not Significant ($p > 0.05$)

Table 4: Antifungal susceptibility testing patterns

Species	No.	Amp B		FCA		KET	
		S	R	S	R	S	R
C. albicans	8	6	2	7	1	6	2
C. tropicalis	12	11	1	12	0	11	1

Amp B – Amphoterecin B

FCA – Fluconazole

KET – Ketoconazole

S – Susceptible R - Resistant

Discussion

While looking at the species distribution in present study it was worth noting that, among 20 positive cases of vulvovaginal candidiasis, 12 (60%) were caused by non albicans species in which all were Candida tropicalis. The findings were consistent with the previous study conducted by Lata R Patel et al (2012)¹⁶ that demonstrated out of 430 positive culture isolates majority (62.6%) were the non albicans species. Candida tropicalis was isolated from 49% of non albicans. This result also supports the study conducted by Sachin C et al (2014)¹⁷ which also concluded that candida species was isolated from 307 high vaginal swabs in which 72% belonged to non albicans species.³³ The results also revealed that the symptoms of the VVC were common in majority of the cases but the symptoms were more pronounced in the cases caused by Candidal species. The rise in VVC infections and more specifically in non-albicans species could be due to several factors ranging from an increase in over-the-counter antifungal use to an increase in high-risk patient populations (i.e. diabetics and menopausal women).¹² For long, Candida albicans was by far the most common species isolated in candidiasis. But now predominance of non albicans is occurring among which Candida tropicalis is on peak (67-90%) and is the commonest cause of Vulvovaginal Candidiasis.^{11,12}

The finding in the study revealed that, majority of the VVC positive cases belonged to lower socio-economic class (class II or below) with monthly income less than Rs. 5000. This could also be attributed to the location of the tertiary hospital which is located in a rural area. We observed the high rate of VVC which is also supported by similar findings in an

epidemiological study conducted by Jindal et al (2007).¹⁸

Conclusion

The predominant candidal species in vulvovaginitis was non-albicans (Candida tropicalis). Candida was most sensitive to fluconazole.

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