

Study of Vulvo-Vaginal Candidiasis among Patients Visiting Obstetrics and Gynecology Department in a Tertiary Care Centre

Bhavana J¹, Yadukul S.^{2*}, Anitha KB³

¹Assistant Professor, MVJ Medical College, Bengaluru, ³Associate Professor, Dept. of Microbiology, AJ Institute of Medical Sciences, Mangalore, ²Assistant Professor, Dept. of Forensic Medicine & Toxicology, Chamarajanagar Institute of Medical Sciences, Chamarajanagar

***Corresponding Author:**

Email: dr.kooooool@gmail.com

Abstract

Background: Vulvovaginitis is a common day to day problem in gynecological practice. *Candida albicans* is the commonest infectious cause. An estimated 75% of women have at least one episode of Vulvo-vaginal Candidiasis and 40%–45% will have two or more episodes within their lifetime. The main objective of this study is to find out the incidence of Vulvo-vaginal Candidiasis among patients visiting Obstetrics and Gynecology Department of A.J. Institute of Medical Sciences, Mangalore.

Methods: Vaginal swabs were collected from 100 patients visiting the hospital with history of white discharge and subjecting it to microscopy and culture. *Candida* isolates were further identified to species using conventional methods.

Results: *Candida* as the causative agent of Vulvovaginitis was noted in 34% of cases. Majority of the women infected belong to 20-39 years group. The predominant species isolated was *C.albicans* 65%. Non-*albicans* species included *C.tropicalis* 24%, *C.krusei* 5%, *C.glabrata* 3% and *C.parapsilosis* 3%.

Conclusion: The predominant species isolated was *C.albicans*. Other non-*albicans* *Candida* species isolated in this study were *C.tropicalis*, *C.krusei*, *C.glabrata* and *C.parapsilosis*.

Keywords: Vulvovaginal Candidiasis; Incidence; White Discharge.

Access this article online	
Quick Response Code:	Website: www.innovativepublication.com
	DOI: 10.5958/2455-6807.2016.00006.4

Introduction

Candida infections have been reported from all parts of the world and occur in all ages and races. *Candida* species are the most common cause of fungal infections leading to a range of non-life-threatening mucocutaneous to life-threatening invasive diseases. Among *Candida* species, *Candida albicans* is the most common infectious agent, and it is also a commensal that colonizes skin, the gastrointestinal and the reproductive tracts¹. The majority of patients, notably immunosuppressed individuals, experience some form of superficial mucosal candidiasis, most commonly thrush, and many suffer from recurrent infections. In developed countries, where medical therapeutics is commonly used, *Candida* species are now among the most common nosocomial pathogens². Common species that cause disease in human are *C.albicans*, *C.guilliermondii*, *C.krusei*, *C.parapsilosis*, *C.tropicalis*, *C.kefyr*, *C.lusitaniae*, *C.dublinskiensis*, and *C.glabrata*³.

Vulvovaginitis is a common day to day problem. Vaginal candidiasis is known variously as Vulvo-Vaginal Candidiasis (VVC), candidal vaginitis,

monilial vaginitis, monilia infection, vaginal yeast infection or thrush. An estimated 75% of women have at least one episode of VVC, and 40%–45% will have two or more episodes within their lifetime. On the basis of clinical presentation, microbiology, host factors, and response to therapy, VVC can be classified as either uncomplicated or complicated. Approximately 10%–20% of women will have complicated VVC that necessitates diagnostic and therapeutic considerations^{1,4,5}. The incidence increases in particular group, such as pregnancy, diabetic women, those on oral contraceptives, etc⁶. In many women a true vaginal Candidal infection may remain unrecognized. False-positive microscopic examinations are also possible and are probably more common than generally suspected. It is also possible to have *Candida* Vulvovaginitis with a false-negative *Candida* culture. At least 3000 organisms/ml are necessary to obtain a positive culture⁷.

Objectives

1. To calculate the incidence of Vulvo-vaginal Candidiasis among patients visiting Obstetrics and Gynecology Department of A.J. Institute of Medical Sciences, Mangalore.
2. Speciation of the isolate by conventional method and to know the incidence of *Candida* species causing vulvo-vaginitis.

Materials and Methods

The present study was done in the Department of Obstetrics and Gynaecology, AJ Medical College, Mangalore.

Patient: A detailed history was taken with a particular emphasis on the pregnancy, high risk behaviors, contraceptive methods used, recent antibiotic treatment, past history of chronic diseases such as Diabetes mellitus, economical status, hygiene etc. Thorough physical examination was carried out. Vital signs were noted. Presence of signs of other opportunistic infection was looked for and associated diseases if any were recorded. Each woman was subjected to per speculum examination, and the clinical characteristics of the vaginal discharge were noted. Written informed consent was obtained from each patient.

Inclusion Criteria: The inclusion criteria for the study were married women, between 18-65 years of age who presented to the health care center with self-reported symptoms of vaginal discharge and/ genital itching and/or genital burning during the study period.

Exclusion Criteria: Women with severe medical disorders requiring immediate referral to higher level of healthcare, women who were currently menstruating, who have never been sexually active, women who had taken a course of antifungal during past three weeks and who had been previously enrolled in this study were excluded.

Control: Hundred age matched controls were included in the study to know the prevalence of colonization of Candida in healthy individuals. Samples of the vaginal discharge collected were processed as follows:

Sample collection: Two samples of the high vaginal secretions were taken with aseptic precautions with the help of sterile cotton swabs. One swab was used for pH evaluation and for direct microscopy and the other swab was used for inoculation onto Sabourauds Dextrose Agar with Chloramphenicol (Himedia).

Culture: SDA slants were incubated at 37^o C for 48-72 hours and observed for growth. Those slants which were showing no growth after 72 hours were recorded as no growth and discarded. The SDA slants with growth were observed for colony morphology and were further processed for speciation.

Test Procedures

- a. **Direct microscopy:** Examination of 10% KOH preparation of the vaginal discharge was done. Samples were subjected to Gram's stain to look for presence of Gram positive yeast like budding cells with pseudo-hyphae.
- b. **Culture:** The specimen was inoculated onto SDA plates with chloramphenicol and incubated at 37^oC

for 48-72 hrs. The plates were observed for growth and colony morphology was studied.

- c. **Germ Tube Test:** Using a Pasteur pipette, 3 drops of fresh pooled human serum was dispensed into test tubes. With a sterile straight nichrome wire the yeast colonies were taken and emulsify into the serum & Incubated at 35^o C for 2-4 hours. A drop of suspension was placed on clean microscope slide. A sterile glass cover slip was placed over the suspension, and then examined under microscope using low power objective and high power objective. The germ tubes are seen as long tube like projections extending from the yeast cells, without any constrictions at the point of attachment to the yeast, as seen in case of pseudo-hyphae. The Germ tube is formed within 2 hours of incubation in *C.albicans* and *C. dubliniensis* and not in any other species of this genus. The demonstration of Germ tube is also known as Reynolds-Braude Phenomenon.
- d. **Corn Meal Agar Test (Dalmau Plate):** With a sterile straight wire, a heavy inoculum of yeast was streaked across the plate containing medium (ploughing). Cover slip was placed over it in such a way that the streak lines project beyond the cover slip & Incubated at 22-26^oC in dark for 3 days. It is examined under microscope by placing the agar plate without its lid on the stage and observing under the low power and high power objectives. The edge of the cover slip was examined, since this region is the junction of aerobic and relatively anaerobic conditions.
- e. **Sugar Assimilation Test:** Suspension of yeast in sterile distilled water of density McFarland no 4 standard (1% Barium chloride: 0.4ml, 1% Sulfuric acid: 9.6ml, cell density: 12×10⁸ CFU/mL, 21.5% Transmittance) was prepared. Yeast nitrogen based agar plates were covered with this suspension and the excess inoculum was removed, surface of plates were allowed to dry. Dextrose, maltose, sucrose, Lactose, Galactose, Melabiose, Celebiose, Inocitol, Xylose, Rafinose, Trehalose discs were used. With sterile forceps, selected carbohydrate discs were placed on the surface of agar 30 mm apart and incubated at 30^o C for 24 -48 hours. Assimilation of a particular sugar interpreted by the growth around the disc.
- f. **Chrom Agar:** Yeast colonies were streaked onto the CHROM agar and incubated at 37^oC for 72 hours. The various colored colonies produced by different species of Candida on CHROM agar were noted and species were identified accordingly.

The data was initially arranged into tables for discussion under different headings. Descriptive statistical analysis was carried out on these data. Results on continuous data are presented as mean+Standard deviation and results as categorical measurements are presented in number and Percentage.

All the percentages were approximated to the unit value. Conclusions were drawn based on the outcome of these statistical treatments. Chi square analysis, a method of treating the significance difference of two portions was used. This test has an advantage of analyzing more than two groups to be compared.

A total of 100 patients (study group) visiting A.J. Institute of Medical Sciences to the outpatient block and also admitted to Gynecological wards, having complaints of white discharge per vagina were included in this study based on the inclusion and exclusion criteria. Hundred age matched controls were also included for the study. The data was analyzed using appropriate statistical methods and discussed here after.

Results and Observation

The age (in years) ranged from 18 to 65 years. The majority of the cases with white discharge per vagina were seen in the age group of 30 to 39 years (38%) followed by 20-29 years (32%) as shown in (Table 1) The mean+SD of age(in years) in the study group was 33.27+10.7 years and in the control group was 34+10.2 years ($p > 0.05$). Among the locale distribution of study group, about 55% of the patients belonged to rural area, and 45% were from urban area (Table 2). Table 4 depicts that about 29% of the cases had infection due to Candida and 28% showed bacterial causative. 5% of cases showed both mixed growth of Candida and bacteria. About 38% of the cases showed no growth (Table 3). Among the cases positive for vaginal candidiasis, most common clinical symptom in all the study group was white discharge per vagina (100%) followed by pruritus in 23(65%) of cases (Table 4). This study shows that about 24% of the patients with vaginal candidiasis had similar complaints in the past and had been treated for the same.

In this study, 22 pregnant women complaining of white discharge per vagina among which 7 of the cases showed growth of Candida, of 12 women were on oral contraceptives of these 3 women had candidiasis. 2 of 7 of the patients being treated for infertility, of which 2 of the cases showed growth of Candida, 1 out of 5 patients on intra uterine devices, showed candidial growth. 14 patients had undergone Hysterectomy in the past had complaints of white discharge per vagina, of them 4 patients were Candida positive. This study showed one among two patients with confirmed HIV infection had vaginal candidiasis. 3 of 12 patients, who had recently completed or presently on prolonged antibiotic therapy for various causes showed Candida positive (Table 5). In this study, the relationship between multiple sexual partners and the prevalence of vaginal candidiasis could not be analyzed, as none of the patients on history taking revealed any such detail. The most common clinical sign on examination (Table 6) was leucorrhoea

seen in all the patients (100%) followed by excoriation seen in 12 patients (35%).

100 High vaginal swab samples collected from the study group, when subjected to Gram staining 32 samples showed Gram positive yeast like budding cells with/ or without pseudohyphae. On culture 34 samples grew Candida. Of 100 samples collected from the control group 2 sample showed Candida on Gram staining, and 4 samples were culture positive (Table 7). This study shows that there is no significant difference between the Grams staining method and isolation of Candida by SDA culture. ($\chi^2 = 0.431$) $p = 0.5$ which is not significant. The Candida isolates from 34 samples growth on Sabouraud's Dextrose agar were subjected to the Germ tube test, 24 samples (71%) were found to be positive indicating the presence of *C.albicans*/*C.dubliniensis* and 13 samples (29%) remained negative (Table 8).

On inoculating the Candida isolates on the CHROM agar media, 24 isolates showed light green color indicative of *C.albicans*, Dark blue color was seen 9 isolates suggestive of *C.tropicalis*, Pale pink colored colonies of *C.krusei* was seen in 2 cases and Two of isolates showed cream – pale white color colonies, suggestive of *C.glabarata*/*C.parapsilosis*/*C.gulliermondii*/*C.stellatoidea* species. Among these, isolates from 3 samples showed colonies of more than one color, suggestive of mixed infection.

- a. One of these showed light green and pale pink colonies were seen suggestive of *C.albicans* and *C.krusei* mixed infection.
- b. Two of the cases showed light green color colonies and dark blue color colonies indicating mixed infection due to *C.albicans* and *C.tropicalis* (Table 9).

Hence from 34 cases of vaginal candidiasis, total number of isolates was 37. All 37 isolates were subjected to Cornmeal agar test; majority showed presence of chlamyospores (65%) suggestive of *Candida albicans*/*C.dubiniensis*/*C.stellatoidea* (Table 11). The sugar assimilation pattern of the isolates correlated with results as in CHROM agar identification and corn meal test (Table 12).

Table 1: Age (in years) distribution among study and control group

Age(in years)	Study group		Control	
	Number	Percentage	Number	Percentage
Below 20	8	8 %	8	8%
20-29	32	32%	32	32%
30-39	38	38%	38	38%
40-49	12	12 %	12	12%
50 and above	10	10%	10	10%
Total	100	100%	100	100%

Table 2: Locale distribution in the study group

Locale	Number	Percentage
Urban	45	45%
Rural	55	55%

Table 3: Literacy status of the study group

Literacy status	Number	Percentage
Illiterate	13	13%
Literate up to 5 th std	60	60%
Up to 12 th std	21	21%
Graduate and above	06	6%

Table 4: Leucorrhoea due to various causatives based on culture

	Number	Percentage
Candida infection	29	29%
Bacterial infection	28	28%
Mixed infections	5	5%
No growth	38	38%

Table 5: Clinical presentation

Manifestations	Number	Percentage
White discharge	34	100 %
Pruritic	22	65%
Soreness	10	29%
Irritation	17	50%
Dyspareunia	8	23%
Burning micturition	6	17%

Table 6: History

History	Number of cases in the study group	Candidial vaginitis	
		Number	Percentage
Pregnancy	22	7	32%
Oral contraceptives	10	3	30%
Intra uterine devices	5	1	20%
Infertility	7	2	29%
Post Hysterectomy	14	4	28%
Diabetes	9	2	22%
HIV	2	1	50%
Post Antibiotic therapy	12	3	25%

Table 7: Clinical findings in cases with vaginal Candidiasis

Clinical findings	Number	Percentage
Vulvar edema	2	05%
Vulvar erythema	4	12%
Vulvar fissures	0	0%
Leucorrhoea	34	100%
Excoriation	12	35%
Cervical ectopy	0	0%
Vesicles	1	02%
Pustules	0	0%

Table 8: Comparison of direct microscopy and culture

Detection of candida by	Study group n=100 With wdpv		Control group n=100 No wdpv	
	Number=34	Percentage	Number =4	Percentage
Microscopy presence of yeast cells	32	32%	2	2%
Isolation of Candida by SDA culture	34	34%	4	4%

$\chi^2 = 0.431$ df= 1 p= 0.51

Table 9: Germ tube Results

Total	Germ tube test positive		Germ tube test negative	
	Number	%	Number	%
N= 34	21	71%	10	29%

Table 10: Different species of candida on CHROM agar

Colony color on CHROM agar	Species identification	Number	Percentage
Light green	<i>C. albicans</i>	24	65
Dark blue with a halo	<i>C. tropicalis</i>	9	24
Pale pink	<i>C. krusei</i>	2	5
Cream / pale white	<i>C. glabrata</i>	1	3
	<i>C. parapsilosis</i>	1	3

Table 11: Cornmeal agar morphology of species isolated

Cornmeal agar morphology	Species identification	No. of cases	Percentage
Irregular or spherical clusters of blastospores at septa and chlamyospores	<i>C. albicans</i>	24	65%
	<i>C. dubilimensis</i>		
	<i>C. stellatoidea</i>		
Elongated cells forming branched hyphae 'crossed sticks' of septa	<i>C. krusei</i>	2	05%
Fine and coarse pseudohyphae, giant cells blastosphere single or in short chains at septa or distal ends of cells	<i>C. parapsilosis</i>	1	03%
Long filamentous hyphae, blastospores borne singly along the hyphae, sometimes with small chlamydoconidia	<i>C. tropicalis</i>	9	24%
No pseudohyphae, cells are small with terminal budding	<i>C. glabrata</i>	1	03%
Total		37	

Table 12: Sugar assimilation pattern of different species

Sugar discs	dex	mal	suc	lac	gal	mel	cel	ino	xyl	Raf	tre	Identification	No.	%
1	+	+	+	-	+	-	+	-	+	-	+	<i>C. albicans</i>	24	65
2	+	+	+	-	+	-	+	-	+	-	+	<i>C. tropicalis</i>	9	24
3	+	-	-	-	-	-	-	-	-	-	-	<i>C. krusei</i>	2	5
4	+	-	-	-	-	-	-	-	-	+	-	<i>C. glabrata</i>	1	3
5	+	+	+	-	+	-	-	-	+	-	+	<i>C. parapsilosis</i>	1	3

Dex-dextrose; mal-maltose; suc-sucrose; lac- lactose; gal- galactose; mel- melabiose; cel- celebiose; ino- inocitol; xyl- xylose; raf- raffinose; tre-trehalose

Discussion

Our study showed that maximum number of cases presenting with leucorrhoea belonged to the age group of 30-39 years, which is comparable to the study done by Puri KJ et al⁸ (20-40 years). The mean age distribution in our study was 33.27+10.7 years which can be compared to the mean age 30 years in a study done by Norma T. Gross et al⁹. Since this hospital serves free of cost to the patients and is accessible to the rural population, more number of cases from rural cases are seen in our study as compared to the urban population.

In our study Candida was found to be the causative organism in 29 cases (29%) and in 5 (5%) cases both Candida and bacterial causes were identified. Hence the incidence of vaginal candidiasis was 34%. The incidence of Candida in cases with white discharge per vagina is 34% which is significantly higher than Candida isolated from healthy group (4%) ($p < 0.05$). This can be compared with the study done by Puri KJ, Madanet al⁸ (31%), Stephanie Weissenbacher et al¹⁰ (31.1%) and Kamara et al¹¹ (30.7%). In our study about 8 patients out of 34 (24%) with vaginal candidiasis had recurrent attacks of VVC (Vulvo-Vaginal Candidiasis) for which they were treated. This is comparable to the study done by E Rylander et al¹² (22%). In a study done by Marie V. Pirotta et al¹³, incidence of vaginal Candidiasis in patients after antibiotic treatment was 37% which is similar to our study (25%).

In our study among the pregnant women with history of white discharge 32 % had Candida as a causative agent which is similar to studies done by J.A. Simoes et al¹⁴ (42.4%), Neeraja Jindal et al¹⁵ (28.2%). In our study, Candida could be demonstrated by Gram staining in 32 (32%) and 34 samples grew Candida on culture which is similar to the study conducted by Bradshaw et al¹⁶ (15% & 28%).

Total number of isolates in our study was 37. The predominant species isolated in our study was *C. albicans* 24 (65%). Non-albicans species included 19 isolates of *C. tropicalis* (24%), 2 of the isolates of *C. krusei* (5%), one isolate of *C. glabrata* (3%) and one isolate of *C. parapsilosis* (3%). This findings are similar to studies done by E Rylander et al, Neeraja Jindal et al which shows 69.57% were *C. albicans*, 8.7% *C. glabrata*, 6.55% each *C. tropicalis* and *C. krusei*, and 4.3% each *C. parapsilosis* and *C. guilliermondii*. Pei Pei Chong et al shows *C. albicans* was the predominant species detected

70.1%, followed by *C. glabrata* (14.9%), *Candida lusitanae* (5.7 %), *Candida famata* (4.6 %), *C. krusei* (2.3%) and *C. parapsilosis* (2.3%), Bradshaw et al conducted a study on 342 women vaginal swab samples of 30 cultured isolates, predominant were *C. albicans* except for 6 (2%), which were *C. glabrata*¹¹⁻¹⁷.

Conclusion

By this study, it is clear that the incidence of vulvo-vaginal candidiasis among the persons having leucorrhoea is very high with high risk among pregnant women and those who are taking oral contraceptives. There was no discrepancy in the findings of various identification tests done. The Germ tube test, Corn meal test CHROM agar and sugar assimilation tests showed similar results.

Acknowledgement

We thank all the teaching and non-teaching Faculty of Department of OBG and Department of Microbiology, AJ Institute of Medical Sciences, Mangalore.

Conflict of Interest: Nil

Ethical Clearance: Taken from the Institute

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How to cite this article: Bhavana J, Yadukul S, Anitha KB. Study of Vulvo-Vaginal Candidiasis among patients visiting obstetrics and gynecology department in a tertiary care centre. International Journal of Medical Microbiology and Tropical Diseases 2016;2(2):60-66.