Original Research Article

DOI: http://dx.doi.org/10.18203/2349-3933.ijam20200076

Identification of various *Candida* species isolated from patients with vulvovaginal candidiasis attending a tertiary care hospital in North India

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Received: 23 November 2019 Revised: 23 December 2019 Accepted: 27 December 2019

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ABSTRACT

Background: Vulvovaginal Candidiasis (VVC) is a condition diagnosed in a large proportion of women presenting with complaints of abnormal vaginal discharge. The risk of VVC is high in women with diabetes mellitus, pregnancy, using oral contraceptives or broad-spectrum antibiotics and those having Human Immunodeficiency Virus infection. Objective of the present study was to identify the various species of *Candida* isolated from patients with Vulvovaginal Candidiasis.

Methods: A cross-sectional study was done from January to June 2018. A total of 69 clinically suspected VVC cases were included in the study whose high vaginal swabs were collected and subjected to direct microscopy and cultured on Sabouraud Dextrose Agar (SDA). Creamy white pigmented colonies on SDA were confirmed as *Candida* by doing Gram's staining. Speciation of positive cultures was confirmed by conventional methods like Corn Meal agar culture, Germ tube test (GTT), *Candida* CHROM Agar Medium (CAM), sugar assimilation and fermentation test.

Results: Out of 69 clinically suspected VVC cases included in the study, only 14 cultures were positive for *Candida* species. All the 14 isolates were found to be Non-albicans *Candida* (NAC). Most common species isolated were *Candida glabrata* 7(50%), followed by *Candida tropicalis* 3(21.4%), *Candida lusitaniae* 3(21.4%), and *Candida parapsilosis* 1 (7.1%).

Conclusions: Candida albicans was known to be the most common causative agent of VVC as it was isolated in 90% cases. However, this study showed that all isolates from VVC were Non-albicans Candida. Therefore, species identification should be done in all microbiology laboratories for accurate diagnosis of VVC.

Keywords: Candida CHROM agar medium, Corn meal agar, Non-albicans Candida, Vulvovaginal candidiasis

INTRODUCTION

Candidiasis is an infection caused by yeast like fungus called, *Candida*. Vulvovaginal Candidiasis (VVC) refer

to a disorder characterized, by signs and symptoms of vaginal inflammation in the presence of *Candida* species. It is characterized by curd like vaginal discharge and itching or erythema and is caused by overgrowth of

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Candida species in vagina. This condition is commonly known as "Vaginal yeast infection", "Vaginal Candidiasis", "Vulvovaginal Candidiasis" or "Candidal Vaginitis". 1,2

VVC is a gynaecological condition diagnosed in a large proportion of women presenting with abnormal vaginal discharge. VVC is the most prevalent disease among females and is responsible for 10 million clinical visits every year. This disease is the second common genital tract infection among females and is usually caused by *Candida albicans*. Most specialists believe that 90% of cases of vaginitis are secondary to bacterial vaginosis, trichomoniasis and Vulvovaginal Candidiasis.^{3,4}

The risk of VVC is high in women with diabetes mellitus, obesity, Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS), using oral contraceptives, broad spectrum antibiotics, tissue transplant, use of immunosuppressive agents and malnutrition. In pregnancy, vaginal candidiasis is common due to altered pH and sugar content in vaginal secretions, and it also has direct effect on yeast cells, causing it to grow faster and stick more easily with the walls of vagina.^{3,5}

Approximately 75% of woman may have at least one episode of VVC in their lifetime, with 45% suffering from second episode and nearly half of them suffer from multiple episodes.³

Recurrent Vulvovaginal Candidiasis (RVVC) is defined as four or more episodes of symptomatic infection within a year, with at least partial resolution of symptoms between episodes. Longitudinal DNA-typing studies suggest that, in most of the women, recurrent disease is due to vaginal endogenous reinfection with the identical strain of susceptible *Candida* species.⁵

Candida albicans is found as the most common causative agent as it is isolated in 90% cases of VVC. Candida glabrata is usually the second most common pathogen detected in VVC followed by Candida tropicalis, Candida parapsilosis and Candida krusei.³

Treatment of VVC is very mild, short course and consists of azole based antifungal medicine applied inside the vagina as vaginal pessary or drugs taken by mouth. One has to determine susceptibility patterns of vaginal isolates of *Candida* to antifungal drugs including Amphotericin B, Voriconazole, Itraconazole and Ketoconazole etc prior to commencement of treatment. If left untreated, it acts as a potential risk factor for other sexually transmitted diseases.^{6,7}

Keeping the above facts in mind the present study was done to identify the various species of *Candida* isolated from patients with Vulvovaginal Candidiasis.

METHODS

A hospital based cross-sectional study was done over a period of 6 months from January to June 2018, among clinically suspected cases of Vulvovaginal Candidiasis attending Out Patient Department of Obstetrics and Gynaecology of Integral Institute of Medical Sciences and Research, Lucknow, to identify the various species of *Candida* causing VVC among these patients. The study was approved by Institutional Ethical Committee. An informed consent was taken from each female patient included in the study prior to sample collection. A predesigned questionnaire was used to get the information regarding socio-demographic profile of the enrolled patients.

Inclusion criteria

All consenting female patients of reproductive age (including pregnant women) and postmenopausal women, who presented with complaints of abnormal vaginal discharge, were included in this study.

Exclusion criteria

• Female patients below 18 years of age and those who refused to give consent were excluded from the study.

Total of 69 clinically suspected VVC cases whose High Vaginal Swabs were collected and processed in the Department of Microbiology for species identification of *Candida* isolates were included in the study.

Two High Vaginal Swabs (HVS) were collected from each suspected patient of VVC and processed in the Department of Microbiology. One HVS was used for direct microscopic examination by Gram's staining for presence of pus cells and Gram positive oval or round budding yeast like cells with or without pseudohyphae (Figure 1).



Figure 1: Gram stain showing Gram positive budding yeast like *Candida* isolate.

Another HVS from each patient was inoculated on Sabouraud dextrose agar (SDA) with or without cycloheximide and incubated at 37°C for 24-48 hrs. Creamy white pigmented colonies grown on SDA (Figure2) were further confirmed as *Candida* by doing Gram's stain.



Figure 2: Creamy white colonies of *Candida* isolates on SDA.

Species identification of culture confirmed *Candida* isolates was done by conventional methods like Dalmau's plate (Corn Meal agar) culture to detect the presence of chlamydospores and special hyphal structure, Germ tube test (GTT), culture on *Candida* CHROM Agar Medium (Figure 3), sugar fermentation (Figure 4) and sugar assimilation test (Figure 5).8



Figure 3: Pigmented colonies of *Candida* species on *Candida* CHROM agar medium.



Figure 4: Sugar fermentation test for Candida species.



Figure 5: Sugar assimilation test for Candida species.

Statistical analysis

The collected data was analyzed using SPSS Data Editor Software version 20. Chi-square test was performed for categorical variables and p value ≤ 0.05 were considered statistically significant.

RESULTS

Out of 69 samples of high vaginal swabs (HVS) collected from enrolled female patients who presented with complaints suggestive of Vulvovaginal Candidiasis, only 14 samples were positive for *Candida* species, whereas, 55 samples were negative for *Candida* species. Each sample yielded single isolate of *Candida* species, thus the prevalence of positive VVC was found to be 20.3% (Figure 6).

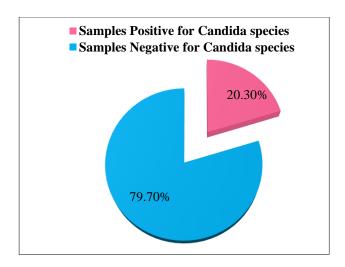


Figure 6: Prevalence of positive cases of VVC.

All 14 isolates were found to be Non Albicans *Candida* (NAC) on the basis of culture characteristics on Corn meal agar, *Candida* CHROMagar, sugar fermentation, sugar assimilation and negative results for Germ tube test. Most common NAC isolated was *Candida glabrata*

(7), followed by *Candida tropicalis* (3), *Candida lusitaniae* (3), and *Candida parapsilosis* (1) (Table 1).

Table 1: Distribution of various *Candida species* isolated from Vulvovaginal Candidiasis cases (N = 14).

Candida Species	Number of isolates (N)	Percentage (%)
Candida glabrata	7	50.00%
Candida tropicalis	3	21.43%
Candida lusitaniae	3	21.43%
Candida parapsilosis	1	7.14%
Total	14	100%

Table 2 shows the age wise distribution of female patients suggestive of VVC enrolled in this study. Majority of patients belonged to reproductive age group, 89.9% (62/69) as compared to postmenopausal women, 10.1% (7/69). Majority of positive cases of VVC was found among reproductive age group, 85.7% (12/14), as compared to postmenopausal women, 14.3% (2/14). However, this difference was not found to be statistically significant (p = 0.565).

Table 2: Distribution of female patients enrolled in the study according to their age group and VVC status (N = 69).

Age group (in years)	VVC	VVC	— Total	Chi-square (χ2)
	Positive N (%)	Negative N (%)	Total	and *p value
15-49 (Reproductive)	12 (19.4%)	50 (80.6%)	62 (100%)	2 0 220
>50 (Postmenopausal)	2(28.6%)	5(71.4%)	7(100%)	$\chi 2 = 0.330$ p = 0.565
Total	14(20.3%)	55(79.7%)	69(100%)	p = 0.303

N = Number of isolates. VVC = Vulvovaginal Candidiasis. * p <0.05 was considered as statistically significant.

Table 3: Distribution of female patients according to their residence and VVC status (N = 69).

Residence	VVC Positive N (%)	VVC Negative N (%)	Total	Chi-square (χ2) and *p value
Rural	1(7.1%)	13(92.9%)	14(100%)	$\chi 2 = 1.877$ p = 0.171
Urban	13(23.6%)	42(76.4%)	55 (100%)	
Total	14(20.3%)	55(79.7%)	69(100%)	

N=Number of isolates. VVC = Vulvovaginal Candidiasis. * p<0.05 was considered as statistically significant.

Table 4: Distribution of female patients according to their education and disease (VVC) status (N = 69).

Educational status	VVC	VVC	Total	Chi-square (χ2)
	Positive N (%)	Negative N (%)		and *p value
Illiterate	8(20.0%)	32(80.0%)	40(100%)	$\chi 2 = 0.005$
Literate	6(20.7%)	23(79.3%)	29(100%)	p=0.944
Total	14(20.3%)	55(79.7%)	69(100%)	

N=Number of isolates. VVC=Vulvovaginal Candidiasis. *p<0.05 was considered as statistically significant.

Table 3 depicts, majority of patients belonged to urban areas, 79.7% (55/69), with highest number of positive isolates, 92.9% (13/14). However, this difference of positivity among isolates from patients of urban areas as

compared to those from rural patients was not found to be statistically significant (p = 0.171).

Table 4 shows that majority of patients were illiterate, 58% (40/69). Maximum number of positive cases of VVC was found among illiterate patients, 57.1% (8/14), as compared to literate patients, 42.9% (6/14). However, this finding was not found to be statistically significant (p=0.944).

DISCUSSION

With the regularly changing lifestyle and dietary habits, the incidence and prevalence of *Candida* species infection has lowered down. Many species of *Candida* usually live as human commensals but it causes Candidiasis in immunocompromised persons. Several researches have shown that the infection increases during two to three last decades.

In this study out of 69 high vaginal swab samples, only 14 samples yielded positive growth for *Candida* species, all of them being Non-albicans *Candida*, thus the overall prevalence of positive VVC was 20.3%. This finding correlates with another study conducted in Bulgaria which also reported prevalence of Non-albicans *Candida* in VVC to be 27.4%. However, in contrast to this finding of 20.3% prevalence of *Candida* species, a study from Yemen reported a very low prevalence of 12.7% *Candida* species in VVC cases. ⁷

In the present study amongst the *Candida species* isolated from VVC, *Candida glabrata* was the most common isolate (50%) followed by *Candida tropicalis* (21.43%), *Candida lusitaniae* (21.43%) and *Candida parapsilosis* (7.14%). Another study done in Puducherry, India, corroborates this finding as it reported an overall prevalence of 23.7% *Candida* species from 211 clinically suspected VVC cases, with *Candida glabrata* (45.1%) as the most common isolate, followed by *Candida tropicalis* (23.5%), *Candida albicans* (17.6%), *Candida krusei* (9.8%) and *Candida parapsilosis* (3.9%).³ While another study from Puducherry, India, reported higher incidence of *Candida tropicalis* (16.4%) as compared to *Candida glabrata* (8%). This is in contrast to present study's finding.¹⁰

This study reported 7.14% isolates of *Candida* parapsilosis among VVC cases. Another study done in Iran corroborates this finding as it reported 1% isolates of *Candida parapsilosis*, which is close to study.¹¹

In this study, author have reported 21.43% Candida lusitaniae and no isolate of *Candida albicans*. Another study at Puducherry, India, reported 17.6% isolates of *Candida albicans* and no isolate of *Candida lusitaniae*.³ This is in contrast with study.

This study reported that majority of *Candida* isolates (23.6%) were from samples of urban patients and only 7.1% isolates were from rural patients. In contrast to this finding a study done in rural Maharashtra reported higher prevalence of VVC among them to be 35%. ¹² Another

study from rural Tamil Nadu reported 10% prevalence of VVC among them, this corroborates present study's finding.¹³

A recent study reported that Non-albicans *Candida* species, particularly *Candida glabrata* were observed in 10-20% of women with recurrent vulvovaginal candidiasis (RVVC). This is in contrast to this finding as author reported 50% isolates of *Candida glabrata*, but no RVVC were seen in this study.

Author reported 2.8% (2/69) positive cases of VVC patients belonging to postmenopausal age. Another study from Amritsar reported 3% of positive cases of VVC among patients belonging to postmenopausal age group.¹⁵ This is in agreement to present study's finding.

Study reported higher incidence of VVC among patients of reproductive age group as compared to postmenopausal age group. VVC was more among patients from urban areas than those from rural areas and VVC was more among illiterate patients as compared to literate patients.

This finding hint that newer species may emerge from time to time even in VVC cases and epidemiological studies are needed to remain update about changing prevalence of *Candida* species.

CONCLUSION

Candidiasis is an infection caused by yeast like fungus called *Candida*. Vulvovaginal Candidiasis (VVC) is the most common type of vaginal infection. 75% females experience at least once in their lifetime. *Candida albicans* was known to be the most common causative agent of VVC. However, this study showed that all isolates from VVC were Non-albicans *Candida*. Therefore, this study has made it imperative that species identification should be done in all microbiology laboratories for accurate diagnosis of VVC for effective timely treatment.

Funding: No funding sources Conflict of interest: None declared

Ethical approval: The study was approved by the

Institutional Ethics Committee

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Cite this article as: Jahan N, Vishnoi P, Gupta B, Srivastava S, Ahmad S, Khatoon R. Identification of various *Candida* species isolated from patients with vulvovaginal candidiasis attending a tertiary care hospital in North India. Int J Adv Med 2020;7:261-6.