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**Original Research Article**

# Isolation and identification of Candida Species in Patients with Vulvovaginal Candidiasis

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**ABSTRACT**

Abnormal vaginal discharge is one of the frequent complaints of women of reproductive age group. This study was carried out to determine the prevalence of vulvovaginal candidiasis (VVC) among the patients attending the Gouri Devi Institute of Medical Sciences and hospital with complaints of suggestive of vaginitis. This study was done in Gouri Devi Institute of Medical Sciences and hospital, Durgapur, West Bengal for a period of 6 months from January 2019 to June 2019. The study included 120 women of the age group 15 – 65 years with complaints suggestive of vaginitis. High vaginal swabs were taken and subjected to direct microscopy, cultured onto Sabouraud Dextrose Agar (SDA) and Hichrome *Candida* differential agar. Candida species were determined by standard microbiological methods and the results were confirmed. Candida species were isolated from 52 patients who had *C.albicans* (23), *C.tropicalis* (20), *C.glabrata* (5), *C.dubliniensis* (3) and *C.krusei* (1). Our study shows higher prevalence of non *albicans* Candida (NAC) causing VVC. Hence, we recommend that the investigations up to species identification of Candida may be routinely followed in the microbiology laboratories.

## INTRODUCTION

Fungal infections have become an alarming problem over the last ten years mainly because of global increase in the number of immunocompromised patients, who are highly susceptible to opportunistic infections, including mycoses. [1] According to a recent study, the incidence of Candidaemia is 6.9 per 1,000 ICU patients of which 7.5% received antifungal therapy [2]. Among the women, vaginal candidiasis has become a common finding worldwide and up to 75% of them have symptomatic vaginal candidiasis at least once [3]. Vulvovaginal candidiasis (VVC) is defined as signs and symptoms of inflammation of the vulva, vagina, or both in the presence of *Candida* spp. and in the absence of other infectious etiology [4]. The clinical features of VVC include severe itching of vulva, abnormal vaginal discharge, edema of vulva, pruritus, hyperemia, vaginal discomfort and leucorrhea, burning, soreness, dyspareunia and vaginal or vulvar erythema, which may cause a problem in marital sexual relations and may even lead to infertility [5].

*Candida albicans* appears to be the cause for 80 to 92 % of vulvovaginal candidiasis [6]. There is an increase in frequency of other candida species nowadays, specifically of *C. glabrata*, may be because of increased use of over-the counter drugs, long-term use of azoles, and the use of short courses of antifungal drugs [7]. The prevalence of candida in India is estimated to be 30% [8]. There are various predisposing factors of VVC, few of which are, hormonal fluctuations in pregnancy, luteal phase of menstrual cycle, use of oral contraceptives, and hormone replacement therapy [9]. Thus, the isolation, identification, characterization and susceptibility testing of Candida species in high vaginal swabs have become increasingly important for management of fungal infections. CHROM agar medium is an easy, rapid and reliable method to isolation and for differentiation of types of Candida species. In the

present study, we explored the characterization of Candida species using CHROM agar and showed the susceptibility pattern of Candida isolates from high vaginal swabs.

## MATERIALS AND METHODS

This research was conducted at Gouri Devi Institute of Medical Sciences and hospital, Durgapur, West Bengal over a period of 6 months. Patients were enrolled in the study by the sequential sampling method. The participants were married women aged 15 - 65 years, who presented erythema and itching of vulva, vagina, or both and cheesy vaginal discharge. The patients filled out a consent form to participate in the research, which was approved by the ethical committee of Gouri Devi Institute of Medical Sciences and hospital. Concomitant with each obtained specimen, a questionnaire was completed for each patient enquiring about their age, marital status, and duration of symptoms, comorbidities, signs and symptoms of current condition, methods of pregnancy prevention, prior parturitions, and history of antibiotic consumption. Patients with four or more discrete attacks of VVC per year were considered as having Recurrent Vulvovaginal Candidiasis (RVVC).

### Sample Collection and Laboratory Diagnosis

Vaginal secretions were obtained in lithotomic position using a speculum and sterile swabs. Two specimens were collected simultaneously under sterile conditions, one for light microscopic examination and the other for fungal culture. For each of the samples, a slide was prepared for Gram staining.

The sample for fungal culture was inoculated into Sabouraud Dextrose agar supplemented with chloramphenicol and Gentamycin and incubated at 30°C. The identification of the Candida species was done by morphological and physiological methods such as culture on CHROM agar Candida media (CHROM agar), germ tube test, chlamydospore forming test on corn meal agar media (HiMedia), growth at 45°C and sugar assimilation test with the HiCandida identification kit (HiMedia). HiCandida identification kit was applied for precise identification of Candida species as per the manufacturer's instructions. The plastic strip had twelve wells with sterile medium for different biochemical tests as follow: well 1, medium for the urease detection test, and well 2 - 12, medium for carbohydrate utilization test (with eleven different sugars in respective wells, including, melibiose, lactose, maltose, sucrose, galactose, cellobiose, inositol, xylose, dulcitol, raffinose and trehalose). In brief, the test was performed as follows; at first a homogenous yeast suspension (1 to 5 × 106 cell/mL) was prepared and inoculated into kit wells and incubated for 24 - 28 hours at 22.5 ± 2.5°C. A standard sample of C. albicans (as confirmed by the molecular method) was also used as the control. After the incubation period the change of color in the kit was noted: well 1 containing urease was considered positive if the yellow color transformed to pink. Wells 2-12 were considered positive if their orange red color changed to yellow; these pits were left for 72 hours and if the color was still orange, the result was considered negative. Interpretation of the results was based on the manufacturer's literature.

### Statistical Analyses

Chi-square test was performed using the SPSS software (version 18.0) and differences were considered significant at $P < 0.05$.

## RESULTS

During 6 months, we studied 120 consecutive patients suspected of VVC at Gouri Devi Institute of Medical Sciences and hospital, Durgapur, West Bengal (Table 1).

The mean age of the patients was 26-35 years. Out of 120 patients with vulvovaginitis, 46 (38.3%) patients showed VVC. Of these patients, 16 (24.2%) had RVVC. The mean age of VVC patients was 31.82 ± 10 years. The age group of 26 - 35 year-olds had the highest frequency of VVC (34.6%). No significant correlation was found between age and occurrence of VVC ($P = 0.137$). Figure 1 shows vulvovaginal symptoms and signs in patients suspected of VVC and VVC patients. Erythema concomitant with itching (40.9%) was the most prevalent sign in VVC patients and patients suspected of VVC. No significant correlation was observed between occurrence of the disease and type of symptoms ($P = 0.608$).

Table 2 shows the distribution of studied patients based on the use of contraception methods. Most patients with VVC (48.0%) did not use any method of contraception. In total, 27.8% of patients

suspected of VVC had positive results for the culture method. Out of 52 patients with VVC, 98.5% had positive results for Candida growth in culture. Of these patients, 48 (92.3%) had positive results for both microscopic examination and culture. 4 patients (7.6%) showed positive results in culture but not in microscopic examination. Out of 48 patients with positive results in microscopic examination, 30 cases (62.5%) showed yeast and budding yeast and 18 (37.5%) pseudohyphae and budding yeast. In total, 52 colonies of Candida spp. were isolated from patients with VVC. The most frequent species of Candida were C. albicans (44.2%), C.tropicalis (38.4%), C. glabrata (9.6%), C. dubliniensis (5.7%) and C.krusei (1.9%). Out of a total of 52 patients with VVC, 16 (24.2%) cases showed RVVC. In 16 patients with RVVC, Candida albicans was responsible in (44.2%). On the other hand, non-albicans species of Candida (55.7%) were frequent species in patients without recurrence. There was no significant correlation between Candida species and recurrent or non-recurrent pattern of disease (P = 0.073).

## DISCUSSION

Vaginitis is a universal problem affecting millions of women globally. Vulvovaginal candidiasis (VVC) is defined as signs and symptoms of inflammation of the vulva and vagina in the presence of *Candida* spp. and in the absence of other infectious etiology [1]. Candidiasis is one of the most diverse fungal infections that can lead to superficial, such as vaginitis, to systemic and potentially life-threatening diseases. Genital involvement in women is one of the most common presentations due to Candida. Vulvovaginal candidiasis results from abnormal growth of Candida in the genital tract mucosa and has increased dramatically in the recent years [10]. Candida albicans was reported as the most common agent of VVC yet it seems that we are recently encountering changes in the pattern of Candida species in VVC. This is why we designed a study to evaluate VVC and the incidence of different species of Candida in patients from Durgapur, West Bengal. In our study out of 120 women, 52 high vaginal swabs (43.3%) were culture positive and grew candida species. This data is higher than to reports by Kumari V *et al.* [1] (30.6%), but lower than reports from Namrata Kalia *et al* (47%) and ranks second as the cause for vulvo vaginal infections [11]. This variation may be due to inaccuracies in pathogen detection, mismanagement, drug resistance, incomplete therapeutic course, self-treatment, lack of appropriate health habits and intestinal infestation[5]. However, Achkar and Fries [4] suggested that VVC is not a reportable disease and is often diagnosed without confirmatory tests and treated with over-the-counter (OTC) medications, and thus its exact incidence is unknown.

In the present study the age group of 26 - 35 year-olds had the highest frequency of VVC, which is concordant with the findings of Mahmoudi Rad et al.[12] and Asadi et al.[13] from Chennai and from Nepal. In our study, similar to the study of Aalei et al [14] no statistical significance was found between age and occurrence of the disease. This may be due to higher vaginal discharge, physiological and hormonal changes, higher sexual activity, vaginal flora changes, the childbearing age and use of various contraceptive facilities in this age group. In the present study, VVC was mostly observed in those who used natural methods for pregnancy prevention. There was a significant correlation between contraceptive method and disease acquisition (P = 0.004). These results are in agreement with the study of Torabi and Amini[15] from Zanjan, however, in contrary with some previous studies [14, 16, 17].

It may be assumed that this non-protective method increases the chances of VVC. In our study, the most common symptom was erythema concomitant with itching in VVC patients and there was a statistically significant correlation between this symptom and VVC. However, typical VVC signs, such as cheesy discharge, erythema and itching were not significantly related to VVC. The same result was reported by some other previous studies [18, 19]. Michigan university researchers also reported itching as the most common symptom in VVC [20].

The most patients who seek medical attention have complaints of white discharge, itching and pain. In our study 52 women had complaints of white discharge (43%) which was the major complaint. It was followed by complaints of itching which was seen in (29%) and complaints of pain by (13%). The clinical presentation was slightly different from reports by Latha Ragunathan et al.[3], where itching (31%) was the major complaint followed by white discharge (29.4%) and pain (15.6%).

In our study, out of 52 patients with VVC, 92.3% of samples showed Candida growth in culture. Of these patients, 7.6% had positive culture results yet negative microscopic examination results. These results indicate the strength of the culture method in comparison with microscopic examination for VVC diagnosis. In this study, the prevalence of C.albicans and non-albicans species of Candida was 44.2% and 55.7%, respectively. According other studies from different countries, C. albicans was the most involved species of Candida in VVC patients [12, 14, 21, 22]. Grigoriou et al.[23] attributed this to the greater ability of C. albicans in adhesion to vaginal mucosa, which is the primary step in establishment of a fungal infection. In our study C.tropicalis was the second leading species that caused infection, and this finding was consistent with many previous studies [24, 25].

Although in our study, C. albicans was the most prevalent isolated species of Candida yet in comparison to non-albicans species, the latter was predominant. During the last decade, different studies have shown an increase in isolation of non-albicans species in VVC patients [21, 26]. Sobel et al.[25] suggested that this pattern may be due to incomplete local or systemic therapeutic regimens, or self-prescribed anti-fungal agents and the increasing use of prolonged anti-fungal courses to prevent recurrence of VVC.

Some of the non-albicans species such as C. glabrata respond poorly to azole agents, especially fluconazole, which can be a reason for the increased prevalence of non-albicans species of Candida in VVC patients. [21, 26]

In our study, C. glabrata was isolated from 9% of VVC patients without recurrences; a finding which was different from other previous studies. Savage and Dubos [27] have shown that C. glabrata is the normal flora yeast of rodents. In the present study CHROM agar Candida medium was applied to differentiate Candida species phenotypically.

This method detects only two major Candida species. In the recent years, non-albicans species, which are concomitant with some yeast species and produce similar colors, have expanded leading to inaccurate diagnosis and treatment failure. In addition, colorimetric techniques are expensive for routine use.

## CONCLUSION

In our study we found that C.albicans was the predominant species followed by *C.tropicalis*. Yet, there is higher prevalence of non-albicans candida species in the study responsible for VVC. Hence, screening of all women with vulvovaginal infections for different species of candida would be helpful in providing better care. Thus, complete identification of causative agent of Vulvovaginal candidiasis up to species level in all the microbiology laboratories is highly recommended as it helps in optimum selection of the therapeutic agent and use of CHROM agar is a simple, rapid and inexpensive method for identification of Candida species especially in the laboratory with limited resources.

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Table 1. Distribution of Patients Based on age Groups

| Patients' Age Group | Patients Suspected of VVC | Patients with VVC |
|---|---|---|
| 15 – 25 | 30 (25.0) | 12 (23.1) |
| 26 – 35 | 30 (25.0) | 18 (34.6) |
| 36 – 45 | 28 (23.3) | 10 (19.2) |
| 46 – 55 | 24 (20.0) | 09 (17.3) |
| 56 – 65 | 08 (6.6) | 03 (5.7) |
| Total | 120 (100.0) | 52 (100.0) |

Table 2. Distribution of Patients Based on the Use of Contraception Methods

| Patients Contraception Method | Patients Suspected of VVC | Patients With VVC |
|---|---|---|
| No contraceptive method | 46 (38.3) | 25 (48.0) |
| Natural | 32 (26.6) | 09 (17.3) |
| Condoms | 14 (11.6) | 08 (15.3) |
| Oral contraception | 10 (8.3) | 05 (9.6) |
| IUD | 06 (5.0) | 03 (5.7) |
| Tubectomy | 12 (10.0) | 02 (3.8) |
| Total | 120 (100.0) | 52 (100.0) |

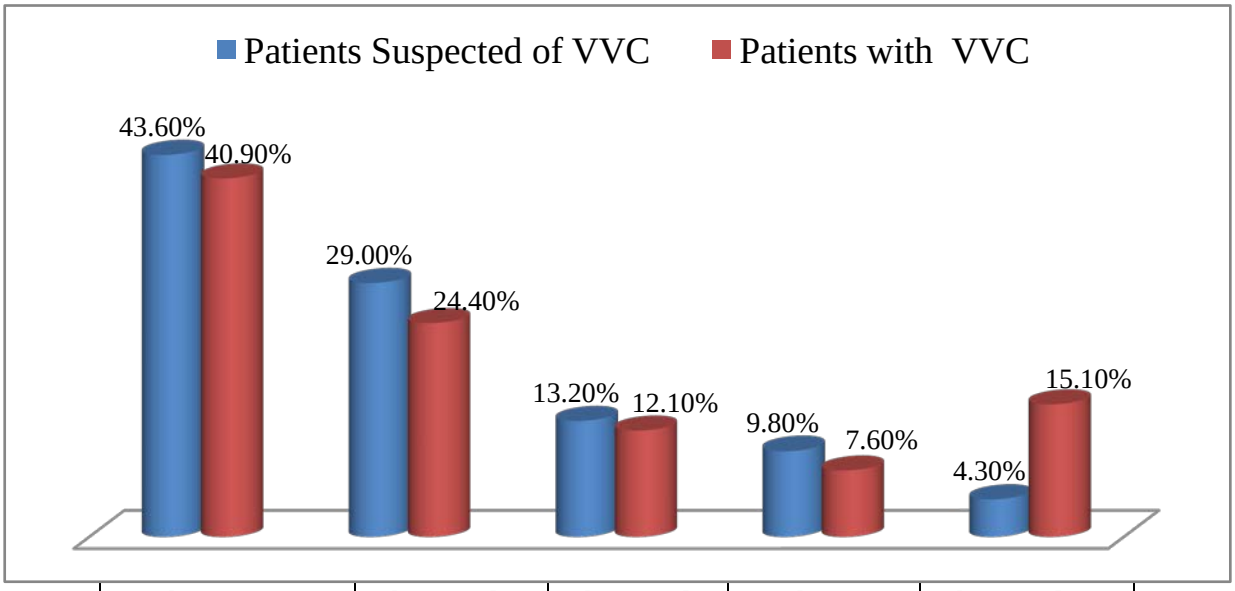


Figure 1: Vulvovaginal Symptoms and Signs in Patients Suspected of Vulvovaginal Candidiasis and Vulvovaginal Candidiasis Patients.

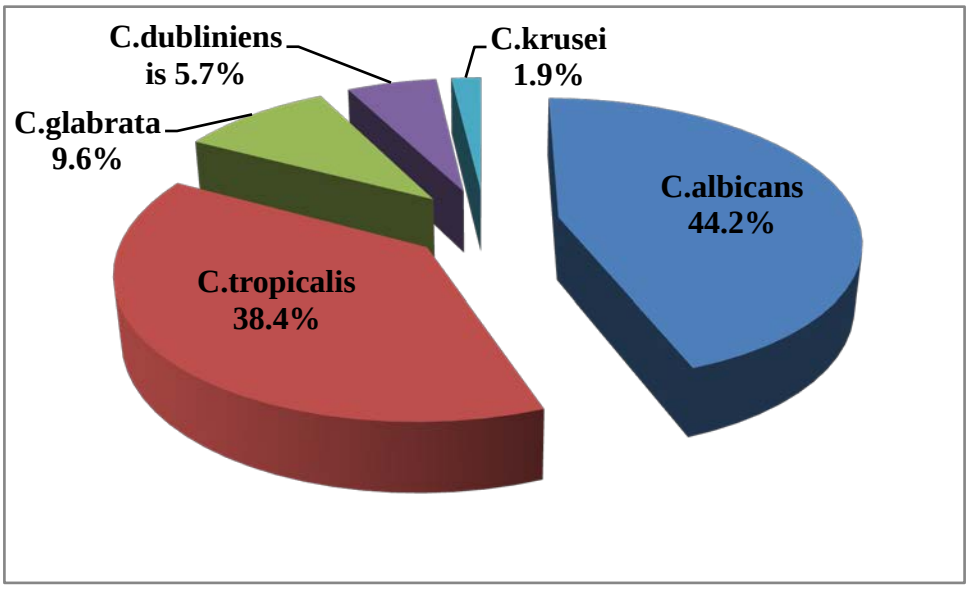


Figure 2: Distribution of Isolated Candida Species from Patients with Vulvovaginal Candidiasis

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