



## RESEARCH ARTICLE

# Study of Vulvo-vaginal candidiasis and in vitro anti-fungal susceptibility pattern

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### Abstract

Vulvovaginal Candidiasis (VVC) mostly affects women of childbearing age and it is the second most common form of vaginitis. The range of VVC manifestations may be from asymptomatic colonization to severe acute symptomatic infection. 10%-20% women with VVC have asymptomatic colonization with *Candida* species. The aim of the study was to identify the species of *Candida* causing Vulvo-vaginal candidiasis (VVC) in the study population & perform the anti fungal susceptibility of the fungal isolates. Two High Vaginal Swabs were collected from suspected cases. Wet mount, culture on Sabouraud's dextrose agar (SDA) and Blood agar were done for identification of *Candida* infection. Isolates from the culture plates were inoculated on HiCrome differential candida agar for speciation. All isolates were tested for antifungal susceptibility to Voriconazole, Ketoconazole, Fluconazole, Amphotericin, Nystatin and Itraconazole using Disk Diffusion method. Out of the 28, 9 cases (32.14%) were positive for *Candida* species of which 4(44.4%) were *Candida albicans*. Nonalbicans *Candida* (NAC) were 5(55.5%) of which 2(22.22%) were *C. tropicalis*, 1 (11.11%) was *C. glabrata* and 2(22.22%) were combination of *C. glabrata* and *C. tropicalis* mixed infection. *Candida albicans* was the most isolated species. *C. albicans* isolates were 100% sensitive to all antifungals under study. *C. tropicalis* was sensitive to antifungals under study with a range of 25% to 75%. While *C. glabrata* isolates were sensitive to Voriconazole, Fluconazole, Itraconazole and Ketoconazole they were resistant to Nystatin and Amphotericin. NAC species were mostly resistant to antifungals. With decreasing susceptibility to Ketoconazole, the use of azoles should be shifted to Voriconazole, Fluconazole and Itraconazole.

**Keywords:** Fungal infection, Vulvovaginal Candidiasis, *Candida* speciation, Antifungal Susceptibility, Sabouraud's dextrose agar, HiCrome candida differential agar, Disk Diffusion method.

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## 1 | INTRODUCTION

Vulvovaginal Candidiasis (VVC) mostly affects women of childbearing age and it is the second most common form of vaginitis (1). Approximately 70-75% of women in the reproductive age group have at least one episode of vulvo-vaginal candidiasis, around 40-45% of women have chances of recurrence and around 5-8% recurrent vulvovaginal candidiasis (RVVC; four or more episodes of VVC in twelve months) (2), (3), (4). Vulvo-vaginal yeast infection occurs due to overgrowth of *Candida* species (5), (6), (7), (8), (9). Anti-fungal therapy is started by most gynaecologists during the patient's first visit without any culture and sensitivity testing (5). Risk of infection is higher in women having diabetes mellitus, on oral contraceptives, Human Immuno-deficiency Virus (HIV)/ Acquired Immuno-deficiency Syndrome (AIDS), using broad spectrum antibiotic therapy and pregnant women (10), (11), (12). Women who are pregnant have a two-fold increase in the prevalence of vaginal colonization by *Candida* species compared to that of non-pregnant women (13). Women having high sugar intake have higher risk of getting vulvo-vaginal candidiasis. vulvo-vaginal candidiasis can be due to several different species of *Candida*, but *C. albicans* is considered as one of the true vaginopathic agents (14), (15).

The range of VVC manifestations may be from asymptomatic colonization to severe acute symptomatic infection. 10%-20% women with VVC have asymptomatic colonization with *Candida* species (16). According to Centers for Disease Control and Prevention (CDC), the diagnosis of *Candida* vaginitis is suggested by the presence of the following symptoms: external dysuria and vulvar pruritus, pain, swelling, and redness. Vulvar edema, fissures, excoriations, and thick curdy white vaginal discharge are the signs of vulvo-vaginal candidiasis. Incorrect diagnosis results in persistence and worsening of the symptoms or recurrence. Isolation of the various *Candida* species in decreasing order- *C. Albicans* (85%-90% cases of VVC), *C. glabrata* (usually the second most common pathogen detected in VVC), *Candida tropicalis*, *Candida para-*

*psilosis* and *Candida krusei* (17), (18), (19). Recently, studies show that there has been a shift from *Candida albicans* to Non-*Albicans Candida* species (20), (21), (22), (23).

Studies done have shown that the percentage of *Candida albicans* isolates showing susceptibility to azoles was significantly lower in recurrent vulvo-vaginal candidiasis than in vulvo-vaginal candidiasis and recurrent vulvo-vaginal candidiasis is mostly caused by non *albicans* species (24), (25). Amphotericin B (polyene) and fluconazole (azole) are most commonly used for treating Candidiasis (26).

## 2 | BODY TEXT

### 2.1 | METHODOLOGY

The study was conducted from January- June, 2019 in the Department of Microbiology of a tertiary care Hospital with samples being collected from the gynaecology department and regional Primary Health Centre. The study was explained to the patients and consent was taken before the collection of sample.

#### 2.1.1 | Sampling

2 High Vaginal Swabs from 28 clinically suspected cases of vulvo-vaginal candidiasis were collected from outpatients and inpatients in the Gynaecology and Obstetrics Department. Samples were collected with strict aseptic precautions. Patients were from the age range of 25-54 years. Complete medical history of the patient was recorded.

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**Supplementary information** The online version of this article (<https://doi.org/10.15520/arjmcs.v8i02.408>) contains supplementary material, which is available to authorized users.

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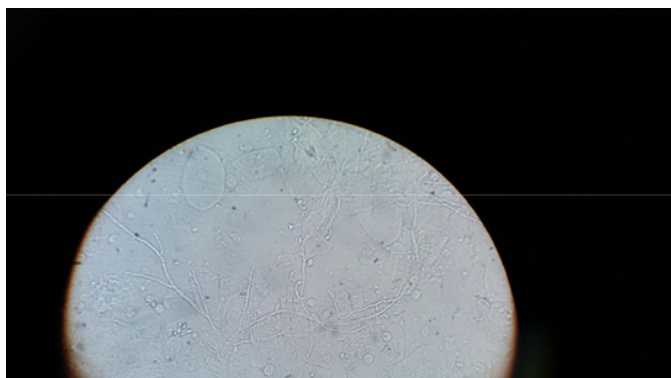
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## 2.1.2 | DIRECT EXAM

**Wet Mount:** The vaginal discharge was mixed with a solution of 10% Potassium Hydroxide (KOH). The solution was covered with a cover slip and was observed under low power and high power for budding yeast cells, pus cells, etc (Image 1).



**FIGURE 1:** *Candida Pseudo hyphae on KOH mount*

## 2.1.3 | Culturing:

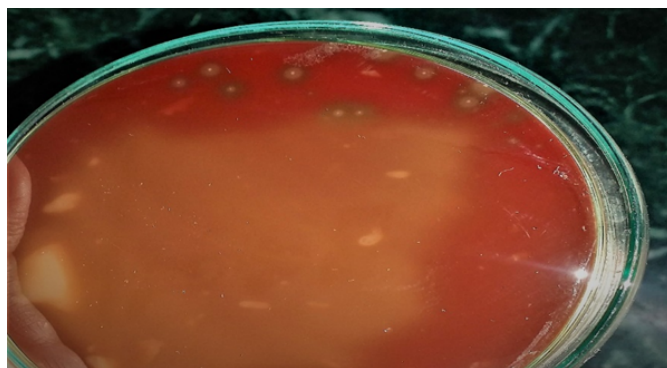
The swab was streaked on Sabouraud's dextrose agar (SDA) (Image 2) and Blood Agar (Image 3) in aseptic environment. The plates were then incubated at 30- 35°C for 48 hours. The colonies having dry, pasty, white to creamy colour, opaque growth were identified as *Candida* species.



**FIGURE 2:** *Candida on SDA*

## 2.1.4 | Identification/Microscopy:

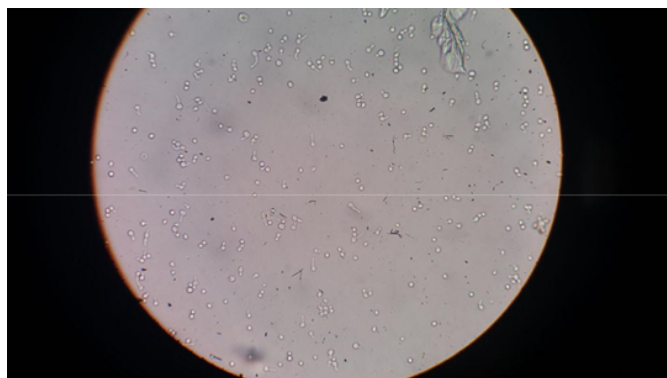
From the colonies, Gram staining was done. Under oil immersion microscope, Gram positive budding



**FIGURE 3:** *Blood agar showing haemolysis caused by Candida isolates*

yeast cells (blastoconidia) and pseudohyphae were seen.

**Germ Tube Confirmatory Test:** Germ tube test was conducted from the isolates on SDA. The *Candida* colony was suspended in human serum and incubated at 37°C for 2 to 4 hours. Presence of germ tubes on direct microscopy (wet mount) differentiated *Candida albicans* from other isolates (Image 4).



**FIGURE 4:** *Candida Germ Tube.*

## Speciation:

HiCrome candida differential agar was used for speciation. The colonies formed on Sabouraud's dextrose agar (SDA) were streaked on the HiCrome Agar (Image 5). The Plates were then incubated at 30-35°C for 48 hours. colony color and colony morphology was observed.

*Candida albicans*- Light green colonies

*Candida glabrata*- Pale pink colonies

*Candida tropicalis*- Blue colonies



**FIGURE 5:** Different Species of Candida on HiCrome candida differential agar

### 2.1.5 | Anti-fungal Susceptibility Testing

#### Inoculum Preparation-

Inoculum was prepared using five 1mm diameter 24 hour colonies formed on Blood Agar for individual species of each sample. Colonies were suspended in 5mL saline and vortexed for 15 seconds while adjusting the turbidity visually.

#### Inoculation of Test Plates-

Sterile cotton swab was dipped into the suspension, rotated several times and pressed firmly against the inside wall of the tube above the fluid level. The swab is then evenly streaked on the SDA Plates. The Plates were left to dry for 3-5 minutes.

#### Application of Disks to Inoculated plates-

Once the surface was dried, disks of Voriconazole, Ketoconazole, Fluconazole, Amphotericin, Nystatin, Itraconazole were placed at equal distance from each other. The plates were then inverted and placed inside an incubator at 30-35°C for 48 hours. The growths around the disks were noted for further analysis of Sensitivity/Resistance to the drugs.

## 3 | RESULTS & DISCUSSION

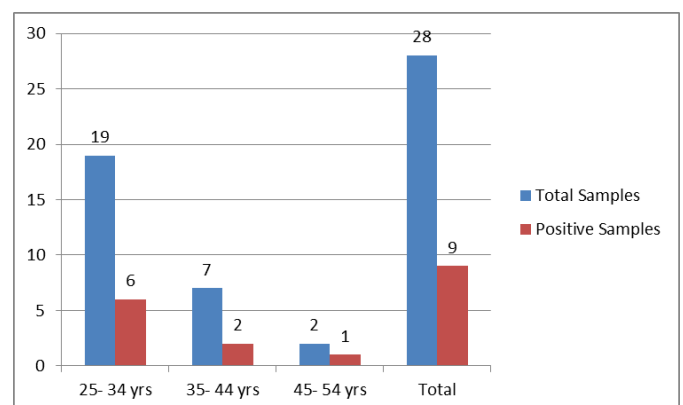
A total of 28 clinically suspected cases of VVC had visited the OPD during January to June 2019.

The 28 suspected cases were in the age range of 25-54 years. Out of the 28, 19 were in the 25-34 yrs age group, 7 in 35-44 yrs age group and 2 in the 45-54 yrs age group. The positivity in each age group

is depicted in (Table 1 & Graph 1). 9(32.14%) were positive for Candida species.

**TABLE 1:** Age wise distribution of samples positive for Candida

Age Group	Total Samples	Positive Samples	Percentage
25- 34 yrs	19	6	31.57%
35- 44 yrs	7	2	28.57%
45- 54 yrs	2	1	50%
Total	28	9	32.14%



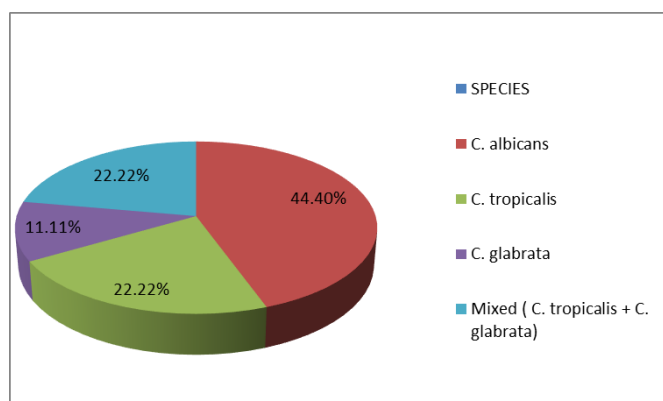
**Graph 1:** Depicting Age wise distribution of samples

Out of the 28, 9 cases (32.14%) were positive for candida, of which 4(44.4%) were *Candida albicans*, NAC were 5(55.5%) of which 2(22.22%) were *C. tropicalis*, 1 (11.11%) was *C. glabrata* and 2(22.22%) were combination of *C. glabrata* and *C. tropicalis* mixed infection (Graph 2)

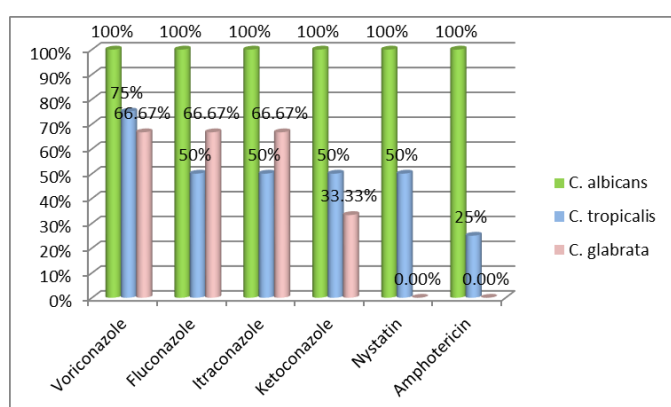
Anti-fungal susceptibility using the disk diffusion method revealed that *C. albicans* isolates were 100% sensitive to all antifungals under study. *C. tropicalis* was sensitive to the antifungals under study with a range of 25% to 75%. *C. glabrata* isolates were sensitive to Voriconazole, Fluconazole, Itraconazole and Ketoconazole but were resistant to Nystatin and Amphotericin (Graph 3).



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Graph 2: Depicting the different species of Candida isolated.



Graph 3: Antifungal susceptibility testing

## 4 | CONCLUSION

In recent years, Candida infections have increased due to increase in the predisposing factors like diabetes mellitus, use of oral contraceptives, HIV/AIDS, using broad spectrum anti-biotic therapy, pregnancy (10), (11), (12). Prevalence of VVC in the present study population was 32.14% which is in correlation with few studies where the prevalence was of range 14%-30% (27), (28), (29), (30). While some Indian studies reported VVC rates of 48%-50% (29), (31). 98% of cases have one causative agent of VVC as reported by other studies. (32), (33), (34), (35) In the present study 77.77% were caused by one species, while only 22.22% (2) cases had mixed infection with NAC species identified on Chromagar candida; which is similar to Richter SS et al., who identified the mixed causative agent in 14.9% of the vaginal samples by using chromogenic culture medium while Fan SR et al.,

identified the mixed causative agent in 2.2% of cases (36), (37). Hence, using Candida Chromagar in the culture medium helps in the detection of mixed infection in an effective way.

Most studies showed C. albicans as the commonest species causing VVC. In the present study, non-albicans Candida species together are of higher incidence than C. albicans. Although C. albicans continues to be the most common Candida species but incidence of non-albicans Candida species are showing a rising trend. Non-albicans Candida species were isolated in significant numbers (55.55%); with 44.44% C. tropicalis and 33.33% C. Glabrata. The increased incidence of NAC species may be due to the excessive use of antifungal agents which usually inhibits the growth of C. albicans, but fails to suppress the growth of few non-albicans Candida species. According to the study of Agarwal et al. (2004) C. tropicalis was more frequent which was in accordance with the present study.

It must be noted that all C. albicans isolates were 100% susceptible to all the antifungal agents used in the present study namely, Voriconazole, Ketoconazole, Fluconazole, Amphotericin, Nystatin, Itraconazole. C. tropicalis was sensitive to all the antifungals under study with a range of 25% to 75%. C. glabrata isolates were sensitive to Voriconazole, Fluconazole, Itraconazole and Ketoconazole but were resistant to Nystatin and Amphotericin.

Our results of antifungal sensitivity showed that amphotericin was least sensitive against all species of Candida. This is not in consistence with a study from India (Bhaumik et al., 2012). While All C. albicans were sensitive to amphotericin B in our study, correlating with the study done by Babin D. et al. (38). Also, higher resistance was observed for NAC as compared with C. albicans against antifungal drugs like previous studies (Almeida et al., 2013; Yang et al., 2008).

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