# Fungal Profile of Vulvovaginal Candidiasis in a Tertiary Care Hospital

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## ABSTRACT

**Introduction:** Vulvovaginal Candidiasis (VVC) is a common medical health problem of adult women. It is most commonly caused by *Candida albicans*. But there is a change in fungal profile. Sabouraud's Dextrose Agar (SDA) is the most common culture medium used where mixed fungal infection may be missed. It can be detected easily by using chromogenic culture medium.

**Aim:** To know the fungal profile of vulvovaginal candidiasis using *Candida* CHROMagar and antifungal susceptibility pattern in patients attending tertiary care hospital.

**Materials and Methods:** Culture confirmed cases of VVC presented at Department of Obstetrics and Gynaecology of Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India, from July 2015 to December 2015 were included in the cross-sectional study. Two high vaginal swabs were collected and inoculated on SDA and *Candida* CHROMagar (Hi-Media, Mumbai, India). After overnight incubation the colonies were counted and colour of the colonies were identified by sugar fermentation and assimilation tests and other conventional tests. Antifungal susceptibility tests were performed by the disc diffusion method using fluconazole (25

μg) and voriconazole (1μg) as per the Clinical and Laboratory Standards Institute (CLSI - M44-A2) guidelines.

**Results:** A total of 50 culture confirmed (23.7%) cases were detected from 211 clinically suspected VVC cases. *Candida glabrata* (45.1%) was the most common isolate, followed by *Candida tropicalis* (23.5%), *Candida albicans* (17.6%), *Candida krusei* (9.8%) and *Candida parapsilosis* (3.9%). One mixed infection of *C. glabrata* and *C. albicans* was identified on *Candida* CHROMagar. Mixed fungal infection was observed in 2% of positive culture and 0.5% of VVC cases. The antifungal susceptibility testing revealed that 15.7% and 9.8% isolates of *Candida* spp. were resistant and Susceptible Dose Dependent (S-DD) respectively to fluconazole. The increase resistant against fluconazole was because of increased isolation of *C. glabrata* strains. All strains of *Candida* spp. were susceptible to voriconazole.

**Conclusion:** *C. glabrata* was the most common causative agent of VVC in a tertiary care hospital. Chromogenic culture medium facilitates detection of mixed fungal infection. In vitro susceptibility testing should be used to guide the treatment especially in cases of non-*albicans* Candidiasis.

Keywords: Antifungal susceptibility testing, Candida CHROMagar, Fungal infection

# INTRODUCTION

VVC is a gynaecological condition diagnosed in a large proportion of women presenting with a complaint of abnormal vaginal discharge. It is caused by overgrowth of Candida species in the vagina [1]. It is clinically characterized by curd like discharge, itching dyspareunia, dysuria, oedema and vulvovaginal erythema [1]. The manifestations of VVC may range from asymptomatic colonization to severe acute symptomatic infection. Although it has a wide clinical presentation but there is no sign or symptoms which is pathognomonic of WC [2]. Therefore, laboratory support may be required for accurate diagnosis. Asymptomatic colonization with Candida species are found in 10%-20% of women [3]. The risk of VVC is high in women with diabetes mellitus, Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS), oral contraceptives, using broad spectrum antibiotics therapy, pregnant women and women involved in receptive oral sex [4-6]. During lifetime, about 75% of woman may experience at least one episode of WC and second episode may experience by 40%-45% of initially infected women. Recurrent Vulvovaginal Candidiasis (RVVC) is defined as four or more episodes of symptomatic WC within a year [7]. It has been observed that less than 5% of women with VVC may develop RVVC [7]. VVC is classified into two different categories - uncomplicated case and complicated case. Sporadic episodes of mild to moderate infections mostly caused by C. albicans among immunocompetent women are known as uncomplicated case. Complicated cases are cases of VVC caused by Non-albicans Candida (NAC)/severe infection/ recurrent infection/VVC during pregnancy or VVC associated with immunosuppression or diabetes [8].

Most of the time there is only one causative agent of VVC. SDA is the most common culture medium. Different species of yeast may not be differentiated on SDA. Now commercial chromogenic medium are available which easily detect mixed fungal infection [9]. Candida albicans is considered as the most common causative agent. It is isolated in 85%-90% cases of VVC [10]. C. glabrata is usually the second most common pathogen detected in VVC followed by other NAC- Candida tropicalis, Candida parapsilosis and Candida krusei [11,12]. C. tropicalis was found to be a predominant isolate next to the Candida albicans in some of the Indian study [13]. Interestingly isolation of C. glabrata from India varied widely from the most common isolate (61.3%) to less than 2% [12-14]. VVC by NAC are gradually increasing as a causative agent [13]. The fungal profile of WC varies from one geographical area to another. There should be a periodic study to know the fungal profile and antifungal susceptibility pattern of VVC. Very limited studies are available from India about the fungal profile of WC using chromogenic culture medium. This study was undertaken to know the fungal profile of WC using Candida CHROMagar and antifungal susceptibility pattern in our geographical location.

## MATERIALS AND METHODS

A total of 211 clinically suspected cases of VVC presented at Department of Obstetrics and Gynaecology, from July 2015 to

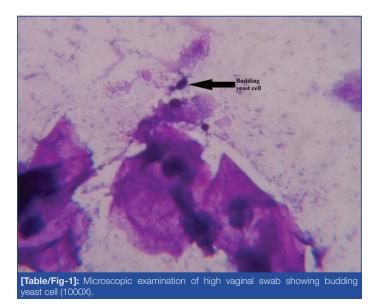
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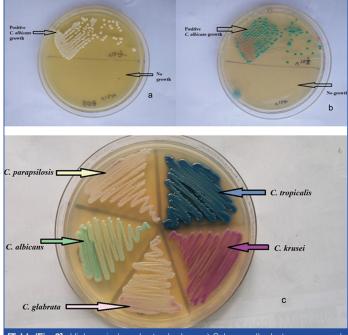
December 2015 in a tertiary care hospital. Patients were from 16-68 years of age with mean age of 33.4 (±8.7) years. Culture confirmed cases of VVC were included in the study which was approved by the Institute Ethics Committee. There were no exclusion criteria. Two High Vaginal Swabs (HVS) were collected from each patient and sent to the Department of Microbiology. Gram's stain was performed on one HVS and observed microscopically for the presence of pus cells and budding yeast cell etc. Another HVS from each patient was emulsified in 1 ml of sterile saline and 1 µl of the suspension was inoculated on to the SDA and Candida CHROMagar (Hi-Media, Mumbai, India). SDA and Candida CHROMagar plates were incubated at 25°C. After overnight incubation the colonies were counted and colour of the colonies were recorded from Candida CHROMagar. Growth was confirmed as yeast by doing Gram's stain. Candida spp. were identified by conventional tests like germ tube test, morphology on corn meal agar, sugar fermentation and assimilation tests [15,16]. Antifungal susceptibility tests were performed by the disc diffusion method using fluconazole (25 µg) and voriconazole (1 µg) (Hi-Media, Mumbai, India), on Muller Hinton Agar (MHA) (Hi-Media, Mumbai, India) supplemented with 2% Glucose and Methylene Blue dye 0.5 µg/ml (GMB) as per the CLSI-M44-A2 guidelines [17]. The categorical variables were expressed as frequency and percentages and the continuous variables were expressed as mean with standard deviation.

## RESULTS

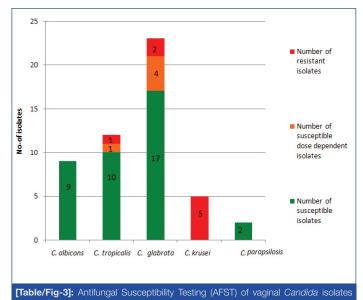
A total of 211 clinically suspected cases of WC were presented during the study period. Fifty HVS (23.7%) yielded positive yeast on culture. All positive yeasts were inoculated on both the culture medium - SDA and Candida CHROMagar. Culture confirmed cases of VVC were from 21-50 years of age with mean age of 33.1 (±7.9) years. Twenty two patients (44%) were 21-30 years of age followed by 19 (38%) were 31-40 years and 9 were (18%) 41-50 years. It was observed that 16 (32%) patients were suffering from pelvic inflammatory disease, 1 (2%) had history of four years of infertility and only one patient (2%) was diabetic. Direct microscopic examinations of the Gram's smear of HVS revealed budding yeast cell in only 7 (14%) cases [Table/Fig-1]. All of them yielded Candida on culture. Majority of high vaginal swabs - 40 (80%) revealed few pus cells (< 5 pus cells/HPF). Greater than 25 pus cells per HPF was observed in 2 (4%) HVSs, 10 to 15 pus cells/HPF were observed in 3 (6%) of them and 5 to 10 pus cells/HPF were seen in 5 (10%) HVSs.

SDA and *Candida* CHROMagar were able to isolate yeast in all positive cases [Table/Fig-2]. But one HVS yielded two *Candida* spp. which was appreciated on *Candida* CHROMagar. A total of 20 HVSs (40%) gave a colony count  $\geq 10^5$  colonies/ml, 20 swabs (40%) gave a count between  $10^4$ - $10^5$  colonies/ml and 10 swabs (20%) showed





[Table/Fig-2]: High vaginal swab streaked on; a) Sabouraud's dextrose agar and; b) *Candida* CHROMagar where upper half showed yeast growth and lower half – no growth; c) Morphology and colour of yeast on *Candida* CHROM agar.



against fluconazole.

colony count between  $10^3$ - $10^4$  colonies/ml. A colony count of  $\ge 10^5$  colonies/ml were seen in both yeast isolates, in the mixed culture.

*Candida glabrata* was the most common isolate and observed in 23 cases (45.1%), followed by 12 cases (23.5%) of *Candida tropicalis*, 9 cases (17.6%) of *Candida albicans*, 5 cases (9.8%) of *Candida krusei* and 2 cases (3.9%) of *Candida parapsilosis* infections. One mixed infection of *C. glabrata* and *C. albicans* was identified. It was observed in 2% of positive yeast culture and 0.5% of WC cases. All nine isolates of *C. albicans* were germ tube test positive.

The antifungal susceptibility testing revealed that, 38 isolates (74.5%) were susceptible to fluconazole. A total of 8 (15.7%) isolates of *Candida* spp. were fluconazole resistant – which include all five strains of *C. krusei*, two strains of *C. glabrata* and single strain of *C. tropicalis* [Table/Fig-3]. All isolates of *C. krusei* were considered resistant to fluconazole as it is intrinsically resistant to fluconazole. There were five isolates (9.8%) of S-DD to fluconazole which include four of *C. glabrata* and one of *C. tropicalis*. All isolates of *C. albicans* and *C. parapsilosis* were susceptible to fluconazole. All *Candida* spp. were susceptible to voriconazole. In case of *C. glabrata*, 2 out of 23 isolates were resistant (8.7%) to fluconazole, 4 showed S-DD

(17.4%), and other 17 were susceptible to fluconazole (73.9%). Among the *C. tropicalis* isolates 10 were susceptible (83.3%) and one isolate (8.3%) each of S-DD and resistant to fluconazole.

# DISCUSSION

VVC is a common public health problem of women presenting with a complaint of abnormal vaginal discharge. A total of 50 participants (23.7%) yielded positive yeast isolates from 211 clinically suspected cases of VVC which is in correlation with other studies [18-20]. It was predominantly seen in sexually active adult females and 44% of the participants were from 21 to 30 years of age in the study which is in correlation with other similar study [18,20]. Associated co-morbid condition and the risk factors were recoded from the requisition proforma on the first visit of the patient. It revealed only one diabetic patient. Risk factors would have been improved if all the patients had been screened for the blood sugar level, detailed clinical history would have been taken and follow up visits of the patients would have been done in the study.

Direct microscopic examinations of the Gram's stained smear of high vaginal swabs revealed budding yeast cell only in 7 (14%) cases and all of these cases yielded *Candida* on culturing. It again reemphasizes the fact that culture is more sensitive than microscopy [21]. Majority of high vaginal swabs – 40 (80%) revealed few pus cells i.e., < 5 pus cells/ HPF. A study from Sulaiman SP et al., observed that funguria was not associated with the pus cells [22]. Here VVC was not associated with presence of vaginal pus cells.

There was only one causative agent of VVC in 98% of cases in the study as reported by other studies [13,14,23,24]. One mixed infection of *C. glabrata* and *C. albicans* was identified on *Candida* CHROMagar. It was observed in 2% of positive culture and 0.5% of VVC cases. Fan SR et al., identified the mixed causative agent in 2.2% of cases which is similar to the current study while Richter SS et al., identified the mixed causative agent in 14.9% of the vaginal samples by using chromogenic culture medium [25,26]. Incorporation of *Candida* CHROMagar in the culture medium helps in the detection of mixed infection. Twenty high vaginal swabs (40%) gave a colony count  $\geq 10^5$  colonies/ml. High colony count help in differentiating between causative pathogen and colonizers.

NAC (82.4%) were isolated more than C. albicans (17.6%) in the study. C. glabrata (45.1%) was the most common species isolated, followed by C. tropicalis (23.5%), C. albicans (17.6%), C. krusei (9.8%) and C. parapsilosis (3.9%). Similar to our results, various studies have reported C. glabrata as the most common pathogen isolated from VVC [14,27]. A study from Goswami R et al. observed C. glabrata as the most common species isolated from diabetes mellitus patients and its frequency was higher when compared to control group [6]. In contrast to our study, a number of studies identified C. albicans as the most common causative agent; 85.2% in Argentina [23], 73.9% in Kuwait [24], 67% in Iran [28], 89% in Australia [29] and 35.5-66% in India [13,30]. C. glabrata in India varied widely from the most common isolate (61.3%) to less than 2% [13,14,31]. The current study is among the few study from India which depicted isolation of C. glabrata as more than 50% of VVC. Mohanty S et al. and Ray D et al., from AIIMS, New Delhi, India, observed 50.4% and 61.3% VVC by C. glabrata respectively [14,31]. The high isolation of C. glabrata in our hospital may be due to the fact that complicated cases, chronic cases or unresponsive cases of VVC may be presented at this tertiary care hospital. AIIMS, New Delhi, India is also one of the tertiary care hospitals as our hospital. Therefore our finding is in correlation with the finding from AIIMS. In several studies C. glabrata was the second most common causative agent of VVC after C. albicans [32]. Similar to our study Vijaya D et al., reported 26.4% C. tropicalis and 3.8% C. krusei [13].

Antifungal susceptibility testing of *Candida* species plays a vital role to establish the susceptibility patterns of isolates recovered from different regions in order to guide empirical therapy. Similar

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to our study, several studies have been reported on fluconazole resistance in C. glabrata [33,34]. Mohanty S et al., performed AFST of representative vaginal isolates and observed similar observation that 30% of Candida spp. were S-DD while none of the Candida spp. were found resistant to fluconazole [14]. Raghunathan L et al., recently studied AFST of 40 vaginal Candida isolates at Puducherry by E-test method and observed that 87.5% were susceptible to fluconazole, 7.5% were S-DD and only 5% were resistant [18]. The increased resistant against fluconazole in the current study was mainly because of high isolation of C. glabrata and C. krusei strains which have increased resistance against fluconazole. Hence, it is recommended to have laboratory based treatment of VVC at tertiary care hospital rather than syndromic management. It will prevent development of resistant cases in the population. It was also observed that all strains of C. albicans were found to be fluconazole susceptible. The WC infections caused by fluconazole resistant C. albicans have been rare [26]. Hence, it is important to know at least the causative agent as C. albicans or NAC so that accordingly fluconazole based regimen or non-fluconazole based azole regimen respectively can be provided [8,26]. Vaginal isolates from patients with recurrent VVC remain susceptible to fluconazole but may show increased resistance if the patient is on long-term exposure [35]. Further studies are is required to evaluate whether fluconazole resistance was due to infection of primary resistant strains of Candida spp. or acquired resistance due to long term repeated exposure of fluconazole among recurrent vulvovaginal candidiasis.

It is one of the few studies from Southern part of India which is describing voriconazole susceptibility of VVC isolates. None of the Candida species were found to be resistant to voriconazole. Similar to our results, Nguyen MH and Christine Yu CY [36] showed voriconazole in vitro activity against fluconazole-susceptible or fluconazole-resistant Candida isolates. Marcos-Arias C et al., observed that voriconazole as a useful alternative for recurrent oral candidiasis [37]. Ying C et al., observed only 81% of C. albicans strains were susceptible to voriconazole in three maternity hospitals in Shanghai [10]. Vijaya D et al., also observed all strains of C. parapsilosis and C. glabrata, 91.4% of C. albicans, 85.7% of C. tropicalis and 50% of C. krusei were susceptible to voriconazole [13]. Hence, continuous monitoring of antifungal susceptibility testing should be done to guide empirical treatment and in vitro susceptibility testing should be required for NAC isolates to guide appropriate treatment of VVC.

# CONCLUSION

The study strongly recommends laboratory based diagnosis and in vitro susceptibility testing specifically for NAC isolates in a tertiary care hospital. Chromogenic medium facilitate the detection of mixed fungal infection and may be included especially among recurrent vulvovaginal candidiasis. Antifungal susceptibility testing plays an important role in the treatment of VVC because recurrent vulvovaginal candidiasis is difficult to treat.

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