

# Characterisation of *Candida* in Vulvovaginitis Patients with Special Reference to Non-*albicans* from a Tertiary Care Hospital in Mumbai, India

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## ABSTRACT

**Introduction:** Vulvovaginal Candidiasis (VVC) is one of the commonest vaginal infections caused by *Candida* spp. affecting women of reproductive age group. Though *Candida* spp. is of endogenous origin several host related and behavioural factors can contribute to the development of infection. *Candida albicans* is the commonest spp. responsible for infection. However, an increasing emergence of Non-*albicans* *Candida* (NAC) has led to the development of recurrent infections, treatment failure and complications.

**Aim:** To characterise *Candida* spp. isolated from clinically suspected cases of VVC.

**Materials and Methods:** It was an observational prospective study done during the period of May 2017 to Oct 2018. A total of 150 vaginal isolates from samples of vaginal discharge were included in the study. *Candida* spp identification was done by

various methods like gram stain, germ tube test, culture on Sabouraud Dextrose Agar (SDA), Dalmau culture on corn meal agar, colour differentiation on CHROMagar (Chromogenic agar) and sugar assimilation tests. Statistical Package for Social Science (SPSS) was used for statistical analysis.

**Results:** Women of age group 26-35 years were most commonly affected population. *Candida albicans* (49.3%) and NAC (50.7%) both were present in almost equal number. The commonest isolate found was *Candida albicans* (49.3%) followed by *Candida glabrata* (29.3%) and *Candida tropicalis* (21.4%).

**Conclusion:** Highest infection rate (54%) was seen in the women of age group 26-35 years. *Candida albicans* being the commonest spp. As NAC are emerging rapidly, speciation will play a vital role in appropriate selection of antifungal agents for the treatment of fungal infections prior to the initiation of therapy.

**Keywords:** Chromogenic agar, Fungal infections, Speciation, Vaginal candidiasis

## INTRODUCTION

The VVC is the second most common vaginal infection affecting millions of women of reproductive age every year [1]. VVC is defined as signs and symptoms of inflammation in the presence of *Candida* spp. and in the absence of other infectious aetiology [2]. *Candida* spp. can be isolated from genital tract in upto 25% of asymptomatic healthy women of child bearing age [3]. Nearly 70-75% of women over 25 years of age report to have atleast one episode of VVC during lifetime. The recurrence rate is 40-50% and 5-8% have Recurrent VVC (RVVC) which is characterised by four or more episodes of the disease over a period of one year [4]. Certain physiological changes in the body like pregnancy, hormones, use of oral contraceptives, intra-uterine devices may lead to *Candida* vaginal colonisation. Use of immunosuppressants, glucocorticoids, can also lead to *Candida* infection [5]. VVC is diagnosed clinically in a large proportion of women in reproductive age group presenting with complaints of curdy white vaginal discharge accompanied by vulvovaginal pruritis, dyspareunia and red swollen vulva and vaginal mucosa. However, accurate diagnosis can be made by microscopic examination and culture [6]. The morbidity associated with VVC causes mental distress, altered self-esteem, anxiety, impairing work performance and interfering with sexual relations [7].

*Candida albicans* is the most common pathogen in 80-90% of cases of VVC [8]. However, there is an alarming increase of NAC species over last decade. Empirical use of antibiotics, over the counter use of antifungals, immunosuppressive conditions like Human Immunodeficiency Virus (HIV), autoimmune conditions or other severe illness may be responsible for the growing NAC [9]. The prevalence of NAC has been found to be 10-

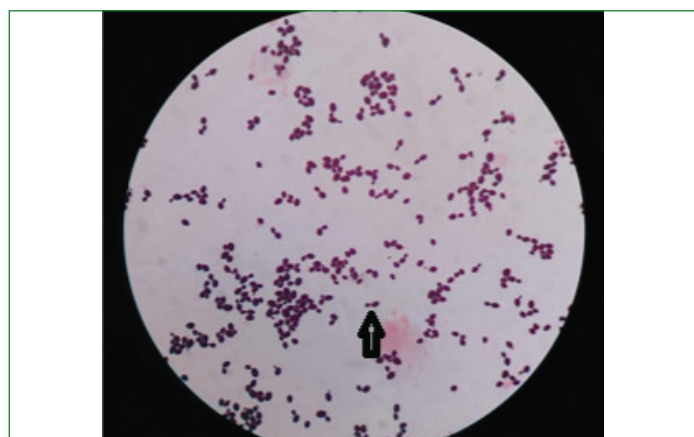
30% in patients with VVC [10]. Most commonly implicated NAC species include *Candida glabrata*, *Candida tropicalis*, *Candida krusei* and *Candida parapsilosis* [11]. VVC can be classified into uncomplicated and complicated cases. Uncomplicated VVC is characterised by sporadic or infrequent VVC with mild to moderate severity caused by *Candida albicans* in apparently healthy non-immunocompromised women. Complicated VVC include episodes due to NAC species or severe cases caused by any *Candida* spp. Moreover, both RVVC which is characterised by four or more episodes of the disease over a period of one year and VVC are classified as complicated when associated with recognised risk factors like diabetes, immunocompromised conditions like HIV or immunosuppressive therapy like corticosteroids [12]. Azoles are the most frequent class of antifungals used to treat *Candida* infections. Since fluconazole is also used in the syndromic management of Reproductive Tract/ Sexually Transmitted Infections (RTI/STI) program, in the kit for vaginal discharge, this may be a cause of recurrent infections and treatment failure by NAC. Further, if VVC is not treated on time, many complications occur, such as pelvic inflammatory disease, infertility, ectopic pregnancy, pelvic abscess, spontaneous abortion and menstrual disorders. Prevention, early diagnosis and prompt treatment of VVC are therefore essential to avert the complications [10].

Since, VC is frequently encountered in women visiting the gynaecology clinic, this study was initiated to characterise *Candida* spp. isolated from clinically suspected cases of VVC. Early speciation would help the clinician in selecting proper antifungal drug and thereby reducing the emerging resistance. Hence this study was carried out.

## MATERIALS AND METHODS

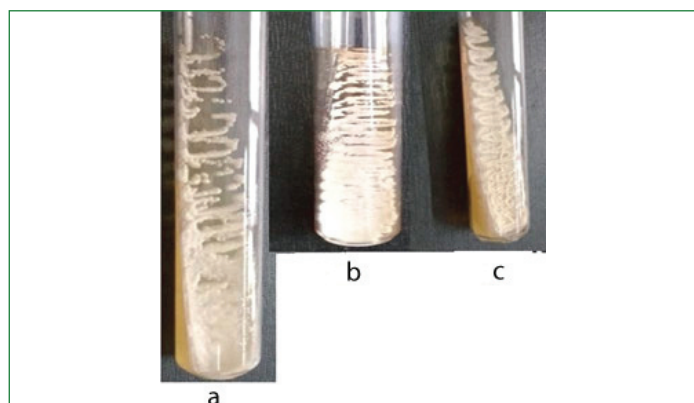
The present study was an observational prospective study which was carried out in the microbiology laboratory of Topiwala National Medical College, Mumbai, Maharashtra, India from May 2017 to October 2018 after approval from the institutional ethics committee, ECARP/2017/02. A total of 150 vaginal *Candida* isolates from samples of vaginal discharge received during this period were included in the study. As the study was carried on laboratory isolates, there was no patient history/neither informed consent was needed. Patients who had any other fungal infection and were on antifungals were excluded from the study.

The isolates received were processed by standard mycological tests [13]. Age of all the patients whose sample were received was recorded to know the common occurrence. Gram smear was performed on all the isolates for the presence of gram positive budding yeast cells [Table/Fig-1].

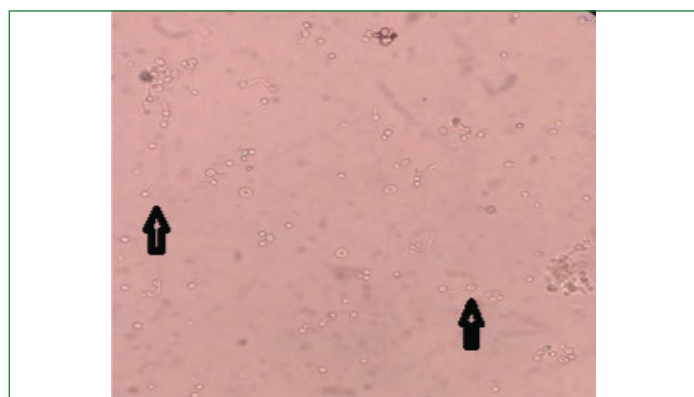


[Table/Fig-1]: Budding yeast cells on gram smear (100x) (shown by arrow).

The isolates were subcultured on Sabouraud Dextrose Agar (SDA) with antibiotics to observe different morphologies of *Candida* spp. as shown in [Table/Fig-2]. To differentiate between *Candida albicans* and NAC germ tube test was performed [Table/Fig-3] [14].



[Table/Fig-2]: Sabourauds Dextrose agar showing growth of *Candida albicans* (a), *Candida glabrata* (b) and *Candida tropicalis* (c).



[Table/Fig-3]: Germ tube test (40x) shown by *Candida albicans* (shown by arrow).

Further speciation of NAC spp was done by Dalmau culture method on corn meal agar. The production of chlamydo-spore, arrangement of blastospores and pseudohyphae were observed extensively by this method [Table/Fig-4]. CHROMagar was used to identify species on the basis of colour [14]. Sugar assimilation test was performed with Maltose, Sucrose, Lactose, Cellobiose, Trehalose and Dulcitol sugars, presence of growth around the disc indicating a positive test [14].



[Table/Fig-4]: Corn meal agar Dalmau culture of *Candida* spp.

## STATISTICAL ANALYSIS

SPSS version 25.0 was used to analyse the data. Data was entered and percentages were calculated.

## RESULTS

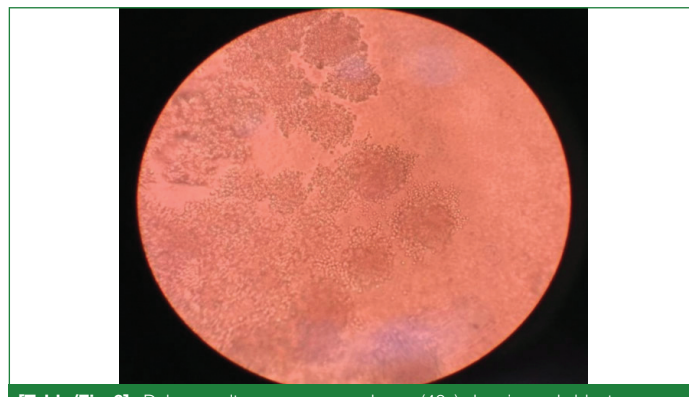
A total of 150 isolates were received. The women in the study were divided into different age groups. Highest infection rate of 54% was seen in age group of 26-35 years as shown in [Table/Fig-5].

Age (years)	Number of females	Percentage
18-25	31	20.6%
26-35	81	54.0%
36-45	34	22.7%
46-55	04	2.7%
Total	150	100%

[Table/Fig-5]: Age wise distribution of *Candida* infection in females.

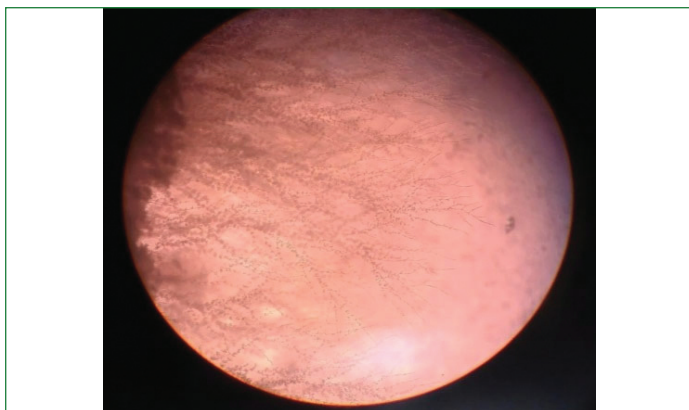
Of the total number of isolates (n=150), *Candida albicans* (49.3%) and NAC (50.7%) were in almost equal number.

Different colony morphology were seen on Corn meal agar as described in [Table/Fig-6-8]. On CHROMagar medium, light green colour was shown by all the *Candida albicans* isolates (49.3%) whereas all the isolates of *Candida glabrata* (29.3%) and *Candida tropicalis* (21.4%) showed pink and blue purple colour respectively.



[Table/Fig-6]: Dalmau culture on corn meal agar (40x) showing only blastospores without pseudohyphae characteristic of *Candida glabrata*.





**[Table/Fig-7]:** Dalmau culture on corn meal agar (40x) showing blastospores singly and in small groups along the pseudohyphae characteristic of *Candida tropicalis*.



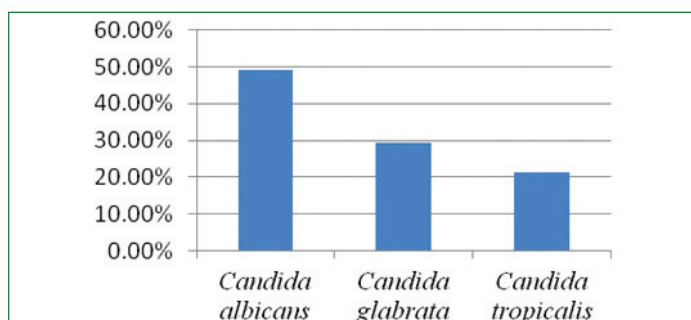
**[Table/Fig-8]:** Dalmau culture on corn meal agar (40x) showing clusters of blastospores, septate and pseudohyphae with large thick walled chlamydospores at the ends as shown by *Candida albicans*.

[Table/Fig-9] shows sugar assimilation results given by different *Candida* spp. All the isolates of *Candida glabrata* were positive for dulcitol whereas *Candida albicans* and *Candida tropicalis* were differentiated by lactose sugar.

<i>Candida</i> spp.	Maltose	Sucrose	Lactose	Cellobiose	Trehalose	Dulcitol
<i>Candida albicans</i>	+	+	+	+	+	-
<i>Candida glabrata</i>	-	-	-	-	+	+
<i>Candida tropicalis</i>	+	+	-	+	+	-

**[Table/Fig-9]:** Sugar assimilation results of different *Candida* spp.  
(+): Positive reaction; (-): Negative reaction

Hence, on the basis of the above mentioned tests, NAC isolated were *Candida glabrata* and *Candida tropicalis*. Amongst the total NAC spp (n=76), *Candida glabrata* (n=44) (57.9%) was commonest followed by *Candida tropicalis* (n=32) (42.1%). Among the total isolated *Candida* spp (n=150), *Candida albicans* (n=74) was the predominant species followed by *Candida glabrata* (n=44) and *Candida tropicalis* (n=32) [Table/Fig-10].



**[Table/Fig-10]:** Species distribution of *Candida* isolates.

## DISCUSSION

The VVC affects females of reproductive age group causing abnormal vaginal discharge. Changes in vaginal flora, acidity of vaginal fluid, hormonal variation and various host related and behavioural factors cause *Candida* spp to induce pathological changes and thereby cause infection in the vagina [2].

Although *Candida albicans* is responsible for the largest number of symptomatic episodes of vaginal discharge, the occurrence of NAC species is increasing. NAC spp cause various complications like pelvic inflammatory disease, infertility, ectopic pregnancy, spontaneous abortion and menstrual disorders [6]. Thus, to avoid such complications and recurrence and to give an effective therapy to patients it is necessary to speciate *Candida*.

In this study, the common age group affected was 26-35 years followed by 36-45 years. This suggests that women in reproductive age group were more vulnerable to vaginal candidiasis. Similar findings were reported by Lakshmi N et al., who showed 45% infection rate in 26-35 years age group followed by 25% in 36-45 years age group [11]. Also, Babin D et al., reported 49.58% as the highest infection rate in the age group 26-35 years [15]. The spp isolation in different studies is shown in [Table/Fig-11] [11,15-18].

Author name [Reference no.]	Place and year of publication	<i>Candida albicans</i>	NAC
Babin D et al., [15]	Kerala, 2013	35.5%	64.5%
Muthusamy S and Elangovan S, [16]	Puducherry, 2016	45.8%	54.2%
Lakshmi N et al., [11]	Andhra Pradesh, 2015	22.3%	77.7%
Gandhi TN et al., [17]	Gujarat, 2015	66.4%	33.6%
Das KH et al., [18]	Tamil Nadu, 2019	42%	58%
Present study	Mumbai, 2021	49.3%	50.7%

**[Table/Fig-11]:** Species isolation of *Candida* in different studies [11,15-18].

The above studies show a preponderance of NAC, however in present study both had an equal isolation.

NAC were further speciated by colony morphology on corn meal agar, colour differentiation on CHROMagar and sugar assimilation tests. The NAC species found in this study were *Candida glabrata* that predominated with 57.9% followed by *Candida tropicalis* (42.1%). This finding correlated with the report of Gandhi TN et al., [17] in which *Candida glabrata* (15.65%) was the commonest NAC spp followed by 9.85% *Candida tropicalis*. Also, Muthusamy S and Elangovan S, reported 22.91% *Candida glabrata* as the commonest NAC species [16]. Distribution of *Candida* isolates in this study included *Candida albicans* (49.3%) followed by *Candida glabrata* (29.3%) and *Candida tropicalis* (21.4%). Similar findings were reported by Gandhi TN et al., showing 66.4% *Candida albicans* followed by 15.65% *Candida glabrata* and 9.85% *Candida tropicalis* [17]. Muthusamy S and Elangovan S, reported *Candida albicans* (45.8%) as the predominant species followed by 22.91% of *Candida glabrata* followed by 14.58% *Candida parapsilosis* and 8.33% *Candida tropicalis* [16]. However, *Candida parapsilosis* was not reported in this study. Thus, in this study, both *Candida albicans* and non-*albicans* spp were isolated.

## Limitation(s)

As the study was carried out on laboratory isolates, clinical history of the patients was not taken and hence clinical parameters were not included in this study.

## CONCLUSION(S)

This study showed highest infection rate in females of reproductive age group. The findings of the study suggest that the *albicans* and non-*albicans* species of *Candida* are both associated with vaginal infections. Speciation of *Candida* will help the clinicians in advising proper antifungal therapy as NAC infections do not respond to

the commonly used drugs. This will prevent recurrent infections in patients and also prevent resistance in the organisms due to improper use of antifungals.

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### PLAGIARISM CHECKING METHODS: <sup>[Jain H et al.]</sup>

- Plagiarism X-checker: Oct 22, 2020
- Manual Googling: Jan 22, 2021
- iThenticate Software: Mar 04, 2021 (11%)

### ETYMOLOGY: Author Origin

### AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Oct 21, 2020**

Date of Peer Review: **Nov 26, 2020**

Date of Acceptance: **Jan 29, 2021**

Date of Publishing: **Apr 01, 2021**