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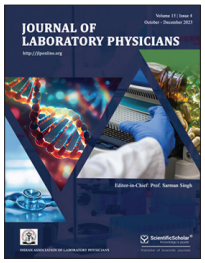


*Original Article*

# Antifungal susceptibility profile of *Candida* species isolated from women with vulvovaginal candidiasis

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## ABSTRACT

**Objectives:** The objective of this study was to study the occurrence of vulvovaginal candidiasis (VVC) among women with vaginitis and to determine the antifungal susceptibility testing (AFST) pattern of *Candida* spp. isolated.

**Materials and Methods:** A prospective study was conducted in a tertiary care setting. The two high vaginal swabs were collected and subjected to Gram's stain, wet mount examination, and cultured on blood agar and sabouraud dextrose agar with chloramphenicol. The cultures were incubated at 37°C for 18–24 h. The *Candida* spp. was identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS), and AFST was performed according to the Clinical and Laboratory Standards Institute guidelines M27-A3.

**Statistical Analysis:** The data was collected in excel sheet. The qualitative variables are presented as frequencies and percentages. The quantitative variables are presented as mean with SD and median. p value of less than 0.05 was considered statistically significant. Analysis will be done by using Epi InfoTM statistical software.

**Results:** A total of 961 women with symptoms of vaginitis were screened. *Candida* spp. was isolated in 108 (11.2%) patients. The median age of affected women was 29 years, and the majority belonged to 20–30 years (60.2%). Age <40 years was the significant risk factor ($P$ = 0.016). Vaginal discharge was the most common complaint (82.4%),followed by itching (55.6%), vulvar edema, dyspareunia, and dysuria. *Candida albicans* was the predominant species (46.3%),followed by *Candida glabrata* (36.1%), *Candida krusei* (6.5%), and *Candida tropicalis* (6.5%). Ninety-six percentages of *C. albicans* were sensitive to fluconazole, while only 22.4% of non-albicans *Candida* spp (NAC) were susceptible. *C. albicans* showed 8% resistance to itraconazole and 8% intermediate resistance to caspofungin, whereas *C. glabrata* showed resistance to fluconazole, itraconazole, and caspofungin.

**Conclusions:** The study showed a higher incidence of NAC and higher antifungal resistance leading to treatment failure. It is, hence, crucial to send fungal cultures, speciate, and perform AFST for all symptomatic patients of vulvo-vaginal candidiasis (VVC).

**Keywords:** Vulvovaginitis, Candidiasis, Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry, Antifungal susceptibility testing

## INTRODUCTION

Vaginitis is the most common syndrome in women presenting to the gynecology outpatient department (OPD).[1] Patients usually show signs of inflammation of the vaginal mucosa, such as abnormal vaginal discharge, itching, soreness, burning, and pain during coitus.[1] The

bacterial vaginosis accounts for 40–50% of cases, followed by vulvovaginal candidiasis (VVC) accounting for 20–25% and trichomoniasis for 15–20%.[2] Nearly, 75% of women of reproductive age will experience VVC at least once in their lifetime, and about 40%–50% will have one additional episode. About 5–8% of women will have four or more episodes of VVC, which is referred to as recurrent VVC (RVVC).[3] Among VVC, *Candida albicans* accounts for 80–95%. Recently, infection due to non-albicans *Candida* spp. (NAC) is on the rise (10–30%).[4]

*Candida* spp. is part of the normal vaginal flora in about 20% of asymptomatic women. It becomes an opportunistic pathogen when the immunity is lowered.[5] The risk factors for VVC include pregnancy, the use of antibiotics, age, uncontrolled diabetes, oral contraceptives, hormone replacement therapy, immunosuppression, and frequent sex.[1] Recurrent vulvovaginal candidiasis (RVVC) can occur in women colonized with *Candida* spp. in the oral cavity and lower gastrointestinal tract.[4,5]

VVC is now considered a global public health problem associated with direct or indirect economic loss.[6,7] It not only causes morbidity in women but also poses a negative impact on work and social life.[8] Hence, the present study was conducted with the aim (i) to study the occurrence of VVC among women with vaginitis attending the Gynecology OPD at a tertiary care teaching hospital in South India and (ii) to speciate and determine the antifungal susceptibility profile of the *Candida* spp. isolated from them.

## MATERIALS AND METHODS

A prospective study was conducted on women attending the gynecology outpatient clinic with symptoms of vaginitis from April 2020 to March 2021. After obtaining informed consent, two high vaginal swabs (HVSs) were collected from each patient by a Gynecologist. One HVS was used for Gram stain and wet mount, while the other was used for culture on sheep blood agar (SBA) and Sabouraud dextrose agar (SDA) with chloramphenicol. Epithelial cells, pus cells, Gram-positive budding yeast cells with or without pseudohyphae, and Gram-positive bacilli resembling lactobacilli were recorded on Gram stain. For wet mount preparation, the swab was placed in 0.5 mL sterile saline and gently mixed and examined under the microscope for the presence or absence of motile trophozoites of *Trichomonas vaginalis*, pus cells, and red blood cells.

For culture, the swab was inoculated onto 5% SBA and SDA (HiMedia, Mumbai, India) incubated at 37°C for 18–24 h in an ambient air incubator. Any growth of white cream colonies on the SDA was further characterized.

### Phenotypic characterization of *Candida* species

The *Candida* isolates were preliminarily identified with Gram's stain, germ tube test, growth on SDA with cycloheximide, sugar assimilation test using Yeast Nitrogen Base (HiMedia, Mumbai, India), and HiChrome Candida Differential medium (HiMedia, Mumbai, India).[9]

### Confirmatory test for speciation

Yeast isolates were identified using matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS). A loop full of overnight growth of yeast isolate was suspended in 1 mL of double-distilled water, vortexed, and centrifuged for 3 min at 13,000 rpm. The supernatant was discarded, and the pellet was washed, centrifuged, and resuspended in 100 µL of distilled water. From this suspension, 1 µL was loaded on the MALDI target plate and allowed to air dry. The dry spot was overlaid with 0.8 µL of 98% formic acid and again allowed to dry. The spot was superseded using 1 µL of MALDI matrix α-cyano-4-hydroxycinnamic acid solution saturated with organic solvent (2.5% trifluoroacetic acid and 50% acetonitrile) and air-dried thoroughly before loading the plate onto MALDI-TOF MS instrument (Bruker Daltonics, Bremen, Germany). Protein mass fingerprint product ion spectra were obtained automatically with Microflex acquisition control software (Flex control 3.4; Bruker Daltonics, Bremen, Germany). Processing of raw spectra was done by MALDI BIOTYPER software (Bruker Daltonics, MC, and Italy) using default settings. The results obtained were categorized using modified score values propounded by the manufacturer: a score ≥2 indicates the identification of species, a score in the range of 1.7–1.99 indicates the identification of genus, and a score below 1.7 denotes no reliable identification.

### Antifungal susceptibility testing (AFST)

Antifungal susceptibility of *Candida* spp. was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines M27-A3 broth microdilution method using RPMI 1640 buffered to a pH of 7.0 with morpholine propane sulfonic acid against eight antifungal drugs (Sigma Aldrich, Bengaluru, India). The control strains used were *Candida krusei* American Type Culture Collection (ATCC) 6258 and *Candida parapsilosis* ATCC 22019. Isolates were categorized as sensitive, intermediate, and resistant according to the breakpoints suggested in CLSI M27-S4 guidelines.[10]

### Treatment of cases

The diagnosis of vaginal candidiasis was confirmed when heavy growth of *Candida* spp. was isolated from vaginal samples. Such patients were treated as per National AIDS Control Organization guidelines on sexually transmitted infection/reproductive tract infection syndromic case management kit-2 that contains secnidazole 2 g and fluconazole 150 mg.

## RESULTS

A total of 961 patients with vaginitis were included during the study period. The *Candida* spp. was isolated in 108 vaginal samples making the overall prevalence 11.2%. The median age of women suffering from VVC was 29 years (range 20–73 years). The majority belonged to the 20–30 years age group (65/108; 60.2%), followed by 31–40 years (30/108; 27.8%) and above 40 years (13/108; 12%). Age <40 years was the significant risk factor for VVC ($P = 0.016$).

Vaginal discharge was the most common presenting complaint ($n = 89$; 82.4%), followed by itching ($n = 60$; 55.6%), vulval edema ($n = 21$; 19.4%), dyspareunia ($n = 16$; 14.8%), and dysuria ($n = 13$, 12%). Out of 108 cases, 30 (27.8%) women were pregnant at the time of the study. Twelve percentages of women had a history of prior antibiotic usage, and 6.5% ($n = 7$) had diabetes mellitus.

One hundred and eight isolates from the vaginal samples represented eight different species. *C. albicans* was the predominant species (50/108, 46.3%), followed by *Candida glabrata* (39/108, 36.1%), *C. krusei* (7/108, 6.5%), and *Candida tropicalis* (7/108, 6.5%). The phenotypic method could correctly identify 99 (91.7%) isolates to the species level. The nine isolates which had discrepant results between the two methods were re-tested with MALDI-TOF MS, and results consistently showed a MALDI score of >2. The phenotypic method identified *Candida inconspicua*, *Candida nivariensis*, and *Candida viswanathii* as *guilliermondii*, *C. glabrata*, and *C. albicans*, respectively. The correct identification rates of *C. albicans*, *C. glabrata*, *C. krusei*, and *C. tropicalis* by the phenotypic method were 98% (49/50 isolates), 92.3% (36/39 isolates), 100% (7/7 isolates), and 100% (7/7 isolates), respectively.

Prior use of antibiotics ($P = 0.017$) and pregnancy ($P = 0.028$) was significantly associated with NAC infection. The association between different symptoms and the species type was not statistically significant.

The antifungal susceptibility testing results by minimum inhibitory concentration (MIC) are shown in Table 1. All the isolates were susceptible to most of the antifungals tested except *C. glabrata* and *C. krusei*. Ninety-six percentages (48/50) of *C. albicans* isolates were sensitive to fluconazole, while only 13/58 (22.4%) NAC were sensitive to fluconazole. If we exclude the *C. krusei* and *C. glabrata*, which are intrinsically resistant, all other NAC were susceptible to fluconazole.

The MICs of azole and echinocandin antifungals were higher for *C. glabrata* and *C. krusei* than those of *C. albicans*. The *C. albicans* showed 8% resistance to itraconazole (>0.25 μg/mL). *C. glabrata* showed 100% and 92.3% dose-dependent susceptible MICs for fluconazole (>2 μg/mL)

**Table 1:** Antifungal susceptibility testing of *Candida* spp. by MIC (mg/L) using CLSI-BMD method.

| Drug | Amphotericin B | | Fluconazole | | | Voriconazole | | Itraconazole | | | Posaconazole | Caspofungin | | | Anidulafungin | Micafungin | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Organism (*n*)** | **R** | **S** | **R** | **S** | **SDD** | **I** | **S** | **R** | **S** | **SDD** | **S** | **I** | **R** | **S** | **S** | **R** | **S** |
| *Candida albicans* (50) | 0 | 50 | 1 | 48 | 1 | 0 | 50 | 0 | 46 | 4 | 50 | 4 | 0 | 46 | 50 | 0 | 50 |
| *Candida glabrata* (39) | 0 | 39 | 0 | 0 | 39 | 3 | 36 | 12 | 3 | 24 | 39 | 18 | 2 | 19 | 39 | 1 | 38 |
| *Candida inconspicua* (2) | 0 | 2 | 0 | 2 | 0 | 0 | 2 | 0 | 2 | 0 | 2 | 0 | 0 | 2 | 2 | 0 | 2 |
| *Candida krusei* (7) | 0 | 7 | 7 | 0 | 0 | 0 | 7 | 0 | 3 | 4 | 7 | 5 | 0 | 2 | 7 | 0 | 7 |
| *Candida nivariensis* (1) | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 |
| *Candida parapsilosis* (1) | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 |
| *Candida tropicalis* (7) | 0 | 7 | 0 | 7 | 0 | 0 | 7 | 0 | 7 | 0 | 7 | 0 | 0 | 7 | 7 | 0 | 7 |
| *Candida viswanathii* (1) | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 |
| Total | 1 | 107 | 6 | 61 | 41 | 3 | 105 | 12 | 62 | 34 | 108 | 27 | 2 | 79 | 108 | 1 | 107 |

R: Resistant, S: Sensitive, I: Intermediate, SDD: Susceptible-dose-dependent, CLSI-BMD: Clinical and laboratory standards institute-Broth microdilution, MIC: Minimum inhibitory concentration

and itraconazole (>0.25 μg/mL), respectively. Persistent symptoms were observed among 25 women (23.1%) infected with *C. krusei* and *C. glabrata*. Such patients were treated with prolonged fluconazole therapy.

## DISCUSSION

Despite advances in diagnosis and treatment, VVC remains a significant health problem among immunocompetent women. Minimal epidemiological information is available from India, and most of the data available are from observational studies. The present study estimated the prevalence of VVC as 11.2%, which was comparable to the results of Pereira *et al.*[11] The previous data from India showed a varied prevalence of 14–21%.[12] The signs and symptoms of VVC cases in our study were almost comparable to the previous studies.[13] The most common age group affected was between 20 and 40 years (88%). Yano *et al.*[13] and Bitew and Abebaw[6] had similar findings in their study. The risky sexual behavior, age at first sexual intercourse <20, hormonal effects, educational status, menstrual hygiene, eating sweet foods, and wearing tight undergarments were found to be the risk factors for VVC.[5,14]

Limited data are available regarding the distribution of yeasts causing the VVC in India and their drug susceptibility. Eight different species of *Candida* were isolated from vaginal samples. *C. albicans* was the most common isolate (46.3%), but the NAC was more frequent (53.7%). The isolation rates of NAC species from various studies ranged from 19.8% to 65%.[6] In the present study, *C. glabrata* (36.1%) was the most common NAC species, followed by *C. krusei* (6.5%) and *C. tropicalis* (6.5%).

Other studies also report *C. glabrata* as the most common NAC species in VVC.[6] NAC species cause milder disease compared to *C. albicans*.[15] However, inherent or acquired resistance to azole antifungals can complicate the management of VVC caused by NAC species.[6] For infections with NAC species, prolonged antifungal therapy is required to clear the infection. Most studies reporting high proportions of NAC species were from tertiary care hospitals, focusing on patients who failed the conventional treatment.[16] The data from these studies may overestimate NAC prevalence if extrapolated to the general population. The other reason for increased NAC prevalence is the use of over-the-counter azole antifungals, which leads to the selection of NAC species. Hence, accurate identification up to the species level is essential for successful therapy, especially in the age group between 20 and 40 years and in women in the perimenopausal and postmenopausal stages.

*C. albicans*, *C. glabrata*, *C. krusei,* and *C. tropicalis* were the four most common species of VVC cases. The phenotypic method identified *C. albicans* (98%) with greater accuracy, while it had a lower correct identification rate for NAC (86.2%). Although MALDI-TOF MS has excellent sensitivity and specificity in identifying the species, the equipment is expensive, and not always accessible for diagnosis.

The *C. glabrata* and *C. krusei* had higher azole MIC and were difficult to treat, often requiring prolonged therapy. Fluconazole resistance was 4% with *C. albicans* isolates, whereas *C. glabrata* exhibited higher MIC for azoles and echinocandins than those for *C. albicans*. Similar findings were also reported earlier by Shi *et al.*[17] and Castanheira *et al.*[18]

All the patients were clinically diagnosed by obstetricians and gynecologists and treated with a prescription of antifungal drugs. Almost 60% of patients reported control or relief of symptoms on standard treatment with topical and oral azoles. Relapse was reported among the women infected with NAC. Hence, speciation and antifungal susceptibility testing will help clinicians decide on the antifungal drug and the duration of therapy.

The limitations of the present study were relatively of a small sample size. Moreover, follow-up of cases could not be done, and relief and cure were based on self-reported data. A large and multicentric study to identify the species distribution and antifungal susceptibility testing (AFST) data is the need of the hour. This is mainly to manage this important public health problem affecting many young women in India.

## CONCLUSIONS

The age group between 20 and 40 years was a significant risk factor for VVC. Compared to the previous studies from India, the prevalence of NAC was higher in the present study. In addition, antibiotic use in the past, pregnancy, and diabetes was all strongly linked to NAC infection. Therefore, the management of VVC should be guided by culture, accurate species identification, and antifungal susceptibility testing, particularly for recurrence or relapses.

### Ethical approval

ESIC MC PGIMSR and MH, Rajajinagar, Bengaluru No. 532/L/11/12/Ethics/ESICMC&PGIMSR/Estt. Vol.III Dated: March 2020.

### Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Nil.

### Conflicts of interest

There are no conflicts of interest.

**Use of artificial intelligence (AI)-assisted technology for manuscript preparation**

The author confirms that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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