

# A study of Candida species from vaginal discharge specimens of clinically symptomatic women of reproductive age group

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## Abstract

**Background:** Candida species are part of the lower genital tract flora of healthy asymptomatic women. They eventually become pathogenic under some prevailing conditions. Confirmation of yeast species helps to initiate early appropriate antifungal therapy thereby reducing the morbidity. **Aim:** To isolate and identify Candida species from vaginal discharge specimens of clinically symptomatic women of reproductive age group. **Material and Methods:** From 184 symptomatic women, two high vaginal swabs were collected from posterior vaginal wall with aseptic precautions. First swab was used for microscopic examination. Second swab was used for fungal culture on Sabouraud's dextrose agar (SDA) slant. Any growth suggestive of yeast was subjected to the CHROM agar test, corn meal agar test, germ tube test, urease test and sugar assimilation test as per standard protocol for further identification. **Results:** Out of the total 184 high vaginal swab specimens, Candida species was isolated in 42 cases (22.8%). Significantly higher percent of cases showed *C. albicans* 52.4%, followed by *C. tropicalis* 35.7%, *C. krusei* 7.1% and *C. glabrata* 4.8%. **Conclusion:** Candida albicans predominated in this study. Presumptive identification followed by confirmation of yeast species helps in initiating an early and appropriate antifungal therapy thereby reducing the morbidity.

**Key Word:** symptomatic women, vaginal discharge, Candida species, Candida albicans

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## INTRODUCTION

Vulvovaginal candidiasis (VVC) is a fungal or yeast infection of lower genital tract of the female caused by Candida species. This can be referred to as Candidiasis or Moniliasis. Candida species are part of the lower genital tract flora in 20%–50% of healthy asymptomatic women.<sup>1</sup> They are normal flora of the vagina that eventually become pathogenic under some prevailing conditions, and thus presents as a common etiology of

vulvovaginitis. When prompt recognition and laboratory confirmation is not achieved, this could lead to devastating genital discomfort and a major reason for frequent hospital visits.<sup>1</sup> *Candida albicans* is the most common species causing VVC. Nevertheless, over last few years research evidence has demonstrated an increment in frequency of cases caused by non- albicans Candida species which could be due to indiscriminate usage of antimycotics which eliminates the more sensitive *C. albicans*.<sup>1</sup> Diagnosis of VVC based only on patient history and genital examination is not possible because of the low specificity of symptoms and signs, since other causes like leukorrhea and pruritus vulvae, mimic VVC. Therefore, in order to have a definitive diagnosis of VVC, cultural isolation and identification of Candida spp. are crucial.<sup>1</sup> Confirmation of yeast species helps to initiate early appropriate antifungal therapy thereby reducing the morbidity. Thus, the present study was carried out to isolate and identify Candida species from vaginal discharge specimens of clinically symptomatic women of reproductive age group.

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## MATERIAL AND METHODS

This prospective cross-sectional study was carried out in Department of Microbiology at a tertiary care hospital over a period of one and a half year. The study commenced after Institutional Ethics Committee approval.

**Sample size:** Sample size was calculated by the Absolute Precision Formula: Sample size (n) =  $Z^2 \alpha \times PQ/d^2$  Where, a) P=prevalence, b) Q=100-P, c) Confidence level=95%, therefore Z = 2, d) Absolute precision required on either side of the proportion (in percentage points) d=10 percentage points i.e. 10% confidence interval. Thus, a total of 184 women from reproductive age group were included in the study.

### Inclusion criteria

- Women in 15 to 45 years age group with symptoms of vaginal discharge with or without itching around the vaginal region or erythema or pain.
- Women who were sexually active.

### Exclusion criteria

- Women with history of local or systemic antifungal treatment in the past one month.
- Pregnant women with medical disorders.
- Women who have never been sexually active.
- Women currently menstruating.
- History of hysterectomy.

A detailed clinical history was taken from the patients regarding age, use of contraception, associated

comorbid conditions like diabetes, infertility, pelvic inflammatory disease and previous history. Following clinical examination, two high vaginal swabs were collected from posterior vaginal wall with aseptic precautions and sent to Microbiology department for further processing.

**Processing of the sample:** First swab was used for microscopic examination. Wet mount and smear for Gram staining was prepared from this swab as per standard protocol. Second swab was used for fungal culture. It was streaked on both Sabouraud's dextrose agar (SDA) slant with chloramphenicol and gentamycin and also on Sabouraud's dextrose agar with chloramphenicol, gentamycin and cycloheximide. They were then incubated at 37°C and 25°C respectively for 4 weeks. These slants were examined for growth, daily for the first week and twice a week for subsequent period. Any growth suggestive of yeast was subjected to the CHROM agar test, corn meal agar test, germ tube test, urease test and sugar assimilation test as per standard protocol for further identification.<sup>2</sup>

**Statistical analysis :** Statistical analysis was performed with the software package: SPSS statistic 20 for Windows. The significance of difference of proportion of categorical variables among groups was examined by the chi-square test (large samples) and Fischer's exact test (small samples). The difference in mean was examined by student's t test. A value of P of  $\leq 0.05$  was considered significant for all statistical analyses and is marked with an asterisk(\*).

## RESULTS

Out of the total 184 high vaginal swab specimens, Candida species was isolated in 42 cases (22.8%) while in the rest 142 (77.2%) specimens no fungal growth was seen. Thus the prevalence of VVC in the present study was 22.8%.

Table 1: Correlation of different age group with growth of Candida species

Age group	Diagnosis			Total	Test	P value
	Growth of Candida	No growth of Candida				
21-25yr	Count	2	10	12	Fischer's Exact test value: 13.257	0.006*
	%	16.7%	83.3%	100%		
26-30yr	Count	16	24	40		
	%	40%	60%	100%		
31-35yr	Count	19	65	84		
	%	22.6%	77.4%	100%		
36-40yr	Count	4	42	46		
	%	8.7%	91.3%	100%		
41-45yr	Count	1	1	2		
	%	50%	50%	100%		
Total	Count	42	142	184		
	%	22.8%	77.2%	100%		
Mean age in years		31.71±3.73	33.13 ±4.26	32.81 ±4.18	T= 3.797	0.053

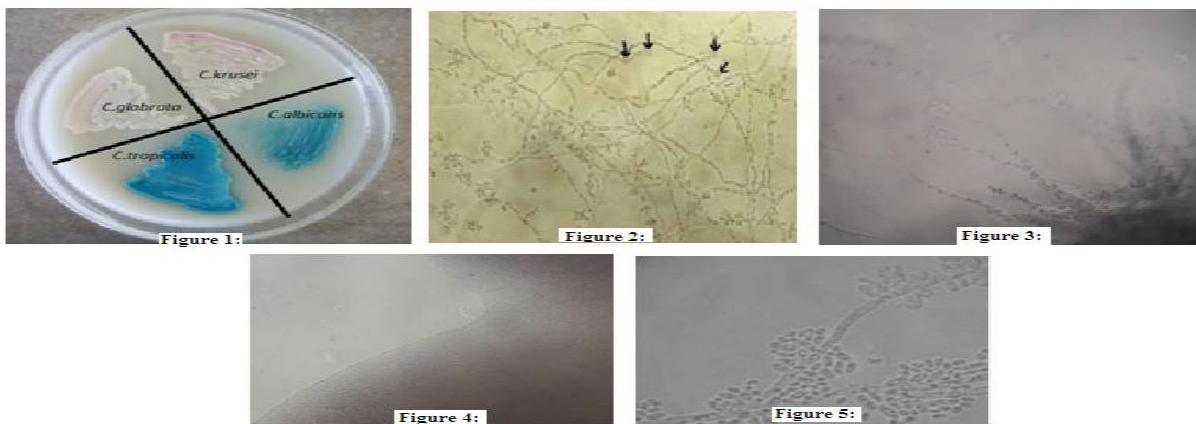
Candida positive and negative cases were compared in various age-groups. Amongst various age groups, 26-30yr (40%)

followed by 31-35yr (22.6%) and 21-25yr (16.7%) showed higher percent of Candida positive cases as compared to other age groups. This variation seen in culture positive cases over different age groups was found to be statistically significant. Mean ages of Candida positive and negative cases were compared. This was not found to be statistically significant. In 42.9% of cases both pus cells and yeast cells were seen on gram stain whereas in 57.1% cases, pus cells and yeast cells were not seen on gram stain. There was no significant difference seen on gram stain findings of pus cells and yeast cells.

**Table 2:** Distribution of Candida species in VVC cases

Species of candida	No. of patients	Percentage (%)
<i>C. albicans</i>	22	52.4
<i>C. tropicalis</i>	15	35.7
<i>C. krusei</i>	3	7.1
<i>C. glabrata</i>	2	4.8
Total	42	100

Cases positive for Candida were divided into groups based on the species isolated. Significantly higher percent of cases showed *C. albicans* 22 (52.4%) followed by *C. tropicalis* 15 (35.7%). (Chi square value: 26.762; P value: <0.001\*).



**Figure 1:** CHROM agar showing different coloured growth of Candida species; **Figure 2:** Chlamydoconidia of *Candida albicans* seen on corn meal agar; **Figure 3:** *Candida tropicalis* showing blastospores singly or in very small groups all along the pseudohyphae in cornmeal agar; **Figure 4:** *Candida glabrata* showing small spherical, highly compacted yeast cells without pseudohyphae on corn meal agar; **Figure 5:** *Candida krusei* showing pseudomycelia with elongated blastospores forming "tree-like" appearance

## DISCUSSION

In spite of several treatment modalities and application of new effective drugs, VVC is a complex and considerable problem in gynecology and obstetrics.<sup>3</sup> On the other hand, different studies in different countries have illustrated that the Candida species involved in this disease along with their susceptibility to antifungal agents, is also changing in recent years.<sup>4-7</sup> In the present study, the most common Candida species isolated was *Candida albicans* which accounted for 22/42 (52.4%) of total Candida species isolated whereas Non-albicans Candida (NAC) accounted for the rest 20/42 (47.6%) of the growth. Among NACs most common species isolated was *Candida tropicalis* 15/42 (35.7%) followed by *Candida krusei* 3/42 (7.1%). The least isolated species was *Candida glabrata* accounting for 2/42 (4.8%) of total growth. In the study reported from Amritsar by Jindal *et al*,<sup>8</sup> *C. albicans* was the most common isolate (69.57%) which is similar to the present study. Among 30.43% of NACs in this study, the most common

species was *C. glabrata* (8.7%) while *C. tropicalis* predominated in the present study. In Vijaya *et al* study<sup>9</sup> conducted in Karnataka, *C. albicans* was the most common species isolated 35/53 (66%) followed by NACs 18/53 (34%). Among NACs commonest species was *C. tropicalis* (26.4%) followed by *C. krusei* (3.8%) and the least was *C. glabrata* (1.9%). This study was consistent with the present study. In the study conducted by Vijaya *et al*<sup>9</sup> and also in the present study, *C. tropicalis* was the most common NAC species isolated unlike in other studies where *C. glabrata* or *C. krusei* was the major isolate.<sup>10-12</sup> On comparison of various studies, it was observed that in most of the studies, including the present study, *C. albicans* was the predominant species isolated. *C. albicans* adheres to vaginal epithelial cells in significantly higher numbers than other Candida species which could be the reason for the relative infrequency of the latter in vaginal candidiasis.<sup>12</sup> In contrary to the present study, the study conducted by Kalaiarasan *et al*,<sup>13</sup> showed non albicans candida as the predominant species

causing VVC. Kalaiarasan *et al* reported *C.glabrata* (45.1%) as the most common isolate followed by *C.tropicalis* (23.5%), *C.albicans* (17.6%) and *C.krusei* (9.8%). Various other studies have also reported *C.glabrata* as the most common pathogen isolated from VVC.<sup>14-16</sup> An increase in the prevalence of NAC vaginitis is seen in many studies.<sup>12,17-20</sup> The possible reason for this may be the increased indiscriminate usage of antimycotics which removes the more sensitive *C.albicans* and selects more azole-resistant NAC species.<sup>17,20</sup>

## CONCLUSION

*Candida albicans* predominated in this study followed by *C. tropicalis*, *C. krusei* and *C. glabrata*. Presumptive identification followed by confirmation of yeast species helps in initiating an early and appropriate antifungal therapy thereby reducing the morbidity.

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