

SPECIES DISTRIBUTION OF CANDIDA ISOLATES FROM THE PATIENTS WITH VULVOVAGINAL CANDIDIASIS IN TERTIARY CARE CENTRE, BIHAR

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Abstract

Background: Vulvovaginal candidiasis here is defined as isolation of *Candida* species in culture from study participants with sign and symptom of vaginal abnormalities. Vulvovaginal candidiasis, bacterial vaginosis, and trichomoniasis are the most common cause of vaginitis of which vulvovaginal candidiasis is the second most common after bacterial vaginosis. **Materials and Methods:** A cross sectional study was conducted from August 2021 to July 2022 at Department of Microbiology, SKMCH, Muzaffarpur, Bihar. Age groups of patients were classified following WHO guideline. Prior to sample collection, patients with genital tract problem were clinically investigated by gynecologists on duty and signs and symptoms of vaginal abnormalities were recorded. All vaginal swabs were then transferred without delay to the Department of microbiology, SKMCH Muzaffarpur, Bihar. Each Vaginal swab specimens were subjected to direct 10% KOH smear examination as well as cultured on CHROMagar™ *Candida* plates, incubated at 35 °C for 4 days to determine the co-infection rate of yeast species and distinguish between *C. albicans*, *C. tropicalis*, *C. glabrata* and *C. krusei* and other yeast species according to the manufacturer's instructions. All agar plates were evaluated for the yeast growth and colony color every day. The presence of yeasts on agar plates was confirmed by the presence of budding yeast in wet preparations with 0.85% saline. All data from the investigation were coded, doubleentered, and analyzed using SPSS version 20. All ethical considerations and obligations were duly addressed, and the study was conducted after the approval of the Departmental Ethical Review Committee. SKMCH, Muzaffarpur, Bihar. **Result:** In the present study we assessed yeast carriage associated with vaginal candidiasis symptoms. The overall prevalence of vulvovaginal candidiasis was 44.1%. Younger women, between 15 and 24 years had a somewhat lower prevalence (31.5%) of vulvovaginal candidiasis, while in the 25 years and older group, the prevalence was between 45.0 and 50%. The adjusted odds ratio showed that vulvovaginitis was not significantly associated with age ($p = 0.33$). **Conclusion:** High prevalence rate of vulvovaginal candidiasis and observation of a high prevalence rate of non-albicans *Candida* species in the present study warrants, the importance of conducting continuous epidemiological surveys to measure changes in species distribution from *C. albicans* to non-albicans *Candida* species.

INTRODUCTION

Vulvovaginal candidiasis here is defined as isolation of *Candida* species in culture from study participants with sign and symptom of vaginal abnormalities. Vulvovaginal candidiasis, bacterial vaginosis, and trichomoniasis are the most common cause of vaginitis of which vulvovaginal candidiasis is the second most common after bacterial vaginosis.^[1,2] Nearly 5–10 million females seek gynaecologic

advice for vaginitis every year world-wide.^[3] Many studies,^[3–8] have reported that three fourth (75%) of women will experience an episode of vulvovaginal candidiasis in their lifetimes, 50% of these will experience at least a second episode, and 5–10% of all women experience recurrent vulvovaginal candidiasis i.e., ≥ 4 episodes of vulvovaginal candidiasis per year.

Although the rate of vulvovaginal candidiasis is frequent, the reasons for its occurrence and

recurrence are often unclear. Socio-demographic characteristics, use of antibiotics and oral contraceptives, diabetes mellitus, dietary practices, personal hygiene, sexual activity, and specific immunological defects have been identified as potential risk factors.^[3,9-11] However, the data supporting each of these factors are conflicting.

In most developing countries such as India, vulvovaginal candidiasis is still received little attention since it is considered to be a trivial disease. However, vulvovaginal candidiasis has been identified as one of a global issues of concern due to its association with direct and indirect economic costs,^[12,13] sexually transmitted infections, particularly HIV,^[14] and ascending genital tract infection.^[15]

Despite *Candida albicans* is the most common cause of vulvovaginal candidiasis, the frequency of vulvovaginal candidiasis caused by other *Candida* species, such as *C. tropicalis*, *C. glabrata*, and *C. krusei* is increasing, especially in HIV-infected women.^[16] Yeast infections resistant to antifungal agents have been increasing and their frequency will likely continue to increase. Resistance to azoles antifungal therapy in *C. albicans* and non-*albicans* *Candida* species has become more common.^[17] Against this background, the aim of this study was to identify and determine the yeasts implicated in causing vulvovaginal candidiasis from patients attending at Tertiary Care Centre, Bihar. Apart from precise identification and susceptibility testing; shortened turnaround times, improved specimen handling, enhanced quality control, reproducibility and the ability to track results are further benefits of the system.^[18]

MATERIALS AND METHODS

A cross sectional study was conducted from August 2021 to July 2022 at Department of Microbiology, SKMCH, Muzaffarpur, Bihar. Socio-demographic, sexual behaviour, and reproductive health characteristics such as age, education, marital status, number of life time male sex partner, history of abortion, previous history of genital tract infection, frequency of vaginal bathing, and frequency of changing underwear were collected by face- to- face interviews using a structured questionnaire. Age groups of patients were classified following WHO guideline.^[19]

Prior to sample collection, patients with genital tract problem were clinically investigated by gynecologists on duty and signs and symptoms of vaginal abnormalities were recorded. Vaginal swabs were collected from study participants having different vaginal abnormalities such as vaginal discharge, itching, vulvar pruritus, irritation, pain during intercourse, and during urination using sterile rayon-tipped applicator stick swabs by experienced nurses. All vaginal swabs were then transferred without delay to the Department of microbiology,

SKMCH Muzaffarpur, Bihar. Each Vaginal swab specimens were subjected to direct 10% KOH smear examination as well as cultured on CHROMagar™ *Candida* plates, incubated at 35 °C for 4 days to determine the co-infection rate of yeast species and distinguish between *C. albicans*, *C. tropicalis*, *C. glabrata* and *C. krusei* and other yeast species according to the manufacturer's instructions. All agar plates were evaluated for the yeast growth and colony color every day. The presence of yeasts on agar plates was confirmed by the presence of budding yeast in wet preparations with 0.85% saline. If yeast species were the same, it was considered to be a single isolate. All yeast isolates were initially subcultured on Sabouraud dextrose agar (SDA) (Merck, Germany) supplemented with 0.02% chloramphenicol and kept in tryptic soy broth medium (TSB, Himedia, India) containing 2.5% glucose, 3% peptone, and 20% glyceron at -80 °C for further use. Germ tube tests in serum at 37 °C for 2-3 h were also used for the differentiation of *C. albicans* and NAC species.

Statistical analysis

All data from the investigation were coded, doubleentered, and analyzed using SPSS version 20. Descriptive statistics and logistical regressions were used to estimate crude ratio with 95% confidence interval to the different variables. P-value < 0.05 was considered significant.

All ethical considerations and obligations were duly addressed, and the study was conducted after the approval of the Departmental Ethical Review Committee. SKMCH, Muzaffarpur, Bihar.

RESULTS

In the present study we assessed yeast carriage associated with vaginal candidiasis symptoms. The overall prevalence of vulvovaginal candidiasis was 44.1%. Younger women, between 15 and 24 years had a somewhat lower prevalence (31.5%) of vulvovaginal candidiasis, while in the 25 years and older group, the prevalence was between 45.0 and 50%. The adjusted odds ratio showed that vulvovaginitis was not significantly associated with age ($p = 0.33$). The prevalence of vulvovaginal candidiasis varied with education and marital status. Women who are illiterate were more affected than those patients with primary school education and above. Similarly, vulvovaginal candidiasis was higher among divorced study subjects (56.2%) compared to unmarried (45.1%) or married (34.7%) study subjects. Vulvovaginitis also varied with selected reproductive health history characteristics. It was more in patients with previous genital tract infection (56.2%) than in those patients with no previous genital tract infections (31.2%). The adjusted odds ratio showed that vulvovaginal candidiasis was significantly associated with previous genital tract infection ($p = 0.004$). The magnitude of infection was more or less the same in

study subjects with history of abortion and with no history of abortion. The magnitude of vulvovaginitis was statistically significant with the number of lifetime male sex partners ($p = .037$) and number of male sex.

Partners in 12 month ($p = 0.001$). The prevalence of vulvovaginal candidiasis was less than in those patients who changed underwear more frequently (two per day; 36.3%) than those who change their pants less frequently (one pant for 2–4 days; 44.7%). Similarly, patients who bathed their vaginal region more frequently were less affected than that did not

bath their vaginal area more frequently (prevalence rate 42.0% versus 41.2%). Species distribution of Candida species is depicted in [Table 1]. A total of ten species of Candida were isolated from 200 women. *C. albicans* was the commonest isolate accounting for 58.6% of the total yeast isolate. The percentage of non-albicans Candida species (41.4%) was less than that of *C. albicans* (58.6%). Of the non-albicans Candida species, *C. krusei* was predominantly isolated (17.2%), whereas *C. lusitaniae* and *C. inconspicua* were found to be the least prevalent isolates (1.1%).

Table 1: Species distribution of Candida isolates from 200 patients with vulvovaginal candidiasis

Species of Candida	No. Of Isolates	% of Isolates
<i>C. albicans</i>	45	56.8%
<i>C. krusei</i>	10	12.7%
<i>C. dubliniensis</i>	7	8.2%
<i>C. glabrata</i>	2	4.3%
<i>C. inconspicua</i>	1	2.1%
<i>C. tropicalis</i>	1	2.1%
<i>C. kefir</i>	1	2.1%
<i>C. guillieromondii</i>	1	2.1%
<i>C. lusitaniae</i>	1	2.1%
<i>C. parapsilosis</i>	1	2.1%
Total isolates 70		2.1%

DISCUSSION

Vulvovaginal candidiasis (VVC) is the second most common vaginal infection in reproductive age women. The results from different studies indicate that the prevalence of vulvovaginal candidiasis varies between countries, depending on the country, region, and population. In cases of complicated VVC, vaginal cultures are necessary to confirm clinical diagnosis and identify unusual species, because patients are more likely to have an infection with non-albicans Candida strains, which may require different treatment but in Vietnam this issue has received very little attention. Therefore, the current study has been performed to determine the prevalence, species distribution of yeast causing vaginal discharge and antifungal susceptibility pattern of *C. albicans* among symptomatic non-pregnant women of reproductive age group.

Vulvovaginal candidiasis is not a reportable disease and the diseases are routinely diagnosed by sign and symptom without the support of laboratory diagnosis. As the result, the spectrum of yeasts implicated in causing the disease and their drug susceptibility profile is not known in the country. The prevalence of vulvovaginal candidiasis varies from one study to another. It is the second most common infection of the vulvovaginal area of symptomatic women accounting for about 17% to 42.%.^[2,3,9] Although the prevalence rate of infection in our study (44.1%) was within the reported range, it was slightly higher than the prevalence rates reported by Ahmed et al,^[3] and Olowe et al,^[9] but lower than the prevalence rate reported by Erylander et al.^[20,21] Differences in socio-demographic characteristics, immune– status of patients,^[5] treating patients with broad spectrum

antibiotics and immune suppressive drugs,^[22] and hormonal influences,^[23] have been identified as some of the factors for differences in the prevalence of the occurrence and/or recurrent vulvovaginal candidiasis among studies. Age, level of education, and marital status as possible risk factors for vulvovaginitis were investigated in the present study. We did not find strong evidence about the associations between socio-demographic characteristics and the prevalence of vulvovaginal candidiasis. Among socio-demographic characteristics, age seems to be an important factor in the overall occurrence of vulvovaginal candidiasis. Out of 70 patients with vulvovaginal candidiasis, 75 (86.1%) patients were in their 2nd to 4th decade of life. Our result was comparable with previous study. Sobel et al,^[11] indicated that vulvovaginal candidiasis is infrequent at puberty (the first occurrence of menstruation), but its frequency increases towards the end of the second decade of life (10–19 years of age) reaching its peak in the third (20–29 years of age) and fourth (30–39 years of age) decade of life. Even though the crude odds ratio revealed that the association of age and vulvovaginal candidiasis was statistically significant the association was not statistically significant as far as the adjusted odds ratio was considered. This may indicate that the association was influenced by other variables. The infection was more in women that were illiterate than in those patients with primary school education and above, and the association of vulvovaginal candidiasis and level of education was statistically significant ($p = 0.0.021$). Improvement in personal hygiene and/or in economic status resulted from education may possibly explain the difference in the rate of infection between illiterates and those with better education. Our finding was consistent

with the findings of Rathod et al.^[24] but in contradiction with the conclusion reached by Vadav and Prakash.^[25] Similarly, it was higher in divorced study subjects (56.2%) than unmarried (45.1%) or married (34.7%) study subjects. The association of vulvovaginitis with marital status was not statistically significant, but it was statistically associated with previous genital infection ($p = 0.04$). Our result was consistent with the findings of Rathod et al.^[24]

Little attention has been given to reproductive health, behavioral factors, and personal hygiene as a risk factor for vulvovaginal candidiasis. In this study, the prevalence of vulvovaginal candidiasis by selected sexual behavior, reproductive health and personal hygiene was assessed. The results of the logistic regression analysis with the adjustment for potential confounders showed that vulvovaginal candidiasis was significantly associated with an increase in the number of life time male sex partner ($p = 0.037$) and male sex partners in 12 months ($p = 0.001$). Our finding was not in line with other previous reports. Sobe et al.^[11] indicated that number of years women had been with their sex partners is not associated with vulvovaginal candidiasis. Furthermore, the role of frequency of coitus as a risk factor to vaginitis remains controversial.^[26] The study of Janković et al.^[27] showed that vulvovaginal candidiasis was statistically associated with continual wearing of panty liners and use of vaginal tampons during menstruation, a finding which is inconsistent with our result ($p = 0.054$). The association of the infection with the frequency of vaginal bathing was not statistically significant ($p = .078$).

CONCLUSION

High prevalence rate of vulvovaginal candidiasis and observation of a high prevalence rate of non-albicans *Candida* species in the present study warrants, the importance of conducting continuous epidemiological surveys to measure changes in species distribution from *C. albicans* to non-albicans *Candida* species. Although, fluconazole still appeared to be active against all isolates of *C. albicans* and non-albicans *Candida* species high resistance rate of *C. krusei* against the drug may support a search for alternative antifungal drugs when treating vulvovaginal candidiasis caused by *C. krusei*.

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