

A study on Vulvovaginal candidiasis among non-pregnant women

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Abstract

Introduction: Candida species are opportunistic pathogens which cause a wide variety of infections in humans ranging from trivial intertriginous infection to fatal candidemia. Vulvo vaginal candidiasis (VVC) is an acute inflammatory disease and a frequent reason for gynaecological consultations. The present study aimed at determining prevalence of Candida species from VVC and to speciate isolated Candida with antifungal susceptibility profile among non pregnant women. **Materials and Methods:** This was a cross sectional study conducted over a period of six months in the department of microbiology and Obstetrics and Gynecology. Swabs collected from patients were subjected to microscopy (Gram s stain) and culture on Sabouraud's dextrose agar. Species level was identified by using chrome agar. Antifungal susceptibility testing: Antifungal susceptibility testing was performed by NCCLS M44-A Disc diffusion method. **Results:** A total of 209 high vaginal swabs were collected from non pregnant women. 71 patients high vaginal swabs yielded growth of Candida species and accounted for 33.97%. Most commonly isolated species were Candida albicans 31 (43.66%) followed by Candida tropicalis 27(38.03%) and Candida glabrata 13 (18.31%). All species were found to be susceptible to Amphotericin B. Among commonly used antifungals, clotrimazole was found to be most susceptible. But, C.glabrata species was least susceptible to frequently used antifungals. **Conclusion:** Candida albicans was the most commonly isolated species. CHRO Magar is rapid, technically simple and cost effective compared to time consuming technically demanding expensive conventional methods. Performing antifungal susceptibility is useful in choosing appropriate antifungal in treating vulvo vaginal candidiasis.

Key words: Vulvo vaginal candidiasis, Candida albicans, CHROM agar

Introduction

Vulvo vaginal candidiasis (VVC) is an acute inflammatory disease and a frequent reason for gynecological consultation as it can affect up to 75% of women of child-bearing age [1]. Clinical signs and symptoms include intense pruritus, vaginal discharge, anerythematous vulva and dyspareunia [2]. Until recently, the problem of vaginal candidiasis was often ignored, or treated as an insignificant problem for the female population. It received more focus only after Herman Gardner said: "Vaginitis can cause more inconvenience than any other gynaecological disease. In addition, many mental and emotional problems are associated with vaginitis" [3].

There are many risk factors for development of vulvo vaginal candidiasis, like vaginal ecosystem, pregnancy, hormonal contraception, diabetes, stress recent antibiotic use, dietary practices, gastrointestinal colonization by the organism, clothing and weakened immune-compromised system [4].

VVC is most often caused by *Candida albicans*, however, other species of *Candida* such as *Candida glabrata*, *Candida parapsilosis*, and *Candida tropicalis* are emerging [5]. Azole antifungals are being frequently used to treat infections caused by *Candida* species. Recently studies reported that intrinsic azole resistance in some *Candida* species as well as development of high-level azole resistance is a problem of critical importance in the clinical setting [6]. Most of the studies done previously focused on immunocompromised

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subjects, especially pregnant women, with few studies on other wise immunocompetent women.

The present study aimed at determining prevalence of Candida species from VVC and to speciate isolated Candida with antifungal susceptibility profile among non pregnant women.

Material and Methods

This was a cross sectional study conducted over a period of six months in the department of microbiology in collaboration with the department of Obstetrics and Gynaecology of Vinayaka mission's medical college and hospital. Study population consisted of non pregnant women attending OPD of Obstetrics and Gynaecology. Verbal consent was obtained from the women before sample collection. Clinical examination was performed of each participant and recorded signs of vaginal abnormalities. A pair of high vaginal swabs were obtained from the posterior vaginal fornix of the subjects aseptically with the help of a vaginal speculum. Swabs collected from patients were subjected to microscopy (Gram s stain) and culture on Sabouraud's dextrose agar.

Species identification: CHRO Magar was used for preliminary identification of yeast and to detect mixed infections. Candida species were differentiated by colonial morphology and colors, which were generated by a chromogenic in the agar, as described by Wortman et al [7].

Antifungal susceptibility testing: Antifungal susceptibility testing was performed by NCCLS M44 - A Disc diffusion method.

Inoculum was prepared by picking five distinct colonies of approximately 1 mm in diameter from 24 h old culture of Candida species. Colonies were suspended in 5 ml of sterile saline and its turbidity was adjusted visually with the transmittance to that produced by a 0.5 McFarland standard. Inoculation of test plates were done with a sterile cotton swab dipped into the suspension. The dried surface of a sterile Mueller-Hinton + GMB (glucose and methylene blue) agar plate was inoculated by evenly streaking the swab over the entire agar surface. Anti fungal disks were dispensed onto the surface of inoculated agar plate. Plates were incubated at 35^o C and examined after 20-24 hours. The zone of inhibition was measured and the results were recorded as susceptible/resistant [8]. Patients aged between 15-45 years and non-pregnant were included in the study. Immunocompromised patients, menstruating women and patients who were taken antifungal therapy in past two weeks (before sample collection) were excluded. Statistical analysis was done by simple percentage method.

Results

A total of 209 high vaginal swabs were collected from non pregnant women aged between 15-45 years attending with any vulvovaginal candidiasis symptoms (itching, soreness, vaginal secretions, inflammation, rashes etc.). Of the 209 patients included in the study, 71 patients high vaginal swabs yielded growth of Candida species and accounted for 33.97%. Majority of the patients yielded growth were ranged between 26-35 years and accounted for 54% followed by 16-25 years which showed 30%. Least growth was seen in the patients between the age group of 36-45 years which accounted for 14% (Table.1).

Table-1: Age wise distribution of Candida growth on SDA medium.

| Age | Growth on SDA |
|-------|---------------|
| 15-25 | 22 (30%) |
| 26-35 | 39(54%) |
| 36-45 | 10 (14%) |

Table-2: Candida species isolated from high vaginal swabs.

| Candida species | Number (%) |
|--------------------|-------------|
| Candida albicans | 31 (43.66%) |
| Candida tropicalis | 27 (38.03%) |
| Candida glabrata | 13 (18.31%) |
| Total | 71 (100%) |

Most commonly isolated species were Candida albicans 31 (43.66%) followed by Candida tropicalis 27(38.03%) and Candida glabrata 13(18.31%) (Table.2)

Table-3: Antifungal susceptibility of Candida species isolated.

| Candida species | Clotrimazole | Fluconazole | Miconazole | Amphotericin B |
|---------------------------|--------------|-------------|------------|----------------|
| Candida albicans (n=31) | 22(70.96%) | 11(35.48%) | 19(61.29%) | 100(100%) |
| Candida tropicalis (n=27) | 15(55.55%) | 7(25.92%) | 13(48.14%) | 100(100%) |
| Candida glabrata(n=13) | 3(23.07%) | 3(23.07%) | 5(38.46%) | 100(100%) |

Discussion

In the present study infection rate of vulvovaginal candidiasis was found to be 33.97%. But according to the study conducted by Enweani et al [9] VVC in non-pregnant women was 40.6% which is high compared to the present study. Eckert et al [10] found 25% of VVC among non pregnant women. The prevalence of vaginal candidiasis reported by different studies was 16.5%, 21.31%, and 19% [11,12,13]. This relatively low prevalence of vaginal candidiasis among women in other studies probably due to adequate knowledge, good personal hygiene, and normal levels of estrogens and corticoids [14].

In the present study, Candida species was predominantly isolated in the age group of 26-35 years which accounted for 54%. This may be due to high sexual activity, poor personal hygiene, the use of contraceptives, and drug abuse among this age-group. Alo et al[15] reported a higher prevalence of *C. albicans* (33.33%) within the age bracket of 36-40 years, while those between 20 and 25 years had the lowest prevalence (20.42%).

In the present study, low Candida infection was found in age group 36-45 years which accounted for 14%. This finding is in line with a previous report by Okungbowa et al[16] who reported prevalence of 10% and 2% within the age-groups of 36-45 and over 46 years, respectively. They reported that this was probably due to the possible increase in vaginal immunity with age as they have decreased levels of estrogen and corticoids, and thus are resistant to *Candida* infections.

In the present study, *Candida albicans* 31 (43.66%) was the most predominant species isolated followed by *Candida tropicalis* 27(38.03%) and *Candida glabrata* 13(18.31%), similar to other study by Grigoriou et al, the most commonly isolated species from the patients was *C. albicans*.

According to Habibipour R, [17]*C. albicans* (81.3%) predominantly and then *C. glabrata* (11%), *C. tropicalis* (4.4%) and *C. kuresi* (2.2%) were isolated from VVC. A similar distribution of *Candida* spp. Similar observation was found by other authors [18,19].

C. albicans adheres to vaginal epithelial cells in significantly higher numbers than do other *Candida* species [20]. Vaginitis induced by non-*albicans* species is clinically indistinguishable from that caused by *C. albicans* [21]. The reason for the increase in incidence of VVC caused by non-*albicans* species is thought to be single-dose antifungal treatment, low-dosage azole-maintenance regimens, and the use of over-the-counter antimycotics[22].

In the present study, all three species of *Candida* were least susceptible to fluconazole and no *Candida* species showed resistance to amphotericin B. Few strains of *Candida* species exhibited multi drug resistance to routinely used antifungals. Relatively low levels of fluconazole resistance were observed in all species of *Candida* isolated. Resistance to fluconazole is of great concern as it is the most common azole used for the treatment of candidiasis including VVC. Fluconazole is available in both intravenous and oral formulations with high bioavailability and is more cost-effective than other antifungal agents.

As per study by Mishra et al[23] all *C. glabrata*, 50% *C. tropicalis* and 12% *C. albicans* isolates were found to be resistant to fluconazole. But, another study by Khan M et al observed, *C. krusei* showed 100% resistance to fluconazole [24]. It is worth mentioning that the non-*albicans* species had the highest levels of resistance compared to *C. albicans*. *C. glabrata* was the least susceptible to the tested antifungals. Although amphotericin B is effective against most strains of *Candida* spp., its usage is limited due to the nephrotoxicity associated with it.

Conclusion

Candida albicans was the most commonly isolated species followed by *C. tropicalis*. Among commonly used antifungals, clotrimazole was found to be most susceptible. But, *C. glabrata* species was least susceptible to frequently used antifungals. Hence, species level identification is required to choose appropriate antifungal and chrom agar medium will be helpful to mycology laboratories for rapid identification of clinically important *Candida* spp.

Contributions by Authors

1. Dr. Harish Babu: Clinical examination of patients and specimen collection.
2. Dr. Kavita: Study design and microbiology work up.
3. Dr. Anandi and Dr. Mohan: Collection of review literature and manuscript preparation.

Information added to existing knowledge: Usually involvement of *C. albicans* is frequent in causing vulvovaginal candidiasis and other species cause vulvovaginal candidiasis infrequently. But in our study, isolation rate of *C. tropicalis* is almost equal to *C. albicans*.

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