ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 15, Issue 8, 2022

Online - 2455-3891
Print - 0974-2441

Research Article

DETERMINATION OF VULVOVAGINAL CANDIDIASIS IN TERTIARY CARE HOSPITAL

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Received: 02 February 2022, Revised and Accepted: 27 May 2022

ABSTRACT

Objectives: Infections of vulva and vagina are commonly encountered in gynecology practice. These infections are predominant in the women of reproductive age group so, this study was done to determine the prevalence of vaginal candidiasis and identify, differentiate *Candida* species isolated from the patients. Three high vaginal swabs per patients were collected using sterile cotton-tipped swabs from Gynecology Department and processed in Department of Microbiology, MLBMC, India. *Candida* was identified using standard guidelines. The study was done on 350 women in reproductive age group (18–49 years), 63.4% were non-pregnant and 36.5% pregnant. One hundred and sixteen/350 (33.1%) samples showed pure growth of *Candida* species. *Candida* positivity among pregnant women (42.9%) was higher than in non-pregnant women (27.1%). Isolation of non-albican *Candida* was higher (26.7%) than *Candida albicans,* this difference was statistically significant ($p<0.05$) The most common isolated species by conventional methods was *C. albicans* (36.3%) followed by *Candida glabrata* (24.1%), *Candida tropicalis* (22.5%), *Candida krusei (10.3), and Candida parapsilosis* (7.7%). In case of *C. albicans*, Fluconazole and Amphotericin B were found to be most sensitive drugs followed by Nystatin, voriconazole and ketoconazole and among non-albicans candida, Nystatin was the highly sensitive drug. Vulvovaginal candidiasis (VCC) is the most common infection in the women reproductive age group. Several predisposing factors such as HIV, diabetes mellitus, oral contraceptive, IUCD usage, antibiotics, and immunosuppressive drug increase the VCC, *C. albicans* was the most prevalent species followed by *C. glabrata* and *C. tropicalis.*

Keywords: Non-albicans *Candida* Species, Sabouraud dextrose agar, Voriconazole, vulvovaginal candidiasis.

 DOI: http://dx.doi.org/10.22159/ajpcr.2022v15i8.44302. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Infections of vulva and vagina are commonly encountered in gynecological practice. These infections are predominant in the women of reproductive age group, and often clinically manifest as discharge per vaginal. Many bacterial and fungal organisms are responsible for vulvovaginal infections, out of which candidiasis is the most common one. Vulvovaginal candidiasis (VCC) contributes to over one-third of all the vaginal infections, and more than 70% of the women in reproductive age group present with VVC at least once during their lifetime. Moreover, it has been estimated that about 8% of the women experience recurrent VVC infections [1] The burden of VVC is often underreported as very few population-based epidemiological studies have been pursued in this regard. Further, since VVC is a common problem, most women fail to report to health-care facility for management, as a result of which the available estimates on the burden are grossly inaccurate. However, a few risk factors have been identified to predispose women to VVC of which immunosuppressive states such as HIV, diabetes mellitus, oral contraceptives, extensive use of antibiotics, corticosteroids, and imbalance in hormones due to pregnancy are common. Since, the disease is common and recurrent, the diagnosis of VVC often remains a clinical challenge. More often, undiagnosed leads to chronic VVC certain patients may develop primarily vulvae symptoms instead of vaginal manifestations of VVC. Vulval pruritus, burning, and thick curdy white discharge are the main symptoms, other clinical features are irritation, soreness, dyspareunia, and dysuria. Erythema, edema, and fissures of vulval and vagina [3]. These fungi cause two major types of infections – superficial surface infections including oropharyngeal and VCC and systemic infections which can be serious and life threatening, with a mortality rate as high as 30% [14]. This study was done to study the prevalence of vaginal candidiasis and to identify and differentiate *Candida* species isolated d demo graphic variation of VCC patients. The aim of the study was to study the antifungal susceptibility of *Candida* isolates by Kirby Bauer disc diffusion method.

METHODS

The study was conducted in the Department of Microbiology, MLBMC, India. Total 350 high vaginal swabs were collected from Gynecology Department from December 2019 to September 2020.

Sample collection procedure

After explaining the procedure of sample collection and taking patients consent, sample was obtained.

The patient complaining with vulvovaginal infection is made to lie in lithotomy position. After urination, Cusco's bivalve speculum is applied to visualize the cervix and vagina.

Three samples were collected, sterile cotton swab is inserted in the post-vaginal fernix and rotated to obtain a sample of discharge. Subsequently, speculum is removed. Sample is sent to Microbiology Department for identification of VCC.

Patients having curdy white vaginal discharge, itching, and irritation with or without pain were included in the study whereas; Patients who do not have any fungal vaginitis symptoms and vaginal swabs showing evidence of bacterial and protozoal infection were excluded from the study.

Microscopic examination

KOH mount

First swab was used for yeast cells identification by wet mount preparation.

Gram staining

Using second swab, Grams staining was prepared for round to oval Gram-positive budding yeast cells with or without pseudohypae (Fig. 1).

Culture and identification of *Candida*

Third swab was used for culture on SDA supplemented with chloramphenicol (0.05 mg/mL) incubated at 37°C for 24 to 48 h.

Smooth and white to cream colored colonies were seen as those of *Candida* as shown in Fig. 2 and finally colonies showing round to oval Gram-positive budding cells with or without pseudohyphae on Grams staining were considered as positive for *Candida*.

Germ tube test

For species identification, the Germ tube was done which is a screening test to differentiate *C. albicans* from other yeast.

Chromagar Candida medium

It is another selective and differential chromogenic medium used for identification of various *Candida* species. *Candida* colonies were inoculated onto CHROMagar (HiMedia) incubated at 37°C for 48 to 72 h. Plates were examined for the color of colonies for presumptive identification. Table 1 and Fig. 3 showing different colors of colonies of *Candida* species on CHROMagar (HiMedia).

Antifungal susceptibility testing

Antifungal susceptibility testing was performed and interpreted for all the isolates of *Candida* using disc diffusion method as recommended by Clinical and Laboratory Standards Institute guidelines. Antifungal discs containing fluconazole (25 µg), ketoconazole (25 µg), Variconazole (01 µg), Amphotericin B (20 µg), and Nystatin (20 µg) were placed on the inoculated media. Zone of inhibition around the disc was measured after incubating the media at 37°C for 24 h.

RESULTS AND DISCUSSION

The present study was carried out in the Department of Microbiology in women of reproductive age group presenting with signs and symptoms of vulvovaginitis in the Department of Obstetrics and Gynaecology in NCRIMS, a tertiary care hospital.

A total number of 350 samples were included in the study, out of them 116 (33.14%) samples showed pure growth of Candida species.

The isolation of non-Albicans Candida (NAC) was higher (62.9%) than *C. albicans* (36.2%) among the study subjects.

Rural area counts 65.51% of *Candida* cases as compare to urban area (34.48). This difference in positivity among rural and urban area women was significant statistically.

Total of the 42 culture positive cases of *C. albicans*, the isolates were subjected to drug sensitivity by disc diffusion method. Forty cases (95.2%) were sensitive to Amphotericin B and fluconazole, 35 cases (83.3.3%) were sensitive to voriconazole, 32 cases (80.9%) were sensitive to ketoconazole, and 30 cases (71.4%) are sensitive to nystatin.

Total of the 74 culture positive cases of *NAC*, the isolates were subjected to drug sensitivity by disc diffusion method. Seventy cases (94.5%) were sensitive to nystatin, 58 cases (78.3%) were sensitive to fluconazole and voriconazole, and 45 cases (60.8%) were sensitive to ketoconazole.

Table 1: Different color colonies of *Candida* species on CHROMagar (HiMedia)

Candida species	Color of colonies
Candida albicans	Light green
Candida dubliniensis	Dark green
Candida glabrata	Pink to purple
Candida krusei	Pink large rough spreading colonies with pale edge
Candida parapsilosis	Cream to pale pink
Candida tropicalis	Blue with pink halo

DISCUSSION

VCC is the most common infection of women in lower genital tract in the reproductive age group, most of the women will have the infection at least once in their life [1].

In our study, the prevalence of the infection of symptomatic women was 33.14%, this study correlates very closely with the study done by Kalia *et al*. (31%) [5]. Studies have reported the low prevalence rate of VVC

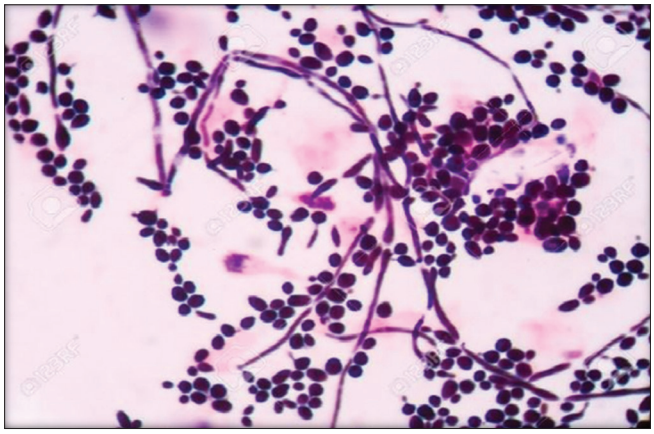

Fig. 1: Gram-positive budding yeast cells with pseudohyphe on Gram's staining (×100)

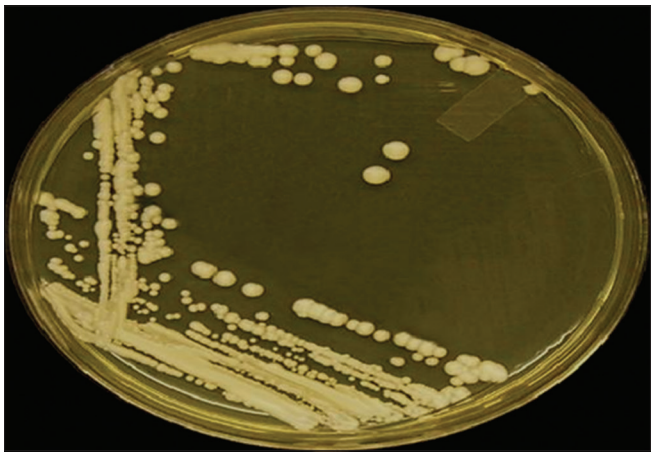

Fig. 2: Growth of *Candida* species on SDA

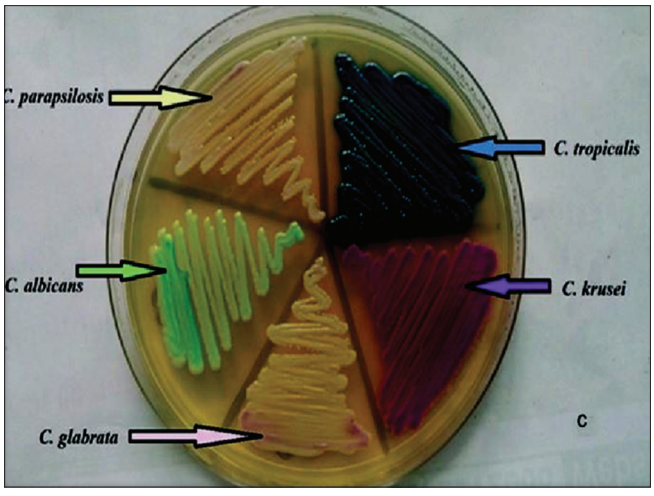

Fig. 3: *Candida* species grown on CHROM agar medium

from Mirza *et al*. [6] 24% and Otero *et al*. [7] 18.5%. The prevalence rate is very higher as comparison to our study done by Ugwa (84.5%) in North-west Nigeria.[8] These differences are assigned by several factors, for example, low socioeconomic status, less education, sample size, improper hygiene, and presence of risk factors for high prevalence.

Most of the studies shows the prevalence of VCC is highest among women in their reproductive age groups. It has been reported that the prevalence of infection was mostly occurs in age groups of 21–30 years in this age group, women are sexually active.

There are some risk factors which are responsible for prevalence of vaginitis infection that are pregnancy (important risk factor of VVC), diabetes mellitus, use of intrauterine device, use of contraceptive pills, and other protective family planning devices to prevent pregnancy, and immunodeficiency such as HIV infection are most common in this age group. All these factors are promoted to VCC infection. In this age group, the ovary produces high amount of estrogen, which favors the growth of *Candida* by maintaining the acidic pH and enhancing the yeast adherence to vaginal epithelial cells.

Species identification of *Candida* is very important. Now in this time, *NAC* species have been isolated with increasing frequency, as compared to *C. albicans*. emergence of new pathogenic species and variable resistance to azoles, the drug most effective against *Candida*, due to inappropriate use of antifungal medications, long-term treatments, and the use of over-the-counter antimycotics. To avoid the determination of less susceptible *NAC* species by anti-fungal treatment, species speciation of *Candida* isolates is most important in routine specimen processing.

The present study showed a significant variation in the distribution of *Candida* species. The most predominant species was found to be *C. albicans* (36.3%), followed by *C. glabrata* (24.1%), *C. tropicalis* (22.5%), *C. krusei* (10.3%), and *C. parapsilosis* (7.7%), respectively. Our findings are similar to the previously reported data by Zarei-Mahmoudabadi *et al*. [9] and Chakrabarti *et al*. [10] reported the prevalence rate (39% and 25%) of *C. albicans*.

In the present study, *C. albicans* was the most common species isolated 36.3%. The previous report from Egyp El-sayed and Hamouda (86.6%) [11] has also reported the highest isolation rate of *C. albicans in* VVC. Worldwide, rates of the isolation of *C. albicans* in cases of VVC range between 47% and 89% in studies from Babin D *et al* India [12].

In the present study, the isolates of *NAC* were higher (62.9%) compare to the isolates of *C. albicans* 36.2%. The highest isolation of *NAC* over *C. albicans* has also been reported by Kotigadde *et al*. [12] (64.5%) Kikani *et al*. [13] (55.6%), and Kalia *et al.* [14] (53% vs. 47%).

The study done by Muni S *et al*. [15] from Bangalore observed higher rate of isolation of *NAC* species as compared to *C. albicans*. Nayman Alpat *et al*. [16] However, Jain *et al*. [17] showed predominance of *NAC* species in urine specimens.

In the present study after *C. albicans, C. glabrata* (24.1 %) was the second most common isolate. (11%) It has been reported to be the second most common isolates in case of VVC from other study in India [12].

In the present study, *C. tropicalis* (22.5 %) has been reported to be the third most common isolate after *C. albicans and glabrata.* Kotigadde *et al*. [12] (29.4%) and Kalia Kalia *et al*.[14] (24.1%) from India have also reported similar findings.

Some other species were also isolated in our study, these were *C. krusie* (10.3%), *C. parapsilosis* (7.7%). The isolation rates of *C. Krusie* have been reported to the range between 3% and 15.7% [14].

In the present study, 18 (15.5%) *Candida* isolates were resistant to fluconazole by disk diffusion method. This finding is close to the reports of resistance by Lee *et al*. [18] (17.1%) reported a higher rate of resistance while, Kikani *et al*. [13] (8.2%) showed a lower rate as compared to our study.

Table 2: *Candida* positivity among study subjects

Total number of case (n)	*Candida* positive, n (%)	*Candida* negative, n (%)
350	116 (33.14)	234 (66.85)

Table 3: *Candida albicans* and non-albicans *Candida* distribution among study subjects

Total number of case (n)	*Candida albicans*, n (%)	Non-albicans, n (%)	*Candida* positive, n (%)	χ^2	P-value
350	42 (36.2)	74 (62.9)	116 (33.14)	9.1	0.014*

#Probability, *Statistically significant difference. χ^2: Chi-squared test

Table 4: Demographic variations (residence) of vulvovaginal candidiasis

Residence	Symptomatic (n=116), n (%)	Asymptomatic (n=234), n (%)	χ^2	P-value
Urban	40 (34.48)	124 (52.99)	11.07	0.014*
Rural	76 (65.51)	110 (47.00)		

Table 5: Analysis of drug sensitivity pattern of *Candida albicans* in vulvovaginal candidosis

Drug	Fluconazole, n (%)	VRC, n (%)	Ketoconazole, n (%)	Nystatin, n (%)	Amp.B, n (%)
Sensitivity	40 (95.2)	35 (83.3)	32 (80.9)	30 (71.4)	40 (95.2)
Resistance	2 (4.7)	7 (16.6)	10 (23.8)	12 (28.5)	2 (4.7)

Amp.B: Amphotericin B, VRC: Voriconazole

Table 6: Analysis of drug sensitivity pattern of non-*Candida albicans* in vulvovaginal candidosis

Drug	Fluconazole, n (%)	VRC, n (%)	Ketoconazole, n (%)	Nystatin, n (%)	Amp.B, n (%)
Sensitivity	58 (78.3)	58 (78.3)	45 (60.8)	70 (94.5)	59 (79.7)
Resistance	16 (21.6)	16 (21.6)	29 (39.1)	4 (5.4)	15 (20.2)

Amp.B: Amphotericin B, VRC: Voriconazole

Resistance to fluconazole among *C. albicans* in our study was to the tune of 7.1%. Our findings are close to that reported by Doddaiah *et a1*. [21] (8.6%) reported it *C. albicans* isolates.

C.krusei was recovered in our study and it was found resistant to fluconazole (12.5%). However, (Doddaiah *et al*. [21]).

Resistance to fluconazole has been reported in *C. tropicalis* (25%) and *C. glabrata* (11.5%) by several workers [21], though none of our isolates were resistant.

Deorukhkar *et al.* [22] reported this to be in 50% of their *C. krusie* isolates. Intrinsic resistance in *C. krusei to* fluconazole is a known fact.

CONCLUSION

VCC is the most common infection in the women reproductive age group. Several predisposing factors include HIV, diabetes mellitus, IUCD usage, antibiotics, and immunosuppressive drug users, immunocompromised patients also influences to VCC. Species identification done by culture method showed *C. albicans* was the most common prevalent species followed by *C. glabrata* and *C. tropicalis.* Specification of *Candida* using CHROM agar needs to be used with caution, particularly identification of *NAC.* Due to emergence of *NAC,* species identification and antifungal susceptibility are to be done as a necessary part of laboratory evaluation of VVC. In case of *Candida albicans,* fluconazole was found to be most sensitive drugs and in case of NAC fluconazole to be found the highly sensitive drugs.

ACKNOWLEDGMENT

We would like to thank the entire team who supported us during the research and also to all patients and their family for allowing us to publish this article.

AUTHORS' CONTRIBUTIONS

All the authors have contributed equally to the data collection, its interpretation, and preparation of the manuscript.

CONFLICTS OF INTEREST

The authors have no conflicts of interest.

AUTHORS' FUNDING

No funding was received for this case report.

PATIENT'S CONSENT

For the publication of this case report, written informed consent was obtained from the patient.

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