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Research Paper

Vulvovaginal candidiasis, an increasing burden to women in the tropical regions attending Bharatpur Hospital, Chitwan

Anisha Subedi[1,a], Milan Kumar Upreti[1,a], Jid Chani Rana[b], Ram Prasad Sapkota[b], Upendra Thapa Shrestha[c,*]

[a] Department of Microbiology, Goldengate International College, Battisputali, Kathmandu
[b] Bharatpur Hospital, Chitwan
[c] Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu

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ABSTRACT

Vulvovaginal candidiasis is a yeast infection commonly caused by the overgrowth of *Candida* species in and around the vulva and vagina. Abnormal vaginal discharge, itching and irritation, swelling and redness of the vaginal area, pain during sexual intercourse, and dyspareunia are important clinical findings of the infection. Currently, the infection is one of the growing burdens to married women. Moreover, the infection with antifungal-resistant *Candida* species adds challenges to managing the disease. Hence, this study was conducted to identify the different *Candida* species causing vulvovaginal candidiasis and to determine its susceptibility pattern against different antifungal drugs. A hospital-based cross-sectional and quantitative study was conducted for the period of six months from September 2022 to March 2023 among symptomatic married women in the Gynecology and Obstetrics Department of Bharatpur Hospital, Chitwan. A total of 300 symptomatic cases were enrolled in the study. *Candida* species were isolated from vaginal swabs following standard microbiological procedures and antifungal susceptibility testing was performed with different antifungal agents. The total prevalence of vulvovaginal candidiasis was found to be 37.3 % (112/300). Among different isolates, *Candida albicans* was found to be the most predominant (52.6 %), followed by *Candida glabrata* (29.3 %) among non-*albicans*. Women from the age group 25–35 years were found to be more infected (47.3 %) and the relationship between contraceptive use and vulvovaginal candidiasis was found to be statistically significant ($p < 0.05$). *Candida* species showed higher susceptibility toward Amphotericin-B (68.1 %), followed by fluconazole (Diflucan), and Clotrimazole (50.9 %). Whereas the least susceptibility was observed to Voriconazole (27.6 %) and Itraconazole (35.30 %). *Candida albicans* was comparatively more susceptible to different antifungal drugs than non-*albicans* species. *Candida parapsilosis* was only susceptible to Amphotericin-B and the increasing incidence of vaginal candidiasis due to non-*albicans Candida* indicates the need for routine speciation of *Candida.*

Introduction

Vaginitis is an inflammation of the vagina that can be infectious or noninfectious. It affects women of all ages. Vaginitis is frequently caused by three etiologies including bacteria, yeasts, and parasites; *Trichomonas vaginalis* respectively known as bacterial vaginosis, vulvovaginal candidiasis, and trichomoniasis [1]. Vulvovaginal candidiasis (VVC) is a form of vaginitis commonly associated with the infection of *Candida* species predominately *Candida albicans* [2]. *Candida* species are normally present in the vagina as normal flora. However, *Candida* can grow and cause an infection if the balance of normal flora like bacteria and yeast in the vagina is disturbed [3]. The risk factors associated with VVC are antibiotic therapy that destroys the vaginal beneficial bacteria causing overgrowing of yeast, fluctuation in hormone levels during menstruation, pregnancy, or hormone therapy, immunocompromised conditions like HIV/AIDS, or other medications, sexual interactions with multiple partners and uncontrolled diabetes [4,5]. An estimated 75 % of females experience VVC once in their lifetime with, 40 - 50 % experiencing recurring episodes [6]. Itching, burning, soreness, and redness of the vulva and vaginal mucosa are the main symptoms of Candidiasis, which is frequently accompanied by abnormal vaginal discharge [7].

Candida species act as both commensals and opportunistic pathogens of the human body [8,9]. They are commonly found as invaders

* Corresponding author at: Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal.
E-mail address: upendrats@gmail.com (U. Thapa Shrestha).
[1] These authors equally contributed to the study

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in cutaneous, vaginal, and gastrointestinal areas. *Candida* species generally do not cause significant harm to healthy individuals [10]. However, the number of *Candida* infections has been increased recently. Certain species within the *Candida* genus have been identified as opportunistic pathogens, primarily affecting immunosuppressed or vulnerable individuals [11]. Among the approximately 150 known species [12], 92 % of human infection is commonly caused by *C. albicans, C. glabrata, C. krusei, C. parapsilosis,* and *C. tropicalis* [13]. Although *C. albicans* continues to be the leading cause of candidiasis [14,15] and is responsible for approximately 50 % of all cases, non-*albicans Candida* species (NACS) are responsible for the remaining cases [16]. The ability to alter its morphology and biofilm production plays a crucial role in the pathogenesis of *C. albicans* [17].

In developing countries like Nepal, diagnosing vulvovaginal candidiasis can be particularly challenging due to various factors like inadequate healthcare facilities, limited awareness and education among women, cultural and social barriers, limited number of female consultants, and the cost of treatment, etc. While there have been some studies done on candidiasis in Nepal taking overall clinical samples [18-20], that said very few of them were done on vulvovaginal candidiasis [21,22]. Previous studies done in Nepal had reported varying prevalence of *Candida* spp in high vaginal swabs. A study at Janaki Medical College and Teaching Hospital, Janakpur had reported a 35 % prevalence of Vulvovaginal candidiasis. In addition, the increasing incidence of multi-drug resistant non-*albicans* species of *Candida* was reported in different studies [18,19,23]. Hence, this hospital-based study was conducted to evaluate the prevalence of vulvovaginal candidiasis and susceptibility of isolated *Candida* species to antifungal agents among symptomatic women attending the Gynecology and Obstetrics Department of Bharatpur Hospital, Chitwan. The study findings would help in the proper diagnosis of their problems, and prescription of appropriate antifungal drugs depending on the susceptibility and quick management of infection among the women of respective areas. In addition, this study employed various methods of *Candida* identification and overcame the study gap in research carried out in VVC which includes women of all ages, pregnant and non-pregnant, under medication, or women with diabetes mellitus.

Methods

Study design

A hospital-based cross-sectional and quantitative study was done among symptomatic married women.

Study area and period

This study was conducted in the Gynecology and Obstetrics Department of Bharatpur Hospital, Chitwan for the period of six months from September 2022 to March 2023.

Ethical approval

The study was approved by the Institutional Review Committee, Institute of Science and Technology, Tribhuvan University (Regd. No. IRCIOST-23–2023).

Inclusion and exclusion criteria

Female married patients of reproductive age attending Bharatpur Hospital, Chitwan with the symptoms of vulvovaginal candidiasis who gave written consent to participate in the study were enrolled in this study. Whereas, unmarried women and patients living with Human Immunodeficiency Virus related acquired immunodeficiency syndrome (HIV/AIDS) were excluded from the study.

Sample collection and transportation

A convenient sampling method was used. The sample for this study was high vaginal swabs. Two high vaginal swabs were collected by medical officers from individual symptomatic patients. A total of 300 high vaginal swabs were collected from symptomatic women and transported to the laboratory using Ame's transport medium as soon as possible (Isenberg 2004).

Microscopic examination and culture of the specimen

Of the two vaginal swabs collected, one was directly employed for microscopic examination to observe the presence of budding yeast cells using the Gram's stain technique. The second specimen was inoculated on Sabouraud Dextrose Agar (SDA) with 0.05 mg/L chloramphenicol and HiCrome *Candida* differential Agar. Then, the plates were incubated for 24–48 h at 35–37 °C [24,25].

Identification of the isolates

Candida species were identified by using standard operating procedures which involve the morphology of colonies, Gram stain reaction, germ tube formation test, sugar fermentation test, and the color of their colonies on HiCrome *Candida* differential agar [24,25]. The distinctive colony colors as per the manufacturer's instructions: C. albicans (light green), C. tropicalis (blue to metallic blue), C. glabrata (cream to white), C. krusei (purple and fuzzy), and C. parapsilosis (cream) were used to identify *Candida* species. Confirmation of C. albicans versus non-albicans strains was achieved using the germ tube test, while further classification of non-albicans species relied on sugar assimilation tests [26].

Germ tube formation test

A small inoculum of the test organism from pure culture was suspended in 0.5 ml fresh human serum and incubated for 2–3 h at 37 ° C. The result of the germ tube test was interpreted under a microscope based on the protocol of the University of Adelaide [24,25].

Sugar fermentation test

A carbohydrate medium (pH 7.4) containing glucose, maltose, sucrose, and lactose separately with Andrade's indicator (Andradea Peptone water) in the tubes containing Durham's tube was inoculated with the test and incubated at 37 °C for 5–10 days. The results were interpreted based on the ability of the organism to ferment the sugar indicated by the change in color of the medium to pink and gas-trapped in the Durham's tube [24,25].

Antifungal susceptibility testing

The antifungal susceptibility testing of *Candida* species was performed using the Kirby-Bauer disc diffusion technique, following the CLSI guidelines [27]. The five different kinds of antifungal agents from different groups; Amphotericin B (20mcg), Clotrimazole (10mcg), fluconazole (Diflucan), Itraconazole (10mcg), and Voriconazole (30mcg) were used (Hi-Media, India) [27].

Quality control

Candida albicans ATCC 10,231 for Gram's stain test and biochemical tests and *C. albicans* ATCC 10,231 (positive germ tube test) and *C. tropicalis* ATCC 750 (negative germ tube test) were used for germ tube formation test. *C.* albicans ATCC 10,231 and *C. tropicalis* ATCC 750 were used as quality control for antifungal susceptibility testing.

Data analysis

Data analysis was done by using the SPSS 22.0 version. The chi-square test was used to determine the significant association of dependable variables like the age of the patient, condition of pregnancy, and contraceptive use. The p-value <0.05 was assumed to be significant for the analysis.

Results

Prevalence of Vulvovaginal Candidiasis

Of 300 high vaginal swabs, 112 (37.3 %) specimens were found to have growth and the remaining 188 (62.7 %) showed no growth. Out of 112 growths, 4 (3.6 %) of them were mixed growth of different *Candida* species and 108 (96.4 %) were single growth.

Age-wise distribution of patients

The majority of the infection was observed in the age group of 25−35 years (47.3 %), followed by 35−45 years (30.4 %). Whereas, the least infection was observed in the women of age group 55−65 years (0.9 %) (Fig. 1).

Vulvovaginal candidiasis occurrence with the practice of contraceptive

Among 109 participants who used different types of contraceptives, 49 (43.8 %) were infected. People using the non-barrier method were comparatively higher (71.6 %) than the barrier method (31 %). The use of contraceptives was found to be significantly associated with a decrease in infections. Likewise, vulvovaginal candidiasis was found to be significantly higher among unemployed patients ($p < 0.05$) (Table 1).

Occurrence of vulvovaginal candidiasis with different symptoms

The common symptoms of vulvovaginal candidiasis were abnormal vaginal discharge and itching or burning of the vulva or vaginal opening (Table 2).

Distribution of Candida species isolated

Five different *Candida* species were identified from the high vaginal swab specimen. *Candida albicans* was the most predominant species accounting for 61 (52.6 %) followed by *Candida glabrata* 34 (29.3 %), *Candida krusei 14* (12.1 %), *Candida tropicalis* 5 (4.3 %) and *Candida parapsilosis* 2 (1.7 %) (Fig. 2, Fig. 3 and Fig. 4).

Antifungal susceptibility patterns of Candida *species*

Of the total 116 *Candida* species, most of them were susceptible to Amphotericin-B (68.1 %) whereas the least susceptibility was observed to Voriconazole (27.6 %) (Fig. 5).

The highest number of *C. albicans* were resistant to Voriconazole (59 %) followed by Itraconazole (44.3 %). Similarly, a higher number of *C. glabrata* were resistant to Clotrimazole (88.2 %) followed by Voriconazole (85.3 %). A total of 85.7 % *C. krusei* were resistant to Clotrimazole and Voriconazole. Likewise, 100 % of *C. tropicalis* and *C. parapsilosis* were resistant to Clotrimazole and Voriconazole. However, *Candida albicans* ATCC 10,231 and *Candida tropicalis* ATCC 750 were susceptible to all antifungal agents used in this study (Table 3, Fig. 6)

Discussion

With reference to the flow of patients complaining of gynecological problems, this study was conducted in the microbiology laboratory of Bharatpur Hospital, Chitwan, a tropical metropolitan city outside the capital city. In this study, we observed a 37.3 % prevalence of VVC among symptomatic married women similar to different

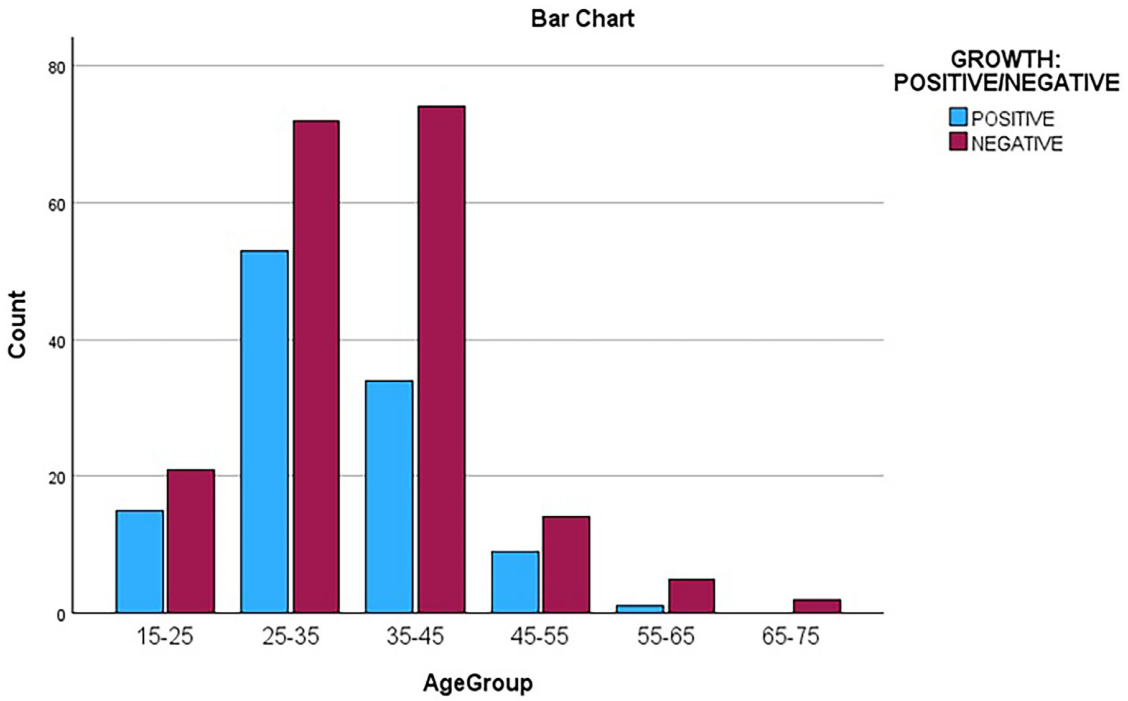

Fig. 1. Distribution of candidiasis among different age groups of the patients.

Table 1
Vulvovaginal candidiasis with the practice of contraceptive use and employment.

Contraceptive users / Employment		Growth No. (%)	No Growth No. (%)	Total	p-value
Contraceptive uses	Users	49 (45.0)	60 (55.0)	109	0.047
	Non-users	63 (33.0)	128 (67.0)	191	
Total		112	188	300	
Employment	Employed	34 (30.4)	103 (54.8)	137	0.00004
	Unemployed	78 (69.6)	85 (45.2)	163	
Total		112	188		

Table 2
Occurrence of vulvovaginal candidiasis with different symptoms.

Symptoms	Growth number (%)	No Growth number (%)	Total
Itching or burning of the vulva or vaginal opening	106 (39.3)	164 (60.7)	270
Soreness redness and swelling of the vulva	44 (43.6)	57 (56.4)	101
Pain or discomfort when urinating	50 (38.5)	80 (61.5)	130
Pain during sexual intercourse	47 (45.6)	56 (54.4)	103
Abnormal vaginal discharge	112 (37.3)	188 (62.7)	300

studies carried out in Nepal (35 %), India (31 %, 37.3 %), Pakistan (31.6 %), Lebanon (39 %), UAE (31.6 %) and other nations worldwide [22,28-33]. Whereas a much higher (73.3 %) prevalence was observed in a study done by Raja et al. 2023 in Pakistan [34] and a low prevalence was observed in some studies conducted in Nepal, Iran, Argentina, and other countries as well [35-40]. The difference in the prevalence might be due to the location of the study, seasonal variation, and environmental conditions of the study site.

The percentage of women infected with candidiasis was observed to be highest in the age group of 25–35 (47.3 %) similar to the studies by Siddiqui R (2020) in India, Alsudani et al. (2022) in Iraq, Cetin et al. (2007) in Turkey, Gaddhar et al. (2019) in Lebanon, Venugopal et al. (2021) in Saudi Arabia and Mbakwem-Aniebo et al. (2020) in Nigeria [41-46]. Whereas the least infection was observed among the age group 55–65 years. The highest incidence of vulvovaginal candidiasis in the age group 25–35 years may be due to the reproductive age of women, the maximum sexual involvement, and the use of contraceptives. The prevalence is minimum in the age group 55–65 years and null in 65–75 years might be due to the less enrollment of women of that particular age group and dryness of the vagina.

We observed 49 (43.8 %) women infected with vulvovaginal candidiasis among 109 patients using different contraceptive users. On the other hand, 63 (56.2 %) women were infected among 191 non-users. It suggests that there is a chance of getting candidiasis after the use of contraceptives as well. Although the percentage of infected individuals not using any type of contraceptive can not be underscored, the use of proper contraceptives can reduce the risk of infections. The relationship between infection and contraceptive users was statistically significant. In the study done by Cetin et al. (2007), they reported the prevalence of candidiasis was 44.2 % in contraceptive users [43]. Among the contraceptive users, a higher growth rate of 28 (57.1 %) was observed in the patients using the non-barrier method whereas 21 (42.9 %) growth rate was observed in patients using the barrier method. Consistent with the study done by Alsudani and Al-Awsi [42]. It might be due to the high glucose content in oral contraceptives which favors the growth of yeast and the comparatively high number of people using condoms as a contraceptive participating in this study. Likewise, we observed that 30.4 % of employed and 69.6 % of unemployed women were infected. The relationship between education and VVC was statistically significant (p-0.00004). Most of the unemployed women enrolled in our study were illiterate and lacked proper knowledge about vaginal health, use of contraceptives, and reproductive health. In the study, the women complaining about abnormal vaginal discharge and itching or burning of the vulva or vaginal opening were relatively higher i.e. 300 (100 %) and 270 (90 %) respectively compared with women complaining about other symptoms. Most of the people had multiple symptoms whereas few of them were complaining about some particular one. It might be due to the most common symptoms of vulvovaginal candidiasis being abnormal vaginal discharge and itching or burning of the vulva or vaginal opening. The findings of this study are in line with different studies done in India by Kombade et al. 2021, Ponnaluri A 2021, and Mariyah et al. 2022 also in Tunisia by Mtibaa

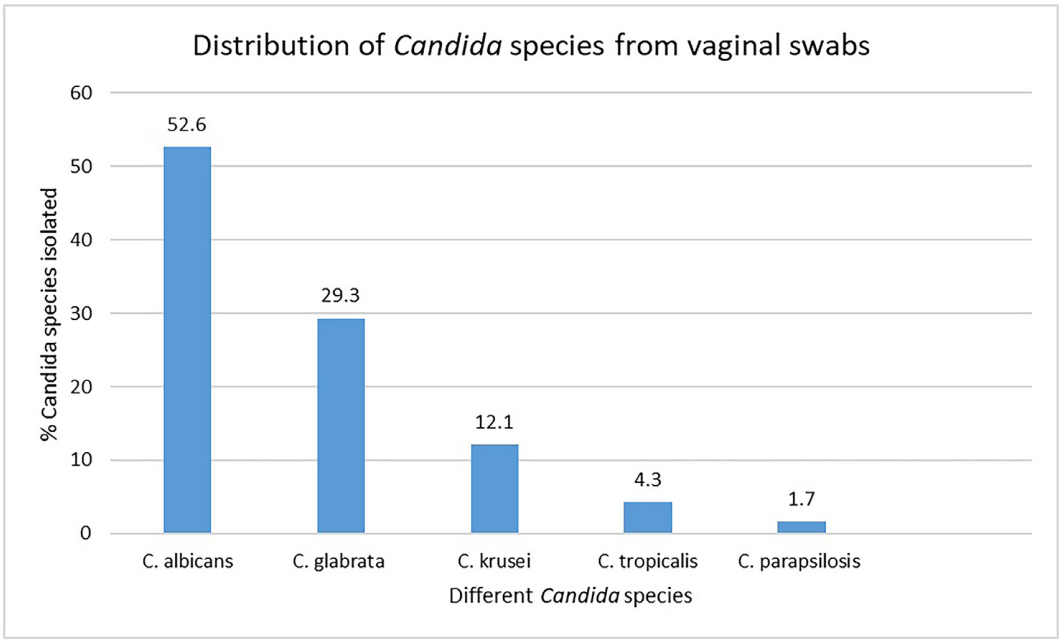

Fig. 2. Distribution of *Candida* species.

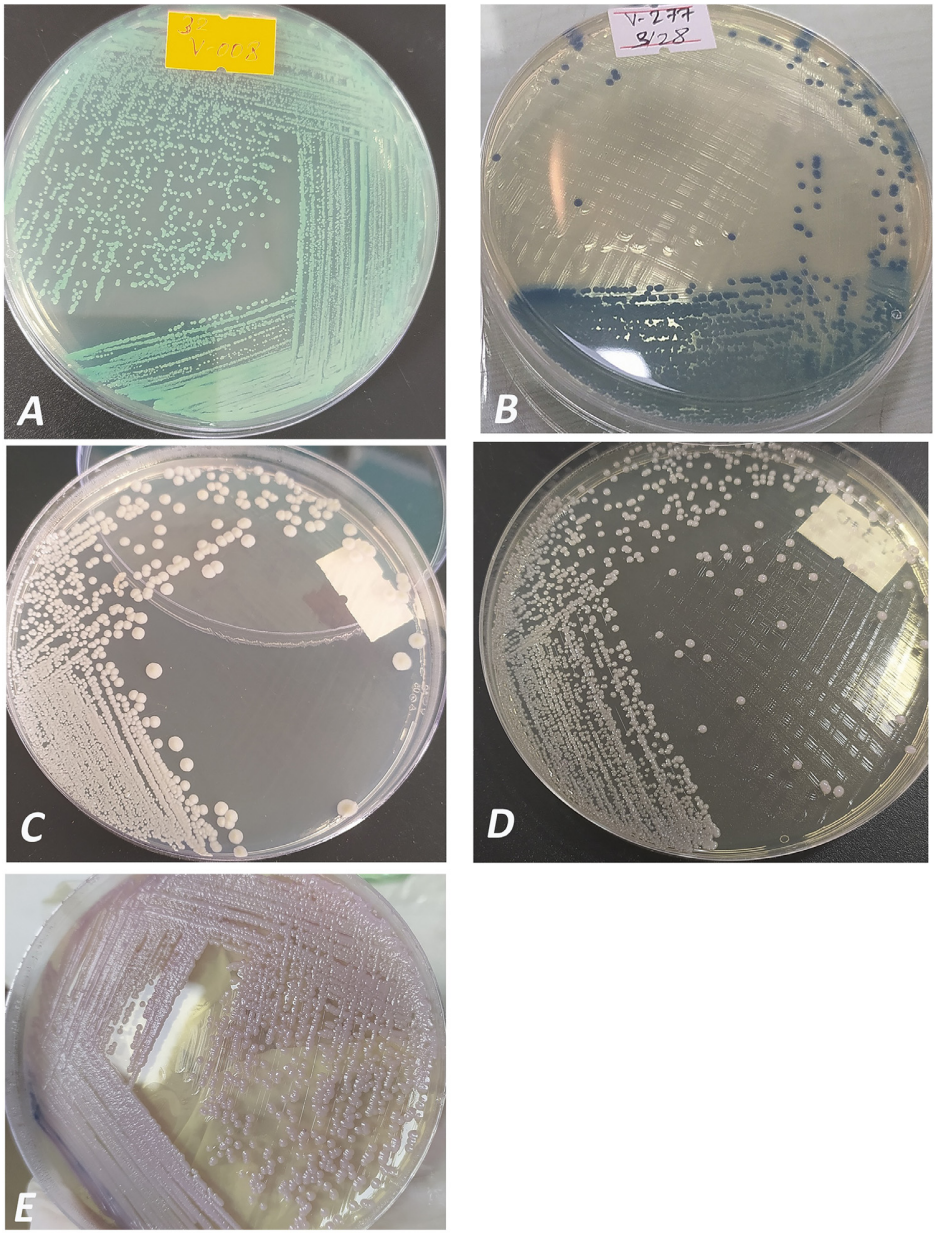

Fig. 3. Colony characteristics of *Candida* species in HiCrome *Candida* Differential Agar from HiMedia (A) *C. albicans* (B) *C. tropicalis* (C) *C. parapsilosis* (D) *C. glabrata* (E) *C. krusei*.

et al., 2017 in which the most common symptom was Leucorrhea followed by vulvar pruritis [47-50]. We also observed a higher number of patients complaining of vulvovaginal candidiasis with different symptoms but no growth for *Candida* species. There are several factors influencing the occurrence of symptoms in patients who do not test positive for *Candida*. Test findings may be affected by the administration of antibacterial or antifungal agents before obtaining high vaginal swabs. Furthermore, individuals might have bacterial or viral infections as well that were not included in this study. Hormonal changes during menstruation and pregnancy can alter the vaginal environment, potentially causing symptoms even in the absence of infection. Variations in the medium when switching from in-vivo to in-vitro conditions or technical mistakes made during sample collection could potentially be another factor in inconsistent test results.

According to the findings of this study, *C. albicans* (52.8 %) was the most predominant species followed by *C. glabrata* (28.7 %) among non-*albicans Candida*. Several studies from Nepal and the neighboring countries including India, China, Lebanon, Khuzestan, Iran, and Bangladesh had reported a higher predominance of *C. albicans* in their respective settings [22,51,52,25,31,40]. Although we found *C. glabrata* as the predominant among non-*albicans Candida*, the other studies from Nepal and Pakistan reported *Candida krusei* as the most common non-*albicans Candida* species [21,53].

The higher resistance to Voriconazole (72.4 %) and least resistance to Amphotericin-B were observed among 116 isolates of *Candida.* Similar to our study, Ghimire et al. also revealed the least resistance to Amphotericin-B (97.1 %), against *Candida* spp [23]. by in vitro susceptibility testing. Likewise, a study from India by Lavanya et al., 2019 found 100 % susceptibility to Amphotericin-B, and 100 % resistance to fluconazole (Diflucan) and Ketoconazole by *C. krusei* and *C. glabrata* [54]. In addition, Gupta and Gadekar, Bilal et al., Mohammadi et al. and Maraki et al. reported the higher susceptibility of *Candida* spp against Amphotericin-B and Voriconazole and higher resistance to fluconazole (Diflucan) and Itraconazole in their previous studies

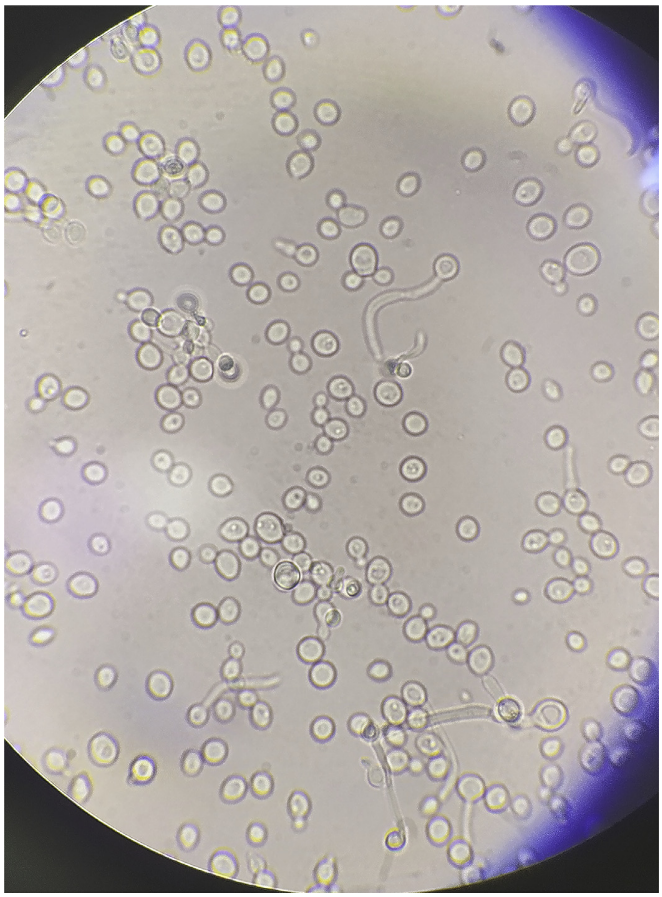

Fig. 4. Germ tube test of *C. albicans*.

done in India, China, Iran and Greece [55-58]. Macura and Skora (2012) published the rising resistance towards Itraconazole [59]. In contrast, Ketoconazole and Amphotericin-B were highly effective antifungal drugs. Non-albicans *Candida* particularly *C. krusei* showed higher resistance towards different antifungal drugs in their study. Likewise, in this survey, it was observed that the resistance pattern of non-*albicans Candida* was comparatively high, especially in *C. parapsilosis* and *C. tropicalis* compared to *Candida albicans*. It might be due to the use of strong and broad-spectrum antibiotics which results in antifungal-resistant mutated strains of *Candida* species. A similar result was obtained by Makanjuola et al. [60]. where *non-albicans Candida* was less sensitive to fluconazole (Diflucan). In contrast with this study, Yassin et al. reported *Candida glabrata* as the most resistant strain and *C. tropicalis* as the most susceptible strain of *Candida* [61]. Likewise, in a study done by Seyoum et al., *C. kuresi* was found to be 100 % resistant to different antifungal drugs [62].

As a study limitation, we performed a conventional method including a germ tube test, sugar fermentation tests, and colony characteristics on HiCrome Candida differential agar for the identification of *Candida* species. We determined antifungal-resistant patterns using the disc diffusion method. So, it would not be significant to generalize the results of AST based on this method. Regular screening of resistant genes should be done using advanced molecular tools. Although the use of HiCrome agar is conventional, the use of HiCrome agar is a rapid and convenient method to identify the species of *Candida* from mixed growth in the absence of other special tools. Although the study was conducted at Bharatpur Hospital in Chitwan, patients included in this study were not limited to the local area. The Hospital is situated in the central region of Nepal, making it the most accessible healthcare facility for people from surrounding tropical areas. It is recognized as the second most popular gynecological hospital in Nepal, following Thapathali Hospital in Kathmandu. Bharatpur Hospital sees a significant number of patients presenting with symptoms of vaginitis. The absence of previous research on vulvovaginal candidiasis at this hospital even emphasizes the importance of our investigation. Furthermore, We recommend a longitudinal and multi-center study in different geographic regions that could provide more comprehensive insights into VVC prevalence and resistance patterns. Lastly, our findings highlight the need for enhanced education on vaginal health, particularly among unemployed women, regular screening for antifungal resistance to guide efficient treatment strategies, and preventive measures for further spread of resistant

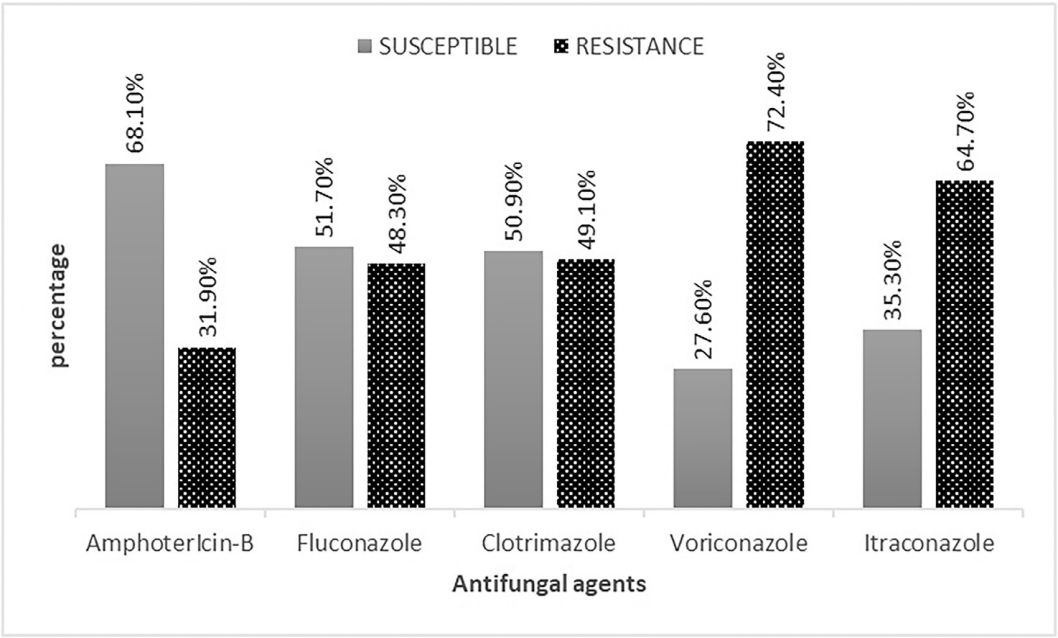

Fig. 5. Antifungal susceptibility patterns of *Candida* species.

Table 3
Antifungal susceptibility patterns of different Candida species.

Candida species	No. of *Candida* species resistant (%) to Antifungal agents				
	Amphotericin-B	Clotrimazole	fluconazole (Diflucan) (Diflucan)	Itraconazole	Voriconazole
C. albicans (*n* = 61)	11 (18.0)	26 (42.6)	20 (32.8)	27 (44.3)	36 (59)
C. glabrata (*n* = 34)	14 (41.2)	30 (88.2)	20 (58.8)	17 (50)	29 (85.3)
C. krusei (*n* = 14)	8 (57.1)	12 (85.7)	10 (71.4)	7 (50)	12 (85.7)
C. tropicalis (*n* = 5)	3 (60)	5 (100)	4 (80)	4 (80)	5 (100)
C. parapsilosis (*n* = 2)	1 (50)	2 (100)	2 (100)	2 (100)	2 (100)
Total (*n* = 116)	37 (31.9)	75 (64.7)	56 (48.3)	57 (49.1)	84 (72.4)
ATCC *Candida* spp	Susceptibility to Amphotericin-B	Clotrimazole	fluconazole (Diflucan) (Diflucan)	Itraconazole	Voriconazole
Candida albicans ATCC 10,231	Susceptible (18 mm)	Susceptible (24 mm)	Susceptible (20 mm)	Susceptible (18 mm)	Susceptible (26 mm)
Candida tropicalis ATCC 750	Susceptible (20 mm)	Susceptible (20 mm)	Susceptible (22 mm)	Susceptible (16 mm)	Susceptible (20 mm)

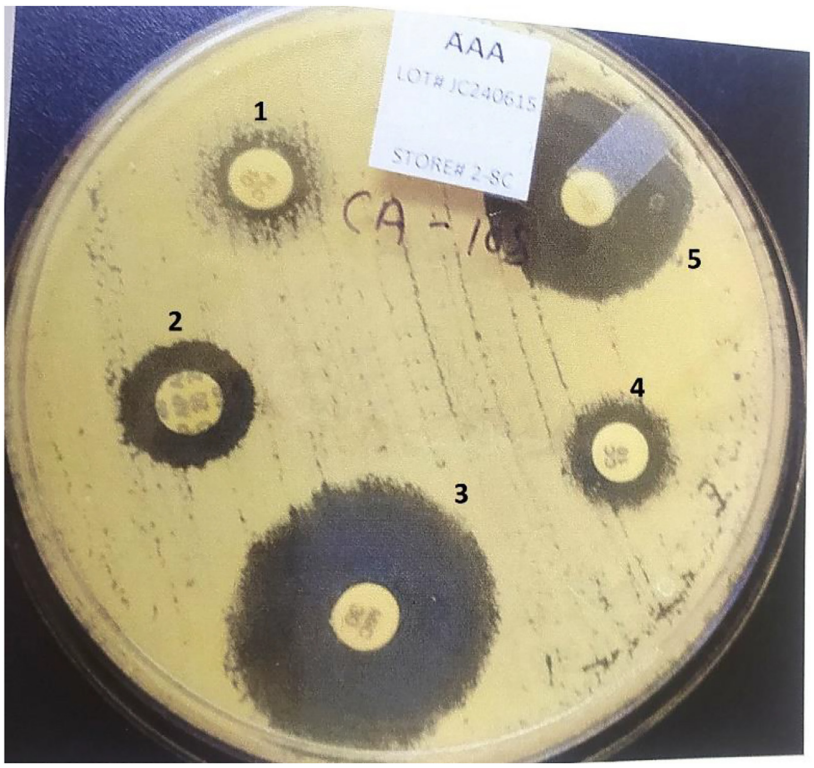

Fig. 6. *Antifungal susceptibility testing of C. albicans* [1- fluconazole (Diflucan), 2- Amphotericin-B (R), 3- Voriconazole(S), 4- Clotrimazole (R), 5- Itraconazole (S)].

Candida strains. By addressing these issues, we intended to provide a deeper and more thorough understanding of VVC, its risk factors, and its implications for women's health.

Conclusion

The rate of vulvovaginal candidiasis among symptomatic women attending the Gynecology and Obstetrics Department at Bharatpur Hospital, Chitwan is quite high. Although *Candida albicans* was the most commonly isolated species, the increasing rate of non-*albicans* species in VVC infection can't be ignored. Different factors like age, pregnancy, contraceptive use, and types of contraceptives contributed to the risk of causing vaginal candidiasis, hence special precautions should be followed during pregnancy and while using contraceptives. The antifungal susceptibility pattern of this study showed higher resistance to Voriconazole and Itraconazole by non-*albicans* species which might result in treatment failure. The increasing incidence of vaginal candidiasis due to non-*albicans Candida* indicates the need for routine speciation of *Candida.*

Declaration of competing interest

The authors declare that they have no competing interests.

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