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Evaluation of Vulvovaginal Candidiasis and Susceptibility Patterns of Isolates Among Married and Unmarried Women of Childbearing Age and the Associated Risk Factors in Calabar, Nigeria

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Abstract
Vulvovaginitis is an infection of the vulva and vagina. *Candida* species are common etiologic agents of vulvovaginitis. The aim of this study was to evaluate vulvovaginal candidiasis susceptibility patterns of isolates and the associated risk factors among women of childbearing age. This study was a cross-sectional study which took place between August 2023 to February 2024. A total of 250 women aged 16-45 years were randomly recruited for the study. Subjects were recruited from gynaecology and sexually transmitted infection clinic. High vaginal swab (HVS) was obtained from all the symptomatic subjects. Samples obtained were subjected to microscopy and culture. The isolates were identified by gram staining, germ tube test and Chrom agar *Candida*. Isolates were subjected to antifungal susceptibility testing using the Kirby-Baur Method. The antifungal agents tested include; Nystatin, voriconazole, fluconazole, amphotericin-B and Itraconazole. The prevalence of vulvovaginal candidiasis was 39.2%. The age groups 16-20 years and 36-45years had 0.0% and 0.0% infection rate respectively. Pregnant women had a higher infection rate 6(5.9%) than the non-pregnant women 7(4.7%). There was no statistically significant association between pregnant and non-pregnant women (X^2= 2.42; p=0.99). Similarly, subjects with multiple sexual partner and those who had unprotected sex had the higher rate of infection, 9(8.8%) and 8(10.5%) respectively. There was statistically significant relationship between multiple sexual partners and unprotected sex with infection rate. (x^2=4.58; p=0.03; and x^2 = 6.28; p=0.01). *Candida albicans* was the predominant species with the prevalence of 67(68.3%) followed by *C. tropicalis* 16(16.3%). The range of susceptibility of the isolates to antifungal agents was 0.0% - 87%. Isolates of *C. albicans* were more susceptible to Nystatin (87.0%), followed by voriconazole (70.0%). The non- *albicans* species showed varying susceptibility rates ranging from 0.0% - 100%. The rank of potency of the antifungal agents against *Candida* isolates was Nystatin, > Voriconazole,> fluconazole,> amphotericin B> itraconazole.

Keywords: Vulvovaginal Candidiasis, Isolates, Susceptibility, Childbearing, Women, Vagina

Introduction

Vulvovaginitis is a common gynaecological infection among women of reproductive age. Women are always susceptible to this infection due to hormonal changes associated with pregnancy. *Candida* species have been reported as common pathogens responsible for vaginitis especially in women of child- bearing age. These pathogens possess serious complication in women and has become an important public health problem for its medical, economic, and social implications (Frobenius & Bogdan 2015). Vulvovaginal candidiasis occurs when the vaginal *Candida* species grow faster than normal. *Candida* species infect the vulva and vagina, most commonly in women of reproductive age (Puranjoy, 2018). Out of the 200 species of *Candida*, the most commonly

encountered in medical practice are *Candida albicans*, *Candida dubliniensis*, *Candida parapsilosis, Candida glabrata, Candida krusei,* and *Candida tropicalis*. It is hypothesized that women colonized by *Candida* species in the anal region have more likelihood of having vulvovaginal candidiasis (Emeribe *et al.,* 2015). The indirect and direct expenses of sexually acquired illnesses make vaginal *Candida* infections a global issue. Research found that 70-75 percent of women of reproductive age will get vulva and vaginal *Candida* infections (Watson *et al.,* 2017). Vulvovaginal candidiasis is caused by *Candida albicans* (80-90%) and *Candida glabrata* (10-20%). Vulvovaginal arises when the host's immune system is weakened and unable to fight against *Candida* which may result in Obstetric infections, newborn infections, pelvic inflammatory disease, increased vulnerability to HIV infection, and infertility in women (Maxwell *et al.,*2022). The anus is also closer to the vagina, allowing intestinal microbes, particularly *Candida* species to enter the vagina (Wolters, 2015). Symptoms of vulvovaginal candidiasis include vulvovaginal pruritus, erythema, dysuria, dyspareunia and edema.

Despite the high prevalence and a large number of risk factors (RFs) associated with the infection, the pathogenic of VVC, mechanisms and recurrent vulvovaginal candidiasis (RVVC) have not yet been fully explained. *Candida* yeasts migrate from the lower gastrointestinal tract to the adjacent vestibule and vagina, a route similar to that taken by vaginal Lactobacillus species (Lívia *et al*., 2021). During pregnancy, there is an increase in progesterone and oestrogen levels, especially in the last trimester. Progesterone has an inhibitory effect on the anti-*Candida* activity of neutrophils (Sayanika *et al*., 2020). On the other hand, oestrogen reduces the ability of vaginal epithelial cells to inhibit the growth of *Candida albicans* on them. However, about 75% of women harbor this fungus without it causing harm to them (Sayanika *et al*., 2020). During normal pregnancy, candidiasis is frequently encountered without significant risk for the foetus. Nevertheless, pregnancy may be negatively affected by VVC. If untreated, vaginal candidiasis can lead to white curd-like vaginal discharge, burning sensation in the vagina and vulva, itching, rash on the vulva and surrounding skin, vulval oedema, fissures, abrasion, chorioamnionitis with subsequent abortion and congenital infection of the neonate and pelvic inflammatory disease (PID) resulting in infertility in non-pregnant women (Pappas *et al.,* 2016; Sayanika *et al*., 2020). *Candida* thrives better at pH values between 3.8 and 5.0, when pH is less than 4.5. VVC could be a risk factor for candidemia in preterm neonates during normal pregnancy. Amongst the *Candida* species isolated from vaginal specimens, *C. albicans* is the most predominant, followed by other non-*albicans Candida* (NAC) such as *C. glabrata, C. tropicalis, C. dubliniensis* and *C. krusei* with the increase in frequency of non-*albicans Candida* being isolated from clinical specimens. Recent studies indicated that non-*albicans Candida* are now considered pathogens (Sayanika *et al*., 2020).

Materials and Methods

Study Design

The study was a cross-sectional study which lasted for a period of six (6) months with three main components; collection of high vaginal swab (HVS) samples, laboratory analysis of collected samples and statistical analysis. Ethical approval was obtained from the Ethical Research Committee of University of Calabar Teaching Hospital and State Ministry of Health, Cross River State. Informed consent was obtained from all the subjects. The Inclusion criteria were symptomatic female subjects attending gynaecology and sexually transmitted infection (STI) clinic who gave consent were enrolled for the study. The exclusion criteria were subjects on antibiotics for the past 2 weeks and those who did not give consent. A structured questionnaire was administered to all the subjects, to obtain their biodata, information on history of infection, hygiene practice and socio-economic status.

Ethics approval and consent to participate.

Ethical approval was obtained from the Ethical Research Committee of University of Calabar Teaching Hospital and State Ministry of Health, Cross River State. Informed consent was obtained from all the subjects.

Sampling Methods

A total of 250 women aged 16-45 years were

randomly recruited for the study. Subjects were recruited from gynaecology unit and sexually transmitted infection clinic. High vaginal swab (HVS) was obtained under aseptic condition by gynaecologists using sterile swab stick containing transport media. The specimens were subjected to various diagnostic tests according to World Health Organization (2020) guidelines on the management of vaginal discharge. The high vaginal swab specimens were inoculated on Sabouraud dextrose agar (SDA) and incubated at 37^0C for 24 hours. Culture plates were examined after 24 – 48 hours for growth, the colonies from Sabouraud dextrose agar were sub-cultured to obtain pure culture. Colonies were picked from overnight culture plates of SDA using inoculating loop. A smear was made on a clean grease free slide and allow to air dry. The smear was covered with crystal violet and allowed to stand for one minute followed by rinsing it with water. Iodine solution was applied and allowed for one minute then rinsed with water. Acetone was applied and rinse immediately with water after ten seconds. The stained slide was lastly stained with safranin for one minute and rinsed with water and then examined using 100X objective with oil immersion. The isolates were identified on CHROMagar which is a differential and selective medium.

Candida colonies on SDA were sub-cultured onto CHROMagar using an inoculating wire loop and incubated at 37°C for 24 hours. Identification of *Candida* was based on the colour of individual colonies which was matched with the colour chart of different species. The following *Candida* species were identified: *Candida albicans* (green colonies), *Candida krusei* (pink colonies), *Candida glabrata* (cream colonies) and *Candida tropicalis* (blue colonies). *Candida albicans* were confirmed using a germ tube test technique. 0.5ml of human serum was pipette into a test tube. A Pasteur pipette was used to touch a colony of the yeast and was gently emulsified in the serum. The tube was incubated at 37^0c for 2 to 4 hours. A drop of the incubated yeast in the serum were applied on a clean glass slide and covered with the coverslip and was examine on the microscope under 10 X objective lens.

A suspension was prepared by picking colonies from the 24-hour old culture of *Candida* species on SDA plate. The colony was inoculated in 5ml of sterile saline and its turbidity was adjusted to 0.5 McFarland standards visually. A sterile cotton swab was moistened in the inoculum suspension, and then, excess fluid was removed by rolling the swab on the inside surface of the tube above the fluid surface. Müller-Hinton agar (MHA) surface was inoculated with the moist swab to make a lawn of the isolate. Antifungal susceptibility testing was done by the disk diffusion method. Using disk dispenser (Oxoid™), fluconazole disk (10 µg), itraconazole (50 µg), voriconazole (1 µg), amphotericin B (100 µg) and nystatin (100 µg) antifungal discs (Thermo Scientific Oxoid, UK) were applied on MHA as recommended by the Clinical Laboratory Standard Institute (CLSI), (2014). The plates were incubated at 37°C for 24 hours. The diameter of zones of inhibition were measured in millimeters using a meter rule for each antifungal disk (Lass *et al.* 2018).

Statistical Analysis

Data obtained in the study were analysed using SPSS statistical version 20.0 software. Descriptive statistics was done. Frequencies were calculated for categorical variables. Dependence with each variable was analysed using Chi-square test. P-value of $\leq$ 0.05 was considered statistically significant.

Results

Socio-demographic Characteristics of Participants

A total of 250 subjects were recruited for the study. Subjects comprised married and unmarried women of childbearing age between 16 to 45 years. Most of the subjects were aged 26 to 30 years (33.6%). The mean age of the subjects was 27.7 ± 5.2 years with minimum age of 16 years and maximum age of 45 years. The ratio of single (154; 61.6%) to married (88; 35.2%) women was 3:2, while the rest of the women were all divorced (8; 3.2%). Nearly half of the subjects were students (117; 46.8%), others were occupied with businesses (81; 32.4%), civil service (37; 14.8%), and farming (10; 4.0%). Only a few were housewives (5; 2.0%). Most of the subjects had attained secondary level of education (131; 52.4%) while two in five subjects attained tertiary level of education (102;

40.8%). Primary and informal education was observed in 15(6.0%) and 2(0.8%) of the subjects respectively (Table 1).

The prevalence of vulvovaginal candidiasis among subjects in the study was 98(39.2%) (Figure 1). Out of the 250 subjects enrolled into the study, those aged 26-30 years had the highest vulvovaginal candidiasis infection rates (51.2%) followed by subjects aged 21-25 years (40.2%). Those aged 16-20 years had the lowest infection rates (18.8%). There was statistically significant relationship between age and infection rates ($\chi 2 = 11.6$; $P = 0.02$) (Table 2). Subjects who were single had the highest rates of vulvovaginal candidiasis (42.2%) while the lowest infection rates (12.5%) occurred among subjects who were divorced, but there was no significant association between marital status and infection rates ($\chi 2 = 3.2$; $P = 0.19$) (Table 2).

Table 1: Socio-Demographic Characteristics of Subjects

Variables	No. (%) of subjects enrolled in the study (N = 250)
Age	
16-20	16(6.4)
21-25	72(28.8)
26-30	84(33.6)
31-35	52(20.8)
36-45	26(10.4)
Marital status	
Single	154(61.6)
Married	88(35.2)
Divorced	8(3.2)
Occupation	
Student	117(46.8)
Civil servant	37(14.8)
Business	81(32.4)
Farmer	10(4.0)
Housewife	5(2.0)
Educational level	
Primary	15(6.0)
Secondary	131(52.4)
Tertiary	102(40.8)
Informal	2 (0.8)

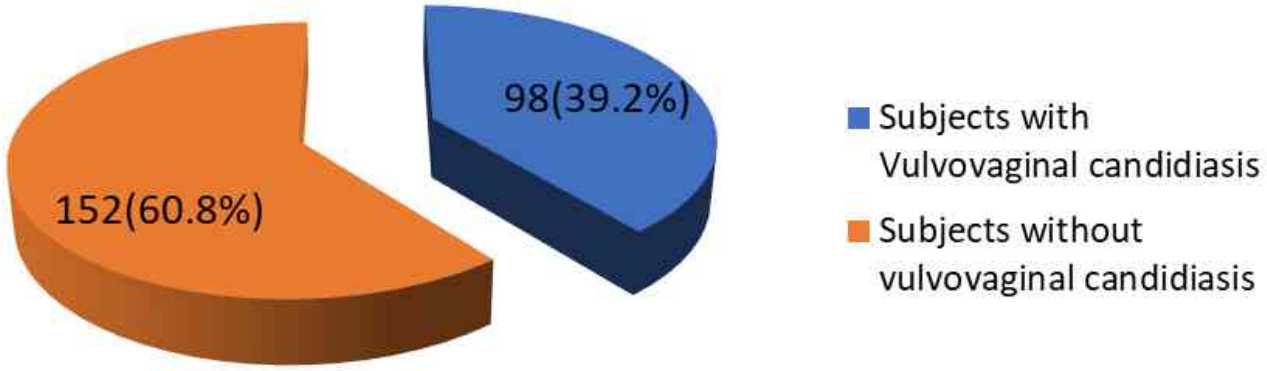

Figure 1 Prevalence of Vulvovaginal Candidiasis among Subjects

Table 2: Distribution of Vulvovaginal Candidiasis by Socio-Demographic Characteristics of Subjects

Age				
16-20	16 (6.4)	3(18.8)	11.6	0.02*
21-25	72 (28.8)	29(40.2)		
26-30	84 (33.6)	43(51.1)		
31-35	52 (20.8)	17(32.6)		
Marital Status				
Single	154 (61.6)	65(42.2)	3.2	0.195
Married	88 (35.2)	32(36.3)		
Divorced	8 (3.2)	1(23.0)		
Occupation				
Student	117 (46.8)	41 (35.0)	3.3	0.497
Civil servant	37 (14.8)	17 (45.9)		
Business	81 (32.4)	32 (39.5)		
Farmer	10 (4.0)	6 (60.0)		
Housewife	5 (2.0)	2 (40.0)		
Educational Level				
Primary	15 (6.0)	7 (46.6)	0.6	0.881
Secondary	131 (52.4)	49 (37.4)		
Tertiary	102 (40.8)	41 (40.1)		
Informal	2 (0.8)	1 (50.0)		

Those who had informal education had the highest rates of vulvovaginal candidiasis (50.0%), followed by those with primary education (46.6%). The lowest infection rate was among subjects with secondary education (37.4%). There was no significant association between occupation and vulvovaginal candidiasis among subjects ($\chi 2 = 3.37$; P=0.49).

Risk Factors Associated with Vulvovaginal Candidiasis among Subjects

Table 3 shows the relationship between vulvovaginal candidiasis and the associated risk factors among subjects. Out of 101 pregnant women enrolled in the study, (46.5%) suffered vulvovaginal candidiasis compared to those who were not pregnant (34.2%). There was significant association between pregnancy and Vulvovaginal candidiasis ($\chi^2 = 3.2$, $p = 0.05$). Vulvovaginal candidiasis was more prevalent among the HIV negative subjects (39.8%) than the HIV positive subjects (28.6%) but there was no association between VVC infection rates and HIV status ($\chi^2 = 1.42$, $p = 0.26$).

Subjects who practiced unprotected sex had higher rates of infection (42.9%) than those who did not practice unprotected sex 65(37.6%). There was no association between VVC infection rates and unprotected sex habit ($\chi^2 = 0.50$, $p = 0.47$). Subjects who were not diabetic, had higher infection rates (39.9%) than those who were diabetic (31.8%). Vulvovaginal candidiasis was higher among subjects who have multiple sexual partners (43.7%), than subjects with single sexual partners (36.1%) Table 3.

Susceptibility Pattern of *Candida* isolates to Antifungal Agents

Table 4 shows the susceptibility patterns of the isolated *Candida* isolates to commonly used antifungal agents. *C. krusei* was the most susceptible (100%) susceptible to Voriconazole, followed by *C. tropicalis* (75%). The least susceptible isolates to Voriconazole were *C. albicans* (54%) followed by *C. glabrata* (63.64) respectively. *C. tropicalis* (87.5%) was the most susceptible to Nystatin followed by *C. krusei* (75%), the least susceptible isolates to Nystatin were *C. albicans* (58%) followed by *C. glabrata* (63.64). Itraconazole was the most resisted antifungal agent with (0%) susceptibility to *C. krusei, C. albicans*, *C. glabrata* and *C. tropicalis.* However, *C. krusei* was (100%) susceptible to

Fluconazole followed by *C. albicans* (70%). The least susceptible isolates to Fluconazole were *C. glabrata* (36.36%) followed by *C. tropicalis* (43.75%). *C. krusei* (75%) was the most susceptible to Amphotericin B followed by *C. albicans* (74%). The least susceptible isolates to Amphotericin B were *C. tropicalis* (31.25%) followed by *C. glabrata* (45.45%) respectively. Furthermore, *C. krusei* was the most susceptible isolate to all the antifungal agents used in the study followed by *C. tropicalis* and *C. albicans* respectively. Itraconazole was the most resisted antifungal agent used in the study with (0%) susceptibility to all the isolates tested.

Table 3: Risks Factors Vulvovaginal Candidiasis among Subjects

Risk factors	No. (%) of subject tested. N=250	No. (%) of subjects infected with VVC (N=98)	$\chi 2$	P-value
Pregnancy				
Negative	149(59.6)	51(34.2)	3.2	0.05*
Positive	101(40.4)	47(46.5)		
HIV				
Negative	236(94.4)	94(39.8)		0.26
Positive	14(5.6)	4(28.6)	1.42	
Unprotected Sex				
No	173(69.2)	65(37.6)	0.50	0.47
Yes	77(30.8)	33(42.9)		
Diabetes				
Negative	228(91.2)	91(39.1)	1.01	0.31
Positive	22(8.8)	7(31.8)		
Multiple Sex Partners				
No	147(58.8)	53(36.1)	1.36	0.24
Yes	103(41.2)	45(43.7)		

Table 4: Antifungal Susceptibility Pattern of *Candida* isolates

Antifungal agent.	*Candida* species	Susceptibility rate
Voriconazole	*C. albicans*	27(54%)
	C. tropicalis	12(75%)
	C. glabrata	7(63.64%)
	C. krusei	4(100%)
	C. albicans	29(58%)
	C. tropicalis	14(87.5%)
	C. glabrata	7(63.64%)
Nystacin	*C. krusei*	3(75%)
	C. albicans	0(0.00%)
	C. tropicalis	0(0.00%)
	C. glabrata	0(0.00%)
Itraconazole	*C. krusei*	0(0.00%)
	C. albicans	35(70%)
	C. tropicalis	7(43.75%)
	C. glabrata	4(36.36%)
Fluconazole	*C. krusei*	4(100%)
	C. albicans	37(74%)
	C. tropicalis	5(31.25%)
	C. glabrata	5(45.45%)
Amphotericin B	*C. krusei*	3(75%)

Discussion

This study evaluated the prevalence of vulvovaginal candidiasis, antibiotic susceptibility patterns of isolates and the associated risk factors among married and unmarried women of childbearing age in Calabar metropolis. The prevalence of vulvovaginal candidiasis of 39.2% recorded in this study is consistent with the 38.0% reported by Mbim *et al.* (2017) in Calabar, but lower than the 55.4% reported in Cameroon by Toua *et al.* (2018). Hidalgo *et al.* (2014) also reported a high prevalence of 75.0% in the United States. The 39.2% prevalence recorded in this study may be due to unprotected sexual activities and poor personal hygiene among subjects. Subjects aged 26-30 years had the highest rates of vulvovaginal candidiasis. This finding agrees with previous reports (Alo *et al.*, 2014; Babin *et al.,* 2015 and Nelson *et al.,* 2016) who obtained a high prevalence among this age group. This may be due to the secretion of high reproductive hormones which can suppress the immune system and create favourable condition for *Candida* colonization.

There was significant association between vulvovaginal candidiasis infection and maintenance of multiple sexual partners. Having multiple sexual partners may encourage indiscriminate contraceptives use which suppresses the growth of normal bacterial flora thereby enabling *Candida* to have opportunity to invade the vaginal walls.

This study showed that marital status also influenced the rate of vulvovaginal candidiasis among subjects. The single subjects had the highest infection rates (42.2%) compared to the married (36.3%) and divorced women (12.5%). However, Okungbowa *et al.* (2016) reported higher incidence of vulvovaginal candidiasis among the married (69.6%) compared to the unmarried (30.4%). The high prevalence of VVC among single women in this study may be due to maintenance of multiple sex partners and indiscriminate use of antibiotics which enables *Candida* to overgrow their niche.

This study revealed significant associations between the risk factors of vulvovaginal candidiasis among women of childbearing age. These were unprotected sex ($\chi^2 = 6.28$, $p = 0.01$) and multiple sexual partners ($\chi^2 = 4.58$, $p = 0.03$). The use of condom which is a means of sexual protection has been shown to reduce the prevalence of sexually transmitted diseases which candidiasis is not an exception. Our findings agree with the work of Nester *et al.* (2001) who noted that having multiple sexual partners increases the chances of getting *Candida infection.* Previous report by Neeria *et al.* (2019) reported that poor personal hygiene increases the risk of getting STDs.

In this study, *C. albicans* (68.3%) was the most frequently isolated species followed by *C. tropicalis, C. glabrata* and *C. krusei* respectively. This is in agreement with previous report by Maria *et al.* (2018) which indicated that *C. albicans* (73.7%) was the most prevalent species. In the present study, *C. tropicalis* and *C. glrabrata* were the second and third most encountered isolates which may points to a gradual shift in the causative agents of VVC.

In this study, *Candida* species were highly resistant to itraconazole 94.0% followed by fluconazole 18.0%. This finding was similar to the work of Dota *et al.* (2014) in Brazil. However, 70.0% of the isolates were susceptible to Voriconazole and Nystatin followed by fluconazole which contradicts the study done in Northern India by Mohanty *et al.* (2017) with high susceptibility to Fluconazole. The present study showed that Voriconazole and Nystatin where the most effective antifungal agents.

Conclusion

The outcome of this study showed a high prevalence of vulvovaginal candidiasis among the study subjects. *Candida albicans* was the predominant isolates causing vulvovaginal candidiasis, followed by *C. tropicalis* and the least was *C. krusie.* Most *Candida* isolates in this study were susceptible to voriconazole, nystatin and fluconazole which favours its use in their empiric treatment considering its broad spectra. The existence of the *Candida* infection is dependent upon certain factors such as sexual activity among women with higher number of sexual partners and having unprotected sex

during their sexual intercourse. The study has shown that the age range of 21-25years are mostly susceptible to the infection and that Voriconazole, Nystatin and Fluconazole were the most effective antifungal drugs when compared to other drugs use in the study. The outcome of this study has revealed that lack of knowledge about personal hygiene. Lifestyle plays a vital role in acquiring vaginal infection. The use of condom, not having multiple partners is recommended to reduce Vulvovaginal Candidiasis.

Competing Interests

Authors have declared that no competing interests exits

Author`s Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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