



## **Antibiogram of Vulvovaginal Candidiasis amongst Pharmacy Undergraduates in a Nigerian University**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Authors ANO, CBU, EOO, CBO and GUE designed the study, and wrote the protocol. Authors ANO, CBU, EOO and GUE performed the statistical analysis, and wrote the first draft of the manuscript. Authors ANO, GUE, FNO and MCU managed the analyses of the study. Authors ANO, CBU, CBO, GUE, FNO and MCU managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Objectives:** To assess the knowledge, perception, incidence and the antibiogram of vulvovaginal candidiasis (VVC).

**Methods:** Validated questionnaires were used to collect demographic information of consenting study participants and to test the participants' level of knowledge and perception of VVC among

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pharmacy undergraduates. High-vaginal swab specimens were also collected for isolation of *Candida* species and speciation using standard yeast identification protocol and CHROMagar respectively.

**Results:** Among the 160 participants, yeasts were detected in 43.75% and 27.50% by culture and direct microscopy respectively. Having multiple sexual partners (at least two), poor vaginal hygiene and antibiotic usage were shown to significantly increase the incidence of VVC ( $p < 0.05$ ). Yeasts other than *Candida albicans* were predominant (66.67%), especially *Candida tropicalis* (40.00%). Although participants' knowledge about VVC was good (80.49%), their perception was only average (54.10%). Susceptibility test showed that the isolates were susceptible to voriconazole ( $84.37 \pm 5.70\%$ ), fluconazole ( $71.54 \pm 7.66\%$ ) and nystatin ( $65.70 \pm 12.33\%$ ). The commonest isolate (*Candida tropicalis*) was fairly susceptible to voriconazole (77.00%) and fluconazole (63.00%) but resistant to nystatin (36.00%).

**Conclusion:** There is high prevalence of VVC despite good knowledge and average perception. *Candida tropicalis* was predominant in the study. Based on antibiogram, Voriconazole was the most effective drug/antibiotic and drug of choice for therapy.

**Keywords:** Vulvovaginal candidiasis; knowledge and perception; asymptomatic mycosis; drug resistance.

## 1. INTRODUCTION

Candidiasis is the most common opportunistic yeast infection caused by species of the genus *Candida*, predominantly by *Candida albicans* [1,2]. Of the several forms of candidiasis, cutaneous candidiasis is the most common and affects the skin, the vagina, the esophagus and the mouth especially in immunocompromised individuals [3,4]. *Candida* species are opportunistic pathogens that exploit impaired immune status to gain access into the systemic circulation and deep tissues. Invasive candidiasis is a serious illness and may eventually spread internally throughout the body despite intake of recommended antibiotics [5].

Vulvovaginal candidiasis (VVC) - also known as vaginal thrush or moniliasis - is a syndromic inflammatory disease marked with the irritation of the vulvovaginal areas [6]. It is less common in pre-pubertal girls and post-menopausal women. Studies showed that the reproductive hormone, oestrogen, enhance the proliferation and attachment of yeast to the vaginal inner lining [7-9]. The disease may persist asymptotically for long periods. *Candida* spp. and many organisms that normally live in the vagina keep each other in balance [9,10].

About half of the women experience vaginal candidiasis at some points in their life, and may have to visit a gynaecology clinic at least twice before the age of 30 years [11]. Candidaemia, a possible complication of VVC, can result in septicaemia if untreated. With the high global prevalence of HIV infection and immunosuppression, VVC is expected to

increase if not prevented [12]. Among all the fungal infections, vaginal candidiasis is the most common one that affects women of childbearing age [13,14]. During the reproductive years of a healthy woman, the vagina maintains a moist environment that is in constant fluctuation. The secretion of an alkaline transudate from the vaginal epithelium and cervical glands maintains this moist environment to a pH ranging from 3.8 to 4.5 [13]. The vaginal microbiota is characterized by relatively few microbial species compared with the intestinal microbiota. The stability of this microbial ecosystem is greatly affected in some women by the physiological variations of the menstrual cycle and hormones, and changes dramatically after menopause. Studies suggested that the vaginal microbiota contributes to protection against microbial pathogens [15,16]. Zhou et al. [17] reported the absence of a clear difference between the vaginal microbiota of women with a history of recurrent vulvovaginal candidiasis (RVVC) and healthy controls, and suggested that the vaginal microbiota does not protect against infection but exist as commensals in healthy humans. However, some members of the vaginal microbiota may cause systemic infection, candiduria and/or VVC, when the host immunity is low.

A good understanding of the prevalence, knowledge and perception of vulvovaginal candidiasis in apparently normal healthy women of reproductive age will help provide information that may be used in instituting health promotion interventions aimed at reducing the infection rate and prevent complications in the future. To the best of our knowledge, no previous study has

reported the pattern and antibiogram of VVC amongst Pharmacy undergraduates in a Nigerian University, so the knowledge too will ultimately add to our current medical knowledge.

The present study aims at assessing the knowledge, perception of VVC among 160 apparently healthy Unizik pharmacy undergraduates as well as determines the therapy of choice for such infections.

## 2. MATERIALS AND METHODS

All the agars and reagents used were produced by HiMedia Laboratories Pvt. Ltd. A-516, Swastik Disha Business Park, Via Vadhani Ind. Est., LBS Marg, Mumbai-400086, India. The microscope was the product of Olympus Cooperation, Tokyo, Japan. Model number: CX22RFS1.

## 3. PROCEDURE

### 3.1 Study Population and Sample Size Determination

Pharmacy undergraduate program in Unizik is currently a 5-year program. The first year is spent in the main campus – Awka, while the remaining four years are spent in Agulu. The studied population consisted of female pharmacy students in Unizik, Agulu. The sample size was calculated using a study conducted by Lennox et al in Calabar, Nigeria among university students [18], the prevalence of vaginitis in students aged 15 to 35 years was 70%, taking 0.05 margin of error, 95% CI. For the total population of less than 10, 000 and 10% attrition, it was adjusted and the final sample size was 153 [18].

One hundred and sixty consenting students were then finally recruited in accordance with the populations in their different levels of study. Selection within levels was done purposively. Pre-enrolment briefing about the study was carried out for each participant, and thereafter, written informed consent was obtained in all participants.

### 3.2 Inclusion Criteria

The participants were included if they have mostly experienced in the past six months (or less), any of these symptoms prior to recruitment: itching, vaginal discharge, urinary frequency and/or urgency, painful urination, pains around the waist areas and vaginal rashes.

### 3.3 Exclusion Criteria

These include pregnancy, refusal to participate in the study and history of diabetes mellitus.

### 3.4 Sample Collection and Questionnaire Analysis

Following passage of sterile disposable Cusco's speculum, high-vaginal swab specimens were collected from the study participants using sterile swab stick and transported in sterile containers to the Pharmaceutical Microbiology laboratory for microscopy and culture. Pre-tested questionnaires were used to obtain data on age, marital status, antibiotic and contraceptive usage, material used during menses and on information about knowledge and perception. The overall percentage of level of knowledge and perception were obtained by calculating the percentage of positive answers to the questions asked on knowledge and perception of the infection respectively. The study subjects were then examined and vaginal samples collected.

### 3.5 Microscopic Examination of Samples

Within an hour of collection, specimens were mixed with 10% w/v saline preparation and KOH preparation in a 5 ml test tube, to expose the fungi. A drop of this mixture was viewed with low power objectives (10× and 40×) after placing on a clean grease-free slide.

### 3.6 Microbiological Analysis

All the specimen samples were processed within 1 hour of collection according to standard technique [19]. Sabouraud dextrose agar (SDA) plates were prepared according to manufacturer's instruction and swabs samples streaked onto them, and incubated for 48 hr at 25°C. *Candida* species were identified based on cultural characteristics and cell morphology and further subcultured onto sterile MacConkey agar plates for purification [19].

### 3.7 Fungal Identification

#### 3.7.1 Sugar fermentation test

Five milliliters of a 1% (w/v) concentration of the various sugars (maltose, glucose, lactose and sucrose) was prepared in a test tube. Three (3) drops of phenol red was added to each tube to serve as an indicator. The tube containing glucose was sterilized by autoclaving for 15

minutes while the ones containing maltose, sucrose and lactose were autoclaved for 3 minutes to avoid being broken down on prolonged heating [20]. One loopful of the culture from a freshly prepared SDA plates was aseptically inoculated into each test tube using sterilized inoculating needle. The tube was incubated at 37°C for 7 days and any tube that did not show discoloration was regarded as a negative result [20,21]. This is done to exclude the presence of bacterial cells in the test specimens as bacteria show positive results. Fermentation is noted by acid and gas production by bacterial cells.

### **3.7.2 Germ tube test**

From a freshly prepared Sabouraud dextrose agar plates, a loopful of a yeast colony was inoculated into 0.5 ml freshly prepared human serum and incubated at 35°C for 3 hr. A wet smear of the yeast suspension was made and mounted on a microscope and viewed with the 400 magnification lens. Sprouting non-septate germinating hyphae confirms the presence of *Candida albicans*.

### **3.7.3 Detection of yeast isolates using CHROMagar candida**

CHROMagar candida (Becton, Dickinson and Company, Maryland, USA) plates were prepared according to manufacturer's instructions (47.7 g/L). Positive isolates on Sabouraud dextrose agar plates were streaked on to the media, for species differentiation, and incubated for 48 hr. The different candida species were recognized by their different colours according to manufacturer's instructions.

### **3.7.4 Antifungal susceptibility testing**

Antifungal susceptibility testing was performed using Mueller Hinton Agar supplemented with 2% glucose and 0.5 mg/mL methylene blue. 0.5 McFarland standard was prepared by mixing 0.05ml of 1.175% barium chloride dehydrate ( $BaCl_2 \cdot 2H_2O$ ) with 9.95ml of 1% sulphuric acid ( $H_2SO_4$ ) together. Five distinct colonies was picked from a 48-hours old culture and inoculated into sterile water. The suspension was diluted to a 0.5 McFarland standard. With sterile cotton swab stick, the suspensions were streaked on to Mueller Hinton Agar (supplemented with 2% glucose and 0.5mg/mL methylene blue) plates and allowed to dry and diffuse for about 5 minutes. The three antifungal discs (voriconazole, nystatin and fluconazole)

were picked (separately) aseptically and dispensed on to the surface of the agar containing each isolates. The plates were then incubated for at 35°C for 24 h. The assay was done in duplicates and Inhibition Zone Diameters (IZD) formed were measured and recorded. Susceptibility/resistance was interpreted according to Clinical and Laboratory Standard Institute (CLSI) [22] guidelines.

## **3.8 Statistical Analyses**

Frequency of categorical variables and mean  $\pm$  SD of continuous variables are reported. Independent t-test was used to compare mean of continuous variables. Categorical variables were compared using chi-square test and Fisher's exact test where appropriate. Statistical analysis was performed using SPSS version 21, IBM Company, USA.

## **4. RESULTS**

The age range of the participants was 17 – 31 with a mean age of 22.4 years. Out of the 160 high vaginal swab samples collected, representing the 160 female students, 70 showed visible fungal growths after the incubation period - giving an incidence rate of 43.75% (Table 1) while direct microscopy (saline preparation and 10% KOH preparation) revealed existence of yeast in 44 (27.5%) patients. All the 44 direct microscopy positive samples were culture positive. The sugar fermentation test showed that the SDA isolates were pure fungi and had no bacterial contamination. The incidence was lowest among the youngest age group (17 – 19 years) followed by the most elderly age group (29 – 31 years). Having multiple sexual partners and poor perineal hygiene during menstrual flow were shown to significantly increase the incidence ( $p < 0.05$ ). Antibiotic usage also affected significantly the incidence positively ( $p < 0.0001$ ). Contraceptive usage did not significantly increase the incidence ( $p > 0.05$ ). The incidence, however, was significantly correlated with participants' hygienic practices as revealed by the materials they use during menstrual cycles ( $p$  value = 0.036). There were no significant differences in the incidence rates in relation to marital status ( $p = 0.6322$ ).

There were mixed candida species in 5 (7.6%), *C. albicans* in 25 (33.3%), *C. tropicalis* in 30 (40%), *C. krusei* in 7 (9.3%), *C. glabrata* in 5 (5.3%) and unidentified fungi in 9 (13.6%) (Table 2). Non-*Candida albicans* were the predominant aetiological agent (66.67%) of the vulvovaginal

candidiasis. *Candida tropicalis* (40%) was the predominant non-*Candida albicans* fungal isolate.

As shown in Table 3, the knowledge of the respondents (judged by correct answers to questions that relates to knowledge) about the syndrome was good 80.49%. However, their idea about it (perception) was only average (54.10%). More than half of the participants opined that it is contracted from the toilet (56.3%). The level of knowledge correlated with the students' levels of study. The respondents showed an average perception (54.10%) of the syndrome showing the need to widen awareness.

The result of the susceptibility test showed that the isolates were susceptible to voriconazole (84.37 ± 5.70%) to fluconazole (71.54 ± 7.66%) and nystatin (65.70 ± 12.33%) – Fig. 1a. However, the performance of the drugs was not statistically different (p value = 0.36). Fig. 1b at the performance of the drugs on individual species of the *Candida* showed that *Candida albicans* responded equally well to any of the three drugs (range = 88- 96) while the most common isolate (*Candida tropicalis*) was marginally susceptible to voriconazole (77%) and fluconazole (63%) but resistant to nystatin. Of the three antifungal agents screened, the isolates recorded the highest resistance to nystatin (36%).

**Table 1. Prevalence of vulvovaginal candidiasis amongst the students (n = 160)**

Variable	Number of participants (n = 160)	Number positive (n = 70)	%	Correlation co-efficient (r <sup>2</sup> )	P value	X <sup>2</sup> (positivity and negativity) P value
<b>A: Age (years)</b>						
17-19	36	11	30.56	[Age versus % positive] = 0.0618	0.687	0.349
20-22	88	42	47.73			
23-25	16	9	56.25			
26-28	4	2	50.00			
29-31	16	6	37.50			
<b>B: Marital status</b>						
Single	156	69	44.23			Fisher's exact test p = 0.6322
Married	4	1	25.00			
<b>C: Number of sexual partners</b>						
0	122	42	39.34	[# of partners versus % positive] = 0.9489	0.005**	0.0002***
1	17	10	58.82			
2	12	9	75.00			
3	3	3	100.0			
>3	6	6	100.0			
<b>D: Usage of antibiotics in the past 3 months</b>						
Yes	110	68	61.82			Fisher's exact test p< 0.0001***
No	50	2	4.00			
<b>E: Usage of contraceptive in the past 3 months</b>						
Yes	25	13	52.00			Fisher's exact test P = 0.388
No	135	57	42.22			
<b>F: Materials used during menstruation</b>						
Sanitary pad	133	51	38.35	[positivity versus negativity due to materials used for menses] = 0.998	0.036*	0.0024**
Toilet tissue	21	13	61.90			
Clean piece of cloth	6	6	100.00			

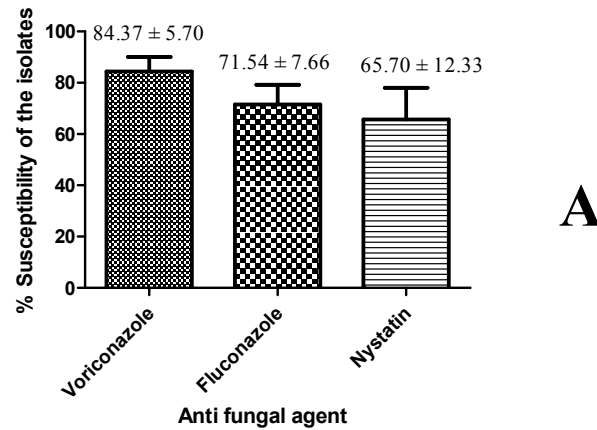
\* means significance; the number of asterisks denotes the level of significance

**Table 2. Speciation of the isolates using CHRO Magar**

Fungal isolate	Culture positive N	Culture negative N	P value (X <sup>2</sup> )
<i>Candida albicans</i>	25	135	< 0.0001
<i>Candida tropicalis</i>	30	130	
<i>Candida krusei</i>	7	153	
<i>Candida glabrata</i>	4	156	
Undifferentiated <i>Candida spp</i>	9	151	

**Table 3. Knowledge and perception of vaginal candidiasis among the students (n = 160)**

<b>A</b>																			
<b>Knowledge = 80.4875%</b>																			
<b>Variables/ Questions asked</b>	<b>1: Have you heard of candidiasis</b>		<b>2: Is Vaginal candidiasis also known as vaginal thrush</b>			<b>3: It is sexually transmitted?</b>			<b>4: It causes intense itching, swelling and irritation of the vulva</b>		<b>5: It causes burning sensation during urination</b>		<b>6: Candidiasis is considered an infectious disease</b>			<b>7: Which pathogens cause candidiasis?</b>		<b>8: Type of organism causing candidiasis</b>	
<b>Response</b>	Yes	No	Yes	No	No idea	True	False	No idea	True	False	True	False	Agree Strongly	Agree	Disagree Strongly	<i>Candida</i> spp	No idea	Fungi	Bacteri a
	160	0	99	51	10	108	52	0	154	6	127	33	92	53	15	136	24	154	6
<b>% Response</b>	100	0	61.9	31.8	6.3	67.5	32.5	0	96.3	3.7	79.4	20.6	57.5	33.1	9.4	85.0	15.0	96.3	3.8
<b>B</b>																			
<b>Perception = 54.10 %</b>																			
<b>Variables/ Questions asked</b>	<b>9: It is curable?</b>		<b>10: It can cause abortion</b>		<b>11: It is mainly contacted from toilet seat</b>		<b>12: It can be common in diabetics</b>		<b>13: It is caused by pregnancy</b>		<b>14: It causes painful intercourse</b>		<b>15: It causes infertility</b>		<b>16: Is it common among your classmates</b>				
<b>Response</b>	True	False	True	False	True	False	True	False	True	False	True	False	No idea	True	False	Yes	No	No idea	
	139	21	45	115	90	70	90	70	34	126	94	51	15	118	42	68	62	30	
<b>% Response</b>	86.875	13.125	28.1	71.9	56.3	43.8	56.3	43.8	21.3	78.8	58.5	31.9	9.3	73.8	26.3	42.5	38.75	18.75	



Antifungal agent	Number of the isolates susceptible to commercial antibiotics (%)					
	<i>C. albicans</i>	<i>C. tropicalis</i>	<i>C. glabrata</i>	<i>C. krusei</i>	Undifferentiated	Total
	N = 25 (%)	N = 30 (%)	N = 4 (%)	N = 7 (%)	N = 9 (%)	N = 75 (%)
Voriconazole	24 (96)	23 (77)	4 (100)	5 (71)	7 (78)	63 (84)
Fluconazole	23 (92)	19 (63)	2 (50)	6 (86)	6 (67)	56 (75)
Nystatin	22 (88)	15 (50)	4 (100)	4 (57)	3 (33)	48 (64)

A large letter 'B' is positioned to the right of the table.

Fig. 1. Susceptibility profile of the *Candida* species

### 5. DISCUSSION

*Candida* species inhabit the vagina as a part of normal flora, but may induce a disease outcome; either as urinary tract infections or vulvovaginitis (vulvovaginal candidiasis) or even both when favorable conditions arise. Vulvovaginal candidiasis is the second most common cause of vaginitis in women [10,23].

From our study, the incidence of the vulvovaginal candidiasis was high, indicating the possibility of other STIs in the study community. Preventive measures for VVC and other STIs need to be urgently addressed. This study shows that pregnancy, sexual activity, antibiotic and contraceptive usage and the choice of materials used during menses all contribute positively to the incidence of VVC among women of child bearing age. The finding correlated well with another study done in Italy and in Nigeria [1,24].

Although *Candida albicans* occupied one third of the detected *candida* spp, most of the isolates were non-*Candida albicans* in the present study, suggesting that they are also becoming important pathogens in VVC [12,14,25]. It is important to take note of this trend and plan to curb it because some studies have shown that infections due to non-*Candida albicans* are often difficult to treat [26,27]. Also, oral and vaginal anti-fungal agents that are frequently used in our locality have been found to be less effective against the non-*Candida albicans* infections [23,24] possibly due to observed high miss-use of fluconazole and nystatin (personal communication). Both drugs are prescription drugs but are easily obtained over-the-counter and from "open-markets". They are often taken in sub-therapeutic dose by the users. Several other researchers also reported the development of resistance to nystatin, imidazole and triazole antifungal agents [28-30]. The mechanisms for the development of

resistance have been suggested to be the activation of multidrug efflux pumps of the ATP-binding cassette (ABC) transporter superfamily and the major facilitator superfamily (MFS) [31,32].

In some parts of the country, *Candida albicans* was reported to be more common [10,23] probably because the studies were based on pregnant women and other studies confirmed that *Candida albicans* is more common in pregnancy than non-*Candida albicans* [27,33]. Our study focused on non-pregnant women and this could be the reason why non-*Candida albicans* was more prevalent. There were also, significantly higher culture negative samples than positive samples because the population tested was apparently healthy individuals but from the prevalence study, the number of positive samples showed a high prevalence.

The knowledge of the respondents concerning vulvovaginal candidiasis was judged by correct answers to questions that related to knowledge about the syndrome. The high knowledge observed in this study could be attributed to the respondents being in para-medical profession. Other studies in Nigeria [10,14,23,24] showed a poor knowledge of the study subjects about the infection possibly because they comprised mainly of non-health professional. Since many of our study population (56.3%) still believed or thought that VVC is a kind of toilet disease, it is then expected that the general (uneducated) population may even have worse perception. This is confirmed by a similar study in the study environment [24] and in other regions of Nigeria [10,14,23]. This actually calls for the need for increased awareness on VVC via all available social media, schools and churches. This will help to reduce possible complications of untreated cases [34].

## 6. CONCLUSION

This study has demonstrated a high prevalence of vulvovaginal candidiasis among the studied population although they have good knowledge and perception of the infection. Non-*candida albicans* dominated the etiology in the studied population. Voriconazole appeared to be the drug of choice in the treatment of vaginal candidiasis in this locality. Aggressive health education via all available social media is urgently needed to help bring about positive attitudinal change in sexual practice. Health education should also include vaginal hygiene

and importance of regular screening for all WBA in every obstetric and/or gynecological clinic visit. These will help forestall the ravaging infection among women of childbearing age and so prevent complications.

## 7. LIMITATIONS OF THE STUDY

The study did not distinguish which study participant has symptomatic or asymptomatic VVC. No investigation was also made on the kind of antifungal agents used by the study participants.

## CONSENT

The participants gave consent to participate in the study after the reasons for the study were explained to them. No information on the participants' names was collected. The instruments for data collection were identified with number.

## ETHICAL APPROVAL

Ethical approval for the study was obtained from the ethics committee of Nnamdi Azikiwe University Teaching Hospital with reference number NAUTH/CS/66/VOL.6/34. This study followed every necessary international, national, and/or institutional ethical guideline and obeyed the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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