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**EPIDEMIOLOGIC AND BIOLOGIC INTERACTIONS BETWEEN VULVOVAGINAL CANDIDIASIS (VVC) AND STAPHYLOCOCCAL INFECTIONS AMONG WOMEN ATTENDING A TERTIARY HEALTH CENTRE IN MAKURDI, BENUE STATE NIGERIA**

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**ABSTRACT**

Vulvovaginal candidiasis (VVC) is an inflammatory condition caused by yeast predominantly *Candida albicans*. To investigate the epidemiology of vulvovaginal candidiasis. 1116 high vaginal swab samples were collected from female patients who attended the Obstetrics and Gynecology unit of the Federal Medical Centre, Makurdi within a twelve month period and were cultured on chocolate blood agar and sabouraud dextrose agar. Three hundred and thirty five (30.0%) of those examined had VVC infections. *Candida albicans* predominated 280 (83.6%), followed by *Candida tropicalis* 34 (10.1%) and *Candida glabrata* 21 (6.3%). One hundred and thirty three (39.7%) of the Vulvovaginal candidiasis patients were co-infected with Staphylococcal infection. VVC was significantly associated with months of the year ( $r = -.108$ ;  $p < 0.05$ ), diabetes ( $r = 0.060$ ,  $p < 0.05$ ), pregnancy ( $r = 0.194$ ;  $p < 0.05$ ), antibiotics ( $r = 0.108$ ;  $p < 0.05$ ) and use of contraceptive pills ( $r = .160$ ;  $p < 0.05$ ). VVC was not associated with Staphylococcus infection ( $r = .027$ ;  $p < 0.05$ ), season ( $r = -0.034$ ;  $p < 0.05$ ), age ( $r = -0.024$ ;  $p < 0.05$ ) and occupation ( $r = -0.022$ ;  $p < 0.05$ ).

**Keywords:** *Vulvovaginal Candidiasis (VVC), Staphylococcal infections, Sabouraud dextrose agar, Candida glabrata, Candida tropicalis.*

**INTRODUCTION**

Vulvovaginal candidiasis (VVC) is a yeast infection of the vulva and vagina. It is commonly called "thrush" and sometimes monilia (Mendling and Seebacher, 2003). Candidiasis is a low grade infection caused by *Candida species*, the most common being *Candida albicans*, a weakly infective fungus which lives in warm, moist conditions. Candidiasis flourishes when the body's immune system is at low ebb. Classically it mainly affects the vaginal. It can also affect the mouth, skin and gastro intestinal tract (Jose, 2002). VVC is a common fungal infection in women of child-bearing age, (Rodgers and Beardall, 1999; Sobel *et al*, 1996). Most women experience at least one or two episodes of VVC in their lifetime. Candida infection of the external genitalia affects the vulva and vagina, presenting clinically with thick discharge, white deposits on the vaginal wall that can be wiped off, and marked erythema of the vulva and the adjacent inguinal region. Subjective symptoms include soreness of the vaginal vestibule and the perianal region, pruritis, and vaginal discharge. Discharge characteristically has a whitish-creamy to crumbly and curdlike consistency (Mendling and Seebacher, 2003). Vulvovaginal candidiasis (VVC) occurs widely in adult women, especially those who are taking oral contraceptives, antibiotics or those who are diabetic or pregnant. These are conditions that can disrupt the normal vaginal flora. Candidal vaginitis poses a risk for neonates as they can be infected during childbirth. It can also be transmitted to male

partners during sexual intercourse (Kathleen and Arthur, 2002). The findings of Paulitsch *et al*, (2006) showed that *Candida albicans* was the most prevalent cause of most cases of VVC. Non-*albicans candida* yeast *C. glabrata* and *C. tropicalis* were detected in few cases.

Geiger *et al*, (1995) observed that the frequency of first diagnosis among university students increases rapidly after age 17, with a large number of women experiencing the condition by age 25. In Tanzania, Namkinga *et al*, (2005) reported that VVC was positively associated with HIV. *Candida albicans* is the leading cause of VVC, accounting for most of the cases (Sobel *et al*, 1998). In recent years, a change in epidemiological trends has been observed showing an increase in vaginal infections attributable to yeasts other than *C albicans*, particularly *C. glabrata* and *C. tropicalis*.( Horowitz *et al*, 1992). Vulvovaginal candidiasis generally presents with marked itching, watery to curdlike discharge, vaginal erythema with adherent white discharge, dyspareunia, external dysuria, erythema, and swelling of labia and vulva with discrete pustulopapular peripheral lesions. The cervix usually appears normal. Symptoms typically exacerbate the week preceding menses with some relief once menstrual flow begins. Vaginal candidiasis frequently is associated with pregnancy, high-estrogen oral contraceptives, uncontrolled diabetes mellitus, tight-fitting clothes, antibiotic therapy, dietary factors, intestinal colonization, and sexually transmitted disease. Specific additional risk factors for recurrent vulvovaginal candidiasis have not been identified (Sobel, 1992). Female-to-female transmission remains questionable, although male sexual partners may experience a transient rash, erythema, pruritus, or burning sensation of the penis minutes to hours after unprotected sexual intercourse. Occasionally, *Candida* balanitis may occur. Although a lot of work has been done on VVC, information is lacking on epidemiology of VVC in Benue State.

The aim of this study is to:

- Isolate and identify *Candida species* in patients with culture-confirmed vulvovaginal candidiasis;
- Evaluate the relationship between risk factors such as pregnancy, diabetes mellitus, antibiotic and contraceptive pills use and vulvovaginal candidiasis; and
- Determine the age distribution of VVC in young women.

This study may provide relevant information on epidemiology of vulvovaginal candidiasis.

## **MATERIALS AND METHODS**

### **Study Area**

The study was carried out in Makurdi, the Benue State capital. Makurdi is located along the banks of the Benue, a major river in Nigeria. It occupies an area of approximately 25sqkm with a population of 239,889 (NPC, 2002). Makurdi has two major seasons, the rainy (April – October) and the dry (November – March). Annual rainfall ranges from 150 – 180mm, while temperatures range between 23<sup>0</sup>C and 30<sup>0</sup>C. Makurdi lies between 7<sup>0</sup> (30’– 43’) N and 8<sup>0</sup> (30’– 35’) E. The vegetation belt is guinea savannah, mostly grass and a few scattered trees (Agisui and Ogbu, 2005). The study was carried out at the Federal Medical Centre, the main

government hospital in the capital city of Benue, between the months of January and December, 2006.

### **STUDY POPULATION**

One thousand, one hundred and sixteen outpatients attending the obstetrics and gynecology unit of the Federal Medical Centre participated in the study. The subjects were women from different localities and of a wide age range (17–72 year). Female patients from different works of life civil servants, business women, traders, teachers, farmers, students and Housewives with a common complaint of vaginal itch and discharge were examined in this survey. Risk factors such as pregnancy, *diabetes mellitus*, antibiotic therapy, use of oral contraceptive pill were assessed by the review of medical records.

### **SPECIMEN COLLECTION**

Physical examination began with an inspection of the vulva, looking for areas of inflammation, ulceration or chronic vulva skin changes, with palpation using a cotton-tip applicator to elicit areas of tenderness; this was carried out by a qualified and experienced nursing officer. With the aid of a speculum inserted into the vaginal and cervix, endocervical swab and high vaginal swab specimens were collected from the lateral vaginal wall by passing sterile cotton-tipped plastic swab sticks several times across the vaginal surface including vaginal discharge materials. The swab was then inserted back into the swab tube and labeled. Each specimen was clearly labeled with the date and time of collection, and the patient name, number, ward and health unit. Specimens were immediately carried to the microbiology laboratory of the hospital where the cotton tipped plastic swab was inserted into 0.5ml of saline water in a micro centrifuge tube, the tube was rigorously mixed for 30 seconds with a laboratory top vortex mixer and 0.15ml of the wash was cultured on chocolate blood agar and sabouraud dextrose agar (SAB). Culture plates were incubated at 35–37<sup>0</sup>C for 48 hours. A wet mount of the vaginal fluid was prepared and viewed under the microscope for presence of yeast and *Staphylococcus* cell. The yeast forms were easily recognized in a wet mount preparation of vaginal fluid as round to ovoid cells of 2–4 $\mu$ m in diameter. Yeast like growing colonies on sabouraud dextrose agar were routinely Gram stained and examined under the microscope. Representative distinct colonies from each culture plates were sub-cultured and stored on sabouraud dextrose agar slant for species identification. *Candida albicans* were identified by chlamydospore formation, *Candida glabrata* were identified by the germ tube test while *Candida tropicalis* were identified by the commercial carbohydrate assimilation tests. This was inoculated with the samples on the agar slants and the result was interpreted by following the manufacturer's instruction. Chlamydospore – these spores are large, thick walled, round or irregular structures formed within or terminally on a hypha. It is common to most fungi, but is characteristic of *Candida albicans*. Germ tube test involves inoculation of a suspension of 10<sup>5</sup> or 10<sup>6</sup> yeast cells/ml from the suspected yeast strain in 0.5ml serum. After 2 – 3 hours of incubation at 35 – 37<sup>0</sup>C, if *Candida glabrata* is present, germ tube production is seen on a slide with a cover slip.

## **Laboratory Procedures**

Agar slants contain the same medium as Petri plates, but in a tube in which the agar has solidified while the tube is on a slanted surface.

### **Preparation of Slant Cultures**

1. Place screw cap test tubes in a test tube rack.
2. Prepare a nutrient agar medium and boil it with stirring until the entire agar is melted. This must be stirred very well so that the melted agar is evenly distributed in the medium.
3. A pipette was used to transfer about 5ml of molten agar to each test tube.
4. With all the tubes containing hot agar, the caps were placed loosely on the tubes and the tubes sterilized.
5. While the medium was still hot, the rack was tilted on a thick solid surface, so that the medium in the tubes were slanted. The medium was allowed to harden in that position.
6. When the medium was cool, the caps were tightened.
7. The slant was then inoculated, by using an inoculating loop to transfer cells from a single colony on a plate to the surface of the slant. The loop was moved back and forth across the surface of the slant. The tubes were capped and incubated until growth became evident.

### **Gram Stain Procedures**

Yeast like growing colonies in SAB were routinely gram stained. Gram stains preparation showing gram positive *Candida* yeast were confirmatory that the patient was infected with *Candida*. Gram stain is the most widely used or probably the most important differential stain employed in the study of microorganisms into gram positive and gram negative groups. A differentiation is based upon the color exhibited by the fungus cells after heat smear are treated with four reagents (crystal violet, iodine solution, 95% ethyl alcohol and safranin 'o', a red dye). Each is applied for a specific time ranging from 30 seconds to 1 minute in a sequential manner. If the color is pink or red the organism is said to be gram negative, when cells are observed under a microscope, but cells that stain purple because they retain the crystal violet are said to be gram positive. Usually *Candida albicans* cells show gram positive chlamyospores when viewed under the microscope.

### **STATISTICAL ANALYSIS**

Data was analyzed using Microsoft SPSS for Windows Version 10.0 Software. Pearson Chisquare ( $\chi^2$ ) analysis was used to test relationship between the variables under consideration. Pearson correlation coefficient test was also used to determine association between variables.

### **RESULTS**

A total of one thousand, one hundred and sixteen (1116) female patients who attended the obstetrics and gynecology unit of the Federal Medical Centre, Makurdi were surveyed. Women involved in the survey had a mean age of 21.8 years (age range 17 to 72 years).

The frequency of *Candida species* is shown in Table 1. The number of women who

had VVC was 335 (30.0%). *Candida albicans*, 280 (83.6%) was the predominant species followed by *Candida tropicalis*, 34(10.1%) and *Candida glabrata*, 21 (6.3%). Four hundred and twenty one (37.7%) patients had *Staphylococcal* infection whereas 133 (11.9%) were co-infected with *Staphylococcus* and *Candida species*. Table 2 summaries the rate of general infection in relation to age. *Candida* infection rates were higher in those patients with ages above 60 years 9 (52.9%), although patients within age group 0 – 20 years had the highest infection rate for *Staphylococcal* infections 13 (54.2%) and mixed infections 5 (20.8%). *Candidiasis* showed no association with age, however *Staphylococcal* infection was significantly associated with age.

### Occurrence of Infections in Relation to Occupation

The frequency of *Candidiasis*, *Staphylococcus* and co-infections in relation to occupation are presented in Table 3. The infection rates for *Candidiasis*, 22 (40.0%), *Staphylococcal* infection, 30 (54.5%) and coinfections 17(30.9%) were highest among the student population. *Candida* infection was least among the teachers, 13 (26.0%) whereas *Staphylococcal* infection was least among the farmers 25 (27.5%). Only one teacher (2.0%) was coinfecting with *Candida* and *Staphylococcal* infections. *Candidiasis* and *Staphylococcal* infections were not associated with occupation although co-infections were significantly associated with occupation.

### Frequency of Vulvovaginal candidiasis

Table 4 presents the frequency of VVC within a twelve month period, the incidence of VVC was highest in the month of February, 16 (50.0%) and least in the month of October, 18 (17.0%). *Candidiasis* was significantly associated with months of the year.

### Frequency of Staphylococcal Infections

The frequency of *Staphylococcal* infections within a twelve month period is displayed in Table 5. From June to December more samples were examined in the microbiology laboratory of the hospital than January to May. The infection rate was highest in the month of February, 20(62.5%) whereas it was least in the month of July, 23 (19.3%). *Staphylococcal* infection was significantly associated with season. The infection rate was higher in the dry season 240(50.0%) than in the wet season 181 (28.5%). Fifty percent (50%) of persons having *Staphylococcal* infection had *Candida tropicalis* while only 38.9% had *Candida albicans*. (Table 11).

### Frequency of Mixed infections

The frequency of Vulvovaginal *Candidiasis* and *Staphylococcal* coinfections are shown in Table 6. The rate of infection was highest in the month of February, 11 (34.4%), although it was least in the month of July, 3 (2.5%). Vulvovaginal *Candidiasis* and *Staphylococcal* coinfections were significantly associated with months of the year. Fifty percent (50%) of coinfecting persons had *Candida tropicalis* whereas only 33.3% had *candida glabrata*. (Table 12). *Candidiasis* was significantly associated with coinfections.

**Frequency of General Infections in Relation to Dry and Wet Season**

The seasonal association of general infection rates is displayed in Table 7. VVC infection rate was higher in the dry season 153(31.9%) than in the wet season 182(28.7%). Candidiasis was not associated with season. *Staphylococcal* infection rate was higher in the dry season, 241 (50.0%) than in the wet season 181(28.5%). Staphylococcal infection was significantly associated with season. Mixed infection rates was higher in the dry season 75 (15.6%) than in the wet season 58 (9.1%). Co-infections was significantly associated with season.

**Association of VVC in Relation to Possible Risk Factors**

Table 8 displays the association between VVC and possible risk factors. VVC infection rate was highest among contraceptive pills users 53 (53.5%) and least among patients having diabetes 36 (39.1%). VVC was significantly associated with risk factors.

**Association of Staphylococcal infections in relation to risk factors**

The frequency of Staphylococcal infections in relation to risk factors is shown in Table 9. Staphylococcal infection rate was highest among patients who were on antibiotic therapy, 38 (46.3%) and least among contraceptive pill users. 38 (38.4%). However, *Staphylococcal* infection showed no association with possible risk factors.

**Frequency of Mixed Infections in Relation To Risk Factors**

Table 10 presents the frequency of VVC and Staphylococcal co-infections in relation to risk factors. Mixed infection rates were highest among patients using antibiotics, 19 (23.2%), closely followed by pregnant women 35(21.5%) but was least among patients having diabetes, 11(12.0%). Mixed infections were significantly associated with pregnancy and use of antibiotics whereas mixed infections was not associated with contraceptive pill use and diabetes.

**Table 1: Frequency of Candidiasis Species among patients**

<i>Candida species</i>	<i>Candidiasis</i>	
	<b>N = 1116</b>	<b>n(%)</b>
<i>Candida albicans</i>	280	(83.6)
<i>Candida tropicalis</i>	34	(10.1)
<i>Candida glabrata</i>	21	(6.3)
Total	335	(30.0)

$\chi^2 = 1116.000^a$

r value = 0.810\*

$\chi^2$  – (Chi-square value)

r – Correlation coefficient.

\* –Correlation is significant at the 0.05 level.

**Table 2: General Infection Rates in Relation to Age.**

<b>Age</b>	<b>Candidiasis n (%)</b>	<b>Staphylococcal Infections n(%)</b>	<b>Mixed infections n(%)</b>
0 – 20	9(37.5)	13 (54.2)	5(20.8)
21 – 30	63(37.7)	80(47.9)	26(15.6)
31 – 40	146(26.3)	190(35.0)	61(11.2)
41 – 50	92(31.1)	105(35.5)	30(10.1)
51 – 60	16(23.2)	27(39.1)	9(13.0)
Above 60	9 (52.9)	6(35.3)	2(11.8)
<b>Total</b>	<b>335(30.0)</b>	<b>421(37.7)</b>	<b>133(11.9)</b>

$\chi^2$  – 13.837<sup>a</sup>                      53.409<sup>b</sup>                      5.160<sup>a</sup>  
 r value - .024                      - .063                      - .043  
 $\chi^2$  – (Chi-square value)  
 r – Correlation coefficient.

**Table 3: Frequency Of Candidiasis, Staphylococcus, Mixed infections in relation to Occupation.**

<b>Occupation</b>	<b>Candidiasis n(%)</b>	<b>Staphylococcal Infections n(%)</b>	<b>Mixed Infections n(%)</b>	<b>N</b>
House wife	185(30.2)	231(37.7)	73(11.9)	612
Student	22(40.0)	30(54.5)	17(30.9)	55
Civil servant	36(28.1)	55(43.0)	16(12.5)	128
Trader	45(30.0)	52(34.7)	16(10.7)	150
Farmer	25(27.5)	25(27.5)	6(6.6)	91
Business	9(30.0)	13(43.3)	4(13.3)	30
Teacher	13(26.0)	15(30.0)	1 (2.00)	50
<b>Total</b>	<b>335(30.0)</b>	<b>421 (37.7)</b>	<b>133(11.9)</b>	<b>1116</b>

$\chi^2$  = 3.505<sup>a</sup>                      14.462<sup>a</sup>                      26.362<sup>a</sup>  
 r value = - .022                      - .044                      - .062\*  
 $\chi^2$  – (Chi-square value)  
 r – Correlation coefficient.  
 \* - Correlation is significant at the 0.05 level.

**Table 4: Frequency of VVC within a twelve month period**

Months	Candidiasis		Total n(%)
	- ve n(%)	+ ve n(%)	
January	22(73.3)	8(26.7)	30(100.0)
February	16(50.0)	16(50.0)	32(100.0)
March	41(51.3)	39(48.8)	80(100.0)
April	23(67.6)	11 (32.4)	34(100.0)
May	57(60.6)	37(39.4)	94(100.0)
June	100(67.1)	49(32.9)	149(100.0)
July	90(75.6)	29(24.4)	119(100.0)
August	88(75.2)	29(24.8)	117(100.0)
September	117(75.5)	38(24.5)	155(100.0)
October	88(83.0)	18(17.0)	106(100.0)
November	51(76.1)	16(23.9)	67(100.0)
December	86 (65.6)	45(34.4)	131(100.0)
Total	781(69.9)	335(30.1)	1116(100.0)

$\chi^2 = 40.654^a$

r value = - .108\*

$\chi^2$  – (Chi-square)

r value – Correlation coefficient

\* Correlation is significant at the 0.05 level

-ve (negative ), +ve (positive )

**Table 5: Frequency of Staphylococcal Infections within a twelve month period**

Months	Staphylococcus Infections		Total n(%)
	- ve n(%)	+ ve n(%)	
January	17(56.7)	13(43.3)	30(100.0)
February	12(37.5)	20(62.5)	32(100.0)
March	34(42.5)	46(57.5)	80(100.0)
April	15(44.1)	19(55.9)	34(100.0)
May	71(75.5)	23(24.5)	94(100.0)
June	79(53.0)	70(47.0)	149(100.0)
July	96 (80.7)	23(19.3)	119(100.0)
August	87(74.4)	30(25.6)	117(100.0)
September	120(77.4)	35(22.6)	155(100.0)
October	71(67.0)	35(33.0)	106(100.0)
November	35(52.2)	32(47.8)	67(100.0)
December	56(42.7)	75(57.3)	131(100.0)
Total	695(62.3)	421(37.7)	1116(100.0)

$\chi^2 = 103.921^a$

r value = - .026



-ve (negative)  
 +ve (positive)  
 $\chi^2$  (Chi-square and  
 $r_{value}$ (Correlation coefficient)

**Frequency 6: Frequency of Mixed Infections within a twelve month period.**

Months	Mixed Infections		Total n(%)
	- ve n(%)	+ ve n(%)	
January	28(93.8)	2(6.7)	30(100.0)
February	21(65.6)	11(34.4)	32(100.0)
March	59(73.8)	21(26.3)	80(100.0)
April	27(79.4)	7(20.6)	34(100.0)
May	85(90.4)	9(9.6)	94(100.0)
June	117(78.5)	32(21.5)	149(100.0)
July	116(97.5)	3(2.5)	119(100.0)
August	111(94.9)	6(5.1)	117(100.0)
September	147(94.8)	8(5.2)	155(100.0)
October	98(92.5)	8(7.5)	106(100.0)
November	65(97.0)	2(3.0)	67(100.0)
December	107(81.7)	24(18.3)	131(100.0)
Total	981(88.1)	133(11.9)	1114(100.0)

$\chi^2 = 81.611^a$

$r_{value} = -.109^*$

$\chi^2$  (Chi-square)

$r_{value}$  (Correlation Coefficient)-ve (negative)+ve (negative)\* Correlation is significant at the 0.05 level

**Table 7: Seasonal Association of General Infection Rates**

Season of the year	Staphylococcal infections		Candida infections		Mixed infections	
	-ve n(%)	+ve n(%)	-ve n(%)	+ve n(%)	-ve n(%)	+ve n(%)
Dry	241(50.0)	241(50.0)	329(68.1)	153(31.9)	407(84.4)	75 (15.6)
Wet	454(71.5)	180(28.1)	452(71.3)	182(28.7)	576(90.9)	58(9.1)
Total	695(62.2)	421(37.8)	781(69.9)	335(30.1)	983(88.1)	133(11.9)

$\chi^2 = .000^b$  1.304<sup>b</sup> 10.900<sup>b</sup>

$r_{value} = -.219^*$  -.034 -.099\*\*

$\chi^2$  (Chi-square)

$r_{value}$  Correlation coefficient

-ve (negative)

+ve (positive)

\*\* Correlation is significant at 0.01 level

\* Correlation is significant at 0.05 level

**Table 8: Association between *Candidiasis* and possible risk factors.**

Risk Factors		Candidiasis		Total n(%)	r value	$\chi^2$ value
		-ve n(%)	+ve n(%)			
Antibiotics therapy	No	738(71.4)	296(28.6)	1034(100.0)	.108**	.928 <sup>b</sup>
	Yes	43(52.4)	39(47.6)	82(100.0)		
Pregnancy	No	702(73.7)	251(26.3)	953(100.0)	.194*	42.064 <sup>b</sup>
	Yes	79(48.5)	84(51.5)	163(100.0)		
Contraceptive Pills	No	735(72.3)	282(27.7)	1017(100.0)	.160*	28.602 <sup>b</sup>
	Yes	46(46.5)	53(53.5)	99(100.0)		
Diabetes Mellitus	No	725(70.8)	299(29.2)	1024(100.0)	.060*	3.963 <sup>b</sup>
	Yes	56(60.9)	36(39.1)	92(100.0)		

\*Correlation is significant at the 0.05 level

\*\* Correlation is significant at the 0.01 level

$\chi^2$  (Chi-square)

r value (Correlation Coefficient)

**Table 9: Frequency of *Staphylococcus* Infections in relation to Risk Factors**

Risk Factors		Staphylococcus Infections		Total n(%)	r value	$\chi^2$ value
		-ve n(%)	+ve n(%)			
Antibiotics therapy	No	651(63.0)	383(37.0)	1034(100.0)	0.050	2.798 <sup>b</sup>
	Yes	44(50.7)	38(46.3)	82(100.0)		
Pregnancy	No	599(62.9)	354(37.1)	953(100.0)	0.029	0.928 <sup>b</sup>
	Yes	96(58.9)	67(41.1)	163(100.0)		
Contraceptives	No	634(62.3)	383(37.7)	1017 (100.0)	0.004	12.966 <sup>b</sup>
	Yes	61(61.6)	38(38.4)	99(100.0)		
Diabetes Mellitus	No	641(62.6)	383(37.4)	1024(100.0)	0.022	0.547 <sup>b</sup>
	Yes	54(58.7)	38(41.3)	92(100.0)		

-ve (negative)

+ve (positive) square)  
 r<sub>value</sub> (Correlation Coefficient)

**Table 10: Frequency of Mixed infection in relation to possible risk Factors**

Risk Factors		Mixed infection		Total	r <sub>value</sub>	χ <sup>2</sup> value
		-ve n(%)	+ve n(%)	n(%)		
Antibiotics	No	920(89.0)	114(11.0)	1034(100.0)	0.098**	10.677 <sup>b</sup>
	Yes	63(76.0)	19(23.2)	82(100.0)		
Pregnancy	No	855(89.7)	98(10.3)	953(100.0)	0.122*	16.601 <sup>b</sup>
	Yes	128(78.5)	35(21.5)	163(100.0)		
Contraceptives pills		900(88.5)	117(11.5)	1017(100.0)	0.041	1.864 <sup>b</sup>
	Yes	83(83.8)	16(16.5)	99(100.0)		
Diabetes Mellitus	No	902(88.1)	122(11.9)	1024(100.0)	0.000	12.595 <sup>a</sup>
	Yes	81(88.0)	11(12.0)	92(100.0)		

\*Correlation is significant at the 0.05 level (2- tailed).

\*\* Correlation is significant at the 0.01 level (2-tailed)

χ<sup>2</sup> (Chi-square)

r<sub>value</sub> (Correlation Coefficient)

**Table 11: Frequency of *Candida* Species and *Staphylococcal* Infections**

Candidiasis	Staphylococcus infection		Total
	-ve n(%)	+ve n(%)	n(%)
-ve	494(63.2)	287(36.8)	781(100.0)
<i>C. albicans</i>	171(61.1)	109(38.9)	280(100.0)
<i>C. tropicalis</i>	17(50.0)	17(50.0)	34(100.0)
<i>C. glabrata</i>	14(66.7)	7(33.3)	21(100.0)
<b>Total:</b>	<b>695(62.3)</b>	<b>421(37.7)</b>	<b>1116(100.0)</b>

r<sub>value</sub> 0.039

χ<sup>2</sup> value 3.963<sup>b</sup>

-ve (negative)

+ve (positive)

χ<sup>2</sup> (Chi-square)

r<sub>value</sub> (Correlation Coefficient)

**Table 12: Frequency of *Candida* Species and Mixed infection**

Candidiasis	Mixed infection		Total n(%)
	-ve n (%)	+ve n(%)	
-ve	781(100.0)	-	780(100.0)
<i>C. albicans</i>	171(61.1)	109(38.9)	280(100.0)
<i>C. tropicalis</i>	17(50.0)	17(50.0)	34(100.0)
<i>C. glabrata</i>	14(66.7)	7(33.3)	21(100.0)
Total:	983(88.1)	133(11.9)	1116(100.0)
r value	0.562*		
$\chi^2$ value	356.427 <sup>a</sup>		
-ve (negative)			
*Correlation is significant at the 0.05 level			
$\chi^2$ (Chi-square)			
r value (Correlation Coefficient)			

**Table 13: Correlational association of infections and parameters under Consideration**

OCC	CND	STPH	COINF	CND SP.	MTH	SEASON	AGE	DIBT.	PREG	ATB	CP
CND	1.00										
STPH	.027	1.00									
	.373										
COINF	.562*	.473*	1.00								
	.000	.000									
CND SP.	.810*	.039	.458*	1.00							
	.000	.191	.000								
MTH	-.108	-.026	-.109*	-.122*	1.00						
	.000	.380	.000	.000							
SEASON	-.034	-.219*	-.099*	-.053	-.143*	1.00					
	.254	.000	.001	.078	.000						
AGE	-.024	-.063*	-.043	-.041	-.029	.062*	1.00				
	.428	.036	.150	.171	.325	.040					
DIBT.	.060*	.022	.000	.040	-.201*	-.016	.175*	1.00			
	.047	.460	.990	.185	.000	.604	.000				
PREG.	.194*	.029	.122*	.132*	.161*	-.071*	-.071*	-.013	1.00		
	.000	.336	.000	.000	.000	.018	.018	.658			
ATB	.108*	.050	.098*	.155*	-.154*	-.046	-.048	.078*	.078*	1.00	
1.00											
	.000	.095	.001	.000	.000	.123	.105	.009	.009	.009	

CP	.160*	.004	.041	.173*	.239*	-.002	-.014	.124*	-.058
.057	1.00								
	.000	.887	.172	.000	.000	.942	.648	.000	.054
.057									
OCC	-.022	-.044	-.062*	-.023	-.112*	.129*	.025	.009	-.050
.028	.045	1.00							
	.456	.144	.039	.434	.000	.000	.396	.758	.094
.354	.429								

CND: Candida  
 STPH: Staphylococcal infection  
 COINF: Coinfection  
 CND SP: Candida species  
 MTH : Month  
 SEASON: Season of the year  
 AGE: Age  
 DIBT: Diabetes  
 PREG: Pregnancy  
 ATB: Anti-biotic  
 CP: Contraceptive Pills  
 OCC: Occupation

**DISCUSSION**

Although VVC is a common disease, little is known about the epidemiology of *Candida* species which cause this disease. Therefore, the data collected in this study could be helpful for the clearer comprehension of the epidemiology of *Candida* species causing VVC. It is said that non-*albicans* Vulvovaginal candidiasis is increasing (Corsello *et al.* 2003). In this study *Candida albicans* is the prevalent cause of VVC with a frequency of (83.6%) followed by *Candida tropicalis* (10.1%) and *Candida glabrata* (6.3%). Similar to this study, (Sojakova *et al.* 2004) found *Candida albicans* in 87.7% of cases and *Candida glabrata* in 6.2%. *Candida tropicalis* occurred in 0.9% in their study (Sobel *et al.* 2004) diagnosed *Candida glabrata* in 3% and *Candida tropicalis* in 0.7%. These differences cannot be clarified without further research in this field, but from this work, VVC causing pathogen to a higher percentage occurs in the *Candida albicans*. The overall percentage of non-albican vaginitis (16.4%) closely resembles findings of Spinillo *et al.*, (1995) who reported 17% of non-albicans vaginitis in Italy. Holland *et al.*, (2003) reported non-albicans in 11% of the VVC cases. Nyirjesy *et al.*, (1995) reported a higher rate of *non-albicans species* 32% of patients with Vulvovaginitis. Patients above 60 years had the highest *Candida* infection rates with a frequency of 52.9%. This result is contrary to the findings of Paulitsch *et al* (2006) who recorded that patients between 31 – 40 years were more often culture-positive compared with other age classes. Patients above 60 years may have low immunity due to aging effects

and may have other diseases which can impair their immunity. These reasons could be responsible for the high rate of *Candidiasis* among this group of patients.

Many patients within age 0 – 20 years had Staphylococcal infections, this result corroborates a report (Wood TV) that Staphylococcal infections occurs most frequently in young menstruating women. Only 11.9% of patients were coinfectd with *Candida* and *Staphylococcal* species. Further research is required to define the role of Staphylococcal infections and its pathological effect on the female genito-urinary tract. The student population recorded the highest infection rates for *Candida* (40.0%), Staphylococcus (54.5%) and even mixed infection (30.9%). Most students these days have adopted the use of tight fitting clothings as a usual mode of dressing. Perspiration associated with tightly fitted clothes or poorly ventilated underwear increases local temperatures and moisture. Mechanical irritation of the Vulvovaginal area by clothing may also predispose already colonized areas to infection.

Foxman, (1990) showed association between *Candidiasis* and clothing among student population. Geiger *et al*, (1995) recorded a low *Candida* colonization among students; they suggested that the use of oral contraceptive pills (a predisposing factor) was more infrequently used among the students groups. Also the student groups who stay in hostels use the public toilet facilities which at times are not very clean. This can be suggestive for their having a high infection rate in the current study. The teacher group recorded the least infection rate for *Candida* and only one teacher was co-infected with *Candida* and Staphylococcal infections. Teachers are learned and enlightened and may have a prior knowledge of some personal hygienic practices. However, *Candidiasis* and *Staphylococcal* infections were not associated with occupation. Fifty percent of Vulvovaginal candidiasis cases were diagnosed in the month of February. This may be attributed to dry season in which water scarcity is a characteristic. Lack of adequate water may have led to poor hygienic practices like irregular and improper bathing, laundry this in turn may support *Candida* colonization in patients. *Candidiasis* (31.9%), *Staphylococcal* infections (50.0%) and mixed infections (15.6%) were all predominant in the dry season than in the wet season. Perspiration rates are higher in the dry season; because of the hotness of the weather also since Makurdi is a geographically hot zone, a host of other factors may have come into play to support *Candida* colonization in patients. The number of incoming samples increased from June to December 2006 than January to May. The physician may have simply written some drugs as treatment instead of recommending cultures for some patients. Also it is possible that more patients visited the hospitals from June to December with complaints of vaginal itch and discharge than January to May.

Use of oral contraceptive pills was significantly associated with *Candidiasis* which occurred in most VVC cases agreeing with previous studies (Reed *et al*.1989), Holland *et al*, (2003), Powell *et al*, (1984). Oral contraceptives may promote yeast adhesion and growth through increased nutrient availability or estrogen stimulation (Powell *et al*. 1984). There was also association between VVC and antibiotic therapy. This is because VVC can be caused by an

over abundance or over growth of yeast cells which sets in when the normal bacteria flora present is thwarted as it controls or checks the yeast flora or maintain a balance between the bacteria flora in the vagina (Jose, 2002). Pregnancy was significantly associated with VVC, agreeing with the findings of Nyirjesy *et al*, (1995). Pregnancy can cause an increased level of estrogen in the body. The increased hormone level changes the vaginal environment thus making it perfect for fungal growth and nourishment (Belinda *et al*.2004). Diabetes was associated with VVC in the present study. This agrees with the findings of Sobel, (1993) and Paulitsch *et al*. 2006) who suggested that behavioural factors are important determinants of *candida* colonization among women with diabetes.

## CONCLUSION

Factors known to affect the vagina environment and contributing to vulvovaginal candidiasis (antibiotic therapy, Diabetes, pregnancy and contraceptive pill use) were assessed in this study and were present in a number of patient with positive cultures. A notable part of patient was co-infected with *Candida* species and *Staphylococcus* species. All risk factors were significantly associated with VVC. Staphylococcal infections was significantly related to age, and season (0.05 level of significance); and candidiasis was significantly associated with months of the year and *candida* species (0.05 level of significance). *candida albicans* accounted for most VVC infections followed by *candida tropicalis* and *candida glabrata*. Students were mostly infected with candidiasis and staphylococcus infections. Older patients above 60 years tend to have a higher frequency of *candidiasis* and infection rates were more prevalent in the dry season than wet season. From this study, results shows that it is necessary to undertake further research in the field of VVC, because many woman were affected from these infections. It is important to perform species identification to obtain more information concerning epidemiology and the increase or decrease of particular species causing VVC.

*Candidal vaginitis* pose a risk for neonates, which can be infected during childbirth and it can be transmitted to male partners during sexual intercourse (Kathleen and Arthur, 2002). In the last two decades, *candida species* have progressed from being infrequent pathogens to among the most important and frequent opportunistic microorganisms causing *nosocomial* infection in hospitalized patients (Jose, 2002). A final concern is that larger definitive studies to include a control group and prospective follow-up are planned; also delivering good explanation to women on issues bordering VVC may reduce infection with *candida* yeast colonization.

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