

## ORIGINAL ARTICLE

Nugent Score and *Candida* Colonization Among Women in Uyo, Nigeria

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## DISCLOSURE

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**ABSTRACT**

**Background:** Bacterial vaginosis are among the most common causes of abnormal vaginal discharges and both represent consequences of imbalance in normal vaginal flora and pH.

**Objective:** The aim of this study was to determine and compare the prevalence of Bacterial vaginosis (BV) and colonization with *Candida* among women attending Obstetrics and Gynecology clinics in a tertiary hospital.

**Methodology:** This was a hospital based cross-sectional study. Pregnant and non-pregnant women attending Obstetrics and Gynaecology clinics in Uyo, Nigeria were interviewed using a structured questionnaire and self-administered vaginal swabs were collected by each participant. Nugent score was determined by Gram stain of smears of vaginal swabs while colonization by *Candida* was determined by inoculating each swab onto Sabouraud's agar and checking for growth of characteristic colonies after overnight incubation at 37°C. Chi square test was used to determine associations between variables.

**Results:** Nugent score was positive for bacterial vaginosis in 40% of participants and *Candida* colonization was present in 49.52% of participants. There was significant association between BV status and colonization with *Candida* spp.

**Conclusion:** There is an inverse relationship between BV and *Candida* colonization among participants in this study.

**Keywords:** Bacterial vaginosis, Candidiasis, Vaginal discharge, Uyo.

## INTRODUCTION

Bacterial vaginosis(BV) is the most prevalent cause of abnormal vaginal discharge in women of child-bearing age.<sup>1</sup> It occurs due to

overgrowth of anaerobic organisms in the vagina leading to an increase in the pH.<sup>2</sup> There is also an adherent, grey, homogenous, offensive discharge present although many

cases are asymptomatic.<sup>1,3</sup> Bacterial vaginosis is characterized by lack of vaginal lactobacilli and this is accompanied by overgrowth of *Gardnerella vaginalis*, *Mycoplasma hominis* and *Mobiluncus* species which form an infected biofilm and creating a permissive environment for overgrowth of other anaerobic bacteria.<sup>4</sup>

The cause of BV remains unclear but it is strongly associated with behavioural factors such as frequency of intercourse, douching, pessaries, and cigarette smoking. Other associated factors include black race, intrauterine device, early age of sexual intercourse, new or multiple sexual partners, and sexual activities with another woman.<sup>5</sup> Bacterial vaginosis (BV) has been associated with increased risk for HIV acquisition, pelvic inflammatory disease, preterm delivery and low birth weight.

Vulvovaginal candidiasis (VVC) is also a common cause of abnormal vaginal discharge. Although the presence of *Candida* in the vagina does not necessarily indicate infection, colonization of the vagina usually precedes infection. *Candida* species have been identified in 10–30% of reproductive-aged women and 6–7% of menopausal women.<sup>6</sup> There is a balance between *Candida* organisms and vaginal defense mechanisms against *Candida*, such as presence of *Lactobacilli* and immune responses, that allows the persistence of *Candida* species as vaginal commensals.<sup>7</sup> It has been suggested that normal vaginal flora consisting predominantly of *Lactobacillus* species may protect against the development of VVC, but there is limited data to support this hypothesis.<sup>8</sup> The protective effect of *Lactobacilli* has been attributed to increasing vaginal acidity by breakdown of glycogen to produce lactic acid, production of bacterial toxins like acidolin, lactacin and bacteriocins, decrease in available energy source, and stearic interference with adherence. *Lactobacilli* have also been noted to inhibit germ tube formation and invitro growth of *Candida albicans*, produce

H<sub>2</sub>O<sub>2</sub> which combines with peroxidases and a halide to produce a toxic antimicrobial system.<sup>9</sup>

The aim of this study was to determine the prevalence of BV and colonization with *Candida* among women attending Obstetrics and Gynecology clinics in a tertiary hospital.

#### METHODOLOGY

This was a cross-sectional study carried out at the Obstetrics and Gynaecology clinics in the University of Uyo Teaching Hospital Uyo, Nigeria. This is a 500-bed tertiary hospital and the Obstetrics and Gynaecology clinics have up to 400 visits each week. Pregnant and non-pregnant women attending these clinics were eligible for inclusion in this study. A convenience sample comprising of all consenting patients at the time of survey was used. Patients on their menstrual period were excluded. Participants were interviewed using a structured questionnaire to obtain biodata and details of pregnancy.

Two self-administered vaginal swabs were collected by each participant after verbal instructions by an interviewer. The participant was instructed to insert the swab into the vagina, rotate and hold for a few seconds and place into the transport tube. Adequate privacy was provided at the specimen collection point and specimen were returned to the investigator within 10-15 minutes of collection.

#### Nugent score

Nugent score is a gram stained smear based method for diagnosing bacterial vaginosis which quantifies the number of large Gram positive rods (*Lactobacillus morphotypes*) against small Gram negative to Gram-variable rods (*Gardnerella vaginalis* and *Bacteroides species morphotypes*) and curved Gram-variable rods (*Mobiluncus species morphotypes*). One of the vaginal swabs obtained was rolled over a microscope slide, air dried, fixed and stained

by Gram stain; 5-10 fields were examined by an experienced clinical microbiologist and Nugent score determined. A score of 7-10 was considered positive for bacterial vaginosis, while scores of 0-6 were considered negative.

### Isolation of Candida

The second swab obtained from participants was inoculated onto Saboraud's agar and incubated overnight at 37°C. *Candida* were identified by its characteristic growth on Saboraud's agar, chromogenic agar and Gram staining cell morphology. *Candida* identification and Nugent scoring were done by different investigators to reduce bias.

### Statistical Analysis

Analysis was performed using SPSS version 20 software for Windows. Pearson's chi-square test was used to determine significance of associations between variables and relative risk (RR) to determine the relationship between BV and *Candida* colonization.

### Ethical Considerations

The study was approved by the Health Research Ethics Committee of the University of Uyo Teaching Hospital, Uyo. Written informed consent was obtained from all

participants and patient identifiers were removed from all data records.

## RESULTS

### Participants

Of 225 women recruited, 210 had complete records. Their characteristics are shown in table 1. The ages of participants ranged from 18 to 42 years with a mean age of  $27.82 \pm 4.89$  years. Pregnant women were 97 and non-pregnant were 113.

### Nugent Scores and Candida Isolation

The Nugent score of participants ranged from 0-9 with a median score of 5.50. Nugent score was positive for bacterial vaginosis in 84 (40.0%) women and negative in 77 (36.7%) women while 49 (23.3%) women had intermediate scores.

*Candida* was isolated from 104 (49.52%) participants. There was no significant association between BV status and participants' characteristics (Table 1), however, there was statistically significant inverse relationship between BV status and *Candida* colonization with RR of 0.544,  $p=0.032$ . (Table 2)

**Table 1.** Participants' characteristics and associations with BV status

Characteristic	Total (%)	BV pos (%)	BV neg (%)	P	
Age	<21	21 (10.0)	6 (28.57)	15 (71.43)	0.276
	21-30	136 (64.8)	61 (44.85)	75 (55.15)	
	31-40	50 (23.8)	16 (32.00)	34 (68.00)	
	>40	3 (1.4)	1 (33.33)	2 (66.66)	
Mean age	$27.82 \pm 4.89$	$27.48 \pm 4.671$	$28.05 \pm 5.037$		
Pregnancy	Pregnant	97 (46.2)	42 (43.30)	55 (57.70)	0.366
	Non-pregnant	113 (53.8)	42 (37.17)	71 (62.83)	
Marital	Married	150 (71.4)	58 (38.67)	92 (61.33)	0.533
	Unmarried	60 (28.6)	26 (43.33)	34 (56.67)	
Education	Primary	12 (5.7)	4 (33.33)	8 (66.67)	0.722
	Secondary	99 (47.1)	38 (38.38)	61 (61.62)	
	Tertiary	98 (47.1)	42 (42.42)	57 (57.58)	

BV=bacterial vaginosis;

pos= positive;

neg= negative

**Table 2.** Association between BV status and *Candida* colonization

	BV positive	BV negative	RR (95% CI)	P
Candida positive	34 (32.69)	70 (67.31)	0.544 (0.311-0.952)	0.032
Candida negative	50 (47.17)	56 (52.83)		

BV=bacterial vaginosis

RR= Relative risk

CI =Confidence interval

## DISCUSSION

Schroder in 1921, developed three grades to assess the microbial composition of the vaginal flora, stressing the protective role of *Lactobacilli*.<sup>10</sup> These grades are (i) *Lactobacillus* dominant, the least pathogenic; (ii) intermediate; and (iii) *Lactobacillus* free, the most pathogenic. These grades may be correlated to the Nugent score of bacterial vaginosis. Associations have been drawn over the years between the intermediate flora and *Candida* vaginitis by Siegler and colleagues.<sup>10,11</sup>

We studied bacterial vaginosis and *Candida* colonization and the relationship between them among women in Uyo, Nigeria. The overall prevalence of Bacterial vaginosis in this study was 40%; with 43.30% among pregnant and 37.17% among non-pregnant participants. These rates are higher than those of recent studies across Nigeria ranging from 11.9-38.0%.<sup>1,5,12,13,14,15</sup> However, an older study reported a prevalence of 67.7% among pregnant women in Lagos.<sup>16</sup> Similar rates have been reported elsewhere outside Nigeria.<sup>6,8</sup> The wide range may reflect the differences in the populations surveyed in these studies.

This study assessed bacterial vaginosis by Nugent score. This is a gram stain based method of assessing BV by determining the relative ratios of large gram positive rods (*Lactobacillus*) small gram negative rods and curved gram variable rods.<sup>17</sup> It is broadly accepted as the gold standard for detection of BV and is considered to be superior to the Amsel criteria because of superior sensitivity and reproducibility, and its independence from clinician's acumen.<sup>4,18</sup> It allows for assessment of alteration of the vaginal flora as

a continuum rather than a dichotomy. Nugent score has been shown to have a good correlation with diagnosis of BV by determination of vaginal bacterial counts using culture and Polymerase Chain Reaction methods.<sup>19,20</sup>

BV was assessed in both pregnant and non-pregnant women in this study. We found a higher prevalence among the pregnant women. It has been suggested that the increased prevalence of BV in pregnant women may be related to immunosuppression that occurs in pregnancy.<sup>16</sup> Because of the association of BV with adverse pregnancy outcomes, it has been recommended that all pregnant women should undergo screening for BV. Hormonal factors may also play a role in the increased prevalence of BV in pregnancy as hormonal contraceptives have been found to be a protective factor against BV.<sup>3,21</sup>

The highest prevalence of BV was in the 21-30 age group, and the mean age of participants with BV was lower than that of BV-negative participants. Other studies have shown increasing prevalence of BV with age<sup>16,22</sup> possibly due to increased span of sexual activity which is also an associated factor for BV. Our finding may be due to high number of younger participants. Other factors associated with risk of BV include being unmarried, young age at first intercourse, regular douching and identifying as a commercial sex worker. In this study, prevalence of BV was higher among unmarried women.

In this study *Candida* colonization was found to be 49.52%. While prevalence of vulvovaginal candidiasis ranges between 10.7-

61.7% in Nigeria<sup>14,15,23</sup> and between 5.94-22.90% in other climes,<sup>6,8</sup> fewer studies report colonization with candida which has been put at 10-30%.<sup>6,24</sup>

Development of VVC is usually attributed to the disturbance of balance between *Candida* vaginal colonization and host environment by physiological and non-physiological changes.<sup>24</sup>

Our study showed an inverse relationship between Nugent score and *Candida* colonization of participants (RR: 0.544; *p*: 0.032). While BV is associated with increased vaginal pH, vulvovaginal candidiasis has been noted to occur at lower pH.<sup>6</sup> Some species of *Candida* like *C. glabrata* are, however, associated with higher pH. This protective effect of *Candida* from BV has been reported.<sup>25,26</sup> Both conditions are not considered strictly sexually transmitted infections but may be enhanced by sexual activity. This inverse association has been shown in some studies to occur as a result of treatment for BV with Metronidazole or Clindamycin leading to overgrowth of *Candida*.<sup>27,28</sup> However, this relationship was not explored in this study.

## CONCLUSION

The prevalence of BV among women attending Obstetrics and Gynaecology clinic in Uyo is 40% and the prevalence of *Candida* colonization is 49.52%. There is an inverse relationship between BV and *candida* colonization among women who participated in this study.

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