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PREVALENCE OF BACTERIAL VAGINOSIS AMONG FEMALE STUDENTS LIVING IN AND AROUND TERTIARY INSTITUTIONS IN OWERRI, IMO STATE

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ABSTRACT

Introduction: Bacteria vaginosis (BV) results from the alteration of the vaginal microbiome (dysbiosis). It is a health condition where the bacteria in the vagina (normal flora) have been replaced with pathogenic microorganisms, usually made up of facultative and strict anaerobes. Different factors have been attributed to the causes of bacteria vaginosis. This research was therefore aimed at ascertaining the prevalence of bacteria vaginosis among female students living in and outside institution owned hostel in order to ascertain the role of toilet facility in predisposing women to bacteria vaginosis. **Materials and Method:** A total of one hundred and fifty (150) students from three institution of higher learning- Federal Polytechnic Nekede, Owerri., Federal University of Technology, Owerri and Alvan Ikokwu Federal College of Education Owerri were recruited. Consent forms and interviewer-administered questionnaire were given to each participant to obtain their consent for the study and to collect detailed information on socio- demographic characteristics. High vaginal swabs (HVS) samples were collected from the vagina fornix of each of the participants by a gynaecologist. Two vaginal samples were collected using the cotton-tipped swabs from the posterior fornix of the vagina from each participant. One swab was used for microscopic examination while the other one was preserved in DNA preservation kit for DNA isolation and purification. The subjects were also examined for fishy odour and greyish discharges which adhere to the walls of the vagina by a clinician. Diagnosis of bacteria vaginosis was made using both Amsel's criteria, Nugent's scores and quantitative polymerase chain reaction (q PCR) **Results:** Students living inside the campuses were found to be more predisposed to

bacteria vaginosis than students living outside the campus. Low level of *lactobacillus* species were found in majority of students living inside the school. **Conclusion:** Poor toilet facilities observed in institution owned hostels may have been the major cause of high prevalence of bacteria vaginosis among hostel inmates.

KEYWORDS: Prevalence, vaginosis, hostel, students.

INTRODUCTION

BACKGROUND

Bacteria vaginosis (BV) results from the alteration of the vaginal microbiome (dysbiosis). It is a health condition where the bacteria in the vagina (normal flora) have been replaced with pathogenic microorganisms, usually made up of facultative and strict anaerobes (Ravel *et al.*, 2011). BV may be asymptomatic in up to 50% of cases, but when present, symptoms such as a fishy odour and a greyish discharge trouble the patient (Bilardi *et a*l., 2013).

The vaginal microbiome of an asymptomatic healthy reproductive aged woman is composed of 90-95% *lactobacillus species*. This has been attributed to the presence of oestrogen and glycogen which are metabolized by *lactobacillus* species thereby producing sufficient lactic acid and hydrogen peroxide which acidify the vaginal environment thereby preventing other pathogenic microorganism's survival (Ravel *et al*., 2011)

Lactobacilli presence in the vagina of reproductive age woman improves the health of the vagina which increases the ability of the woman to conceive, carry the foetus to term avoiding preterm birth.(PTB) and recurrent spontaneous abortion. *Lactobacillus* has also been shown to support implantation of embryo to the walls of the vagina. However, absence or low level of *lactobacillus* and an increase level of facultative anaerobes like *Gardnerella*, *Atopobium*, *Prevotella* etc, endangers the vagina and exposes the woman to a lot of danger ranging from bacteria vaginosis, spontaneous abortion, preterm birth and sometimes infertility.

Bacteria vaginosis has been known to be the commonest disease experienced by reproductive aged women worldwide. If this condition is not detected early and treated, its complication may be devastating as more pathogenic bacteria like Human immunodeficiency virus (HIV/AIDS) and human papilo virus (HPV) may be acquired .This will eventually lead to

infertility, PTB and recurrent spontaneous abortion. (Pybus and Onderdonk. 2010), (Cohen *et al.*, 2012)

This research is therefore aimed at determining the prevalence of bacteria vaginosis among female students in tertiary institutions living within and around institutions hostel in Owerri, Imo State, using Amsel's criteria and Nugent's score.

MATERIALS AND METHOD

Subject Recruitments

Consent forms and interviewer-administered questionnaire were given to each participant to obtain their consent for the study and to collect detailed information on the socio-demographic characteristics., gynaecological and menstrual history, the medication used, tobacco and alcohol intake, age, marital status and pregnancy. Presenting symptoms of infections, use of contraceptive, menstrual history, previous vaginal infections episodes, sexual intercourse, medical history, history of using vaginal douches or any vaginal preparations, the douching solutions used and the reason for the choice, and history of taking any antimicrobial agents either systemic or vaginal was included. Simple random sampling technique was be adopted for the recruitment of subjects.

Sample Collection

A total of one hundred and fifty speculum assisted High vaginal swabs (HVS) samples were collected from the vagina fornix of each of the participants by a gynaecologist. Two samples of vaginal samples were collected using the cotton-tipped swabs from the posterior fornix of the vagina from each participant. One swab was used for microscopic examination while the other one was preserved in DNA preservation kit for DNA isolation and purification. The subjects were also examined for fishy odour and greyish discharges which adhere to the walls of the vagina by a clinician.

'Amsel's Criteria Determination

The pH of the individual samples was determined using a pH strip (Sanaisi Company, Shanghai, China). The pH strip was dipped into the vaginal fluid and the pH of each sample was read and recorded. The swab was then extracted into 0.2 mL of physiological saline in a test tube; a drop of the extract was then placed on a glass slide. A drop of 10% potassium hydroxide is placed on another glass slide. The swab is then stirred in the 10% potassium hydroxide and immediately evaluated for the presence of a fishy odour. Both drops are then

covered with a cover slip and examined at 400x magnification with a light microscope. Clue cells are identified as vaginal epithelial cells with such a heavy coating of bacteria that the peripheral borders are obscured. Clinical diagnosis of BV was made using three of these criteria.

Laboratory Testing Using Standard Gram Stained Methods

The diagnostic criteria developed by Spiegel *et a*l 1983 and later modified by Nugent *et al* 1991 which has been a well-reproduced standardized Gram stain scoring method was used (Nugent *et al*., 1991) (Table1 Eschenbach, 1993).

Procedure

The collected vaginal swabs were used to make smears on different microscopic slides. The Smears were allowed to air dry after which they were fixed using flame. Crystal violet was used to flood the slides, which were allowed foe sixty seconds before they were washed. Grams iodine was thereafter used to flood the smear and was allowed for sixty seconds. Acetone was used to flood the slides which were quickly washed off with water. Neutral red was used to flood the slides and was allowed for sixty seconds. The slides were blot dried and examined using oil immersion objective lenses. The stained slides were read, and the numbers of morphotypes were evaluated based on a standardized scoring method. The smear was then evaluated for the following morphotypes under oil immersion (100xmagnification): large Gram-positive rods (lactobacillus morphotypes), small Gram-variable rods (*G vaginalis* morphotypes), small Gram-negative rods (*Bacteroides* species morphotypes), curved Gram-variable rods (*Mobiluncus* species morphotypes) and Gram-positive cocci.

Vaginal cleanliness was thereafter, evaluated according to morphological observations. The criteria of vaginal cleanliness were as follows: Grade I was a large number of large Gram-positive rods (indicative of Lactobacillus spp.), vaginal epithelial cells, and no other bacteria observed with WBC 0–5/HP under microscopy. Grade II was some *Lactobacillus* spp. and vaginal epithelial cells, some pus cells, and other bacteria observed under microscopy with WBC 10–15/HP. Grade III was a small amount of *Lactobacillu*s spp, a large number of pus cells, and other bacteria observed under microscopy with WBC 15–30/HP. Grade IV was no *Lactobacillus* spp. but pus cells and other bacteria observed under microscopy with WBC more than 30/HP. Grades I-II mean normal vaginal cleanliness, while grades III-IV mean abnormal vaginal cleanliness with inflammation.

Quantitative Polymerase Chain Reaction (q PCR) was used as confirmatory test of bacteria vaginosis in samples which showed positive and negative for Amsel criteria and Nugent's scores.

Data were analyzed using SPSS 16.0 version and Microsoft Excel Sheet and interpreted according to frequency distribution and percentage. A chi-square test was used to determine significant association wherever applicable with a *p*-value of less than 0.05 regarded as significant.

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

A total of 150 students were recruited. 50 respondents were recruited from each institution-Federal Polytechnic Nekede, Alvan Ikokwu Federal College of Education and Federal University of Technology, Owerri all located in Imo State. Students within the age range of 18-40years were recruited. A total number of respondents living in institution owned hostels were 78(52%) while those living outside the institution were 72(48%). Respondents with and without symptoms of bacterial vaginosis were also recorded. Details of this information is as documented on table 1

Table 1: Distribution of Respondents by Socio-Demographic Characteristics.

Socio-Demographic Characteristics: Age	**Frequency (N = 150)**	**Percentage (%)**
31-40	40	26.7
20-30	110	73.3
Reasons for choice of accommodation		
Poor toilet facility		
Yes	138	92
No	12	8
Total	150	100.0
State of origin		
Anambra	26	17.3
Abia	20	13.3
Imo	100	66.7
Ebonyi	4	2.7
Total	**150**	**100.0**
Ethnic group		
Yoruba	2	1.3
Igbo	148	98.7
Total	**150**	**100.0**

Marital status		
Not married	122	81.3
Divorced	-	-
Married	28	18.7
Total	150	100.0
Institutions		
Fedponek	50	33.3
AIFCE	50	33.3
FUTO	50	33.3
Total	**150**	**100.0**
Hostel of residence		
Institution owned hostel	78	52
Outside campus	72	48
Total	**150**	**100.0**
Symptoms of infection		
Yes	102	68
No	48	32
Total	**150**	**100**

Of the 50 respondents sampled from each of the institutions, 34 (68%), 35 (70%) and 28(48%) from FPNO, AIFCE, and FUTO respectively, showed evidence of bacteria vaginosis. Again, 63 (80.8%) of respondents living in institution owned hostels had evidence of bacteria vaginosis with percentage prevalence of 80.8 while 30(41.7%) living outside the campuses, showed evidence of bacteria vaginosis with percentage prevalence of 41.7 Details are as presented in table2.

Table 2: Prevalence of Bacteria Vaginosis among Respondents based on Institutions.

Institution	No of Respondents	No living in Institution owned hostel	No with evidence of BV	No living outside campus	No with evidence of BV
FPNO	50	30	22	20	12
AIFCE	50	28	25	22	10
FUTO	50	20	16	30	8
Total	150	78	63	72	30
Percentage Prevalence			80.8		41.7

Out of a total of one fifty (150) respondents, 93 (62%) had thin white homogenous discharge, 100(66.7%), had their pH >4.5, 21(14%) had positive whiff test, while 98(65.3%) had presence of clue cells. Table 3 shows the results in details.

Table 3: Clinical diagnosis of BV based on Amsel's criteria.

Diagnostic Methods	Positive	Negative
Thin white homogenous discharge	93	57
pH> 4.5	100	50
Positive whiff test	21	129
Presence of Clue cells	98	52

The result obtained from the real time PCR showed that, of the 21 samples analysed,(5 of which were from BV positive cases while 16 were from BV negative cases), all(100%) of clinically identified BV positive cases also showed positive BV. *Lactobacill*i species were detected in all of the samples (range from 5.9×10^{6} to 3.2×10^{10} organisms per sample. *Gardnerella. vaginalis* was detected in all the samples except one of the samples. In contrast, only six of the samples had detectable numbers of *M. hominis* which ranged from $<10^{4}$ to 7.5×10^{7}. The number of *lactobacill*i was significantly higher (P=0.012, Mann–Whitney U-test) in the BV-negative group (median number 1.1×10^{9}) than in the BV-positive group (median number 8.5×10^{6}). In contrast, the number of *G. vaginalis* organisms was significantly higher (P=0.004) in the BV-positive group (median number 1.3×10^{10}) than in the BV-negative group (median number 5.4×10^{7}). Only two of the BV-positive women were positive for *M. hominis* and the number of *M. hominis* organisms was not significantly different between the two groups, with p-value of 0.325. Details are as in table 4.0

Table 4.0 Quantity and P-value of microorganisms present in samples using qPCR.

Organism	Total (n=21)	BV+ (n=5)	BV− (n=16)	P value
Lactobacillus	3.4×10^{8} (6×10^{6}–2.7×10^{10})	8.5×10^{6} (6×10^{6}–2.7×10^{8})	1.1×10^{9} (1×10^{7}–2.7×10^{10})	0.012
G. vaginalis	4.2×10^{8} ($<10^{4}$–1.2×10^{11})	1.3×10^{10} (7×10^{9}–1.2×10^{11})	3.75×10^{7} ($<10^{4}$–1.3×10^{10})	0.004
M. hominis	$<10^{4}$ ($<10^{4}$–7.5×10^{7})	$<10^{4}$ ($<10^{4}$–7.2×10^{5})	$<10^{4}$ ($<10^{4}$–7.5×10^{7})	0.325

4.2 DISCUSSION

Bacteria vaginosis, is a common condition that has important clinical consequences. Diverse types and number of bacteria are implicated in this condition. This study sought to determine if living in the school hostel and sharing toilet facilities can predispose individuals to acquisition of this clinical condition. The result showed that the prevalence of bacteria vaginosis is higher (80.8%) among students living in institution owned hostel than those living outside the institution with prevalence of 41.7%. The high prevalence of bacteria vaginosis among students living in institution owned hostel could be as a result of the

crowded nature of the hostels where students are faced with the option of sharing toilet facilities. Dirty toilet facilities have been known as a good transmitter of bacterial infections to vulnerable users. Again, public (hostel) toilets have been discovered to be left unattended to by institution employed cleaners who barely sit up to their responsibility of tidying up the toilets. This result also suggest that reproductive aged women and girls who stay in institution-owned hostels have higher chances of acquiring more severe sexually transmitted infections, predisposition to preterm birth(for pregnant ones)even premature rupture of membrane. This exposure to bacteria vaginosis which is highly communicable, by students living in institution owned hostels could likely be the reason many students prefer living outside the campus where toilet facility usage is restricted to few people.

The result also showed that PCR can be used to detect and quantify several of the genital tract bacteria that are associated with bacteria vaginosis, including *G. vaginalis*, *M. hominis* and *lactobacilli* in genital tract samples. It was also discovered through this research that samples from students with BV that were diagnosed clinically using Amsel's criteria and Nugent's score have significantly higher numbers of *G. vaginalis*, but significantly lower numbers of *lactobacilli*. The low level of *Lactobacillus* species in the genital tract of these respondents could have led to the clinical manifestation of bacteria vaginosis in these subjects. This therefore suggests that if the subjects are exposed to a clean environment with adequate toilet facilities, and are exposed to adequate dose of *lactobacilli* (probiotics), they may recover from this clinical condition. A PCR method has several potential advantages in studying the biology of BV over the two most commonly used methods for detecting BV, the Amsel criteria and the Gram stain (Amsel *et al*., 1983). PCR is very sensitive and can be optimized to pick up very low numbers of bacteria. However, this study and other studies (Hillier *et al* 1993) indicated high number of both *lactobacilli* and *G. vaginalis* organisms in the genital tract and therefore the high level of sensitivity of PCR may not be needed in the diagnosis of BV. However, the ability of real-time PCR to quantify the number of DNA copies over several logs is very useful for quantifying the severity of BV. Although Nugent test has proved useful for quantifying the severity of BV, it cannot identify organisms such as *M. hominis* that do not show up on Gram stains.

The classical diagnostic methods like Amsel's criteria and Nugent's scoring systems remain the most feasible and economical options for the diagnosis of BV especially in developing countries where multiple criteria are used for the confirmation of BV (Nugent *et al* 1991,

Verstraelen and Verhelst 2009). For Amsel's criteria, clinical diagnosis and few easy –to run laboratory tests are used, whereas Nugent's criteria involve assessment of normal flora in the Gram-stained smear of vaginal discharge. Although the use of culture in the diagnosis of most bacterial infections has been regarded as the gold standard, it may not be a good method for the diagnosis of BV, since the bacteria that cause BV are difficult to isolate and are usually present in small number as normal vaginal flora (Udayalaxmi *et a*l 2011).

CONCLUSION

In conclusion, this study indicated that students living in institution owned hostels where toilet facilities are shared have high prevalence of bacteria vaginosis than those living outside the institution (private owned hostels) where toilet facility usage is restricted to few inmates. The use of quantitative PCR is specific, sensitive but may not be needed for the diagnosis of bacteria vaginosis as the results obtained from both the clinical (Amsel's criteria) and laboratory diagnosis (Nugent's score) agree with the results obtained from qPCR. It can also be concluded that, bacteria vaginosis results from low abundance of *lactobacillus* species and high diversity of facultative anaerobes.

Recommendations: Based on the outcome of this research, the following recommendations can be made

1 More hostels should be built for students in higher institutions to help decongest the hostels.

2 Management of institutions should employ more cleaners to assist in cleaning of hostel facilities especially, the toilets. They should also be compelled to clean the facilities regularly.

Further studies: More work should be done on this research to know the role of immunity of individuals in bacteria vaginosis.

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