Clinical epidemiology and diagnosis of Bacterial vaginosis among pregnant women attending clinics in Irrua Specialist Teaching Hospital, Edo State, Nigeria

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ABSTRACT

Background: Bacterial vaginosis (BV) may be common in women of reproductive age group but little is known about the pattern of vaginal flora associated with BV in Nigeria sub-rural population. Objectives: This study was designed to determine the prevalence, etiology, and standard diagnosis of bacterial vaginosis in Irrua Specialist Teaching Hospital Edo State, Nigeria. Material and Methods: This prospective study involved 344 consenting consecutive antenatal patients at the gestational age of 13-20 weeks attending Irrua Specialist Teaching Hospital Irrua, Edo State, Nigeria. Amsel diagnostic criteria was compared with Nugent’s scores, culture of Gardnerella vaginalis and combination of Nugent and culture of Gardnerella vaginalis. Results: The prevalence of 30.23% was found using Amsel criteria, 22.09% using Nugent’s method and 23.26% from culture of Gardnerella vaginalis. No statistical relationship was found between socio demographic characteristics, sexual, social and vaginal hygiene practices and bacterial vaginosis. However, there was statistical relationship between report of fishy odor during and after sexual intercourse, Gardnerella morphotypes, Bacteriodes morphotypes and BV (p<0.02, p<0.05, p<0.01). There was inverse relationship between lactobacilli morphotypes and BV. This study confirmed strong relationship between Amsel criteria and Nugent method in the diagnosis of BV in pregnancy (p=0.00). Conclusion: The prevalence rate of BV was high and the study highlights the polymicrobial nature and endemicity bacterial vaginosis among the studied population. Epidemiological risks commonly associated with BV did not seem to be important in the study.

Introduction

Bacterial vaginosis (BV) is the commonest cause of abnormal vaginal discharge in women of the reproductive age, yet the etiology remains unclear (1, 2). It is a syndrome because no single bacterial agent can be regarded solely responsible for the syndrome and because of the absence of a true inflammatory response in most cases (1-5). The incidence varies in different parts of the world, e.g. 25% in a group of healthy Canadian women (6), 29.9% in Indonesia (7), 15% in rural Brazil (8) and 14.2% in healthy Nigerian women (9). An estimated 25-30% of women have BV at any given time mostly without signs and rises to 85% in prostitute population (10). The reported prevalence of BV among pregnant women ranges from 10-30% and in more than 30% of women that undergo termination of pregnancy in the United Kingdom (11).

Bacterial vaginosis is a polymicrobial superficial vaginal infection involving a reduction in the amount of hydrogen-peroxide producing lactobacillus and an over growth of anaerobic and gram-negative or gram — variable bacteria (12, 13). The reduced number of lactobacillus promote overgrowth of anaerobic bacteria including Gardnerella vaginalis, Mycoplasma hominis, bacteriodes species, Mobiluncus species, prevotela species, Peptostreptococcus Species (12-13). Mobiluncus species is a sensitive marker for the diagnosis of bacterial vaginosis (14). Women with bacterial vaginosis may experience an odorous discharge and/or abnormal vaginal bleeding, with one half of cases being asymptomatic (3-5). On the other hand, Gardnerella vaginalis has been reported in up to 50 percent of women without symptoms or signs of BV making the bacteria not diagnostic of BV; (3, 15). The decrease in lactobacillus may be the most important...
predictor in subsequent BV development (16). Clue cells are formed when Gardnerella Vaginalis, present in high numbers, adhere in the presence of an elevated pH to exfoliated epithelial cells (18).

In pregnancy, bacterial vaginosis is one of the leading causes of preventable preterm birth. A growing body of literature has begun to suggest an increased risk of spontaneous abortion among pregnant women with bacterial vaginosis (19-21). These infection are thought to contribute to preterm birth through complex interactions between microbial organisms and maternal and fetal natural defense mechanisms (22, 23). Sialidases are enzymes that play a role in bacterial nutrition, cellular interactions and immune response evasion (24, 25). Sialidases are secreted from anaerobic gram-negative bacterial rods such as Bacteroides, Gardnerella and Prevotella species (24-29). Pregnant women do not commonly develop bacterial vaginosis after 16 weeks gestation (30, 32). Classically, the diagnosis of BV is based on finding, three of the following four critical criteria (Amsel’s criteria) (31). Homogenous thin vaginal fluid that adheres to the vaginal walls, vaginal pH greater than 4.5, whiff test; (release of amine or fishy odour with alkalination (10% KOH), presence of Clue cell: (presence of vaginal epithelial cells with borders obscured with adherent small bacteria) (33). Amsel criteria has the following limitations: Assessment of vaginal pH lacks specificity because an increase in vaginal pH may be a consequence of many other lower genital tract conditions. Conduct of the Whiff test is subjective for each individual clinician and lacks sensitivity and identification of clue cells may vary according to the microscopist and the quality of sample (33).

Of the diagnostic methods currently available for assessment, the Amsel criteria is the gold standard for the diagnosis of bacterial vaginosis and it reflects both the change in vaginal ecology and the strong microbial association (31). The method was modified by Nugent et al (36) to include the intermediate category that demonstrated the presence of mixed microbial flora with significant numbers of the lactobacillus morphotypes. The Nugent criteria is the test most often used in epidemiology while BV blue is a chromogenic point of care test based on detection of ion of increased vaginal fluid sialidase activity (>7.8IU) (39 41, 42). In non-pregnant women, the presence of bacterial vaginosis is associated with an increased risk of upper genital tract and sexually transmitted infections (2-4), and with the acquisition of HIV (5-9). In pregnancy, BV increases the risk of post-abortal sepsis, early miscarriage, recurrent abortion, late miscarriages preterm pre labour rupture of membrane (PROM), spontaneous preterm labour (SPTL) and preterm births, histological chorioamnionitis and postpartum endometritis.

This study seeks to evaluate the prevalence of bacterial vaginosis in pregnancy in a semi-rural community in Nigeria using three of the known diagnostic methods (Amsel criteria, gram stain and culture), assess the epidemiological profile and clinical correlates of BV, evaluate the vaginal bacterial microflora pattern of BV patients and validate Gram stain and culture diagnostic methods against the gold standard (Amsel Criteria).

Materials and methods
This was descriptive cross – sectional study which was carried out at the Antenatal clinic, Irrua Specialist Teaching Hospital, Irrua, Edo State, a tertiary care hospital and a referral center of Edo, Delta, Kogi and Ondo States in Nigeria. The department has 52 gynecological and 58 Obstetrics beds and undertakes more than 1500 deliveries annually. The three hundred and twenty three (323) patients sampled was guided by the upper limit required to give 95% level of confidence at an expected prevalence of 30%, using the precise prevalence formula: Sample size $N = \frac{Pq}{(E/1.96)^2}$ (78), where (1.96) is a constant, $P$ is a maximum known prevalence of the disease (30%), $q$ is 1-P (proportion of persons free from the disease) and $E$ is the error margin allowable (5%). With the above formula, the minimum epidemiologically significant sample size to be collected was three hundred and twenty three (323). In other to account for sampling error and drop outs, the total sample collected was made up to three hundred and sixty three (363).

The study population was consecutive consenting pregnant women attending the antenatal clinic of Irrua Specialist teaching Hospital, Irrua, Edo State. Consenting consecutive antenatal patients (who enrolled for antenatal care in the early second trimester from 13 up to 20 weeks gestation regardless of symptoms and retroviral status) were recruited from August to December, 2012. Pregnant women were excluded from the study for any of the following reasons; vaginal bleeding, use of lubricants or topical vaginal medications within the previous 72 hours antimicrobial therapy within 4 weeks, cervical cerclage, low lying placenta, steroids use, and pregnancy following assisted reproduction and diabetes mellitus in pregnancy. Gestational age was based on last menstrual period with corresponding height measurement and ultrasound report. Participants were administered a structured interviewer’s questionnaire which had 4 sections: Section A: Assessed the socio demographic characteristics of the enrolled patients such as age, parity, marital status, level of education, husband’s profession/level of education and ethnicity. Section B: Assessed the past reproductive performances eg; previous history of abortion, preterm delivery, puerperal sepsis, perinatal/neonatal infectious morbidity, STIs/HIV infection and intrauterine contraceptive device usage. Section C: Assessed the sexual, social and vaginal hygienic practices and current pregnancy. Section D: Contained the Performa designed for the study to record the results. The patients had genital examination done in dorsal position. Bivalve vaginal speculum was passed. No antiseptic lotions or creams were used for lubrication and where necessary, the vaginal speculum was moistened with sterile water. The vaginal wall was inspected and the presence of vaginal...
discharge and characteristics recorded. A pH paper (1-12
Merck & Co. Inc. Rahway, N.J) was mounted onto a
‘mosquito’ artery forceps which was gently introduced in
the lateral wall/posterior fornix and was wetted with vaginal
secretion. The pH was read and recorded. Two swab
samples of the vaginal secretion were taken from the lateral
wall or posterior fornix of the vagina using plastic swab
tipped with alginate wool in a peel pouch (medical wire and
equipment Co. Ltd; Corsham, Wilts, England). One of the
swabs was used for Microscopy (wet preparation, gram
stain) and the second swab was used for culture.
Following the removal of the vaginal speculum; 0.02ml (a
drop) of 10% Potassium hydroxide (KOH) was added to the
discharge on the speculum. The perception of a fishy amine
odour was recorded as positive diagnosis for bacterial vaginosis (77). The presence of clue cells (>20%) was
observed as the most closely associated criterion for the
diagnosis of bacterial vaginosis. Clinical diagnosis of bacterial
vaginosis was made using Amsel criteria (31).
During the clinical examination, direct smear were prepared
gram stained using the kopelfop modification. Each microbial
morphotype were measured and scored using Nugent’s
identification protocol (52-53) and summary BV score
computed (36) Large gram positive bacilli were called the
Lactobacillus morphotype. Small gram variable bacilli or
cocobacilli were called the Gardnerella morphotype. Other
organism were categorized by morphology and interpreted
accordingly. The swabs for culture were taken to the
laboratory for processing using Amies transport medium.
The transported vaginal swab were inoculated onto various
selective and non-selective media. This solid media include
blood agar, chocolate agar, maconkey agar and saubouraud’s
agar. Columbia blood agar plates were incubated aerobically
at 37°C for 24 – 48 hours to isolate aerobic bacteria including lactobacilli. Columbia human blood agar plates were
incubated at 36°C and read after 48 – 72 hours for Gardnerella
vaginalis isolation. The selective media for recovering gram
negative anaerobes from specimen which may contain
contaminating facultative flora were used i.e. Blood agar
supplemented with neomycin (75ug/ml), Vancomycin
(2.5ug/ml) and or nalidixic acid (10ug/ml). Identification
was by carbohydrate fermentation and morphological
analysis
Data were entered and stored in Microsoft excel Spread
sheet and analyzed using SPSS statistical package.
Proportions were compared by Chi-square where
appropriate and the statistical significance of p-value was
p<0.05. Patients were excluded from the analysis where
clinical information/specimens were not available. Based on
the results, the sensitivity; specificity, the false positive rate,
positive prediction value, negative predictive value and
accuracy will be determined when the Amsel; composite
criteria used as “gold standard” is compared with Gram stain,
culture and combination of Gram stain and culture. All
sample collected for the purpose of this study was treated
with strict confidentiality Approval for the study was
obtained from the ethical committee of the Irrua Specialist
Teaching Hospital Irrua, Edo State, Nigeria
Results:
Three hundred and sixty-three consecutive pregnant women
were enrolled in the study over a five month period between
August and December, 2012 regardless of symptoms after
reviewing the pregnant women with features listed in the
exclusion criteria but nineteen of them were disqualified due
to incomplete data while 344 pregnant women’s data were
analyzed.
The mean age of the pregnant women was 27±4.55 years
(range 17 - 38). The age range distribution shows that
16(1.74%) were in the age range <20years, 76 pregnant
women (22.09%) were in the 20 – 24 years age range, 152
(44.19%) were in the 25 – 29 years age range, 76 (22.09%)
were in 30-34 year age range while 34(9.88%) were in the
age range >34 years. Parity ranged from 0 to 5. The mean
was 1.058±1.19. Majority of the participants were
nulliparous accounting for 136(39.53%), while primiparous
women accounted for 156(45.34%).
Using the Amsel criteria, 104 (30.23%) of the women were
diagnosed as having bacterial vaginosis in the study
population. Age and parity did not significantly influence
the occurrence of bacterial vaginosis in the study population. (X²
00.0104, δf = 4, p> 0.09 for age X² = 0.1515, δf = 4,
p>0.90 for parity). Age less than 25years and low parity (0-
2) also were not significantly associated with the diagnosis
of bacterial vaginosis (X² = 0.00491 δf = 1 p>0.95, for age
<25, X² = 0.13381 δf = 1, p>0.7 for parity <2).
Respondents’ levels of education majority of them had
secondary education as well as post-secondary education.
One hundred and sixty seven 167(48.54%) had post-
secondary education, 104 (30.23%) completed secondary
education, 49 (14.24%) of them had part secondary
education. The level of education did not translate into
improved financial status as majority 112(32.56%) of them
were unemployed. Level of education and occupation bore
no statistical relationship with the diagnosis of bacterial
vaginosis (X² = 0.0012699 δf = 5. P > 0.95 for education,
X² = 0.01516 δf = 6, P>0.80 for occupation.
Majority of the enrolled pregnant women 312(9.70) were
married in monogamous setting. Being single or cohabiting
with a partner appeared to be associated with the diagnosis
of bacterial vaginosis as 12 out of 16 of the single pregnant
women had bacterial vaginosis. All the pregnant women
cohabiting with a partner had bacterial vaginosis but there
were no statistical relationship between marital status and
diagnosis of bacterial vaginosis (X² = 0.000145, δf = 5,
P>0.95). Other socio – demographic characteristics were
ethnicity and husband’s occupation and level of education.
Majority of them were Esan 232(67.44%), others were
Yoruba 20(5.81%), Etsako 24(6.97%), Bini 16 (4.65%),
Igbo 12(3.49%) Own 16(4.65%). Majority of the husband
of the enrolled women had tertiary education and gainfully
employed unlike their wives.
Details about their past reproductive performance were collected through the interviewer questionnaire designed for the study, 196(36.98%) reported at least an abortal process of which 39(11.34%) were spontaneous abortion. 149(43.31%) had induced abortion, 12 (3.49%) had 2 episodes of previous spontaneous abortion while 8(2.32%) had three or more episodes of previous spontaneous abortion. There were no statistical significant relationship was found between acquisition of bacterial vaginosis with previous process ($X^2 = 0.85993, \delta f = 1, P > 0.5$). table1 Only 25/344(7.27%) of the study population had one previous preterm deliveries. Previous post-delivery/post – abortal infection were also reported by 24 (6.98%) of the participants. No statistical relationship was found between previous preterm delivery, post-delivery abortal infections, previous sexually transmitted infection, previous intrauterine contraceptive device use and diagnosis of bacterial vaginosis using Amsel criteria (table 1).

Table 2 showed sexual, social behavior and vaginal hygienic practices in pregnancy in association with clinical diagnosis of bacterial vaginosis. The mean age of sexual debut of the studies population was 18.36years 17.5 ± 4.95 years was the mean age of coitarche for women diagnosed to have bacterial vaginosis while 18.51 ± 2.519 years for negative bacterial vaginosis. Coital activity in pregnancy was high as 308(89.53%) of the enrolled pregnant women had sexual intercourse during pregnancy but no statistical significant relationship was found between sexual activity and clinical diagnosis of bacterial vaginosis ($X^2 = 0.66857, \delta f = 1, P > 0.5$). Twice weekly sexual intercourse was reported 120(34.88%). There was significant statistical relationship between report of malodorous fishy smell during and after sexual intercourse with the diagnosis of bacterial vaginosis ($X^2 = 5.22264, \delta f =1, p<0.02$), forty one (11.98%) had new partner in present pregnancy, more than 40% had 2 or more partners before present pregnancy (table 3). Douching was reported by 111(32.27%) of the participants while 212 (61.62%) reported the use of medical soap or scented soap in washing the vagina (table 2). Vaginal discharge was reported by 103 (29.94%) of participants but on clinical examination abnormal vaginal discharge was found in 160(46.59%) of the participants. The diagnosis of bacterial vaginosis was significantly dependent on finding of vaginal discharge on clinical pelvic examination and complaint of vaginal discharge ($X^2 = 4.7948, \delta f =1, p<0.02, X^2 = 3.8301, \delta f=1, p<0.05$) as shown in table 2. diagnosis of bacterial vaginosis clinically was not significantly dependent on douching, use of medicated/scented soap ($X^2 = 0.69559, \delta f =1, p<0.03, X^2 = 0.001834, \delta f=1, p<0.90$).

Table 3 and 4 showed vaginal microflora pattern in pregnant women with or without bacterial vaginosis as determined by gram stain smear of vaginal fluid and culture. The organisms seen on smears of vaginal fluid showed that gram-positive cocci were seen in 71(20.06%) out of 104(30.23%) with BV and 5 (2.08%) out of 240 patients without bacterial vaginosis ($X^2 = 4.6434, \delta f =1, p<0.05$). Similarly, curved rods were seen in 20(19.93%) of 104(30.23%) with BV and in none of 240 patients without bacterial vaginosis ($X^2 = 2.55591, \delta f =1, p<0.05$). The Gardnerella morphotypes was seen more in cases of patients with BV ($X^2 = 4.38470, \delta f =1, p<0.02$). Small gram negative bacilli resembling bacterides morphotypes were seen in 67(64.42%) out of 104 (30.23%) patients with BV and 14 (7.93%) out of 240 patients without BV ($X^2 = 6.084315, \delta f =1, p<0.01$). The lactobacilli morphotypes was absent or present only in low quality (1 to 2') in 81 (77.88%) out of 104(30.23%) with BV ($X^2 = 4.86223, \delta f =1, p<0.05$) and only 21(8.75%) of 240 patients without bacterial vaginosis.

Table 5: shows the diagnostic composite criteria of Amsel. White thin homogenous discharge vaginosis and yellowish thick vaginal discharge was associated with diagnosis of bacterial vaginosis. Among the diagnostic criteria, the presence of vaginal discharge and clue cell greater than 20% were significantly associated with the diagnosis bacterial vaginosis ($X^2 = 3.54709, \delta f =1, p<0.05, X^2 = 4.79485, \delta f =1, p<0.02$ respectively).

Table 6: shows results of the Nugent’s methods of diagnosis and its relationship with Amsel criteria. Among the enrolled women evaluated 104(30.23%) were diagnosed as positive and 240(69.77%) were negative based on clinical criteria. According to the gram stain using Nugent’s method 76(22.09%) were deemed positive, 112(32.56%) had intermediate and normal finds were regarded as negative in this study. There was a significant relationship between the diagnosis using Amsel’s criteria and abnormal Nugernt’s score of (7-10. ($X^2 = 9.3452, \delta f =1, p<0.001$). Table 7: Using the Amsel criteria as the “Gold Standard” for the diagnosis of bacteria vaginosis in pregnancy, there is evidence that Nugent’s method has better sensitivity, specificity, positive predictive value, negative predictive value and accuracy. 69.58%, 98.30%, 94.74%, 85.07%, 88.37% respectively than culture of G. vaginalis (26.92%) but combination of culture and Nugent’s method had a higher sensitivity (71.67%) but accuracy of diagnosis is not improved. Gestational age for collection of specimen for the study was between 13 and 20 weeks with a mean of 16.24± 2.227 based on last menstrual period where necessary from ultrasound report.
TABLE 1: PAST REPRODUCTIVE PERFORMANCE

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>Total N = 344 (%)</th>
<th>Bacteria Vaginosis Positive (%)</th>
<th>Bacteria vaginosis negative (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Abortion</strong></td>
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<td></td>
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</tr>
<tr>
<td>Yes</td>
<td>196(56.98)</td>
<td>60(17.44)</td>
<td>136(39.53)</td>
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<tr>
<td>No</td>
<td>148(43.02)</td>
<td>44(12.79)</td>
<td>104(30.23)</td>
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<tr>
<td><strong>Type</strong></td>
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<td>Spontaneous</td>
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<td>12(3.49)</td>
<td>27(7.85)</td>
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<tr>
<td>Induced</td>
<td>149(43.31)</td>
<td>48(13.95)</td>
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<td><strong>No of spontaneous</strong></td>
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<td>8(2.32)</td>
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<td>96(27.29)</td>
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<td><strong>Previous post delivery/ abortal infection</strong></td>
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<td>8(2.32)</td>
<td>16(4.65)</td>
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<tr>
<td>No</td>
<td>320(92.02)</td>
<td>96(27.29)</td>
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<td><strong>Previous neonatal morbidity / motality</strong></td>
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<td>40(11.63)</td>
<td>16(4.65)</td>
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<td>319(92.73)</td>
<td>99(28.80)</td>
<td>216(61.04)</td>
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<td><strong>Previous sexually transmitted infection</strong></td>
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<td>99(28.80)</td>
<td>216(61.04)</td>
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<td><strong>Previous IUCD use</strong></td>
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<td>6</td>
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<td>8(2.33)</td>
<td>17(4.94)</td>
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<td>0(0.00)</td>
<td>0(0.00)</td>
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Table 2
Sexual, social behavior and vaginal hygienic practices in pregnancy in relation to clinical diagnosis of bacterial vaginosis (using the Amstel criteria).

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>Total N = 344 (%)</th>
<th>Bacteria Vaginosis Positive (%)</th>
<th>Bacteria vaginosis negative (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vaginal Discharge as presenting complain</strong></td>
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<td></td>
</tr>
<tr>
<td>Yes</td>
<td>103(29.94)</td>
<td>47(13.66)</td>
<td>56(16.28)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>241(70.06)</td>
<td>57(16)</td>
<td>184(53.49)</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td><strong>Vaginal discharge on clinical observation</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>160(46.51)</td>
<td>100(29.07)</td>
<td>60(17.44)</td>
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</tr>
<tr>
<td>No</td>
<td>184(53.49)</td>
<td>1(0.29)</td>
<td>183(53.20)</td>
<td>&lt;0.02</td>
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<tr>
<td><strong>Age at coitache</strong></td>
<td></td>
<td>17.5±4.94</td>
<td>15.3±2.5192</td>
<td></td>
</tr>
<tr>
<td><strong>Coitus in pregnancy</strong></td>
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<td></td>
</tr>
<tr>
<td>Yes</td>
<td>308(89.53)</td>
<td>92(27.44)</td>
<td>216(62.79)</td>
<td></td>
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<td>12(0.87)</td>
<td>24(6.98)</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td><strong>Frequency of coitus</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily</td>
<td>72(20.93)</td>
<td>24(6.98)</td>
<td>48(13.95)</td>
<td></td>
</tr>
<tr>
<td>Twice weekly</td>
<td>120(34.88)</td>
<td>28(8.14)</td>
<td>92(27.44)</td>
<td></td>
</tr>
<tr>
<td>Weekly</td>
<td>68(19.77)</td>
<td>24(6.98)</td>
<td>44(12.79)</td>
<td></td>
</tr>
</tbody>
</table>
Occasionally 84(24.42) 28(8.14) 56(16.28) NS

Malodorous fishy smell during and after intercourse
Yes 36(10.47) 28(8.14) 8(2.33) <0.02
No 308(89.53) 80(25.25) 228(66.28) <0.02

Puritic vulva / vagina
Yes 48 24(8.14) 24(6.98) >0.95
No 308(89.53) 80(25.25) 228(66.28) <0.02

New partner in current pregnancy
Yes 4(1.19) 27(7.85) 14(4.07) >0.3
No 303(88.08) 84(24.42) 219(63.66) >0.3

No of partners before pregnancy
1 204(59.30) 57(16.57) 147(42.73) >0.99
2 80(23.26) 16(4.65) 64(18.61) >0.05
≥3 60(17.44) 33(9.59) 27(7.85) >0.05

Douching
Yes 111(32.27) 32(9.30) 79(22.97) >0.99
No 233(67.73) 72(20.93) 161(49.80) >0.05

Use of medicated/scented soap for washing the vagina
Yes 212(61.62) 77(22.97) 135(39.24) >0.99
No 132(38.37) 27(7.85) 105(30.52) >0.05

Retroviral status-positive 12 12 0 0.00

Table 3: Vaginal microflora pattern in pregnant women with and without bacterial vaginosis as determined by Gram Stain smear of vaginal fluid.

Clinical diagnosis
Morphotypes of organisms seen on gram stain
<table>
<thead>
<tr>
<th>Total N = 344 (%)</th>
<th>Bacteria Vaginosis Positive (%)</th>
<th>Bacteria vaginosis negative (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram positive cocci</td>
<td>76(22.09)</td>
<td>71(68.27)</td>
<td>5(2.88)</td>
</tr>
<tr>
<td>Gram negative bacilli</td>
<td>81(23.54)</td>
<td>67(64.42)</td>
<td>14(5.83)</td>
</tr>
<tr>
<td>Gram variable rods</td>
<td>24(6.97)</td>
<td>18(17.31)</td>
<td>6(2.33)</td>
</tr>
<tr>
<td>Gram coccobacilli</td>
<td>56(16.27)</td>
<td>49(47.11)</td>
<td>7(2.91)</td>
</tr>
<tr>
<td>Curved rods</td>
<td>20(5.81)</td>
<td>20(19.23)</td>
<td>0(0.00)</td>
</tr>
<tr>
<td>Lactobacillus (gram positive rods) morphotypes ≥2+</td>
<td>204(59.30)</td>
<td>3(2.88)</td>
<td>201(61.75)</td>
</tr>
<tr>
<td>Lactobacillus (gram positive rods) morphotypes 0–2+</td>
<td>102(29.65)</td>
<td>81(77.88)</td>
<td>11(3.77)</td>
</tr>
<tr>
<td>Gram negative cocci</td>
<td>5(1.45)</td>
<td>2(1.92)</td>
<td>3(1.25)</td>
</tr>
<tr>
<td>Fusiform (bipolar rod)</td>
<td>8(2.32)</td>
<td>7(6.73)</td>
<td>1(0.49)</td>
</tr>
<tr>
<td>Yeast cells (bud, hyphae)</td>
<td>20(5.81)</td>
<td>8(7.69)</td>
<td>12(5.00)</td>
</tr>
</tbody>
</table>

Table 4: Vaginal microflora pattern in pregnant women with and without bacterial vaginosis as determined by cultivation.

Clinical diagnosis
Culture of organisms
<table>
<thead>
<tr>
<th>Total N = 344 (%)</th>
<th>Bacteria Vaginosis Positive (%)</th>
<th>Bacteria vaginosis negative (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacilli spp</td>
<td>160(46.51)</td>
<td>12(11.53)</td>
<td>148(41.67)</td>
</tr>
<tr>
<td>Gardnerella spp</td>
<td>80(23.26)</td>
<td>41(39.42)</td>
<td>39(11.33)</td>
</tr>
<tr>
<td>Bacteriodes spp</td>
<td>64(18.64)</td>
<td>56(53.84)</td>
<td>8(2.32)</td>
</tr>
</tbody>
</table>

23
Table 5: Nature of vaginal discharge and diagnosis of bacterial vaginosis using Amsel composite criteria

<table>
<thead>
<tr>
<th>Table 5: Nature of vaginal discharge and diagnosis of bacterial vaginosis using Amsel composite criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vaginal discharge on speculum examination</strong></td>
</tr>
<tr>
<td><strong>Nature of vaginal discharge</strong></td>
</tr>
<tr>
<td>Homogenous discharge (white)</td>
</tr>
<tr>
<td>White thick/ curd like discharge</td>
</tr>
<tr>
<td>Yellowish thin discharge</td>
</tr>
<tr>
<td>Creamy discharge</td>
</tr>
<tr>
<td>Brownish discharge</td>
</tr>
<tr>
<td>pH ≥ 4.5</td>
</tr>
<tr>
<td>Whiff test</td>
</tr>
<tr>
<td>Wet preparation clue cell ≥ 20%</td>
</tr>
<tr>
<td>≥ 3 Amsel criteria</td>
</tr>
<tr>
<td>&lt; 3 Amsel criteria</td>
</tr>
</tbody>
</table>

Table 6: The relationship between Gram stain Nugent’s method and Amsel criteria

<table>
<thead>
<tr>
<th>Table 6: The relationship between Gram stain Nugent’s method and Amsel criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram stain</strong></td>
</tr>
<tr>
<td><strong>Nugent score</strong></td>
</tr>
<tr>
<td><strong>Total N = 344 (%)</strong></td>
</tr>
<tr>
<td><strong>Bacteria Vaginosis Positive (%)</strong></td>
</tr>
<tr>
<td><strong>Bacteria vaginosis negative (%)</strong></td>
</tr>
<tr>
<td><strong>P value</strong></td>
</tr>
</tbody>
</table>

Table 7: Comparison of sensitivity, specificity, predictive values, positive and negative rates of gram stain, culture and both with clinical diagnosis using Amsel criteria

<table>
<thead>
<tr>
<th>Table 7: Comparison of sensitivity, specificity, predictive values, positive and negative rates of gram stain, culture and both with clinical diagnosis using Amsel criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity (%)</strong></td>
</tr>
<tr>
<td><strong>Specificity (%)</strong></td>
</tr>
<tr>
<td><strong>PPV (%)</strong></td>
</tr>
<tr>
<td><strong>NPV (%)</strong></td>
</tr>
<tr>
<td><strong>FPR (%)</strong></td>
</tr>
<tr>
<td><strong>FNR (%)</strong></td>
</tr>
<tr>
<td><strong>Accuracy (%)</strong></td>
</tr>
</tbody>
</table>

Discussion

A prevalence of 30.23%, 22.09% using Nugent’s method and 23.26% from culture of *Gardnerella vaginalis* were found in the studied population. This compares to the range of 20-23% in Burkina Faso (54) and Malawi (55) and 20-30% in Kenya and South Africa (58-59). The prevalence rate in this
A study with 10% hypae and chromotubation 30(8.72%). The al risk nce with findings of other on clinical f facultative species of lactobaccil ridal effects - or charge as presenting complaint (,). Following een reported by various sarma hominis and u at erella vaginalis was y found a strong statistical significance between diagnosis over and of sexual trans finding o 4(1.16%) and different species of Candida characteristics by which will warrant further investigatio studied att rods) is some vaginal gram smears. Despite observing M media were not available. Mobiluncus spp were urealyticum were not isolated because appropriate culture pat which ma several logistic problems arose in the course (to the maintenance of a he epis to the attachment of any pathogenic BV with a normal flora was noted in this study. A low pH has been shown to have direct microbicidal and viridical effects (Lactobacillus species can also adhere onto the vaginal epithelial cells blocking the attachment of any pathogenic BV associated bacteria onto these cells. Lactobacilli are known to the maintenance of a healthy vaginal micro environment (70-72).

Several logistic problems arose in the course of this study which may limit interpretation of data on vaginal flora pattern. First mycoplasma hominis and ureaplasma urealyticum were not isolated because appropriate culture media were not available. Mobiluncus spp were not isolated despite observing Mobiluncus species like organism (curved rods) is some vaginal gram smears. These findings could be attributed to a low prevalence of Mobiluncus species in our studied population or to an inadequate isolation procedure which will warrant further investigation.

Other bacteria isolates in this study were Staphylococcus spp 8(2.32%), Coliforms 8(2/32%), Neisseria gonorrhoea 4(1.16%) and different species of Candida characteristics by their buds, hypae and chromotubation 30(8.72%). The finding of Neisseria gonorrhoea 37 is not surprising because increasing data also indicate that BV facilitates the acquisition of sexual transmitted diseases including Neisseria gonorrhoea, HIV, HSV type 2 and Chlamydia trachomatis. Following microbiological analysis, Gardnerella vaginalis was isolated in 80(23.26%) of the enrolled pregnant women but it was only isolated in about 41(39.42%) of the patients diagnosed to have BV using Amsel criteria. This is not surprising because gardnerella vaginalis is also regarded as part of normal vaginal flora and this has been demonstrated by several authors.69-72

The presence of Gardnerella vaginalis was not restricted to women with clinical signs of BV. Gardnerella vaginalis was isolated in both normal and intermediate group in this study. It has been postulated that a synergistic mechanism exists among the bacteria involved in BV (73). Current findings revealed a positive correlation between Gardnerella vaginalis and Prevotella involving ammonia utilization and also between prevoella and Peptostreptococcus species. Using Nugent’s method 112(32.56%) vaginal flora has intermediate grade or scores of 4-6. This has been described as a mixed microbial flora acting as a transitional phase between normal and BV flora. Studies have shown that subsequent sampling of women in this intermediate grade revealed that some transition to normal flora and other acquire BV (74). The emergent of this intermediate phase will require additional study to determine the factors that influence the vaginal microflora that lead to the initial overgrowth of Gardnerella vaginalis and subsequent increase in anaerobic organisms. The presence of intermediate flora has been shown to increase the risk of adverse obstetric outcome and acquisition of HIV (75, 76).

There was a statistical significant relationship between vaginal discharge as presenting complaint (on clinical observation (X² = 4.7949, df =1, p>0.02), vaginal discharge on clinical observation (X² = 5.6302, df =1, p>0.01) and diagnosis of bacteria vaginosis using Amsel Criteria. While 103(29.94%) of the patients reported to no vaginal discharge on questioning, 160(46.51%) had it on observation (X² = 0.2815, df =1, p>0.8) this disagreed with the work done by Apea Kubi et al (63), in which there was significant relationship between presenting symptoms and clinical observation of vaginal discharge. The nature of the discharge does not seem to correlate with the diagnosis of BV. In this study, white homogenous and yellowish thick vaginal discharge was more associated with BV. Gardner et al (34) described a thin grey homogenous discharge with tendency to adhere to the vaginal wall rather than pool in the posterior fornix was found to be associated with BV. However, Thomason et al (37) found homogenous discharge to be of little value in diagnosing BV, while Krohn et al (27) showed that in pregnant women, homogenous discharge was not independently related to bacterial vaginosis. The study further highlights the importance of microbiological examination of vaginal discharge over and above clinical observation.

The presence of clue of cells detected in a wet preparation of vaginal fluid correlated well with clinical diagnosis of BV.
This is not surprising since the presence of clue cells in greater than 20% was one of the four criteria used to define BV. The pH $\geq 4.5$ was reported in this study in up to 148(43.02%) of cases but only 68(19.77%) of cases where diagnosis to have BV clinically. This is because it’s affected by a lot of factors like recent intercourse because of release of alkaline semen, cervical mucus, blood, trichomoniasis. High pH 5 and 6 are said to promote adherence of G. vaginalis and anaerobic organisms to vaginal epithelial cells. Whiff test (positive amine test) was observed in 92(26.74%) of the pregnant women and 84(24.42%) were diagnosed to have BV clinically. This is not surprising because organisms associated with BV produces amino and malic acids which causes irritation of mucus membrane and fishy odor during intercourse or following addition of 10% KOH of vagina fluid, in this study, report of malodor fishy odour during and after intercourse was significantly associated with the clinical diagnosis of bacterial vaginosis ($X^2 = 5.2226$, df =1, $p>0.02$).

In this study, normal gram stain using Nugent’s method correlated with clinical diagnosis in 72(69.23%) of cases diagnosed clinically. Up to 36 (34.64%) with intermediate flora diagnosed clinical to have BV using Amsel criteria. BV was diagnosed in 12(100%) clinically using Amsel criteria while using Nugent’s method, 4(33.33%) have BV and 8(66.67%) had intermediate flora.

Nugent’s method compared with the “Gold standard” as used in this study had a sensitivity of 69.2% specificity of 95%, negative predictive value 85.07%, positive predictive value 94.74%, false positive rate of 1.72%, false negative rate 22.09% and accuracy of 88.37%. This study found a strong association between Amsel criteria and Nugent’s scores, and the isolation of G. vaginalis and anaerobic organisms and inverse relationship with the presence of lactobacilli. However, few cases diagnosed clinically using Amsel criteria were missed by Nugent’s method. The finding of intermediate flora by Gram stain was similar to the corresponding population in Nigeria (89) and other countries in Africa (58, 59) and the pattern of vaginal micro flora associated with BV were also similar. Amsel criteria and Nugent’s method are recommended for use clinically Nigeria especially as they correlated well with the result of polymerase chain reaction technology in developed world (9). Direct Gram staining of smear should facilitate the diagnosis of BV for confirmation by culture, particularly in a third world setting with a few standard laboratory facilities (68).

In conclusion: the finding of the study is instructive in many respects. The incidence of bacterial vaginosis is high among the antenatal population. The overall contribution to adverse pregnancy outcome as it relates to preterm delivery, low birth weight choioamnionitis and neonatal infectious morbidity can only be inferred from findings done by other workers. Amsel criteria still remains the gold standard in making a diagnosis. The study highlights the polymicrobial nature of the condition rather than any specific organism.

The epidemiological risk factors popularly associated with BV did not seem from this study to be important. This may be explained by the subset of the population used. There was also from this study a strong correlation between symptoms and microbiological diagnosis. This makes a compelling case for empirical treatment especially in the third world environment. This is because of the dearth of appropriate laboratory facility and trained personnel. Microbiological confirmation however should always be aimed at and this study has confirmed the high degree of correlation between the Amsel criteria and Nugent’s score. Future longitudinal studies are needed to evaluate adverse pregnancy outcome associated with the especially high incidence of BV in this environment. This strategy will in no small way improve pregnancy outcome in this environment.

CONFLICT OF INTEREST
There was no conflict of intrest

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