

Prevalence Of Facultative Anaerobic Bacteria In Bacterial Vaginosis As A Risk Factor For Pelvic Inflammatory Disease

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ABSTRACT:

Introduction: Bacterial vaginosis (BV) is a condition characterized by raised vaginal pH and milky discharge. This condition is associated with an alteration of the normal vaginal flora by a mixed flora of aerobic, anaerobic and microaerophilic species. This condition is simple to treat, however It can lead on to complications like miscarriage, pre term delivery, low birth weight baby, premature rupture of membranes, chorioamnionitis, postpartum endometritis, vaginal cuff cellulitis and pelvic inflammatory disease.

Objective :To find the prevalence of anaerobic organisms in women suffering from bacterial vaginosis.

Methodology: Hospital based Prospective cross-sectional study has been conducted in the Department of Microbiology. Patients with Bacterial Vaginosis, Visited in Obstetrics & Gynaecology, department of Birat Medical College and Teaching Hospital. Total number of cases included in the study was 613. A high vaginal swabs (HVS) was collected. Amsel's criteria, Nugent score and culture were performed.

Results: The examination of 613 High Vaginal Swab of pregnant and nonpregnant reproductive age group women with symptomatic and asymptomatic vaginal discharge. The prevalence of an anaerobes in BV ware *Gardnerella vaginalis* 172(28.1%) based on Amsel's criteria, Nugent score and culture. *Mobiluncus Spp.*384 (56.8%) based on Amsel's criteria and Nugent score. The highest number of an anaerobes in BV cases was seen among 25-39 Years age group 216 (35.2%) and least number of an anaerobes in BV cases were seen in patients with age groups 40-59 Years 163 (26.6%) and 15-24 Years 156(25.4%).

Conclusion: This finding suggests that the colonization of facultative anaerobes is also more important in vaginal ecology. So, similar studies must be carried out to improve the health status of women and preventing the risk posed towards BV. It is concluded that anaerobic bacteria are important pathogens in the causation of Bacterial Vaginosis along with other organisms.

Keywords: Anaerobic bacteria, Bacterial vaginosis, Gram stain, Nugent score, Amsel's criteria

1. INTRODUCTION

Bacterial vaginosis (BV) is one of the most common lower genital tract conditions. Most are occurring in 35% of women attending sexually transmitted infection (STI) clinics, 15% to 20% of pregnant women, and 5% to 15% of women attending gynecology clinics.¹ Forty-one patients with acute pelvic inflammatory disease were evaluated for the coexistence of bacterial vaginosis.² Bacterial vaginosis (BV) is a clinical entity characterized by a change in vaginal ecology where the normal flora of lactobacillus morphotypes is replaced by a mixed microbial flora consisting of anaerobes. *Gardnerella vaginalis* is considered to be the most common form of vaginal infection among women of reproductive age.³ The most clinically symptoms are malodorous vaginal discharge and a fishy odour. The Various studies have found the prevalence of BV to range from 15 to 30 per cent in non pregnant women and upto 50 per cent in pregnant women.⁴ Bacterial vaginosis is diagnosed by the Amsel's criteria and Nugent score.⁵ Bacterial Vaginosis has been associated with clinically suspected and subclinical PID.^{6,7,8,9,10,11,12,13} Pelvic inflammatory disease (PID) and inflammation of a woman's fallopian tubes (salpingitis) and uterine lining (endometritis) are a frequent and morbid condition among young women. In a 1995 national survey, 8% of all women and 11% of African American women reported that they had received treatment for PID.¹⁴ More than 1 million American women seek treatment for PID annually.^{15, 16} Major reproductive and gynecologic sequelae result from PID including infertility, ectopic pregnancy, recurrent PID, and chronic pelvic pain.^{17, 18} Bacterial vaginosis is a condition characterized by raised vaginal pH and milky discharge in which the normal vaginal flora is replaced by a mixed flora of aerobic, anaerobic and microaerophilic species.¹⁹ It may be diagnosed on clinical grounds if minimum three of the four criteria are fulfilled.²⁰ Microbiology of bacterial vaginosis is complex and involves various an anaerobes such as *Gardnerella vaginalis*, *Mobiluncus spp.*, *Prevotella spp.*, *Peptostreptococcus spp.*, *Bacteroides spp.*, *Eubacterium spp.* and aerobic organisms.¹⁹ The present study was undertaken to find the prevalence of anaerobic organisms in women suffering from bacterial vaginosis. Three days after the delivery, anaerobes return where they can cause endometritis and post-partum sepsis.²¹ The Various methods available for the diagnosis of bacterial vaginosis were Amsel's criteria, Nugent score, Hays/Ison system; Schmidt's scoring system, Spiegel's criteria, anaerobic culture, gas liquid chromatography, sialidase activity and DNA probes for *Gardnerella vaginalis*.²² Among the various methods available for diagnosis of bacterial vaginosis, Amsel's criteria is easy to perform and often used by clinicians for establishing clinical diagnosis. The presence of a homogeneous vaginal discharge, pH > 4.5 of vagina, the incidence of any clue cells in the wet mount of the vaginal discharge and a positive whiff test constitutes Amsel's composite criteria. According to Amsel's, if 3 of the 4 criteria are positive, the patient has bacterial vaginosis.²³ Nugent score is considered as the gold standard method and culture is a specific method where etiological agent is isolated but has its own disadvantages like time, cost and labor constraints.²³ Gram stained smear of vaginal discharge is prepared and examined under oil immersion. Clue cells are the vaginal epithelial cells covered with gram variable coccobacilli. The Presence of clue cells indicates BV. The smear is interpreted by Nugent's score. A score of more than or equal to 7 was diagnostic for BV. After inoculation on an anaerobic blood agar. It is incubated in anaerobic atmosphere using McIntosh-Fildes jar at 37°C for 48 hrs. The Facultative anaerobic bacteria are identified by various biochemical tests and antibiotic discs like metronidazole 5µg discs and Gentamicin 10µg are placed on primary and secondary streaking for observing primary sensitivity and presumptive identification of an facultative anaerobes.¹¹ Obligate anaerobes are identified based on gram stain findings, colony morphology, beta hemolysis, pigment production;

swarming, aerotolerance test and Identified by antibiotic discs used are vancomycin 5 µg, colistin 10 µg and kanamycin 1 mg.²⁴ In addition, culture and identification of bacteria from vaginal specimens has been evaluated to be specific but insensitive and costly to the laboratory.²⁵ *Mobiluncus species* strongly associated with BV, which is very difficult to recover by culture.²⁶

2. METHODOLOGY

A Quantitative Hospital based Prospective cross-sectional study was conducted among patients with Vaginal discharge attending in Obstetrics and Gynaecology, both inpatients and outpatients department of Birat Medical College and Teaching Hospital. The study period was December 2020 to January 2021. Women were pregnant and nonpregnant with symptomatic vaginal discharge. High vaginal swab specimen was sending in Microbiology laboratory. The sample size was 826. Ethical clearance was taken from the institutional Review Committee of Birat Medical College and Teaching Hospital. Data were entered into Microsoft Excel and analyzed by using SPSS version 23. The statistical significance was considered at $p < 0.05$.

Vaginal examination: Per speculum examination was performed by a gynecologist to all patients presented with vaginal discharge. There were two main categories of diagnostic tests for BV, one was clinical criteria and another was laboratory-based testing. The Amsel's criteria were originally published in the American Journal of Medicine in 1983. It was available for the diagnosis of bacterial vaginosis using only four criteria. In diagnosis of bacterial vaginosis Amsel's criteria have been validated as equivalent to Nugent scoring. It was considered particularly helpful in the microscopic diagnosis. There were four parameters used to determine the presence or absence of BV. These were i) Thin, white, yellow, homogeneous discharge, ii) Clue cells on wet mount microscopy, iii) a vaginal fluid pH of over 4.5 when placing the discharge on litmus paper and iv) Release of fishy odor when adding 10% potassium hydroxide (KOH) solution to wet mount - also known as "whiff test." The most widely accepted clinical criteria was 'Amsel's criteria'.²⁷ This clinical diagnosis requires three of the following four criteria First, a vaginal pH of greater than pH 4.5; second, the presence of clue cells in the vaginal fluid; third, a milky, homogeneous vaginal discharge; and finally, the release of an amine (fishy) odour after addition of 10% potassium hydroxide to the vaginal fluid.²⁷

Laboratory investigations: Samples were collected from vaginal walls with a cotton-tipped swab. The High vaginal swabs then inoculated into a tube containing approximately 2ml of saline and transported to the Microbiology Laboratory of the Birat Medial College and Teaching Hospital. The Nugent scoring system was previously considered the gold standard for the diagnosis of bacterial vaginosis. This system was discovered by RP Nugent and published in 1991. Using the Nugent score, vaginal smears were made on a microscopic slide and observe under in oil immersion, and a minimum of 10 high power fields are examined for three bacteria morphotypes such as *Lactobacillus spp.*, *Gardnerella spp.* and curved gram rods (*Mobiluncus spp.*). Each of these three categories was received and score was based on the number of bacteria counted. These three scores are added together for a total score ranging from 0 - 10. The scoring system was as follows 0-3: negative for BV, 4-6: intermediate and 7+: positive for BV. Gram-stained smears was prepared, examined and interpreted for the diagnosis of BV according to the Nugent scoring system.²⁸ A score of ≥ 7 was interpreted as positive for BV.²⁹

Culture: After inoculation, anaerobic blood agar was incubated in anaerobic atmosphere using McIntosh-Fildes jar at 37°C for 48 hrs. Anaerobic bacteria identification was made using Gram stain finding, biochemical test, namely catalase test, oxidase test, indole test and nitrate test. Identification discs like metronidazole 5 µg and Gentamicin 10µg were placed on culture plate after primary and secondary streaking for observation the sensitivity of facultative anaerobes.³⁰ The obligate anaerobes were identified based on colony morphology, beta hemolysis, pigment production, swarming, and aerotolerance test. Identification discs used were vancomycin 5 µg, colistin 10 µg and kanamycin 1 mg.²⁴

3. RESULTS

Among 826 High Vaginal Swabs samples were examined by Amsel's criteria and Nugent's criteria and cultured 613 were positive for common facultative anaerobic bacteria. The different findings were shown in different tables.

Table 1:

Nugent scoring of Gram stained smear for bacterial vaginosis caused by facultative anaerobic bacteria.

Organism Morphotypes	Number /oil immersion field	Score
Lactobacillus –like(parallel sided, Gram Positive rods)	>30	0
	5-30	1
	1-4	2
	<1	3
	0	4
Mobiluncus like(curved, Gram negative rods)	>5	2
	<1-4	1
	0	0
Gardnerella vaginalis like(tiny, Gram variable coccobacilli)	>30	4
	5-30	3
	1-4	2
	<1	1
	0	0

As in table 1, interpretation of Nugent scoring of Gram stained smear and Total score:-0-3 Normal; 4-6 intermediate; 7-10 Bacterial vaginosis.

Table 2:

Prevalence of common facultative anaerobes identified from vaginal swabs (n = 613)

Anaerobic Bacteria	Frequency	Percent
No bacteria	3	0.5
<i>Gardnerella vaginalis</i>	172	28.1
<i>Mobiluncus Spp.</i>	348	56.8
Lactobacilli	88	14.4
<i>Mobiluncus Spp.</i> and Lactobacilli	2	0.3
Total	613	100.0

Table-2 shows the most common facultative anaerobes in High Vaginal Swabs were *Mobiluncus spp.* (56.8%) followed by *Gardnerella vaginalis* (28.1%).

Table 3:

Frequency of common facultative anaerobes in different age group (n=613)

Age-group	Frequency	Percent
Below 15 Years	11	1.8
15-24 Years	156	25.4
25-39 Years	216	35.2
40-59 Years	163	26.6
60 Years and above	67	10.9
Total	613	100.0

Table 3 shows that the most common age group diagnosed with Bacterial Vaginosis in their High Vaginal Swabs sample was 25-39 years.

Table 4:

Age distribution of different common facultative anaerobes identified from High Vaginal Swabs (n =613)

Age group	Anaerobic Bacteria					Total
	No bacteria	<i>Gardnerella vaginalis</i>	<i>Mobiluncus Spp.</i>	Lactobacilli	<i>Mobiluncus and Lactobacilli</i>	
Below 15 Years	0 0.0%	0 0.0%	0 0.0%	10 90.9%	1 9.1%	11 100.0%
15-24 Years	0 0.0%	1 0.6%	85 54.5%	69 44.2%	1 0.6%	156 100.0%
25-39 Years	0 0.0%	7 3.2%	201 93.1%	8 3.7%	0 0.0%	216 100.0%

40-59 Years	2 1.2%	99 60.7%	61 37.4%	1 0.6%	0 0.0%	163 100.0%
60 Years and above	1 1.5%	65 97.0%	1 1.5%	0 0.0%	0 0.0%	67 100.0%
Total	3 0.5%	172 28.1%	348 56.8%	88 14.4%	2 0.3%	613 100.0%

Table 4 shows that among *Mobiluncus spp.*, the most common age group was 25-39 years followed by *Gardnerella vaginalis*, the most common age group was 40-59 years, and *Lactobacilli*, the most common age group was below 15 years.

Table 5:

Frequency of patients with anaerobes identified from High vaginal swabs.

Patients	Anaerobes					Total
	No bacteria	<i>Gardnerella vaginalis</i>	<i>Mobiluncus Spp.</i>	<i>Lactobacilli</i>	<i>Mobiluncus and Lactobacilli</i>	
Inpatient	1 0.4 %	72 25.6%	159 56.6%	48 17.1%	1 0.4%	281 100.0 %
Outpatient	2 0.6 %	100 30.1%	189 56.9%	40 12.0%	1 0.3%	332 100.0 %
Total	3 0.5 %	172 28.1%	348 56.8%	88 14.4%	2 0.3%	613 100.0 %

Table 5 shows that compare the anaerobes identified in Bacterial Vaginosis most common in outpatient *Mobiluncus spp.* 56.9% and *Gardnerella vaginalis* 30.1% and less common in inpatients *Mobiluncus spp.* 56.6% and *Gardnerella vaginalis* 25.6%.

4. DISCUSSION

Bacterial Vaginosis (BV) is a common problem encountered in the hospital. The vaginal ecosystem is established over a number of years. The dynamic environment of the vagina is influenced by factors such as hormonal fluctuations, menstruation, douching, hygiene, pregnancy, breastfeeding and sexual practices.³¹⁻⁴¹

BV is a risk factor for pelvic inflammatory disease, HIV, sexually transmitted infections (STIs), and reproductive and obstetric disorders or negative outcomes. It is possible for sexually inactive persons to develop bacterial vaginosis.⁴²

Bacterial vaginosis may sometimes affect women after menopause. Also, subclinical iron deficiency may correlate with bacterial vaginosis in early pregnancy.⁴³ A longitudinal study published in February 2006, in the *American Journal of Obstetrics and Gynecology*, showed a link between psychosocial stress and bacterial vaginosis persisted even when other risk factors were taken into account.⁴⁴

Bacterial vaginosis is a common disease in outpatient gynecological practice. *Mobiluncus spp.* is one of the bacterial species recently described as associated with bacterial vaginosis. In our study, bacterial vaginosis and *Mobiluncus sp.* morphotypes were identified by means of a Gram-stained smear of the vaginal secretions. Bacterial vaginosis associated with *Mobiluncus species* in general practice.⁴⁵

Bacterial vaginosis is the commonest infection among women visiting reproductive health clinic. It is a simple to treat infection but can lead on to complications like miscarriage, pre term delivery, low birth weight baby, premature rupture of membranes, chorioamnionitis, postpartum endometritis, vaginal cuff cellulitis and pelvic inflammatory disease. Among the various methods available for diagnosis of bacterial vaginosis, Amsel's criteria is easy to perform and often used by clinicians for establishing clinical diagnosis. Nugent score is considered as the gold standard method and culture is a specific method where etiological agent is isolated but has its own disadvantages like time, cost and labor constraints. The Nugent's criteria was used by these investigators as an easy and reliable diagnostic tool to determine the prevalence of BV.

In our study the prevalence of anaerobes in BV was *Gardnerella vaginalis* 172(28.1%) and *Mobiluncus Spp.* 384(56.8%) based on Amsel's criteria, Nugent score and culture. Limited studies on facultative anaerobes in BV have been performed in Nepal. A study from university of Kufa BV showed that *G. vaginalis* was detected in 63% and 71% of high vaginal swab and endocervical swab of women with BV. A wide distribution of *G. vaginalis* (detected in high vaginal swab) among the age group 15–25-year-old.⁴⁶ It is documented that *Mobiluncus spp.* were detected by Gram stained vaginal smear in 21% of 633 STD clinic patients, including 53% of those with and 4% of those without bacterial vaginosis (BV), as diagnosed by clinical criteria reflected in University of Washington, Department of Obstetrics and Gynecology.⁴⁷ The frequency of *bacterial vaginosis patients* with vaginal discharge in Gynaecological department. Frequency of anaerobes in inpatients identified from vaginal swab *Gardnerella vaginalis* was 72(25.6%) and Frequency of anaerobes in outpatients identified from vaginal swab *Gardnerella vaginalis* was 100(30.1%). Frequency of anaerobes in inpatients identified from vaginal swab *Mobiluncus Spp.* was 159(56.6%) and Frequency of anaerobes in outpatients identified from vaginal swab *Mobiluncus Spp.* was 189 (56.9%). Bacterial vaginosis is sexually transmitted disease not the hospital acquired infection. Our study revealed that most of common anaerobes are prevalence in out-patients department of Gynaecological department. The cause of BV is not completely understood, but certain activities, such as unprotected sex or frequent douching, increase the risk of BV.⁴⁶ Many women with bacterial vaginosis have no signs or symptoms. *Mobiluncus Spp.* is highest prevalence in BV then *Gardnerella vaginalis*. A study In University of Washington, Department of Obstetrics and Gynecology, *Mobiluncus* was detected by Gram stain in 20% of women attending the STD clinic, including 110 (53%) of those with and 16 (4%) of those without a clinical diagnosis of bacterial vaginosis.⁴⁷ The highest number of an anaerobes in BV cases was seen among 25-39 Years age group 216 (35.2%) and least an anaerobes in BV cases were seen in patients with age groups 40-59 Years 163 (26.6%) and 15-24 Years

156(25.4%). A study from Swapna Muthusamy et al, the subjects belonged to 15-49 years of age. Among them, more than half of women fell in the decade of 30-39 years.²³ It is found that *Mobiluncus Spp.* More common in BV cases was found among 25-39 years age group 201(93.1%) ,*Gardnerella vaginalis* more common in BV cases were found among 40-59 Years 99(60.7%) and *Lactobacilli* more common in BV cases were found among 16-24 years 69(44.2%).In a study from Kufa University, Iraq a wide distribution of *G. vaginalis* (detected in high vaginal swab) among the age group 15–25-year-old compared with those detected in endocervical, which shows a high percentage of detection among age group 26–36 years old.⁴⁶ Krohn reported finding *Mobiluncus* morphotypes by Gram stain in 28% of 122 pregnant women with BV compared with 3% of 471 pregnant women without clinical signs of BV.⁴⁸

5. CONCLUSION

This finding suggests that the colonization of facultative anaerobes is also more likely significance in vaginal ecology. Limited studies on BV have been found in Nepal. So, similar studies must be carried out to improve the health status of women, therefore, preventing the risk towards the BV. Anaerobic bacteria are important pathogens in the causation of bacterial vaginosis along with other aerobic organisms. Therefore, it is concluded that anaerobic bacteria are also important pathogens in bacterial vaginosis. Their identification would help the clinicians in appropriate treatment of bacterial vaginosis.

6. RECOMMENDATIONS

The difference in the type of rate of isolations of anaerobes reflects the difference in the population under study and the different methods of investigations. It is recommended that antenatal health care facilities should incorporate screening of Bacterial Vaginosis among pregnant women to prevent the complications of pregnancy.

7. LIMITATION OF STUDY

The study is a hospital based prospective study and may not truly reflect population level findings in the rural areas and the entire state. The prevalence of anaerobic bacteria in the laboratory is an in vitro activity and may not exactly reflect the in vivo activity.

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Conflict of Interest

The authors would like to declare no conflict of interest in this study.

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