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Original Research Article

Prevalence, Clinical Presentation and Underlying Etiology of Bacterial Vaginosis: A Hospital-Based Cross-Sectional Study

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Abstract:

Background: Bacterial vaginosis (BV) is the most common vaginal disorder, affecting a significant proportion of women. It's wide spread prevalence and severe health implications make It a critical public health issue.

Aim and Objectives: This study was done to identify the prevalence, microbiological characteristics, and underlying causes of BV.

Materials and methods: This hospital-based case-control study was conducted on patients presenting with abnormal vaginal discharge, pregnant women, women experiencing preterm labor, women with a history of hysterectomy or medical termination of pregnancy (MTP), and women using intra uterine contraceptive devices (IUCDs)

Results: Nearly half (48%) of bacterial vaginosis (BV) cases identified using the Nugent scoring system were found to harbor Gardnerella vaginalis (G.vaginalis) bacteria. A higher prevalence of G.vaginalis isolation was linked to an elevated vaginal pH, exceeding 4.5. Clue cell detection demonstrated a sensitivity of 69.23% and a specificity of 86.95%, while the amine test displayed a sensitivity of 73.07% and a specificity of 66.30%. Metronidazole, ampicillin, and chloramphenicol are effective antimicrobials against G.vaginalis infections.

Conclusion: Bacterial vaginosis is a common health problem in women of reproductive age. Given the prevalence and adverse consequences of BV, implementing routine screening and effective management strategies is crucial to prevent future complications.

Keywords: Bacterial Vaginosis, Gram Stain, Vaginal Culture.

Introduction

Bacterial vaginosis (BV) is the most common disorder of the female reproductive tract (FRT) for which clinical intervention is sought. BV is a microbial shift condition characterized by an alteration in the vaginal microbiome with a decline in Lactobacillus colonization, a healthy vaginal bacterium, and a simultaneous overgrowth of facultative anaerobic bacteria [1]. Neither the presence nor absence of any single bacterial species is sufficient for diagnosis; instead, multifactorial clinical and microbiological criteria are used to diagnose BV [2] Historically, BV was called Gardnerella vaginitis because it was believed that the Gardnerella vaginalis bacterium was the cause of this condition [3].

Although still uncertain, it is thought that most bacterial vaginosis infections start with Gardnerella vaginalis, creating a biofilm that then allows other opportunistic bacteria to grow within the vagina [4]. There are several risk factors behind the acquisition of BV, such as having multiple partners, douching of the vagina resulting in disturbance of natural vaginal flora, lack of lactobacilli due to any reason, and alteration in natural vaginal flora in pregnancy [5].

Although up to half of BV-affected women do not experience symptoms, for those that do, it is the symptoms themselves, including malodor and vaginal discharge that cause significant distress to women and impact their quality of life and relationships [6-7].

This condition affects between 20 and 60% of women worldwide and can pose serious immediate and long-term sequelae [8]. Women who have BV are at a higher risk of developing pelvic inflammatory disease, and pregnant women experiencing BV are significantly more likely to encounter complications, including preterm birth [9]. Furthermore, BV

increases a woman's chance of acquiring sexually transmitted infections, including HIV, whose acquisition rate is increased by 60% in women experiencing BV. These serious clinical consequences of BV, combined with its high prevalence, make this condition an immediate priority [1, 7, 10]. The early and presumed diagnosis is utmost important to prevent further complications. As few population-based prevalence surveys of bacterial vaginosis have been reported from our region, this study was conducted to identify the prevalence rate, microbiology laboratory findings, and underlying etiology of bacterial vaginosis.

Materials and Methods

This hospital-based case control study was conducted in the Department of Microbiology, S.C.B. Medical College, Cuttack, Odisha, over a period of three years.

Patients presenting with abnormal vaginal discharge, pregnancy cases, cases with preterm labor, prior cases for hysterectomy and medical termination of pregnancy (MTP), and cases with intrauterine contraceptive devices (IUCD) who attended the Obstetrics and Gynecology OPD of S.C.B. Medical College, Cuttack, were taken as the study group (150 cases). A matching group of 50 patients without complaints of abnormal vaginal discharge were included as controls. Patient consent was taken.

A detailed history of each patient was recorded with respect to age, marital status, menstrual history, obstetric history, duration of pregnancy, mode of contraception, and recent gynecological procedures.

Three vaginal swabs were collected from each of the above groups of patients by sterile cotton-tipped swabs from the posterior vaginal fornix using aseptic precautions. One of the swabs was put in 0.5 ml of sterile physiological saline. The other two swabs were put in sterile tubes with cotton plugs and transferred immediately to the microbiology laboratory. The characteristics of vaginal discharge, i.e., color, nature, consistency, odor, etc., were recorded.

The pH and amine tests of the vaginal discharge were done. A clinico-microbiological diagnosis of bacterial vaginosis was made as per the standard criteria described by Amsel et al. (1984).

The swabs were transported in sterile capped test tubes to the Microbiology Laboratory for aerobic and anaerobic cultures in MacConkey agar, blood agar, and human blood bilayer media with Tween-80 (HBT) within 2–3 hours of collection. The HBT agar plates and sheep blood agar plates were incubated in a candle jar with 5% CO_2 in a humid atmosphere. Humidity was provided by keeping wet blotting paper or a wet cotton wool swab in a petridish inside the candle jar. Mac Conkey agar plates were incubated aerobically at 37°C and examined after 24 hours. HBT agar plates were examined after 48 hours and were reincubated for another 24 hours if no growth was found.

Another swab was used for making a smear for Gram staining and direct wet-mount microscopy. The Gram-stained slides were examined under an oil immersion objective (1000x magnification). Then, the etiological agent and normal flora on the Gram-stained smear were counted and scored according to the standardized Nugent's scoring method [12].

Any isolate that showed tiny, Gram variable pleomorphic, coccobacillary forms on Gram stain preparation, was nonmotile, β haemolytic on HBT agar, produced pinpoint, convex, grey, opaque colonies, was negative for catalase and oxidase, positive for fermentation of glucose, maltose, starch, and positive for hippurate hydrolysis, was identified as Gardnerella vaginalis. The isolates of G. vaginalis were maintained on H. medium by subculturing every 48 hours and incubating in a candle jar. Any other bacterial pathogens grown were isolated and identified according to Gram stain morphology, cultural characters, and biochemical reactions as per standard procedures. Antibiotic susceptibility testing was performed by the standard disc diffusion method.

Results

In this study, 32 of 150 cases (21.34%) and 5 of 50 controls (10%) revealed both candida and Trichomonas vagianalis. Hence, these cases of non-bacterial causes of abnormal vaginal discharge were excluded from further experimentation (Table 1)

Table 1: Non-bacterial causes of abnormal vaginal discharge

Pathogens	Cases (n = 150)	Controls (n = 50)
Trichomonas vaginalis	30 (20%)	4 (8%)
Candida Species	15 (10%)	3 (6%)
Both Trichomonas and Candida Species	32 (21.34%)	5 (10%)

Taking into consideration any three of Amsel's positive criteria, 49 (41.52%) out of 118 cases were diagnosed as bacterial vaginosis. None of the 45 controls satisfied the three positive criteria together (Table 2).

Table 2: Bacterial vaginosis diagnosed by Amsel's Criteria.

Criteria (Amsel's)	Cases (n = 118)	Control (n = 45)
Homogenous discharge	64 (54.23%)	12 (26.65%)
pH > 4.5	80 (67.79%)	9 (20%)
Presence of clue cells	28 (23.72%)	1 (2.22%)
Positive Amine test	48 (40.67%)	5 (11.11%)
Any 3 criteria positive	49 (41.52%)	0 (0%)

Gram's staining and Nugent's scoring system revealed BV score of 7 -10 in 50 (42.37%) of 118 cases and in 2(4.4%) of 45 controls (Table 3).

Table 3: Bacterial vaginosis diagnosed by Gram's staining (Nugent's scoring)

Scoring of bacterial flora	Cases (n = 118)	Controls (n = 45)
0 – 3	29 (24.57%)	18 (40%)
4 – 6	39 (33.05%)	25 (55.55%)
7 – 10 (BV score)	50 (42.37%)	2 (4.4%)

Maximum numbers of cases of BV fall between 25 – 40 years of age as seen in BV cases diagnosed either by Amsel's criteria or Nugent's scoring (Figure 1)

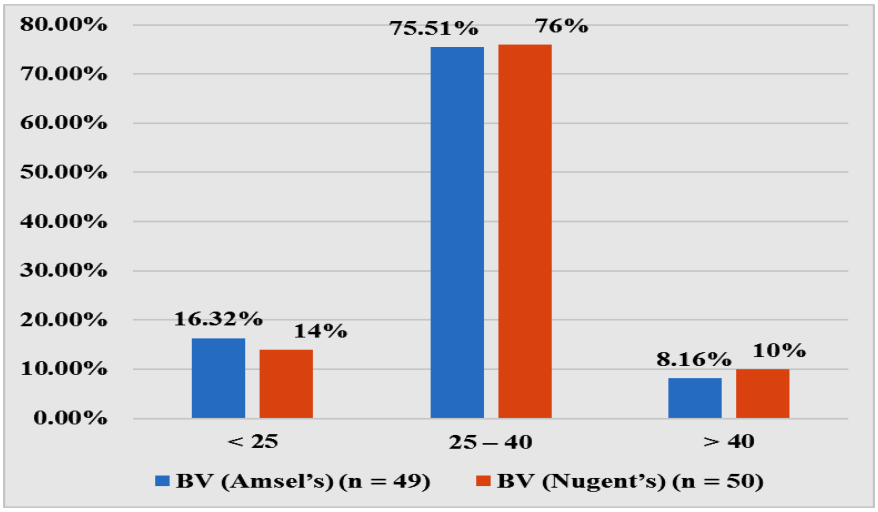

Figure 1: Age distribution in Bacterial Vaginosis

Out of 49 cases of BV diagnosed by Amsel's criteria, in 23 cases, G. vaginalis was isolated. Similarly, 24 isolates of G. vaginalis were obtained from 50 cases of BV diagnosed by Nugent's methods. Of the total isolates of G. vaginalis, 30 (100%) were isolated in HBT media, whereas the rate of isolation in sheep blood agar was only 28%. All 30 isolates of G. vaginalis were catalase-negative, oxidase-negative, and positive for sodium hippurate hydrolysis. The maximum numbers of cases of BV are from patients with abnormal vaginal discharge. Maximum numbers of G. vaginalis were also isolated from the same group. Out of total 118 cases, G. vaginalis was isolated in 26 cases (22.03%) and in 4 (8.8%) out of 45 controls Table 4).

Table 4: Diagnosis of bacterial vaginosis and isolation of *Gardnerella vaginalis* in different study groups

Case	No.	BV (Nugent's)*	*G. Vaginlas*
Abnormal vaginal discharge	52	31 (59.61%)	16 (30.76 %)
Pregnancy	23	6 (26.08%)	3 (13.04 %)
Preterm labour	9	3 (33.33%)	1 (11.11%)
For MTP	13	4 (30.76%)	3 (23.07%)
For hysterectomy	14	3 (21.04%)	2 (14.28%)
With IUCD (CuT)	7	3 (42.85%)	1 (14.28%)
Total (cases)	118	50 (42.37%)	26 (22.03%)
Control	45	2 (4.4%)	4 (8.88%)

** -BV diagnosed by Nugent's Method*

Maximum number of *G. vaginalis* were isolated from specimens of vaginal discharge which were copious 87.5%, grey 81.25%, homogenous 93.75% and malodourous 87.5% (Table 5)

Table 5: Abnormal vaginal discharge and *G.vaginalis* isolation

Character of abnormal vaginal discharge (n =52)		*G. vaginalis* isolated (n =16)
Amount	Copious	14 (87.5%)
	Moderate	2 (12.5%)
Colour	Grey	13 (81.25%)
	White	3 (18.7%)
Consistency	Homogenous	15 (93.75%)
	Non homogenous	1 (6.25%)
Odour	Malodorous	14 (87.5%)
	Normal odour	2 (12.5%)

Both in cases and controls *G. vaginalis* was isolated from vaginal discharge having a pH environment of more than 4.5 (Table 6).

Table 6: Vaginal pH pattern and isolation of Gardnerella vaginalis

Vaginal PH	Gardnerella vaginalis isolated (n=30)	
	In cases (n=26)	In control (n=4)
< 4.5	0%	0%
4.5 – 5.5	17 (65.3%)	4 (100%)
> 5.5	9 (34.61%)	0%

Out of 26 G. *vaginalis* positive cases, 18 (69.23%) showed presence of clue cells and in 8 (30.7%) cases clue cells were absent. Clue cells were also present in 92 *G. vaginalis* culture negative cases (13.04%) Of the 4 controls where *G. Vaginalis* was isolated, 1 (25%) revealed presence of clue cells and 3(75%) did not reveal any clue cells. Of the 26 G. *vaginalis* positive cases, 19(73.07%) were positive for Amine test and 7(26.92%) were negative for the test. Amine test was positive in 31(33.69%) of 92 *G. vaginalis* culture negative cases. Out of the 4 controls where G. *vaginalis* wasisolated, amine test was positive in 2(50%) cases. The sensitivity and specificity of clue cell test was 18(69.23%) and 80(86.95%) whereas the same for Amine test was 19(73.07%) and 61(66.31%)

Majority of isolates (96.67%) were sensitive to Metronidazole (50µg). The sensitivity to Ampicillin, Chloramphenicol, Ciprofloxacin and Tetracycline were 70%,66.67%,40%,46.66% respectively. All the isolates showed resistance to Metronidazole (5µg) (Table 7).

Table 7: Antibiotic Susceptibility of *Gardnerella vaginalis* isolates (n =30)

Antibiotics used	Disc potency (µg/disc)	Sensitivity	Resistance
Ampicillin	10	21 (70%)	9 (30%)
Chloramphenicol	30	20 (66.67%)	10 (33.33%)
Ciprofloxacin	5	12 (40%)	18 (60%)
Tetracycline	30	14 (46.66%)	16 (53.34%)
Metronidazole	5	0 (0%)	30 (100%)
Metronidazole	50	29 (96.67%)	1 (3.33%)

Maximum Aerobic bacterial isolates was E.coli both in cases and controls (Table 8).

Table 8: Isolation of Aerobic bacteria other than Gardnerella vaginalis

Bacteria isolated	Cases (n =118)	Control (n =45)
Escherichia coli	28 (23.72%)	9 (20%)
Klebsiella pneumonae	18 (15.25%)	2 (4.44%)
Staphylococcus aureus	9 (7.64%)	2 (4.44%)
Coagulase negative staphylococcus	8 (6.77%)	6 (13.33%)
Gr. B streptococcus	0 (0%)	0 (0%)
Enterococcus spp.	4 (3.38%)	0 (0%)
Lactobacillus (Faculative spp.)	14 (11.86%)	12 (26.67%)
Mixed organisms	10 (8.47%)	8 (17.78%)
No growth	5 (4.23%)	1 (2.22%)

Discussion

Globally, BV is a common genital problem among women seeking gynecological care. The prevalence rate of BV is found to be 24.4% by Nugent's method [13]. Modak et al.[14] in India reported a similar result, providing a prevalence rate of 24%. But in this present study, higher prevalence rates of BV were observed (43%) than those in the previous reports. The variation in the findings might be due to population size, methods of analysis, geographic distribution, and socioeconomic and behavioral differences in the studied population.

The present study revealed that the prevalence of BV was high among women of the age group 25–40 years (75%) and lowest for 10–20 and >40 years' age groups. Other studies also found similar findings. A study from Nepal observed the highest prevalence of BV among the age group of 30–40 years and the least among those below 20 years of age and the 51–60 age group [13]. Also, Garba et al.[15] in Nigeria found BV to be most prevalent among the 26–30 age group (35.8%) and least prevalent among the >40 age group (10.5%). The highest prevalence in the age group 30–40 years might be due to the age being the most reproductively active age group and high sexual exposure at this age.

In this study, taking into consideration any three of Amsel's positive criteria, 49 (41.52%) out of 118 cases were diagnosed with bacterial vaginosis. None of the 45 controls satisfied the three positive criteria together. Gram's staining and Nugent's scoring system revealed a BV score of 7–10 in 50 (42.37%) of 118 cases and in 2 (4.4%) of 45 controls.

In the previous report, the criteria (Amsel's, Spiegel, Nugent *et al.)* followed for the diagnosis of bacterial vaginosis, in which Amsel's clinical criteria [38/46 (82.6%)] were statistically highly significant compared to two other ones. They concluded that the lower incidence of bacterial vaginosis by Spiegel's and Nugent's criteria can be explained as most of the women fell in the gestational age group from 21 to 30 weeks, or they might have had a chronic infection in which clue cells were absent due to the local immune response to IgA antibodies [16].

The most studied vaginal anaerobe, *Gardnerella vaginalis*, has been recovered from the vaginal samples of almost all women with BV. *G. vaginalis* possesses a number of virulence factors, including the production of sialidase A and the toxin vaginolysin. It is also able to adhere to vaginal epithelial cells and establish a biofilm. Although *G. vaginalis* is associated with various clinical conditions, it has been found in vaginal samples of healthy individuals, albeit often in lower numbers than in BV cases [17].

In this investigation, the most common clinical sign and symptoms of patients with bacterial vaginosis were malodorous vaginal discharge with itching. This finding corresponds with previous studies conducted in different countries [13]. In another study, approximately 50% of patients with bacterial vaginosis did not have any symptoms [18].

The diagnosis of bacterial vaginosis is usually established according to clinical criteria or microbiological tests. Bacterial vaginosis is often misdiagnosed using clinical criteria because the components are subjective and dependent on the performance of the clinician and available equipment. *G. vaginalis* commonly occurs in the vagina of women without bacterial vaginosis, and bacterial vaginosis may be produced by microorganisms other than *G. vaginalis*. The results of the study by Nugent et al. [12] indicated that the criteria for the diagnosis of bacterial vaginosis using Gram stain can be produced reliably by different centers and microbiologists. It is also reliable when evaluating an asymptomatic population [18].

The study also determined that vaginal culture has a sensitivity of 77.8% and a specificity of 97.7% for the diagnosis of bacterial vaginosis when compared to Gram stain. Vaginal cultures for *G. vaginalis* are often the primary laboratory test available for the diagnosis of vaginitis. Although it has a sensitivity of 83–94% among the women who have clinical signs of bacterial vaginosis, the usefulness of these cultures is doubtful. *G. vaginalis* commonly occurs in the vaginas of women without bacterial vaginosis, and bacterial vaginosis may be produced by microorganisms other than *G. vaginalis* [18].

Culture findings from our study revealed the isolation of G. vaginalis in 26 (21.51%) of 118 cases and in 4 (8.88%) of 45 controls. The HBT media employed for the isolation of G. vaginalis was found to be most suitable, rendering satisfactory isolation of G. vaginalis in our study. G. vaginalis did not grow on Mac Conkey agar and did not produce hemolysis on unselective sheep blood agar.

The presumptive identification of *G. vaginalis* colonies on the selective and differential HBT media was easier as it exhibited pin point, diffuse β haemolysis. On Gram staining, they revealed pleomorphic, Gram negative, or Gram variable coccibacilli. They were non-motile, and further biochemical tests revealed catalase- and oxidase-negative properties, showing positive fermentation reactions for glucose, maltose, and starch and positive reactions for hippurate hydrolysis. HBT media is highly selective due to the incorporation of antibiotics, which inhibit the overgrowth of other vaginal flora, making the detection of minute colonies easier than in sheep blood agar. The reason for the high isolation rate on this medium is due to

the availability of all nutrients provided by the Columbia agar base.

The low isolation rate on the non-selective sheep blood agar is due to the overgrowth of commensal flora, which easily obscures the very minute non-haemolytic *G. vaginalis* colonies.

In our study, BV cases diagnosed by both Amsel's and Nugent's methods revealed associations with *G. vaginalis* of 46.93% and 48%, respectively. The above isolation rate in our study is attributable to factors like the inclusion of appropriate and vulnerable cases and the use of the most effective (selective) media. The *G. vaginalis* isolated in our study mostly belongs to the confirmed BV cases of Amsel's and Nugent's methods.

According to our present report as well as our previous report, it seems likely that vaginal culture is an adequate diagnostic criterion when it is positive [18].

A higher percentage of ***G. vaginalis*** isolation is related to a pH value of more than 4.5. Hence, a raised pH can be considered a reliable indicator of *G. vaginalis* infection in a carefully correlated clinical condition. The sensitivity and specificity of clue cell detection are found to be 69.23% and 86.95%, respectively. The amine test shows 73.07% sensitivity and 66.30% specificity. Effective antimicrobials for G. vaginalis infection are Metronidazole, Ampicillin, and Chloramphenicol.

Conclusion

Based on our study findings, we concluded that bacterial vaginosis is a common health problem in women of reproductive age. In view of the significant association of bacterial vaginosis and *G. vaginalis* with the various obstetrical and gynecological conditions, a thorough routine screening for infection and their management have become imperative to avoid future adverse outcomes.

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