

Role of *Gardnerella vaginalis* as an etiological agent of bacterial vaginosis

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ABSTRACT

Background and Objectives: Bacterial vaginosis is a risk factor for obstetric infections, various adverse outcomes of pregnancy and pelvic inflammatory disease. The objectives of this study were to assess the prevalence of bacterial vaginosis in women attending Gynaecology Outpatient Department (O.P.D) and sexually transmitted disease (S.T.D.) clinic and to assess the role of *Gardnerella vaginalis* as an etiological agent of bacterial vaginosis.

Materials and Methods: Two hundred women attending Gynaecology O.P.D and S.T.D. clinic with symptoms suggesting lower genital tract infection were included in the study. pH of the vaginal discharge was measured and three high vaginal swabs were collected. Bacterial vaginosis was diagnosed using Amsel's criteria and Nugent's method. *Gardnerella vaginalis* was isolated and identified by standard methods.

Results: Prevalence of bacterial vaginosis using Amsel's criteria and Gram stain scoring method was found to be 51.5% and 49% respectively. *Gardnerella vaginalis* was isolated in only 8.7% cases of bacterial vaginosis.

Conclusion: Our study showed a relatively high prevalence of bacterial vaginosis in the population under study. Women attending various healthcare facilities should be screened and treated properly to prevent recurrence. Low isolation rate of *Gardnerella vaginalis* may be attributed to factors like poor viability and fastidiousness of the organism to grow in various media.

Keywords: Bacterial vaginosis, *Gardnerella vaginalis*, Gynaecology

INTRODUCTION

Bacterial vaginosis represents a unique upheaval of the complex vaginal bacterial flora with the disappearance of *Lactobacilli* and its replacement by a mixed flora of aerobic, anaerobic and microaerophilic species. It is a polymicrobial condition and it involves various organisms such as *Gardnerella vaginalis*, *Mycoplasma hominis*, *Mobiluncus* species, and other anaerobic bacteria, i.e., *Peptostreptococcus* sp.,

Prevotella sp., *Porphyromonas* and *Bacteroides* (1, 2).

Bacterial vaginosis is the commonest cause of vaginal discharge occurring in women attending gynecological clinics in our country (3). Earlier designated as 'non-specific vaginosis' because of the absence of recognized pathogens, bacterial vaginosis, afflicts as many as one-third of women attending S.T.D clinics and Primary Health Care units. It is a risk factor for obstetric infections, various adverse outcomes of pregnancy and pelvic inflammatory disease (2).

Gardnerella vaginalis is the only species of genus *Gardnerella*. *Gardnerella vaginalis* has been suggested as the principal cause of bacterial vaginosis although the isolation rate of this organism from patients of bacterial vaginosis is variable 6-94%, due to broad diversity in selection of patient's material,

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methods, and criteria for establishment of diagnosis (4, 5).

This study was undertaken to assess the prevalence of bacterial vaginosis in women attending Gynecology O.P.D. and S.T.D. clinic and to study the role of *Gardnerella vaginalis* as an etiological agent in patients suffering from bacterial vaginosis.

MATERIALS AND METHODS

This study was prospectively conducted over a period of twelve months at a tertiary care institute located in North-east India. Two hundred women attending Gynecology O.P.D and S.T.D. clinic with complaints of one or more symptoms suggesting lower genital tract infection (abnormal vaginal discharge, vulvar pruritis, malodour, burning micturition and dysuria) were included in the study after taking informed & written consent from the patient. The sample size was decided on the basis of the findings of the previous study (6).

Approval for conducting the study was taken from the Institutional Ethical Committee. Women that were menstruating or had received any treatment for vaginitis in the preceding six weeks were excluded from the study.

A patient was suspected to be suffering from bacterial vaginosis if at least three or more of the four criteria of Amsel were present. The clinical composite criteria used were increased homogenous greyish-white vaginal discharge, increased vaginal pH>4.5, a fishy smell on addition of 10% KOH to vaginal fluid (Whiff test) and presence of clue cells on a wet mount preparation. Clue cells are vaginal epithelial cells with an overlay of micro-organisms. If less than three criteria were detected, the patient was considered not to have bacterial vaginosis or to be 'normal' (7).

Three high vaginal swabs were collected from the upper part of posterior fornix and lateral vaginal walls using sterile cotton-tipped swabs. pH of the discharge was measured with a narrow range pH paper by placing the paper directly on the vaginal wall.

The swabs were used for amine test, direct microscopy and culture. The wet film was examined microscopically with the 40x objective for the presence of pus cells, clue cells, budding yeast cells and *Trichomonas vaginalis*. The Gram stained smears were examined under oil immersion for the presence of pus cells, clue cells, budding yeast

cells, lactobacilli, Gram variable or Gram negative coccobacilli and were graded as per standardized, quantitative, morphological classification developed by Nugent *et al.* (8). Composite score was categorized into three categories, scores 0–3 being normal, 4–6 being intermediate and 7–10 being definite bacterial vaginosis. Smears showing vaginal epithelial cells with an overlay of predominantly Gram negative or Gram variable bacilli were taken to be positive for clue cells. Swab was used to inoculate MacConkey agar, Sheep blood agar, Chocolate agar, Human blood agar composed of Columbia Agar base, *Gardnerella vaginalis* selective supplement and 5% human blood and Human blood bilayer Tween agar (HBT) composed of Columbia Agar base, *Gardnerella vaginalis* selective supplement, 5% human blood, 1% proteose peptone and Tween 80 (Hi-Media, Mumbai, India).

The plates were inoculated by Semi- quantitative method. MacConkey agar and sheep blood agar were incubated aerobically for 18-24 hours and the organisms identified according to standard methods. Chocolate agar, Human blood agar and HBT agar were incubated for 48 hrs-72 hrs at 35°C in a candle jar (5-10% CO₂). Quantitation of relative number of *Gardnerella vaginalis* colonies on HBT medium was done by semiquantitative technique (9, 10).

Growth from human blood agar was purified by subculture on HBT media and *Gardnerella vaginalis* identified by standard methods based on the characteristics like no hemolysis on sheep blood agar, diffuse β-hemolysis on human blood agar and HBT media, a negative oxidase and catalase test, sugar fermentation reaction, hippurate and starch hydrolysis test, alpha and beta glucosidase activity and sensitivity to bile (10%) discs (10).

Statistical analysis. Data was statistically analysed for significance of association of *Gardnerella vaginalis* with bacterial vaginosis and Amsel's criteria for diagnosing bacterial vaginosis using Chi-square test. Analysis was performed on MS Office Excel and P<0.05 was taken as significant.

RESULTS

Out of 200 patients included in this study, 103 (51.5%) were diagnosed as bacterial vaginosis using the clinical composite criteria as suggested by Amsel *et al* (1983); 97 (48.6%) of the patients had only one

Table 1. Presenting features of bacterial vaginosis

Discharge	Odour	Vulval Irritation	Burning micturition (BM)	Dysuria	No. of patients of BV (%)
+	+	+	-	-	9(8.7)
+	+	-	-	+	18(17.5)
+	-	+	+	+	11(10.7)
-	+	+	-	+	15(14.6)
-	+	-	+	-	12(11.7)
+	-	-	-	-	29(28.2)
-	-	+	-	-	9(8.7)

or two out of four clinical criteria.

The most common presenting feature in patients of bacterial vaginosis was abnormal vaginal discharge (28.2%) only while in 17.5% cases vaginal discharge was associated with vaginal malodour and dysuria (Table 1).

Of the clinical criteria of bacterial vaginosis, abnormal vaginal discharge was present in 67 (65%) cases of bacterial vaginosis while a pH of ≥ 4.5 was found in 97(94.2%) cases of bacterial vaginosis. Amine test was positive in all 103 (100%) cases of bacterial vaginosis. Clue cells were present ($>20\%$) in 85 (82.5%) cases of bacterial vaginosis on wet mount while only 18(17.5%) cases of bacterial vaginosis showed clue cells $<20\%$ (Table 2).

According to Nugent's scoring system, of the 200 cases, 98 (49%) were diagnosed as bacterial vaginosis with a Nugent score of 7 to 10, 42 (21%) as intermediate with a Nugent score of 4 to 6 and 60 cases (30%) were diagnosed as normal with a Nugent score of 0 to 3.

Out of 200 cases, 11 yielded growth of *Gardnerella vaginalis* either alone or in association with other aerobic organisms. Of these 11 isolates, 9 (81.8%) were isolated from bacterial vaginosis and 2 (18.2%) were from non bacterial vaginosis cases. Statistically, isolation of *Gardnerella vaginalis* did not differ significantly between bacterial vaginosis and non bacterial vaginosis cases ($P>0.05$). Quantitative estimation of *Gardnerella vaginalis* colonies from bacterial vaginosis cases revealed that the growth was 4+ in 7 cases and 3+ in 2 cases whereas in non-bacterial vaginosis cases, the growth was 1+ in each case.

DISCUSSION

Bacterial vaginosis is considered as a common vaginal disorder in women of reproductive age. It is a frequently encountered problem confronting practitioners in women's health care. Prevalence of bacterial vaginosis approaches up to 76.8% of women in reproductive age group (11).

Table 2. Frequency distribution of Amsel's criteria

Diagnostic criteria (Amsel's)	Women with bacterial vaginosis (n=103)	Women without bacterial vaginosis (n=97)	P value
a) Homogeneous vaginal discharge	present	67	0.025
	absent	36	
b) pH	≥ 4.5	97	0.005
	<4.5	6	
c) Amine test	Positive	103	0.003
	negative	0	
d) Clue cells $>20\%$	Present	85	0.005
	Absent	18	

The interest in bacterial vaginosis has increased lately because of the evidence of adverse sequel to this disorder, such as amniotic fluid infection, clinical chorioamnionitis, premature rupture of membranes (PROM), preterm delivery, low birth weight and postpartum endometritis. Non-pregnant women with bacterial vaginosis have been reported to get post-abortion pelvic inflammatory disease, post-hysterectomy vaginal cuff cellulitis and plasma cell endometritis. Several publications have also reported an altered vaginal microflora being linked to an increased susceptibility to the acquisition of HIV and other sexually transmitted infectious agents such as *Neisseria gonorrhoeae* and *Chlamydia trachomatis* (12, 13).

Prevalence of bacterial vaginosis in this study was 51.5%. Similar prevalence rates have been reported in previous studies too (14, 15). Factors responsible for higher prevalence of bacterial vaginosis among the study population were lower socio-economic status, improper sanitation, poor hygiene, malnutrition.

Among the individual criteria used to diagnose bacterial vaginosis, raised pH is recognized as the most sensitive but least specific criteria (15). In the present study, the pH of the vaginal fluid was also found to be significantly associated with bacterial vaginosis. Majority of the patients (46.6%) with bacterial vaginosis had a pH between 5.0-5.5, 26.2% and 21.4% of the patients had a pH between 4.5 and 5.5-6 respectively. Amsel *et al.* (1983) also found a pH of more than 4.5 in 81% cases of bacterial vaginosis (7). Errors in pH measurement may be made by sampling cervical mucus rather than vaginal discharge which has a higher pH or due to presence of cervical infection which increases the pH by increasing the flow of cervical secretions into the vaginal canal (3).

Amine test is both highly sensitive and specific. False positive amine test occur rarely (3). Association between amine test and bacterial vaginosis was found to be statistically significant in this study. Detection of amine odour is observer dependant with wide person to person variability. The amine test is easily performed, rapid, inexpensive diagnostic test with good sensitivity and specificity which, as suggested by previous studies, is ideally suited to clinical settings where microscopy is not available (16).

Significant association was found between clue cells and bacterial vaginosis which was in confirmation with earlier studies (9,17). According

to a previous study, the sensitivity and specificity of more than 20% clue cells on wet mount for diagnosis of bacterial vaginosis is 81% and 99%. However, recognition of clue cells in wet mount which is an excellent denominator of bacterial vaginosis is subjected to variability, depending on the quality of microscope, the adequacy of specimen and the skill of observer (17).

Out of 200 samples, 98 (49%) samples were diagnosed as bacterial vaginosis and 42 (21%) were diagnosed as intermediate using Nugent's Gram stain scoring system. According to Rosenstein *et al.*, the intermediate stage is considered a transitional phase and the patients may go on to frank bacterial vaginosis (18).

Gram staining of vaginal secretions is more reliable with sensitivity of 89-93% and specificity of 70-83%. This technique is least expensive, requires the least time to perform, is more widely available than other laboratory methods and is the most interpretative of the laboratory methods (8).

In this study, the prevalence rate of *Gardnerella vaginalis* in 103 bacterial vaginosis patients was studied and a rate of 8.7% (9/103) was found. Prevalence of *Gardnerella vaginalis* reported by various workers varies from 6-94% probably because different authors have studied different types of population and have considered different criteria for selecting the cases of bacterial vaginosis.

These factors along with poor viability and fastidiousness of the organism to grow on different culture media and also since different methods for isolation and identification were used, may explain this variation in isolation rate. Prevalence of *Gardnerella vaginalis* in patients of bacterial vaginosis found in this study is in close conformity with the observation of other workers reporting an isolation rate of 6% and 10.2% of *Gardnerella vaginalis* respectively (4, 5, 19).

Gardnerella vaginalis has been reported to show contradictory biochemical profiles in the literature so the impression has been that *Gardnerella vaginalis* is a species with heterogeneous characteristics. However, in this study, most of the strains were found to be fermenting mannose, maltose, fructose which is in general agreement with the other published reports as are the negative reactions for catalase, oxidase, mannitol, salicin and β -glucosidase (20), (Table 3).

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Table 3. Biochemical reactions of 11 strains of *Gardnerella vaginalis*

Sl. No.	Test	Test result	No. of strains	Percentage
1.	Hemolysis on			
	- Human blood	+	11	100
	- Sheep blood	-	11	100
2.	Catalase	-	11	100
3.	Oxidase	-	11	100
4.	Hippurate hydrolysis	+	10	90.9
5.	Fermentation of:-			
	a) Fructose	+	9	81.8
	b) Sucrose	-	8	72.7
	c) Arabinose	-	9	81.8
	d) Dulcitol	-	7	63.6
	e) Inositol	-	8	72.7
	f) Maltose	+	11	100
	g) Salicin	-	10	90.9
	h) Lactose	-	9	81.8
	i) Mannitol	-	11	100
	j) Xylose	-	7	63.6
k) Mannose	+	11	100	
6.	α -glucosidase	+	10	90.9
7.	β -glucosidase	-	11	100
8.	Starch hydrolysis test	+	9	81.8
9.	Inhibition by:-			
	a) Metronidazole (50 μ g)	+	11	100
	b) Nitrofurantoin (150 μ g)	+	9	81.8
	c) Bile, 10%	+	11	100

appear to have a role in reducing the complications associated with this infection. However, problem with diagnosis continue to dominate clinical practice, although new tests have been introduced. Recurrent bacterial vaginosis might be due to the survival of metronidazole or clindamycin resistant bacteria in the vagina.

Hence, it may be important to explore primary preventive strategies which target the risk factors or behaviours for bacterial vaginosis.

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