ROLE OF *GARDNERELLA VAGINALIS* AMONG SYMPTOMATIC BACTERIAL VAGINOSIS PATIENTS ATTENDING AT CHITTAGONG MEDICAL COLLEGE HOSPITAL

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Abstract

Background : Now a day's bacterial vaginosis is an extremely common health problem for women in the world which causes many complications both in the pregnancy and non-pregnancy states. G. vaginalis is most important cause of bacterial vaginosis. **Materials and methods :** A cross sectional study was conducted to assess the role of Gardnerella vaginalis as an etiological agent of bacterial vaginosis. This study was undertaken to assess the patients attending at the outpatient department of Gynaecology and Obstetrics of Chittagong Medical College Hospital, Chittagong. A total of 170 sexually active female in the age group of 15-45 years, with abnormal vaginal discharge were selected for the study. A detailed history and a thorough clinical examination of all the cases were done. 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 with their drug sensitivity test by standard methods. **Results :** In this study 38(22.35%) Gardnerella vaginalis were isolated by culture and bacterial vaginosis was detected by Amsel clinical criteria (Clinical method) 43(25.30%), Gram stain Nugent criteria (Gold Standard) 45(26.47%).Our study showed a relatively high prevalence of bacterial vaginosis and high isolation rate of Gardnerella vaginalis within the positive bacterial vaginosis patients. **Conclusion :** This result helps proper management and treatment.

Introduction

Bacterial Vaginosis (BV) is a clinical syndrome characterized by shift of protective resident micro organisms as Lactobacillus spp. by opportunistic pathogenic bacteria such as Gardnerella vaginalis and other anaerobic bacteria. 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 bacteroids[1]. In most cases of BV, the predominant bacterial species found is Gardnerella vaginalis. Historically, G. vaginalis was thought to be the sole causative agent of this condition. But its role in the aetiology of BV was downgraded over the years. The biofilm-forming potential and cytotoxic activity of G. vaginalis have renewed interest in the virulence of this organism[2]. So bacterial vaginosis is mostly caused by the synergistic interaction of G. vaginalis with obligate anerobes. Recent evidence has once again placed G. vaginalis in the spot light and has indicated that G. vaginalis is equipped with a number of virulence properties and consequently the idea that it is the aetiological agent of BV is being revisited[3].

Bacterial vaginosis is associated with gynecologic complications, such as cervicitis, salpingitis, endometritis, post-operative infections and pelvic inflmmatory disease and many obstetric complications, such as premature rupture of the membranes, preterm deliveries, chorioamniotitis

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and postpartum endometritis. Despite the fact that Bacterial Vaginosis (BV) is the leading vaginal disorder globally, very little is known about its etiology or pathogenesis.

This study was designed to isolate the causative agent G. vaginalis from bacterial vaginosis patients with their antibiotic sensitivity pattern and showed the role of G. vaginalis in bacterial vaginosis which would guide clinicians and microbiologists for proper handling of this pathogen & prevent unnecessary use of antibiotics.

Materials and methods

This was a cross-sectional comparative study carried out in the Department of Microbiology, Chittagong Medical College, Chittagong, during the period of July 2011 to June 2012. Approval from ethical review committee of Chittagong Medical College was duly taken. A total of 170 women, 50 pregnant and 120 non-pregnant, in the age group of 15-45 years patients attending the Gynae out-patient department of Chittagong Medical College Hospital was enrolled for this study. The results of the experiments were recorded systematically and Data was statistically analysed for significance of association of Gardnerella vaginalis with bacterial vaginosis diagnosed by Amsel criteria and Nugent criteria using Chi-square test. Statistical analysis was done by standard statistical procedure Statistical Package for Social Sciences (SPSS) and p<0.05 was taken as significant.

Inclusion criteria

i) Women of reproductive (15-45 years) age group.
ii) Patient having history of abnormal vaginal discharge.
iii) Patient with or without mild vulver itching or burning.
iv) Patient included pregnant and non-pregnant women.

Exclusion criteria

i) Patient below 15 yrs & over 45 yrs.
ii) Patient associated with known case of malignancy or AIDS.
iii) History of taking antimicrobial agents or vaginal medication for vaginitis within the last one month.
iv) Patient having history of vaginal douche on the day of examination.
v) Menstruating women.

Three vaginal swab samples were collected with all aseptic precaution after taking informed consent from patient or her legal attendant. Samples were collected from each patient by standard technique. First swab sample used for amine test and wet mount preparation. Second swab sample was collected from left lateral vaginal wall for culture of Gardnerella vaginalis. Third swab sample collected from vaginal fornix and used for making Gram's stain for Nugent criteria.

Detection of bacterial vaginosis by –
• Amsel criteria
• Nugent criteria

Isolation and Identification of *Gardenella vaginalis:* Culture in Human blood bilayer Tween 80 (HBT) agar media and other biochemical test.

Procedure of Amsel Criteria: It should require the presence of at least three of the following four criteria.

i) **Physical Examination of Vaginal Discharge:**
Presence of thin, gray, homogenous, malodorous, adherent vaginal discharge.

ii) **pH Measurement of Vaginal Fluid:**
In BV patients vaginal fluid with a pH >4.5.

iii) **Whiff Test or Amine Odour Test:**
At first one or two drops of 10% KOH was added in vaginal secretion on a glass slide and smelled for fishy odour (amine odour).

iv) **Wet Mount Preparation:**
Presence of clue cell on saline wet mount in BV patients.

Procedure of Nugent Criteria: Third swab sample collected from right lateral vaginal wall and was rolled on a glass slides, the smear were air dried and then fixed with methanol for Gram's stain. Then fixed smears were stained Koploff`s modification of Gram's for detection of clue cells and evaluation of bacterial morphotypes under light microscope (At x1000) according to scoring system (Score 0-10) of interpretation by Nugent et al.

The amount of each morphotype detected on the smear was graded and allocated a score as below (Table I).

Table I : Nugent criteria Gram stain interpretation (Score 0 to10)

Score	Lactobacillus morphotypes	Gardnerella & Bacteroides morphotyes	Curved Gram-variable rods
0	4+	0	0
1	3+	1+	1+ or 2+
2	2+	2+	3+ or 4+
3	1+	3+	
4	0	4+	

The score of each morphotype is to be added together to get a total score.

Total score =*Lactobacilli + G. vaginalis and Bacteroides spp.* + curved rods (In each slide).

By using the scoring system, the study cases were grouped into three groups ie, Bacterial Vaginosis (BV) group, intermediate group, normal flora group.

*A slide with a total score of ≥7 is interpreted as "BV".

* A slide with a total score of 4 to 6 is interpreted as "intermediate group".

* A slide with a total score of 0 to 3 is interpreted as "normal flora".

Culture for Isolation of Gardnerella vaginals: The second swab inoculated into a selective and differential Human blood bilayer Tween 80 (HBT) agar media for isolation of G. vaginalis.

Procedure of Culture: Collected vaginal swab was inoculated and the plate was placed immediately in the candle extinction jar containing water soaked cotton. All plates are incubated in 5% CO_2 with increased humidity at 37^0 C for 48 - 72 hrs for primary isolation of G. vaginalis and read at 48 hours and rechecked at 72 hours before discarded. The plates were examined by oblique lighting after 24 hrs, 48 hrs, and 72 hrs.

Colonies on HBT agar media were identified as round opaque, smooth colonies that were pinpoint in size after 24 hrs of incubation and 0.5 mm in diameter at 48 hrs, produce β hemolysis after 48 or 72 hrs of incubation.

The β-haemolytic colonies from HBT agar were examined by Gram's staining to see Gram negative coccobacilli. Subcultures were done on Human blood Columbia agar media and Sheep blood agar media by using β-haemolytic colony for pure isolation and to see the haemolytic character. Colonies were also used for catalase test, oxidase test and fermentation of different sugar.

The Identification of Gardnerella vaginalis, Based on

i. Colonial morphology: Colonies on HBT agar were identified as small white colonies with -hemolysis after 48 to 72 hours of incubation.

ii. Clear -hemolysis with diffuse edges on HBT media, but no hemolysis on sheep blood agar. The zone of hemolysis was 1 to 2 mm wide around the isolated colonies on HBT agar after 48 hours of incubation.

iii. Gram stained smear from a colony: Gram variable or Gram negative coccobacilli or small rods.

iv. Catalase and oxidase test negative.

v. Fermentation of different sugur: Maltose, mannitol, lactose, sucrose.

vi. Susceptibility to different antimicrobial agents.

Antimicrobial Susceptibility: All the isolates of G. vaginalis obtained by culture were tested for antimicrobial susceptibility by the single disc diffusion method against different antimicrobial agents. The organisms were tested against Metronidazole (MTZ) Clindamycine (CD) Ampicilin (AMP) Ceftriaxone (CRO) Erythromycin (E) Ciprofloxacin (CIP) Vancomycin (VA) Cotrimoxazole (SXT) Chloramphenicol (C) and Tetracycline (TE).

Results

A total of 170 clinically suspected cases of Bacterial Vaginosis (BV) aged between 15-45 years were included in this study. Among the study cases, 120 (70.59%) were non-pregnant and 50 (29.41%) were pregnant with gestational age ranging from 6 to 32 weeks.

Table I : Distribution of study population on the basis of Amsel criteria

Pregnancy Status	Total	Amsel Criteria Bacterial Vaginosis	Other than B V
Pregnant	n=50	13 (26.00)	37 (74.00)
Non-pregnant	n=120	30 (25.00)	90 (75.00)
Total	170(100.00)	43 (25.30)	127 (74.70)

● Figures within parentheses indicate percentage

Table II : Frequency distribution of Amsel's criteria

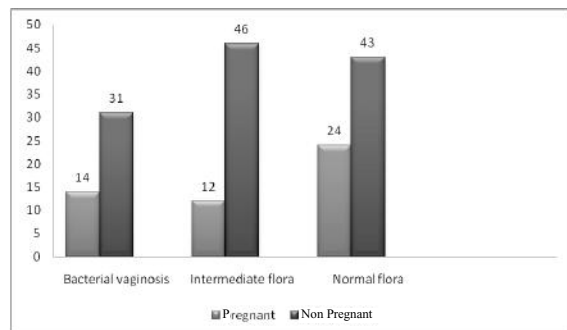| Diagnostic criteria (Amsel's)		Women with bacterial vaginosis (n=43)	Women without bacterial vaginosis (n=127)	χ^2 test Significance
Homogeneous vaginal discharge	Present	41	17	p = 0.000
	Absent	02	110	Highly significant
	pH ≥4.5	40	25	p = 0.000
	<4.5	03	102	Highly significant
Amine test	Positive	42	15	p = 0.000
	Negative	01	112	Highly significant
Clue cells	Present	43	05	p = 0.000
	Absent	00	122	Highly significant

Fig 1 : Distribution of study population on the basis of Nugent criteria.
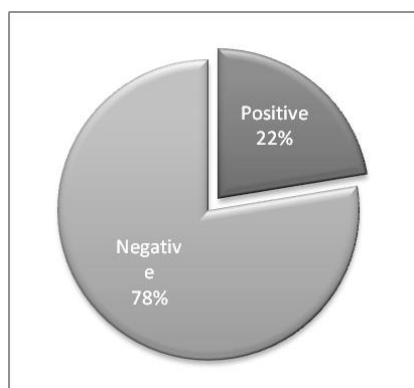(Bar chart showing distribution of Nugent criteria)

Fig 2 : Distribution of study population on the basis of culture of *G. vaginalis*
(Pie Chart : Distribution of isolated G. vaginalis result)

Table III : Association of Amsel clinical criteria and culture of G. vaginalis (n=170)

Culture of G. vaginalis	Amsel clinical criteria		
	Positive	Negative	Total
Positive	38 (22.35)	00 (0.00)	38 (22.35)
Negative	05 (2.95)	127 (74.70)	132 (77.65)
Total	43(25.30)	127 (74.70)	170 (100.00)

- Figures within parentheses indicate percentages
- $\chi^2 = 139.495$, p = 0.000, Highly significant (p < 0.001)

Table IV : Association of culture of G. vaginalis and Nugent criteria (n=170)

Nugent Criteria	Culture of *G. vaginalis*		
	Positive	Negative	Total
Bacterial vaginosis	38 (22.35)	07 (4.12)	45 (26.47)
Intermediate	00 (00)	58 (34.12)	58 (34.12)
Normal Flora	00 (0.0)	67 (39.41)	67 (39.41)
Total	38 (22.35)	132 (77.65)	170 (100.00)

- Figures within parentheses indicate percentages
- $\chi^2 = 154.869$, p = 0.000, Highly significant (p< 0.001)

Table I : On the basis of Amsel criteria (Clinical criteria) 43(25.30%) cases were Bacterial vaginosis (BV) positive and 127(74.70%) BV negative.

Table II: Shows frequency of component of Amsel criteria. Here within 170 patients 58 cases presented homogenous vaginal discharge, 65 cases showed pH 4.5, amine test positive 57 cases and clue cell present only 48 cases. Within these 43(25.30%) cases are BV positive, because we included the presence of at least three of the four criteria. But we included clue cell must. p<0.0001 was taken as significant.

Fig 1: On the basis of Nugent criteria by Gram-staining, study cases were categorized into three groups, Positive bacterial vaginosis (BV) were 45(26.47%) intermediate group were 58 (34.12%) and normal flora were 67(39.41%).

Fig 2: Shows that culture of vaginal fluid yielded growth of G. vaginalis. In which 38(22.35%) cases of G. vaginalis were isolated and 132(77.65%) cases were culture negative.

Table III shows the association of culture of G. vaginalis with Amsel criteria. The Amsel criteria were positive in all 38(22.35%) culture-positive cases. Additional 05(2.95%) cases were positive among culture-negative cases. The difference was highly significant (p<0.001) when Amsel criteria was compared with culture.

Table IV shows the association of culture of G. vaginalis with Nugent criteria (Gram stain). Culture was positive in all 38(22.35%) cases out of 45(26.47%) BV positive cases by Nugent criteria. No more cases were positive in intermediate group and no case in normal flora group of Nugent criteria. The difference was highly significant (p<0.001), when culture of G. vaginalis and Nugent criteria were compared (Here intermediate group and normal flora group were considered as negative).

Discussion

Bacterial vaginosis is considered as a common vaginal disorder in women of reproductive age. 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 micro flora being linked to an increased susceptibility to the acquisition of HIV and other sexually transmitted infectious agents such as Neisseria gonorrhoeae and Chlamydia trachomatis[4,5].

In this study out of 170 study cases, 43(25.30%) cases were diagnosed as bacterial vaginosis by Amsel criteria, 45(26.47%) cases were positive by Nugent criteria.

On the basis of Amsel clinical criteria, a total of 43(25.30%) cases had been identified to have BV which is slightly lower than that of Navarrate P et al., Rangari et al. and Neelam et al. who reported 31.1%, 58% and 38.55% cases of BV respectively[6,7,8]. This slightly lower incidence in our study may be due to mandatory inclusion of clue cells on saline wet mount as a marker of BV

for every case, which makes the Amsel criteria more specific. Higher prevalence rates have been reported in previous studies too[9,10]. Factors responsible for higher prevalence of bacterial vaginosis among the study population were lower socio-economic status, improper sanitation, poor hygiene, malnutrition[11].

Among the individual criteria used to diagnose bacterial vaginosis, raised pH is recognized as the most sensitive but least specific criteria[10]. In the present study, the pH of the vaginal fluid was also found to be significantly associated with bacterial vaginosis. Majority of the patients with bacterial vaginosis had a pH between 5.0-5.5. Amsel et al also found a pH of more than 4.5 in 81 % cases of bacterial vaginosis[12]. 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[11]. Amine test is both highly sensitive and specific. 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 setting where microscopy is not available[13].

Significant association was found between clue cells and bacterial vaginosis which was in confirmation with earlier studies[11]. According to a previous study, the sensitivity and specificity of 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[11]. In our study, among BV cases diagnosed by Amsel criteria, 100% had clue cells positive on vaginal wet smear. That's why we have got the accurate results but the incidence of BV was slightly low.

In our study, the study cases were categorized into three groups according to Nugent criteria. Out of 170 study cases, 45 (26.47%) cases were diagnosed as BV, 58 (34.12%) cases as intermediate

group and 67 (39.41%) cases as normal flora group. The Nugent criteria with mandatory inclusion of clue cells in Gram's smear make the diagnosis reliable and specific. Nugent criteria with inclusion of clue cells had been able to identify 45(26.47%) BV patients. This is slightly higher than that of Udayalaxmi et al and Devi et al who reported 19% and 20.5% in India[14,15].

According to Rosenstein et al, the intermediate stage is considered a transitional phase and the patients may go on to frank bacterial vaginosis[16]. 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[17].

In this study vaginal specimen from study cases were subjected to culture in Human Blood Bilayer Tween (HBT) agar media a highly selective media, yielded growth of *G. vaginalis* from 22.35% of study cases. The isolation was higher than that of Devi et al and Udayalaxmi in India who reported 17.42% and 16.7% respectively[14,15]. Slightly higher rate reported by Gupta et al and Nahar et al might be due to the use of three or more media that were either non selective or enriched for primary isolation of *G. vaginalis* and variable methods for their identification[18,19].

In our study the antibiotic sensitivity pattern of G. vaginalis showed extreme variation. Gardnerella vaginalis showed high (52.63%) resistant to most commonly used metronidazole and 100% sensitive to clindamycin.

The association between incidences (22.35%) G. vaginalis by culture with bacterial vaginosis by amsel criteria (25.30%) and Nugent criteria (26.47%) are highly significant. Begum et al.and Akhter et al from BSSMU in Bangladesh reported similar findings and association[17,20]. But different study showed very low isolation rate of G. vaginalis 6% and 10.2%. Those studies showed slightly association between Gardnerella vaginalis with bacterial vaginosis. But Gupta et al in India, Nahar et al in Bangladesh and Pheirfer et al in UK who reported 54.1%, 38.98% and 91.44% bacterial vaginosis respectively[18,19,21]. They isolated 50%-90% G. vaginalis within positive bacterial vaginosis. They showed G. vaginalis was

the main causative organism of bacterial vaginosis. In our study positive bacterial vaginosis were 43(25.30%) or 45(26.47%). Within this 38(22.35%) cases were G. vaginalis. Only 5/7 cases were G. vaginalis negative. This is the great association between G. vaginalis with bacterial vaginosis. This study showed G. vaginalis had significant role in the bacterial vaginosis.

Conclusion
Early detection of causative agent and treatment of bacterial vaginosis appear to have a role in reducing the complications associated with this infection. Hence, it may be important to explore primary preventive strategies which target the risk factors or behaviours for bacterial vaginosis. Our study showed G. vaginalis is the principle causative agent of bacterial vaginosis. Though G. vaginalis is very fastidious bacteria and our laboratory setting is limited, it needs further trial for diagnosis and evaluation in clinical and laboratory settings. So detection of causative agent of bacterial vaginosis and proper antibiotic treatment will prevent recurrent bacterial vaginosis which will reduce drug resistance.

Disclosure
All the authors declared no competing interest.

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