Detection of bacterial vaginosis by simple method among the reproductive women attending Chittagong Medical college Hospital

NN Ara[1] M Sheran[2] MM Rahman[3], M Sultana[4]

Abstract

A cross sectional study was conducted on patients attending at the outpatient department of Gynaecology and Obstetrics of Chittagong Medical College Hospital, Chittagong during the period of July' 11 to June' 12. A total of 170 sexually active female in the age group of 15-45 years, with abnormal vaginal discharge were selected for the study. Among them 50 pregnant and 120 non-pregnant. A detailed history and a thorough clinical examination of all the cases were done. In this study bacterial vaginosis was detected by Amsel clinical criteria (clinical method), Gram stain Nugent criteria (Gold Standard), culture and by newly developed BV assay test. Out of 170 study cases, 43(25.30%) cases were diagnosed as bactrial vaginosis by Amsel criteria, 45(26.47%) cases were positive by Nugent criteria, 46(27.06%) cases were positive by BV assay test and 38(22.35%) cases were culture positive for Gardnerella vaginalis. Sensitivity of the clinical criteria (Amsel), BV assay test, and culture were 95.5%, 97.8% and 84.4% respectively in response to Gold standard Nugent criteria. Within these procedures BV assay test showed better result and sensitivity and can be done very easily.

Key words: Detection, bacterial vaginosis, reproductive women.

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Introduction

Bacterial vaginosis (BV) is the most prevalent vaginal infection in reproductive age women, and has been consistently associated with adverse outcomes related to the upper genital tract, and with increased risk of HIV acquisition[1]. Microbiologically, BV is polymicrobial disorder characterized by depletion of hydrogen peroxide-producing lactobacilli with profound overgrowth of anaerobic bacteria.

Bacterial vaginosis (BV) causes several complications affecting organs both in the pregnancy and non-pregnancy states. It is associated with numerous upper genital tract complications with significant maternal and fetal morbidity. Bacterial vaginosis during pregnancy has been linked to a increased risk of preterm rupture of membrane, preterm labour, or low birth weight deliveries, chorioamnionitis, postpartum endometritis and pelvic inflammatory disease etc[2]. Bacterial vaginosis is also associated with an increased risk of HIV-1 transmission in non-pregnant women and more susceptible to *Herpes simplex virus, Chlamydia trachomatis, Neisseria gonorrhoe,* and *Human papilloma virus* and post surgical infection[3].

Bacterial vaginosis is characterized by a thin, gray, homogenous, malodorous vaginal discharge in which complex alteration of vaginal flora with loss of the normally acidic (pH <4.5) vaginal environment that is dominated by hydrogen-peroxide producing Lactobacilli and an increase in concentration of other organisms[4], especially anaerobic gram negative rods like *Gardnerella vaginalis, Mobiluncus spp., Prevotella spp., Bacteroides spp., Peptostreptococcus, Fusobacterium* and *Atopobium vaginae* and *Mycoplasma species*[2]. Change in the normal vaginal flora causes change in pH which allows BV associated organisms like Gardnerella vaginalis and other anaerobes to overgrow and cause chronic infection and discharge.

Prevalence of BV varies with age, ethnicity, education and poverty. Among different study population such as US, Europe and South East Asian countries prevalence varies from 5-50%[5].The prevalence of BV in a developing country such as India is 20% to 47% in non pregnant women and up to 31% in pregnant women[6].

1. Dr. Nura Nasrin Rowshan Ara
Assistant Professor, (Microbiology)
Army Medical College, Chittagong.

2. Dr. Mohammed Sheran
Assistant Professor, (Microbiology)
Chattagram International Medical College.

3. Dr. Md. Mostafizur Rahman
Assistant Professor, (ENT)
Cox's Bazar Medical College.

4. Dr Munawar Sultana
Assistant Professor (Obs & Gynae)
Chittagong Medical College.

Correspondence
Dr. Nura Nasrin Rowshan Ara,
e-mail : nasrinsumi@yahoo.com

In Bangladesh the prevalence of bacterial vaginosis (BV) was 22.65%[7] and 23%[8]. Another study the prevalence of bacterial vaginosis was 16% in pregnant women & 30% in non-pregnant females attending primary healthcare-delivery units in Bangladesh[9].

Though bacterial vaginosis has various complications both in pregnant and non-pregnant women, so early diagnosis of BV is very essential for patients and physicians. Besides most of our people are poor and illiterate. We should find out a simple, easy and reliable diagnostic procedure. Various methods available for the diagnosis of bacterial vaginosis are Amsel's criteria, Nugent score, Hays/Ison system, Schimdt's scoring system, Spiegel's criteria, anaerobic culture, gas liquid chromatograpy, sialidase activity, DNA probes for Gardnerella vaginalis and PCR. In our study we tried to find out the simple, easy and accurate test for the clinician and poor patients of our country.

Materials & Methods

This was a cross-sectional study carried out in the department of Microbiology, Chittagong Medical College, Chittagong, during the period of July'11to June' 12. 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 was enrolled for this study.

Inclusion criteria

Women of reproductive age with in 15-45 years, both pregnant and non pregnant, with abnormal vaginal discharge, with or without mild vulver itching or burning are considered as patients.

Exclusion criteria

- Below 15 yr & over 45yrs.
- Known case of malignancy or AIDS patient.
- History of taking antimicrobial agents or vaginal medication for vaginitis within the last one month.
- Menstruating women.
- Patient having history of vaginal douche on the day of examination.

Procedure

Samples were collected with all aseptic precaution after taking informed consent from patient or her legal attendant. Three vaginal swab samples were collected from each patient by standard technique.

First swab sample

This swab sample used for making Gram's stain , amine test and wet mount preparation.

Second swab sample

This swab sample collected from left lateral vaginal wall for culture of 'G. vaginalis'.

Third swab sample

This swab sample collected from vaginal fornix and used for new rapid BV assay test. The second swab inoculated into a selective and differential Human blood bilayer Tween 80 (HBT) agar media and placed immediately in the candle extinction jar containing water soaked cotton at 37º c for 48 - 72 hrs. The plates were examined by oblique lighting after 24 hrs, 48 hrs, and 72 hrs.

Identification of G. vaginalis were done on the basis of their colony morphology, staining characters, Haemolysis production, Oxidase reaction , Catalase reaction , sugar fermentation and other relevant biochemical tests as per standard method.

Antimicrobial sensitivity by disc diffusion technique against different antimicrobial agents.

Detection of bacterial vaginosis by -

1. Amsel criteria
2.Nugent criteria
3. Bacterial vaginosis assay test
4. By culture of Gardenella vaginalis

Amsel clinical criteria

i) Presence of clue cell on saline wet mount.
ii) Positive amine (fishy) odour after adding 10% KOH to the vaginal discharge.
iii) Vaginal fluid with a pH >4.5
iv) Presence of thin, gray, homogenous, malodorous, adherent vaginal discharge.

Nugent criteria (Gram stain)

A standardized 0-10 point scoring system was done based on three bacterial morphotype:

- 1. Lactobacillus morphotypes, Gram positive rods.
- 2. Gardrenella vaginalis and Bacteroides spp. morphotype, small Gram-negative to variable rods.
- 3. Mobiluncus spp. morphotype curved Gram-variable rods.

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 i.e., 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".

Rapid test BV (Bacterial vaginosis) **Assay Test Kit:**

Procedure of BV assay test.

At first 6-8 drops of specimen diluents were added to test tube. Then the specimen swab was placed in test tube and washed thoroughly. After washing, the swab was discarded and the specimen solution was retained. After unwrapping the test tray and pressing the test tube and then the whole content of the specimen solution was added into the specimen window. When the specimen was fully absorbed, 4 drops of Extract Solution were added. The result was displayed in the test window within 5 minutes. For the first time, 4 drops of positive control or negative control were added to the specimen window. After control was fully absorbed, 4 drops of Extract solution was added and the result was displayed in the test window within 5 minutes.

Result

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.

Out of 170 cases (Table-1), the highest 85(50%) cases were within 25-35 years age group, followed by 61 (35.89%) were within >35 years age group and the lowest 24(14.11%) were within < 25 years age group. Mean age was ± SD: 32.44 ± 6.70 years.

Table-I: Distribution of study population according to age groups and pregnancy status (n = 170)

Age in groups	Pregnancy Status		Total
	Pregnant	Non-pregnant	
<25 Years	04 (2.35)	20 (11.76)	24 (14.11)
25 - 35 Years	44 (25.88)	41 (24.12)	85 (50.00)
>35 Years	02 (1.18)	59 (34.71)	61 (35.89)
Total	50 (29.41)	120 (70.59)	170 (100.00

- Figures within parentheses indicate percentages
- Mean ± SD : 32.44 ± 6.70 Years; Median = 34.00 Years; Range : 19 - 45 Years

On the basis of Amsel criteria, the study cases were categorized into two groups: 43(25.30%) were bacterial vaginosis (BV) and 127(70.59%) were other than BV (Table-II).

Table-II: Distribution of bacterial vaginosis in pregnant and non-pregnant 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 percentages

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

Table-III: Distribution of bacterial vaginosis in pregnant and non-pregnant on the basis of Nugent criteria.

Pregnancy Status	Total	Nugent Criteria		
		Bacterial vaginosis	Intermediate	Normal Flora
Pregnant	n=50	14 (28.00)	12 (24.00)	24 (48.00)
Non-pregnant	n=120	31 (25.83)	46 (38.33)	43 (35.83)
Total	170 (100.00)	45 (26.47)	58 (34.12)	67 (39.41)

- Figures within parentheses indicate percentages

Figur 1 shows the results of BV assay test. Out of 170 cases, 46 (27.06%) cases were BV assay test positive and rest 124 (72.94%) were negative.

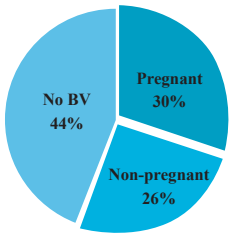

Fig-1: Distribution of bacterial vaginosis by BV assay test among pregnant and non-pregnant

Table-IV shows that culture of vaginal fluid yielded growth of G. vaginalis in 38 (22.35%) cases. The isolation of G. vaginalis was slightly

higher among non-pregnant cases 28 (23.33%) than in pregnant cases 10 (20.00%).

Table-IV: Results of culture of G. vaginalis among the in pregnant and non-pregnant.

Pregnancy Status	Total	Culture of *G. vaginalis*	
		Positive	Negative
Pregnant	n=50	10 (20.00)	40 (80.00)
Non-pregnant	n=120	28 (23.33)	92 (76.67)
Total	170 (100.00)	38 (22.35)	132 (77.65)

• Figures within parentheses indicate percentages

Figur 2 shows the results of the individual methods like Amsel criteria, BV assay test and culture were compared with Nugent criteria (Gold standard) to determine the sensitivity and specificity of each method. The sensitivity of BV assay was higher than that of Amsel criteria (97.8% vs. 95.5%) and culture (97.8% vs. 84.4%). The BV assay test had excellent sensitivity and specificity in respect of Gram-stain. The sensitivity was very high (97.8%) and the specificity was also high (98.1%) and acceptable.

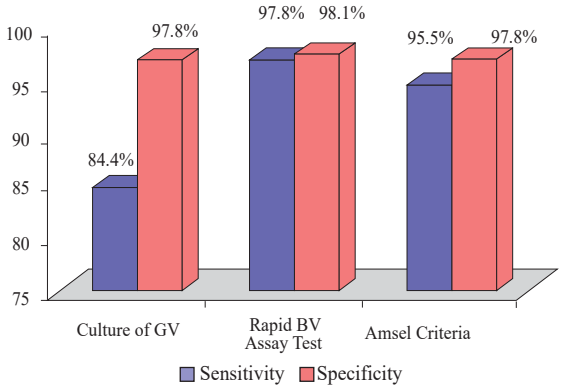

Fig-2: Evaluation of Amsel criteria, rapid BV assay test and culture in respect to Nugent criteria.

Figur 3 shows the distribution of BV cases in different age groups.Total 47 positive bacterial vaginosis were found by all methods. The positive BV cases were found higher 28 (59.57%) in 25-35 years age group, followed by 14 (29.79%) in > 35 years and 5(10.70%) in < 25 years age group.

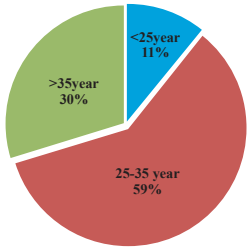

Fig-3: Distribution of bacterial vaginosis cases by all methods in different age groups (n = 170)

Fig: Distribution of BV in different age groups

Discussion

Bangladesh has a high burden of reproductive morbidity. Bacterial vaginosis has been documented as a risk factor for adverse birth outcomes and many other gynaecological complications. Find out a simple, accurate and easy procedure might be useful for the reproductive women.

On the basis of Amsel clinical criteria, among the 170 study cases, a total of 43 (25.30%) cases had been identified to have BV, which is slightly lower than that of Navarrate P et al[10] Rangari et al[11] and Neelam et al[12] who reported 31.1%, 58% and 38.55% cases of BV respectively.

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. Among BV cases diagnosed by Amsel criteria, 100% had clue cells on vaginal wet smear and other associated markers like amine odour and raised pH (>4.5) were present very high percentage of cases (90-95%). Although raised pH is one of the important criteria for Amsel method of diagnosis for BV but a number of normal cases had a pH above 4.5 and a good number of cases had associated homogenous vaginal discharge without showing other criteria and does not fall in BV group.

Ajani et al[13] studied all pregnant women, including those with HIV/AIDS and in the third trimester of pregnancy. In this study, we excluded women with medical conditions including HIV/AIDS and studied women between 15 and 45 years.

In our study, according to Nugent criteria we found 45 (26.47%) cases were 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 easy, reliable and specific. Our result was slightly higher than that of Udayalaxmi[14] et al and Devi et al[15] who reported 19% and 20.5% in India and lower than that of Chawla et al[16] in India, Navarrete et al[11] and Munjoma[17] in USA which were 32.86%, 31.8%, and 34% respectively. In Bangladesh Begum et al[9] and Bilkis[7] in BSSMU reported 23% and 22.63% respectively. A slightly higher rate might be attributed to non-inclusion of clue cells in their study, while a slightly lower rate might probably be due to study on pregnant cases

only[7].

A new rapid test the BV Assay test was done in vaginal fluid for diagnosis of bacterial vaginosis. The test was found 46 (27.06%) positive out of 170 cases. Our result was consistent with those of Carlson[18] and Posner et al[19] who reported 25% and 30% respectively.

In this study we isolated G. vaginalis from vaginal specimen by in Human blood bilayer tween (HBT) agar media, a highly selective media and yielded growth of 22.35%. The isolation was higher than that of Devi et al[15] and Udayalaxmi[14] in India who reported 17.42% and 16.7% respectively, but lower than that of Gupta et al[20] in India and Nahar et al[21] in Bangladesh who reported 54.1%, 38.98% respectively. Begum et al[9], Shameem[8] from BSSMU in Bangladesh reported similar findings 25.5% and 21% respectively. This slightly higher rate reported by Gupta et al[20] and Nahar et al[21] 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.

In our study we found total 47 positive bacterial vaginosis by all methods. Out of them found higher 59.57% in 25-35 years age group, followed by 29.79% in > 35 years age group and 10.70% in < 25 years age group in the present study. By this findings it is clear 25-35 years age group is the highly risk group for the bacterial vaginosis. Similar findings were also reported by Yusuf et al[22], Shameem[8] and Bilkis[7] in Bangladesh and Sangeetha et al[23], Bhalla et al[24] in India.

The results of the individual methods like Amsel criteria, BV assay test and culture were compared with Nugent criteria (Gold standard) to determine the sensitivity and specificity of each method. The BV assay test had excellent sensitivity and specificity in respect of Gram-stain. The sensitivity was very high (97.8%) and the specificity was also high (98.1%) and acceptable. The sensitivity of BV assay was higher than that of Amsel criteria (97.8% vs. 95.5%) and culture (97.8% vs. 84.4%), though a slightly lower specificity had been obtained in Amsel criteria (98.1% vs. 100%) and culture (98.1% vs. 100 %). A similar result was also reported by Miller[25], Carlson[18] and Prosner et al[19].

The most commonly used clinical method is Amsel criteria but all the parameters of this criteria except pH are either subjective or technically difficult[7]. Preparation for Gram-staining is simple compared to most diagnostic laboratory methods, but it still requires a trained personnel for the assessment of the slides, which could be the major drawback. Though bacterial vaginosis polymicrobial disease, so culture of G. vaginalis is not specific. Microbiological confirmation of these organisms are difficult, time consuming and impractical for service laboratories. On the other hand BV assay test is simple, rapid bed side test and can be done within 5 minutes with almost same sensitivity and specificity.

Conclusion

Result of BV assay test is better than other methods. Its sensitivity and specificity are excellent with the advantage of being technically simple and rapid. This assay is rapid, does not require special instrument, is highly sensitive, easy to operate, accurate and the cost is low. Bacterial vaginosis is more prevalent in highly safe child bearing period (25-35). The majority of reproductive women at the greatest risk for the sequelae of BV, they would greatly benefit from access to BV assay test.

Recommendation

Therefore, we recommend as follows: Pregnant and non pregnant women attending outpatient department of Chittagong medical college hospital should be screened routinely for BV to avoid infection sequelae. Adequate laboratory facilities should be provided. This will aid prompt and adequate diagnosis of BV in pregnancy and also in non-pregnant. Effort should be made to discourage promiscuity among sexually-active age group and self diagnosis/medication among pregnant women.

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