

DETECTION OF BACTERIAL VAGINOSIS AND COMPARISON OF CLINICAL & MICROBIOLOGICAL METHODS AMONG WOMEN ATTENDING CHITTAGONG MEDICAL COLLEGE HOSPITAL

Nura Nasrin Rowshan Ara^{1*} Md Anwar Husain² Nasima Akter³ Md Mostafizur Rahman⁴
Abu Hena Md Saiful Karim Chowdhury⁵

Summary

Bacterial vaginosis is a special public health concern in the world because of high burden of reproductive and pregnancy related morbidity. This present study was undertaken to detect the bacterial vaginosis in reproductive women by different clinical and microbiological methods on patients attending at the out patient department of Gynaecology and Obstetrics of Chittagong Medical College Hospital. A total of 170 sexually active female in the age group of 15-45 years, with abnormal vaginal discharge were selected for the study. 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 and compared these methods. 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, 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. All these findings make the BV assay test more accurate and reliable screening procedure for the diagnosis of bacterial vaginosis which help our physicians and patients.

Key words

Bacterial vaginosis; Amsel criteria; Nugent criteria; Bacterial vaginosis assay test; Gardnerella vaginalis.

Introduction

Bacterial vaginosis is the most common lower genital tract disorder among women of reproductive age (Pregnant and non-pregnant) and the most prevalent cause of vaginal discharge and malodour. It has been associated with a significant number of obstetric and gynaecologic complications, such as preterm labour and delivery, preterm premature rupture of membranes, spontaneous abortion, chorioamnionitis, postpartum endometritis, post caesarean delivery wound infections, postsurgical infections, and subclinical pelvic inflammatory disease [1]. 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 (HPV) and post surgical infection [2].

Bacterial vaginosis is a polymicrobial disease. It is a disorder of the vaginal ecosystem characterized by a shift in the vaginal flora from the normally predominant Lactobacillus spp. to one dominated by a mixed flora including Gardnerella vaginalis, Mobiluncus spp. Prevotella spp. Bacteroides spp. Peptostreptococcus, Fusobacterium and Atopobium vaginae and Mycoplasma species [3].

The prevalence of Bacterial Vaginosis (BV) varies widely from 5 to 51 percent in different population [4]. In India the prevalence of bacterial vaginosis is 20% to 47% and in Bangladesh BV were 22.65, 23% and 30% [5-8]. It has an extremely high recurrence rate and in some women it causes relapses and remits spontaneously. Better understanding of the factors that lead to the development of BV is needed to prevent relapse [9]. Though bacterial vaginosis is hazardous, has more complications and causes relapse, so early and accurate diagnosis of BV is very essential for patients and physicians.

1. Assistant Professor of Microbiology
University of Science & Technology Chittagong (USTC).
2. Professor of Microbiology (Retired)
Chittagong Medical College, Chittagong.
3. Professor of Microbiology
Chittagong Medical College, Chittagong.
4. Junior Consultant of Otolaryngology
Upazilla Health Complex, Lohagara, Chittagong.
5. Lecturer of Microbiology
Chittagong Medical College, Chittagong.

*Correspondence: Dr. Nura Nasrin Rowshan Ara
E-mail: nasrinsumi@yahoo.com
Cell : 01819 850606

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 chromatography, sialidase activity and DNA probes for *Gardnerella vaginalis*. In our study we detected the bacterial vaginosis by Amsel's clinical criteria and other microbiological laboratory method like Nugent score, culture and simple laboratory test BV assay test. And by comparing these methods we tried to find out the simple, easy and accurate test for diagnosis of bacterial vaginosis which help the clinicians and poor patients of our country.

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 statistical analysis was done by standard statistical procedure Statistical Package for Social Sciences (SPSS).

Inclusion criteria

Women of reproductive age within 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

- i) Below 15 yr & over 45yrs
- ii) Known case of malignancy or AIDS patient
- iii) History of taking antimicrobial agents or vaginal medication for vaginitis within the last one month
- iv) Menstruating women
- v) 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 was collected from right vaginal wall and used for making Gram's stain, 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 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 37^oc for 48 – 72 hours. 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 was done by disc diffusion technique against different antimicrobial agents.

Detection of bacterial vaginosis by different clinical and microbiological (Laboratory) methods:-

- i) Amsel criteria
- ii) Nugent criteria
- iii) Bacterial vaginosis assay test
- iv) By culture of *Gardnerella vaginalis*

Amsel clinical criteria (Group of clinical parameter)

- 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:

- i) *Lactobacillus* morphotypes, Gram positive rods.
- ii) *Gardnerella vaginalis* and *Bacteroides* spp. morphotype, small Gram-negative to variable rods.
- iii) *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.

Results

A total of 170 women, 50 pregnant and 120 non-pregnant, clinically suspected cases of Bacterial Vaginosis (BV) aged between 15-45 years with abnormal vaginal discharge, with or without mild vulver itching or burning were included in this study.

Out of 170 cases, on the basis of Amsel criteria (clinical criteria), 43(25.30) cases were bacterial vaginosis (BV) positive and 127(74.70%) BV negative. On the basis of Nugent criteria 45(26.47%) were BV positive and 125(73.53%) BV negative. The results of BV assay test shows 46 (27.06%) cases were BV assay test positive and rest 124(72.94%) were negative. Culture of vaginal fluid yielded growth of G. vaginalis in 38(22.35%) cases and 132(77.65%) cases were culture negative (Fig 1).

The results of Amsel criteria and Nugent criteria (Gram stain) are compared. Amsel criteria were positive in all 43(25.30%) 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 Amsel criteria and Nugent criteria were compared (Here intermediate group and normal flora group were considered as negative) [Table I].

Table II Shows the comparison of BV assay test and Amsel criteria. The BV assay test was positive in all 43 positive cases of bacterial vaginosis by Amsel criteria and 03(1.76%) additional cases were positive by BV assay test out of 127(74.70%) Amsel criteria negative cases. The difference was highly significant ($p < 0.001$), when BV assay test was compared with Amsel criteria.

Table III Shows the comparison 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.

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).

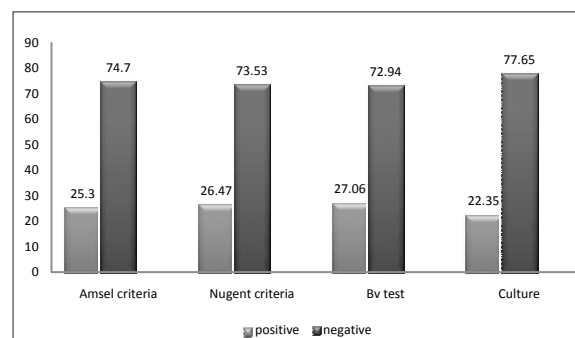


Fig 1: Distribution of study population by different methods

Table I : Comparison of Amsel clinical criteria and Nugent criteria for the diagnosis of bacterial vaginosis (n=170)

Nugent Criteria	Amsel clinical criteria		Total
	Positive	Negative	
Bacterial vaginosis	43 (25.30)	02 (1.17)	45 (26.47)
Intermediate	00 (00)	58 (34.12)	58 (34.12)
Normal Flora	00 (0.0)	67 (39.41)	67 (39.41)
Total	43 (25.30)	127 (74.70)	170 (100.00)

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

Table II : Comparison between rapid BV assay test and Amsel criteria (with χ^2 test significance)

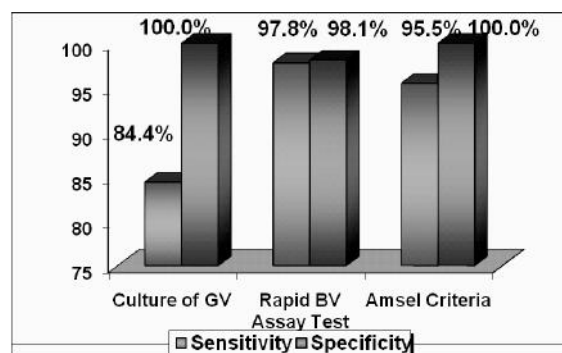
Amsel Criteria	Rapid BV Assay Test		Total
	Positive	Negative	
Bacterial Vaginosis	43 (25.30)	00 (0.00)	43 (25.30)
Other than B.Vaginosis	03 (1.76)	124 (72.94)	127 (74.70)
Total	46 (27.06)	124 (72.94)	170 (100.00)

- Figures within parentheses indicate percentages
- $\chi^2=150.252$, $p=0.000$; Highly Significant ($p<0.0001$)

Table III : Comparison between Amsel clinical criteria and culture of *G. vaginalis* for the diagnosis of bacterial vaginosis (n=170).

Culture of <i>G. vaginalis</i>	Amsel clinical criteria		Total
	Positive	Negative	
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 ($p<0.001$)

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

Discussion

Bacterial vaginosis is associated with significant obstetric and gynaecological complications. The prevalence of BV ranges from 4 to 64%, depending on the racial, geographic and clinical characteristics of the population [10]. So early and accurate diagnosis of BV is essential to control BV and to reduce its complications in the general population. The diagnosis of bacterial vaginosis is difficult and controversial due to its polymicrobial nature. The most commonly used clinical method is Amsel criteria which are known as Amsel clinical criteria. But all the parameters of this criteria except pH are either subjective or technically difficult [7]. Examination of a Gram-stained vaginal smear (Nugent criteria) is a quick and relatively simple means of diagnosis. Although preparation for Gram-staining is simple compared to most diagnostic laboratory methods, it still requires a trained personnel for the assessment of the slides, which could be the major drawback [6]. Though bacterial vaginosis polymicrobial disease, so culture of *G. vaginalis* is not specific. Besides this, *G vaginalis* is present in healthy vaginal environment also. Microbiological confirmation of these organisms are difficult, time consuming and impractical for service laboratories. So it is not possible to identify any single species as the cause of BV. To overcome this situation, we also used a rapid bed side as well as laboratory based test called BV assay test. 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 Rangari et al and Neelam et al who reported 31.1%, 58% and 38.55% cases of BV respectively [11-13].

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.

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 et al and Devi et al who reported 19% and 20.5% in India and lower than that of Chawla et al in India, Navarrete et al and Munjoma in USA which were 32.86%, 31.8%, and 34% respectively [11,14,17]. In Bangladesh Begum et al and Bilkis in BSSMU reported 23% and 22.63% respectively [6,8]. 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 and Posner et al, who reported 25% and 30% respectively [9,18].

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, but lower than that of Gupta et al [14,15,19]. In India, Nahar et al in Bangladesh, and Pheirfer et al in UK who reported 54.1%, 38.98%, and 91.44% respectively [20,21,8,7]. Begum et al Shameem from BSSMU in Bangladesh reported similar findings 25.5% and 21% respectively. This 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.

The results of clinical criteria (Amsel criteria), was compared with Nugent criteria (Gold standard). The difference was highly significant ($p < 0.001$) when Amsel criteria was individually compared with Nugent criteria. Bilkis, Shameem in Bangladesh and Udayalaxmi et al.¹⁴ in India reported similar findings [6,7,14].

When the Amsel criteria (Clinical method) was compared with BV assay test, the BV assay test was found positive 43 (100%) in all 43 BV positive cases. Three cases were positive out of 127 negative cases. The difference was highly significant ($p < 0.001$). This finding represents that BV assay test was better diagnostic tools than clinical method for the diagnosis of bacterial vaginosis.

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, Carlson and Prosner et al [22,9,18].

So out of these tests, in comparison between clinical and different microbiological methods, we found better results and sensitivity by microbiological methods except culture. Clinical methods are group of parameters and technically difficult. Culture is time consuming and difficult. But BV assay test is simple, rapid bed side test and can be done within 5 minutes with almost same sensitivity and specificity. The advantage being that this test can be used for screening large number of outpatient attending hospital.

Conclusion

The majority of women at the greatest risk for the sequelae of BV, they would greatly benefit from access to detect the bacterial vaginosis early and by reliable methods. The BV assay test shows excellent result and is suitable for routine use in laboratory than other clinical and microbiological methods. But its specificity is less than clinical criteria. Our study population is limited and study subject were selected from the Hospital outpatient department which is sometimes may be non-representative of general population. So it needs further trial for evaluation in clinical and laboratory settings. But A rapid and reliable method like BV assay test help the clinicians to detect bacterial vaginosis patients early and to manage them properly.

Disclosure

All the authors declared no competing interest.

References

1. Yudin, MH & Money, DM. Screening and management of bacterial vaginosis in pregnancy. *J of Obstetrics and Gynaecology Canada*, Toronto. 2008; 30(8):702-716.
2. Watts, DH, Fazzari, M, Minkoff, H, Hillier, SL, Sha, B, Glesby, M, Levine, AM & Burk, R. Effects of bacterial vaginosis and other genital infections on the natural history of human papillomavirus infection in HIV-1-infected and high-risk women. *J Infect Dis*. 2005; 191: 1129–1139.
3. Ling, Z, Kong, J, Liu, F, Zhu, H, Chen, X, Wang, Y, Li, L, Nelson, KE, Xia, Y & Xiang, C. Molecular analysis of the diversity of vaginal microbiota associated with bacterial vaginosis. *BMC Genomics*. 2010; 11: 488.
4. Koumans, EH, Sternberg, M & Bruce, C. The prevalence of bacterial vaginosis in the United States, 2001–2004; associations with symptoms, sexual behaviors, and reproductive health. *Sex Transm Dis*. 2007; 34: 864–869.
5. Puri, KJ, Madan, A & Bajaj, K. Incidence of various causes of vaginal discharge among sexually active females in age group 20-40 years. *Indian J Dermatol Venereol Leprol*. 2003; 69: 122-125.
6. Bilkis, B. Rapid laboratory diagnosis of BV by New prolin Aminopeptidase assay and comparison with conventional methods, M.Phil.Thesis, Department of microbiology Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. 2003.
7. Shameem, A. Rapid laboratory diagnosis of bacterial vaginosis by BV Blue test and comparison with conventional methods, M. Phil. Thesis, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. 2006 .
8. Begum, A, Nilufar, S, Akther, K, Rahman, A & Khaton, F. Prevalence of selected reproductive tract infections among pregnant women attending an urban maternal and childcare unit in Dhaka, Bangladesh. *J Health Popul Nutr*. 2003; 21(2):112-116.
9. Carlson, M. The accuracy of Wet Mount compared to Fem Exam in Diagnosing Bacterial vaginosis. Institute of Midwifery, Women & Health ,viewed 05 September 2013. Available from <http://www.instituteofmidwifery.org/MSFinalProj.nsf/82fd56378e8ef>.
10. Guaschino, S, De Seta, F, Piccoli, M, Maso, G & Alberico, S. Aetiology of preterm labour: bacterial vaginosis. *BJOG*. 2006; 113(3): 46-51.
11. Navarrete, P, Dominguez, M, Castro, E & Zelman, R. Evaluation of Nugent and Amsel criteria for the diagnosis of bacterial vaginosis. *Rev Med Chil*. 2000;128(7): 767-771.
12. Rangari, AA, Singh, P & Sharma, VK . Comparison of the Amsel's composite clinical criteria and Nugent's criteria for diagnosis of Bacterial vaginosis: A step towards preventing mis-diagnosis. *J of Advance Researches in Biological Science*. 2013; 5(1): 37-44.
13. Neelam, S & Sohail, I. Rapid Clinical Diagnostic tests for bacterial vaginosis and its predictive value. *International J of Pathology*. 2010; 8(2): 50-52.
14. Udayalaxmi, G, Subbannayya, K & Shalini, S. Comparison of the methods of bacterial diagnosis of bacterial vaginosis. *J of Clinical and Diagnostic Research*. 2011; 5: 498-501.
15. Devi, PS, Rao, Ashriyan, M, Rajaram, K & Jagadish, C. Diagnosis of Bacterial vaginosis in a rural setup. *Indian J of Medical Microbiology*. 2004; 22(1): 47-50.
16. Chawla, R, Bhalla, P, Chanda, S, Grover, S & Garg, S. Comparison of Hay's criteria with Nugent's scoring system for diagnosis of Bacterial vaginosis. *Biomed Res Int*, viewed 02 January 2014. Available from <http://www.ncbi.nlm.nih.gov/pubmed/23841066>.
17. Munjoma, MW. Simple method for the detection of bacterial vaginosis in pregnant women. viewed 01 September 2013. Available from <http://hdl.handle.net/10852/30100>.
18. Posner, SF, Kerimova, J, Aliyeva, F & Duerr, A . Strategies for diagnosis of bacterial vaginosis in a resource – poor setting. *International J of STD & AIDS*. 2005; 16: 52-55.
19. Gupta, BK, Kumar, R, Sofat, R, Khurana, S & Deepinder . The role of Gardnerella vaginalis in non-specific vaginitis in intrauterine contraceptive device user. *Indian J of pathology and microbiology*. 1998; 41(1): 67-70.
20. Nahar, A, Rahman, KM, Hassan, MM & Haq JA. Studied on the anaerobic vaginosis among the Bangladeshi women of reproductive age group. *Bangladesh Med Res Counc Bull*. 1994; 20: 129-138.
21. Pfeifer TA, Forsyth PS, Durfee, Ma Pollock, HM & Holmes KK. Non specific vaginitis, role of Haemophilus vaginalis and treatment with metronidazole. *N Engl J Med*. 1978; 298: 1429-1434.
22. Miller, L. Can Fem Exam card use facilitate bacterial vaginosis diagnosis on data of abortion to prevent post abortion endometritis. *The American College of Obstetricians & Gynecologists*. 2001; 97(4): S58-S59.