
Diagnosis of Bacterial Vaginosis by Amsel Criteria and Gram Stain Method

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Abstract:

Objective: To estimate the prevalence of bacterial vaginosis by Nugent Gram stain in symptomatic women attending Obstetric and Gynaecologic clinic in Tarhuna city- Libya, to evaluate Amsel criteria against Gram stain in diagnosis of bacterial vaginosis, and to examine clinical association between some factors and this disease.

Material and Methods: This study was carried out on patients attending the Obstetric and Gynaecologic clinic in Tarhuna city for 4 months during the period between 1st of May and 31 of August 2006. From 350 women who have the criteria for this study, 300 women were agreed to enter this study. Inclusion criteria includes: age between 16 years and 50 years, pregnant women, and symptoms of vaginal infections as abnormal vaginal discharge, vulval itching, dysuria, and lower abdominal pain. Exclusion criteria were antibiotics and/ or local vaginal treatment within 14 days before taking the sample, hysterectomy, and sexual intercourse within 8 hours before the examination. Clinical data consisting of Whiff test, clue cells, and appearance of vaginal discharge (Amsel criteria) were compared with vaginal fluid Gram stain (Nugent criteria) for diagnosis of bacterial vaginosis.

Results: The prevalence of bacterial vaginosis was 25.1%. The sensitivity and specificity of Amsel criteria and Gram stain were 67.8% and 97.7% respectively. Clinically, bacterial vaginosis was directly related to vaginal douching (P value < 0.001).

Conclusion: Amsel criteria had low sensitivity in compare with Gram stain for diagnosis of bacterial vaginosis.

Key words: Bacterial vaginosis, Diagnosis, Amsel criteria, Gram stain.

Introduction:

Vaginitis is one of the most common problems in clinical practice, and the leading reason for a patient to visit her gynaecologist. It is recognized that some forms of Vaginitis can lead to obstetric and gynaecologic complications.¹ It has been certain that vaginal infections are associated with increased risk of human immunodeficiency virus acquisition.²

Vaginal infection is most often caused by three conditions:

Bacterial vaginosis

Candidacies

Trichomoniasis

Most studies performed in North America and northern Europe have shown bacterial vaginosis to be prevailing cause of Vaginitis, followed by Candidacies while the prevalence of Trichomoniasis is declining.³

History of bacterial vaginosis:

The original phase of bacterial vaginosis arose from advancements in descriptive

microbiology which started in 1894. In that year Doderline described the presence of lactobacilli in normal vaginal flora.⁴ Normal vaginal flora of both pregnant and non-pregnant women is characterized by high concentration of hydrogen peroxide-producing Lactobacilli.

Lactobacilli fulfil a central role in vaginal haemostasis by producing lactic acid which has long been considered to constitute the primary microbiological barrier against infection by genital pathogens. Facultative Lacto- bacilli make up 50% to 90% of aerobic vaginal flora in women. Lactic acid inhibits the growth of micro-organisms by maintaining a low PH. In 1914 Cutis associated black pigmented Bactericides, a curved rod now known as Mobiluncus and anaerobic cocci with abnormal vaginal discharge.⁴

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In 1921 Schroder categorized vaginal flora using gram stain into the least pathologic (lactobacilli), a less frequent intermediate stage, and a stage of vaginal flora considered the most pathogenic (now known as B. V).⁴

Weaver et al.⁴, was also confirmed the absence of Lactobacilli in this syndrome which was known at that time as non-specific Vaginitis.

A non motile, pleomorphoc gram-negative rods was isolated from the urethral discharge of men with prostates by Leopold in 1953.⁴ He also isolated the organism from vaginal discharge of women considered to have cervicitis. In 1954 Moore⁵ discussed characteristic of anaerobic vibrio wet preparations and cultures from vaginal specimens obtained from patients of infertility clinic.

In 1955 Gardner and Dukes published a classic clinical epidemiology paper on Vaginitis. They describe a new vaginal syndrome initially known as 'non-specific Vaginitis', and identified knew organism, called *Haemophilus vaginalis*, which was though to be the causal agent. It was for a short time known as *Corynebacterium vaginale* and is know identified as *Gardeneralla vaginalis* which has been repeatedly associated with this syndrome since its original description.^{6,7} They also described the clinical features of this syndrome that forms the basis of diagnosis today.

However over the next 30 years, particularly since the use of selective culture media, it became apparent that *G. vaginalis* can be detected in up to 40% to 50% of otherwise normal women without signs or symptoms of vaginal discharge⁸ and further that anaerobic organisms (most notably *Bactericides* species and *Mobiluncus* sp.) and *Mycoplasma hominis* were also highly associated with the syndrome described by Gardner and Dukes.⁹

The concentration of *Gardeneralla vaginalis* and anaerobes in vaginal fluid is 100 to 1000 times higher in women with this syndrome than that in control women without signs of vaginal infection.^{10,11}

The term bacterial vaginosis (BV) has been advocated in place of original term non-specific Vaginitis to reflect the complex alteration of bacterial flora and to connote the presence of increased discharge without an apparent inflammatory response.^{4,6}

In 1980 Durieux and Dublanchet reported the first microbiological characteristics of highly motile, anaerobic bacteria isolated from patients with motile rods in their abnormal vaginal discharge. Other investigators have confirmed the presence of highly motile, anaerobic rods in patients with Vaginitis.⁵

Definition and prevalence:

Bacterial vaginosis (B.V) is defined as an alteration of the vaginal ecosystem characterized by an overgrowth of several anaerobic bacteria; including: *Gardeneralla vaginalis*, *Prevotella* spp, *Mobiluncus*, Gram positive cocci, and *Mycoplasma* and by reduction or absence of lactobacilli.¹¹

B. V is a syndrome marked by an increased vaginal PH over 4.5, thin, milk-like vaginal discharge, and amine or fishy odour. Up to 50% of women are asymptomatic. Microbiologically, bacterial vaginosis is characterized by a shift in the vaginal flora from dominant flora of *Lactobacillus* spp. to a mixed flora that includes *G. vaginalis*, *Bactericides*, *Mobiluncus* spp., and *Mycoplasma hominis*.¹²

It thought to be the most prevalent cause of vaginitis among reproductive aged Women. It occurs among 35% of women attending STD clinic,^{4,13} 15 to 20% of pregnant women,¹⁵ and 5 to 15% of women attending Gynaecologic clinic^{6,16} It has been estimated that the prevalence of bacterial vaginosis in the general population in the UK of women in their reproductive years is 10%. This is similar to the prevalence of 12% found in other study.^{11,18} The surveillance of bacterial vaginosis in the UK is restricted to cases diagnosed through genitourinary medicine.

Detection of bacterial vaginosis in pregnancy may be of relevance as this condition has been consistently associated with increased risk of prematurity. In USA, it has been found that the prevalence of bacterial vaginosis is between 15% and 30% in non-pregnant women with African American women being particularly at risk.¹⁹

Complications:

Bacterial vaginosis is associated with adverse pregnancy outcomes as; recurrent miscarriage, premature rupture of membranes, premature birth, chorioamnionitis, and delivery of low birth weight independent of other risk factors.²⁰

Also, B.V is associated with cervical intraepithelial neoplasia, and upper genital tract infections as pelvic inflammatory disease, endometritis, post gynaecologic-surgery infections, cervicitis, and urinary tract infections.²¹

Diagnosis of bacterial vaginosis:

The first standard technique for diagnosing bacterial vaginosis was proposed by Amsel et al.¹⁶ It was widely used because it is simple, inexpensive, and can be easily done by the clinician.

Amsel et al. proposed that B.V was present if three of four criteria were present:

- Homogenous, thin, milk-like vaginal discharge
- Vaginal PH >4.5
- Clue cells
- Fishy odor on alkalization of vaginal Secretions

Alternatively, bacterial vaginosis can be diagnosed by using standardized scoring systems to interpret a Gram stain of vaginal secretion.

The first standardized method of interpreting Gram stained vaginal smears for bacterial vaginosis was proposed by Spiegel et al.²² and later modified by Nugent et al.¹² to include an intermediate classification.

The aim of this study:

To estimate the prevalence of bacterial vaginosis by Nugent Gram stain criteria in

women attending obstetric and gynaecologic clinic in Tarhuna city-Libya, to evaluate Amsel criteria against Gram stain in the diagnosis of bacterial vaginosis, and to examine clinical association between some factors and this infection.

Sitting:

Tarhuna city- Libya

Materials and methods:

The data was collected from patients attended the out-patients clinic at Tarhuna city for four months between 1st of May 2006 and 31 of August. Patients were eligible if they were between 16 and 50 years old.

Among 350 eligible women, 300 (85%) agreed to participate in the study. 50 patients were excluded because they had antibiotics, or used vaginal antimicrobial medication in the previous 14 days, had hysterectomy, or had sexual intercourse within 8 hours before collecting the sample. Another 15 patients were excluded because they were menstruating.

A standardized medical, obstetric, sexual, social, and culture of the vagina and cervix were obtained. Specific complains of vulval itching, presence of unusual odour or discharge, and abnormal bleeding were noted.

Vaginal examination and sample collection:

Patients were interviewed and examined by residents working according to the programmed schedule. Pelvic examination under a septic technique was performed with specific attention to the vulva, vagina, and cervix (erythema, friability of the cervix, colour of the cervical mucus). Characters of vaginal discharge as amount, colour, and odour were noted and recorded.

Three samples of vaginal secretions were obtained with saline solution- moistened Swabs. The samples were prepared and examined by one microbiologist.

The 1st sample was used to measure the vaginal PH by using colourpHas indicator strips (Macherey- Nagel Germany) with a

range of PH from 3.8 to 5.8. Odour was tested by spreading the swab containing vaginal secretion on clean slide and then adding a drop of 10% potassium hydroxide solution and smelling the slide.

The test is considered positive if it has a fishy amine-like odour. The slide then covered with a cover slip and examined under light microscope to check the presence of hyphae.

The second swab was immersed in 0.5ml of 0.9% physiological saline and a drop was located on a glass slide for wet mount preparation. The specimen was examined under-light microscope at x400 for bacterial morphologic types, clue cells, yeasts, and trichomonads. Wet mounts preparation were prepared within 15 minutes of collection and analyzed immediately.²³

Clue cells were identified as vaginal epithelial cells with such a heavy coating of bacteria that the peripheral borders were obscured. The number of clue cells and normal epithelial cells were counted. The specimen was considered adequate if

at least 10 epithelial cells per high power field were seen. The specimen was considered positive if at least one clue cell per high power field (x400).²³

The vaginal gram stains were made of vaginal secretion obtained by 3rd swab. The gram stain interpretation of Nugent et al. was used. The criteria developed by Nugent et al. were a scoring system from 0 to 10 which allowed for gradations in the severity of bacterial vaginosis.

The scoring criteria give the quantitation;

- 0 no morph types
- 1+ less than 1 morph type
- 2+ 1 to 4 morph types
- 3+ 5 to 30 morph types
- 4+ 30 or more

Of the following bacterial group:

Gram positive rods (Lactobacillus)

Gram negative to variable rods (Gardeneralla vaginalis, and Bactericides spp)

Gram negative rods (Mobiluncus spp)

When the total score is 7- 10 bacterial vaginosis was considered, and a total score of 0- 3 was considered normal.

Table1: Calculating system (0- 10) for Gram stain.

Score	Lactobacillus morph types	Gardeneralla and Bactericides spp. morph types	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+	

Results:

After exclusion of 65 cases, the total cases remained in the study were 235 women. Of these, 74 (31.5%) women were pregnant. The mean ages of these patients

were 32.5 ± 6.75 years and the mean gestational ages were 21.18 ± 8.29 weeks. 158 (67.23%) women were non pregnant and between age of 16 and 50 years

Table 2: Shows symptoms of patients.

symptoms	number	percentage
v. discharge	134	60.9%
Vulval itching	67	28.5%
Offensive discharge	67	28.5%
Lower abd. pain	131	55.5%
Dysuria	81	34.5%

Table 3: Results according to Amsel et al. and Nugent et al.

	Number	Percentage (%)
Amsel et al.	44	18.7
Nugent et al.	59	25.1

Our study found that 44 cases (18.7%) according to Amsel et al. and 59 cases were positive for bacterial vaginosis (25.1%) were positive by Gram stain.

Table 4: Comparison between Amsel criteria and Gram stain for diagnosis of B.V

	* Gram Stain		Total
	positive cases	negative cases	
*Amsel positive cases	40 (17%)	4 (1.7%)	44
Criteria negative cases	19 (8%)	172 (73.19%)	191
Total	59	176	235

***p value >0.05**

From 235 cases, we found that 40 cases (17%) were positive for B.V according to Amsel et al. and Gram stain, 4 cases (1.7%) were positive by Amsel and negative by Gram stain, whereas 19 cases (8%) were positive by Gram stain and negative according to Amsel.

Table 5: comparison between Amsel criteria and Gram stain for diagnosis of Bacterial Vaginosis in pregnant women

	* Gram Stain		Total
	positive cases	negative cases	
*Amsel positive cases	9 (12.2%)	2 (2.7%)	11
Criteria negative cases	5 (6.75)	58	63
Total	14	60	74

***p value > 0.05**

Table 6: comparison between PH (>4.5) & gram stain for diagnosis of B.

	* Gram Stain		Total
	positive cases	negative cases	
PH positive cases	53 (38.12%)	86 (61.87%)	139 (59.14%)
Number negative cases	6	90	96
Total	59	176	235

***p value > 0.05**

Regarding the increase in PH >4.5, we found that 139 cases (59.1%) had PH over 4.5. Of these 53 cases (38.1%) were positive for bacterial vaginosis by Gram stain, and 86 cases (61%) were negative by Gram stain.

Table7: comparison between Whiff test and Gram stain for diagnosis of bacterial vaginosis.

	* Gram Stain		Total
	positive cases	negative cases	
*Whiff positive cases	37 (88%)	5 (11.9%)	42 (17.87%)
Test negative cases	22	171	193
Total	59	176	235

***p value > 0.05**

This test was positive in 42 cases (17.87%). Of these, 37 cases (88%) were positive for bacterial vaginosis by Gram

stain also and 171 cases (88.6%) were negative by Gram stain.

Table 8: comparison between presence of clue cells & Gram stain for diagnosis of B.V.

	* Gram Stain		Total
	positive cases	negative cases	
*Clue positive cases	43 (93.4%)	3 (6.52%)	46 (19.57)
Cells negative cases	16	173	189
Total	59	176	235

***p value < 0.05**

The clue cell were found in 46 cases (19.57%). Of these, 43 cases (93.4%) were positive for bacterial vaginosis by

Gram stain and 3 cases (6.5%) were negative by Gram

Table 9: Comparison between presence of vaginal discharge & Gram stain for diagnosis of B. V

	* Gram Stain		Total
	positive cases	negative cases	
*vaginal positive cases	57 (26.1%)	161 (73.9%)	218 (92.8%)
Discharge negative cases	2	15	17
Total	59	176	235

*** P value < 0.05**

Discussion:

Our study explored the diagnostic utility of Amsel criteria for detection of bacterial vaginosis in the women attended the outpatient's clinic with symptoms of vaginal infections. In this study, bacterial vaginosis was detected in 25.1% of cases by gram stain,¹² whereas with Amsel criteria only 18.7% of cases had bacterial vaginosis which represents 74.5% of cases of those detected by Gram stain.

These results were similar to that done by Amsel et al in which bacterial vaginosis was detected in 12 to 25% of the study group.¹⁶ Another study was done by Schwebke et al.⁷ They found that the rate of B. V according to Amsel criteria when used was 31.1%, which represents 78.9% of those detected by Gram stain.

This in clear apposition with study done by Gratacosta et al which compared Amsel criteria with gram stain for diagnosis of bacterial diagnosis. This

study recorded a rate of 1.8% by Amsel which represents only 36% of those detected by Gram stain.²⁴

Decrease sensitivity of Amsel criteria in diagnosis of bacterial vaginosis (67.8%) and the increase in the negative predictive value in compare with Gram stain in this study may be related to un-subjective criteria. This is corresponding to the study done by Schwebke et al. which assessed Amsel criteria vs. Gram stain for diagnosis of bacterial vaginosis in 617 non pregnant women.

Schwebke et al. reported a prevalence rate of 39% and 70% sensitivity when Gram stain was used as the gold standard.⁷ Of note is that; that study was carried out on non- pregnant women attending gynaecologic or STD clinic due to gynaecologic complains.

To our knowledge, only one previous study was comparing clinical criteria of Amsel with Gram stain results performed on a similar patients to that reported in this study.⁷

In our study we found that Whiff test had low sensitivity (62.7%) in diagnosis of bacterial vaginosis when compared with Gram stain. This result supports the finding of previous study done by Eschenbach et al.²⁵

Regarding clue cells in wet mounts preparation, the sensitivity was 72.9% (not significantly different from previous study which was done by Lin et al.). That study was done on 100 women. The sensitivity in that study was 81%.¹⁴ Our result also was similar to Schwebke et al. who reported sensitivity of 79.85%.⁷

Regarding increase PH more than 4.5, the comparison of PH versus Gram stain for diagnosis of bacterial vaginosis in our study showed sensitivity of 89.9%, specificity of 51.1%, positive predictable value (PPV) of 38.1%, negative predictable value of 93.8%, and degree Of accuracy 60.85%. This suggests that some results were false positives and the patient does not fulfill the Nugent criteria.

Conclusion:

We conclude that Amsel criteria had low sensitivity for diagnosis of bacterial vaginosis in women with symptoms of the disease. So use of these criteria alone in compare with Gram stain may result in misdiagnosis of BV due to high false negative results.

Also some factors may increase risk of bacterial vaginosis especially vaginal douching.

References:

1. Sobel J. D. Vaginal infection in adult women. *Med Clin. North America*. 1990; 74: 1573- 1602.
2. Taha T. E. Hoover, D. R. and Kumwenda, N. J. Bacterial vaginosis and disturbances of vaginal flora: association with increased acquisition of HIV. *AIDS*. 1998; 12: 1699- 1706
3. Kent, H.L. Epidemiology of Vaginitis. *Am J Obstet. Gynecol*. 1991; 165: 1168- 1176.
4. Eschenbach D. A. History and review of bacterial vaginosis. *Am J Obstet. Gynecol*. 1993; 169: 441- 445.
5. Thomason, J. L., Achreckenberger, P.C, Spellacy, W. N. and LeBeau, L.J. Clinical and microbiological characterization of patients with non specific vaginosis associated with motile, curved anaerobic rods. *J infec Dis*. 1984; 149: 801- 804.
6. Eschenbach D. A. Hiller, S. L. and Critchlow, C. W. Diagnosis and clinical features associated with bacterial vaginosis. *Am J Obstet Gynecol*. 1988; 158: 819- 828.
7. Schwebke J. R., Hiller S. L., Sobel J. D., Mcregor J. A. and Sweet R.L. Validity of vaginal Gram stain for the diagnosis of bacterial vaginosis. *Obstet Gynecol*. 1996; 88: 573- 576.
8. Totten P., Amsel R. and Hale J. Selective differential human blood bilayer media for isolation of Gardnerella (Haemophilus) vaginalis. *J Clin. Microbiol*. 1982; 15: 141- 147.
9. Paavonen J., Miettinen A. and Stevens C. E. Mycoplasma hominis in non specific vaginitis. *Sex Transm. Dis*. 1993; 10: 271- 275.
10. Pfeiffer T. A, Forsyth P. A. and Durfee M. A. Non specific vaginitis: role of Haemophilus vaginalis and treatment with metronidazole. *N Eng Med*. 1978; 198: 1429- 1434.
11. Spiegel C. A., Amsel R., Eschenbach D., Schoenkenecht F. and Holmes K. K. Anaerobic bacteria in non specific vaginitis. *N. Eng J Med*. 1980; 303: 601- 607.
12. Nugent R. P., Krohn M.A. and Hillier S. L. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of Gram stain interpretation. *J. Clin Microbiol*. 1991; 29: 297- 301.
13. Hill L. H., Ruperlia H. and Embil J. A. Non specific vaginitis and other genital infections in three clinic populations. *Sex Transm Dis*. 1983; 10: 114- 118.
14. Lin, D; Pan, B; Fuh, J. C. and Hung, T. Improving Gram –stained reproducible result by further adding clue cells in diagnosing bacterial

- vaginosis. Kaohstung J Med Sci. 2002; 18:164- 170.
15. Martius J., Krohn M.A., Hillier S.L., Stamm W.E., Holmes K.K. and Eschenbach D.A. Relationship of vaginal Lactobacillus species, cervical Chlamydia trachomatis, and bacterial vaginosis to preterm birth. Obstet Gynecol. 1988; 71: 89- 95.
16. Amsel R., Totten P.A., Chen K.C.A., Eschenbach D., and Holmes K.K. Non specific vaginitis: Diagnostic criteria and microbiological and epidemiological associations. AMJ Med. 1983; 74: 14- 22.
17. Hay P.E., Lamont R. F., Taylor-Robinson D., Morgan D.G., Ison C, and Pearson J. Abnormal bacterial; colonization of the genital tract and subsequent preterm delivery and late miscarriage. BMJ. 1994; 308: 295- 298.
18. Hay P. E, Morgan D. G., Ison C. , Bhide S. A., Romney M., McKenzie P., Pearson J, Lamont R.F. and Taylor-Robinson D. A longitudinal study of bacterial vaginosis and other changes in the vaginal flora during pregnancy. Br J Obstet Gynecol. 1994; 101: 1048-1053.
19. Goldenberg R.L., Klebanoff M.A., Nugent R., Krohn M. A., Hillier S. and Andrews W. W. Bacterial colonization of the vagina during pregnancy in four ethnic groups. Vaginal infection and prematurity study Group. Am J Obstet Gynecol. 1996; 174(5): 1618- 1621.
20. Harmanli O. H., Chang G. Y., Nyirjesy P., Chatwani A., and Gaughan J. P. Urinary tract infections in women with bacterial vaginosis. Obstet Gynecol. 2000; 95: 710- 712.
21. Sweet R.L. Gynecologic conditions and bacterial vaginosis: Implication of non- pregnant patient. Infect Dis Obstet Gynecol. 2000; 8: 184- 190.
22. Spiegel C.A., Amsel R., and Holmes K.K. (1983): Diagnosis of bacterial vaginosis by Gram stain of vaginal fluid. J Clin Microbiol. 18: 170- 177.
23. Thomason J.L. Gelbert S.M., Anderson R. J., Walt A.K., Osypowski P.J. and Broekhuizen F.F. Statistical evaluation of diagnostic criteria for bacterial vaginosis. Am J Obstet Gynecol. 1990; 162: 155- 160.
24. Gratacos E., Figueras F, Barranco M, Ros R., Andreu A., Alosa P.L. and Cararach V. Prevalence of bacterial vaginosis and correlation of clinical to Gram stain diagnostic criteria in low risk pregnant women. Eur J Epidemiol. 1990; 15: 913- 916.
25. Chaim W. Karpas Z. and Lober A. Bacterial vaginosis can be diagnosed using a new and rapid analytical method based on ion mobility spectrometry. Eur J Obstet Gynecol. 2003;111: 83- 87.