ORIGINAL ARTICLE

# Frequency Distribution of Bacterial Vaginosis Using Nugent Score and Culture in Women with Vaginal Discharge

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## Abstract

**Introduction**
Accurate and timely identification of *Bacterial vaginosis* (BV) from high vaginal swab of high-risk patients with vaginal discharge has been found to be a cost effective measure for early diagnosis and treatment of patient, it also decreases the burden of health care cost and complications

**Objectives**
To determine the frequency distribution of BV in women with vaginal discharge using Nugent Score and culture and evaluate agreement between these procedures.

**Study Design**
Descriptive and Cross-sectional study

**Setting and Duration**
This study was carried out at the Department of Microbiology, Fauji Foundation Hospital, Rawalpindi, Pakistan from July 2017 to December 2017.

**Subjects and Methods**
All females with age more than 18 years reporting the hospital outpatient department with complaint of vaginal discharge were included in the study. Sampling technique was purposive and non-probability. At least three high vaginal swabs were taken for Gram staining, culture and wet smear for *Trichomonas* vegetative form. Nugent's Score was assigned as per standard criteria. A score of ≥7 indicated BV.

**Results**
Age range of patients (n=203) was from 20-80 years. Mean age was 44.58 + 10.24 (Mean + SD). In 93 patients (45.81%) Nugent Score was 7-10 whereas culture of high vaginal swabs revealed positive growth in 116 patients (57.14%).Bacterial culture was better in diagnosing BV than Nugent score (p=<0.0001). No Trichomonas was seen in all the specimens examined.

**Conclusion**
Nugent scoring is a good, efficient and rapid method for diagnosis of BV. However, due to low positivity than that of culture negative Nugent score does not rule out the disease and needs verification with culture.

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**Key Words**
Bacterial vagnosis, Nugent Scoring, Vaginal discharge

**Introduction**
Bacterial vaginosis (BV) is a change in flora balance of vagina in which there is increase of anaerobic Gram negative rods like *Gardnerella vaginalis*and *Mycoplasmas*with a decrease in *Lactobacilli*that is primarily responsible for healthy vaginal tissue.[1] In females of reproductive age this problem often observed.[2] BV commonly presents as abnormal vaginal discharge and vulva pruritis.[2] Gram-staining devised by Nugent *et al* is acceptable technique for diagnosis of BV. Amsel's clinical criteria for BVare also quicker method in case of increased workload.[2]

Frequency of distribution is affected by various socioeconomic factors including multiple pregnancies,[3] asymptomatic pregnant women,[4] and poor hygiene,[5] previous history of spontaneous abortions and use of intrauterine contraceptive devices.[5] In most of the studies conducted in different setups most frequent microorganisms involved in BV are *Gardnerella vaginalis* and *Mycoplasma hominis*.[7,8,9] Other frequent organisms are *Escherichia coli* and *Mycoplasma curtisii*.[9]

Diagnosis of BV can be made using several methods, such as Amsel's criteria, Gram stain, vaginal cultures, oligonucleotide probes, molecular methods. The most specific and sensitive of these tests is PCR technique, but it not feasible in our country due to high cost and lack of equipment at various remote laboratories.[9] The most widely used method for diagnosing BV is Nugent scoring, especially for developing countries such as Pakistan where it has proven to be cost effective, reliable and efficient.[10]

As pathogenesis of BV is not clear, the management of BV is a challenging aspect for clinicians and throughout the world, and it is becoming a very common syndrome in women of reproductive age. Recent articles are showing variability in prevalence of BV. Hence determining frequency of distribution will help to assess the actual load of this infection. It will further help the early detection, management and prevention of

complications.

## Materials and Methods

This descriptive, cross sectional study was carried out by Department of Microbiology, Fauji Foundation Hospital, Rawalpindi from July 2017 to December 2017. Females with ages more than 18 years reporting the hospital outpatient department with complaint of vaginal discharge were included in the study. Sampling technique was purposive and non-probability sampling. A sample size of 203 was taken using 95% confidence level and 5% margin of error and taking expected frequency of BV as 15.6%.[5] After taking through clinical history high vaginal swabs were collected. At least three swabs were collected which were used for slide smear and Gram staining, bacterial culture and preparation of wet smear for Trichomonas. The culture was done by inoculating the Blood agar, chocolate agar and MecConkey agar. Media were kept for 24-48 hours at $35^0$C±2 and additional $CO_2$ was provided using candle jar for Chocolate agar. One blood agar plate was kept at $35^0$C±2 an aerobically. The procedures describe by Barrow and Feltham was used for *Giardia lamblia* isolation and identification.[11] However, isolation of *Mycoplsma*, *Mobiluncus* and other fastidious microorganisms could not be adopted because of meager facilities available for them. Women with history of antimicrobial including Metronidazole intake, any oral or vaginal antifungal preparation, women in post-partum period, previously diagnosed with HIV infection, having visible vaginal or cervical mass or suspected cancer were excluded from the study. Contaminated samples were not included.

All vaginal swabs were collected with the sterile swab sticks. Vaginal speculum was used for obtaining the specimen under visual control. Swabbing was done by inserting a dry cotton-wool tipped swab in vaginal furnaces. Swabs were returned to the transport sleeve, labeled and transported to the laboratory.

### Processing

The HVS specimens were considered potentially infectious and masks & gloves were always worn. In the laboratory, the swabs were smeared onto the glass slides. The smears on the glass slides were air-dried. Gram stain was performed and the stained smears were independently examined using the light microscope under oil immersion at 100x magnification lens. Nugent's Score was assigned as per criteria mentioned in Table1. A score of >7 indicated BV.

### Operational Definitions

### Bacterial vaginosis

If the Nugent scoring was >7, it was labeled as bacterial vaginosis (Table 1).

### Nugent scoring

On Gram-staining morphology and number of micro-organism in per smear field is counted that determines the score as per table 1.

### Data Analysis

The data was entered in SPSS (version 21) software. Descriptive statistics was calculated for both qualitative and quantitative variables. Mean and standard deviation (SD) was given for quantitative variables i.e; Age. Frequency and percentage was given for qualitative variables i.e; BV. Effect modifier like age, socioeconomic group, diabetes, multiple pregnancies, spontaneous abortions, intra-uterine contraceptive devices was controlled by stratification. For post stratification chi-square was applied. P-value = 0.05 was taken as significant

## Results

A total of 203 patients met the inclusion criteria during the study period. Age ranged from 20-80 years. Mean age was

**Table 1. Nugent Scoring Criteria for the Microscopic Diagnosis of Bacterial Vaginitis**

| # Lactobacillus (Gram-Positive bacilli-llarge) | Score | # Gardenerella/Baceroides (Gram variable bacilli-small) | Score | Mobiluncus species (Gram-Negative/ variable bacilli-curved) | Score |
|---|---|---|---|---|---|
| ≥30 | 0 | 0 | 0 | 0 | 0 |
| 5-20 | 1 | <1 | 1 | <1 | 1 |
| 1-4 | 2 | 1-4 | 2 | 1-4 | 1 |
| <1 | 3 | 5-20 | 3 | 5-20 | 2 |
| 0 | 4 | ≥30 | 4 | ≥30 | 2 |

**Interpretation:Nugent Score =3** = Smear negative for Bacterial Vaginosis; **Nugent Score 4-6** = Smear indeterminate for Bacterial Vaginosis (Altered vaginal flora and repeat testing of another vaginal smear is recommended); **Nugent Score ≥7** = Smear consistent with Bacterial Vaginosis

44.6+ 10.2 (Mean + SD). Using Nugent scoring 93 patients (46%) had a score of 7-10 making the diagnosis of BV as compared to the results of cultures that tested (n=116, 57%) culture positive for BV. Bacterial culture when compared to Nugent Score for diagnosing BV had a p value of <0.0001 proving it better for diagnosis [Table 2]. The organisms isolated were (n=45, 39%) *Escherichia coli*, (n=21, 18%) *Klebsiella pneumoniae*, (n=8, 7%) *Citrobacterfreundii*, (n=5,4%) *Gardnerellavaginalis*, (n=17, 15%) *Staphylococcus aureus*,(n=11, 10%) *Streptococcus pyogenes*, (n=4, 4%) *Streptococcus* species and 5 (4.3%) *Peptococcus* species. No Trichomonas was seen on direct microscopy of all the specimens. All this makes Nugent Scoring an efficient screening tool for establishing the diagnosis with higher scores, although due to low positivity than that of culture negative Nugent score does not rule out the disease and needs further workup. Presence and absence of *Candida* species in patients with vaginal discharge did not affect the Nugent scoring results (p= 0.257) [Table 3].

**Table 2: Comparison of Nugent scoring with culture results in patients with vaginal discharge ( n=203 )**

| Bacterial culture result | Nugent Scoring* | | | P value |
|---|---|---|---|---|
| | Normal # patients | Intermediate # patients | Bacterial vaginosis # patients | |
| No growth | 23 | 0 | 8 | <0.0001 |
| Normal vaginal flora | 42 | 4 | 10 | |
| Culture Positive@ | 33 | 8 | 75 | |
| Total | 98 | 12 | 93 | |

*Nugent scoring; Normal= 0 – 3; Intermediate= 4 – 6; Bacterial vaginosis= 7 – 10;
@Culture result= Growth of pathogenic bacteria

**Table 3. Association of Nugent scoring with isolation of candida species in patients with vaginal discharge (n=203 )**

| Candida | Nugent Scoring* | | | |
|---|---|---|---|---|
| | Normal # patients | Intermediate # patients # patients | Bacterial vaginosis | Total # patients |
| Present | 19 | 2 | 23 | 44 |
| Absent | 77 | 10 | 72 | 159 |
| P value | 0.257 | | | |
| Spearman's Correlation significance | 0.413 | | | |

*Nugent scoring; Normal= 0 – 3; Intermediate= 4 – 6; Bacterial vaginosis= 7 – 10; @Culture result= Growth of pathogenic bacteria

## Discussion

Bacterial Vaginosis (BV) is a common vaginal disorder in women in reproductive age. Since the initial work of Leopoldo in 1953 and Gardner and Dukes in 1955, researchers have not been able to identify the causative etiologic agent of BV. However, there is increasing evidence that BV occurs when *Lactobacillus* spp., the predominant species in healthy vaginal flora, are replaced by anaerobic and microaerophilic bacteria, such as *Gardenellavaginalis*, *Mobiluncuscurtisii*, *M. mulieris*, other anaerobic bacteria and/or *Mycoplasma hominis*. It is estimated worldwide that 20–30 % of women of reproductive age attending sexually transmitted infection (STI) clinics suffer from BV, and that its prevalence can be as high as 50–60 % in high-risk populations (e.g., those who practice commercial sex work (CSW). According to epidemiological data, women are more likely to report BV if they: 1) have a higher number of lifetime sexual partners; 2) are unmarried; 3) have engaged in their first intercourse at a younger age; 4) have engaged in CSW, and 5) practice regular douching.[12]

In our study about half of the women (45.81%) reporting outpatients with complaint of vaginal discharge were found to be positive for BV using Nugent scoring. However, some other studies revealed lesser positive results using Nugent Scoring; in the study at Agha Khan University Hospital Karachi (Pakistan) frequency was 16.1%,[13] in the study at Bahawal Victoria Hospital Bahawalpur (Pakistan) was 10.8%[14] and at Khyber Medical University frequency of 35.3%[6] was noted. Similarly study conducted at Ethiopia in 2014, revealed prevalence of BV as19.4%.[1] Another study conducted in Yazd city revealed 15.6% frequency of bacterial vaginosis.[15] The variation in results of these different studies appears to be multifactorial. In some of the studies the multiple observers were involved in examination of the Nugent Scoring smear slides. In one study BV was identified if Amsel criteria and Nugent scoring both revealed positivity. The study population in our study was women having discharge whereas in other studies the women were included in the study irrespective of vaginal discharge. Moreover, in almost all these studies only females of reproductive age were included where as in our study older ages were also included.

In our study HVS cultures diagnosed more cases of BV as opposed to Nugent score with a significant p value but about half of the women with vaginal discharge revealed positive Nugent score makes it reliable and efficient way of screening especially when ruling in the disease. However, negative test

cannot rule out the disease but requires further workup.

Presence and absence of *Candida* species in patients with vaginal discharge did not affect the Nugent scoring results. A study from India conducted in 2012 in India[16] showed that the differences in the prevalence of vulvovaginal candidiasis were not observed by the presence or absence of laboratory-confirmed BV which correlates with our study (p=0.257).

**Conclusion**

With appropriate sampling and diagnostics, 45.81% of females with complaints of vagina discharge had BV, making it likely the most common cause of vaginal discharge in our setting. Nugent score is an efficient, cost effective and reliable method for screening of BV making the diagnosis likely if tested positive, but negative testing does not rule out the disease and needs further workup.

**References**


1. Mengistie Z, Woldeamanuel Y, Asrat D, Adera A. Prevalence of bacterial vaginosis among pregnant women attending antenatal care in TikurAnbessa University Hospital, Addis Ababa, Ethiopia. BMC Res Notes 2014; 7: 822-26.
2. TAJ N, Alam USM, Sarah, Bajwa Z, Waheed A, Ehsan ullah. Bacterial Vaginosis in pregnant women and its diagnosis using Amsel's clinical criteria and Nugent's method. *JPMHS* 2014; 8 (1): 133-36.
3. Ibrahim SM, Bukar M, Galadima GB, Audu BM, Ibrahim HA. Prevalence of bacterial vaginosis in pregnant women in Maiduguri, North – Eastern Nigeria. *Niger J Clin Pract* 2014; 17: 154-8.
4. Aslam A, Safdar A, Malik A. Bacterial vaginosis. *JPak Med Assoc* 2009; 59:601-604.
5. Bafghi FA, Hoseizadeh A, Jafari AA, Jouzsheri NM. Frequency distribution of bacterial vaginosis in women reffered to health centres in Yazd city. *J Bio Phar Chem res* 2014; 1: 179-185.
6. Habib A, Siddiqui I. Frequency of bacterial vaginosis in patients with vaginal discharge. Khyber Med Univ J 2016; 8: 32-35.
7. Turovskiy Y, Noll SK, Chikindas LM. The etiology of bacterial vaginosis. *J ApplMicrobiol* 2011; 110: 1105-1128.
8. Kumar N, Behera B, Sagiri SS, Pal K, Ray SS, Roy s. Bacterial vaginosis: Etiology and modalities of treatment – A brief Note. *J Pharm Bioallied Sci*2011; 3: 496-503.
9. Freinkel RK, Shen Y. The origin of free fatty acids in sebum. II. Assay of the lipases of the cutaneous bacteria and effects of pH. *J Invest Dermatol* 1969; 53: 422–7.
10. Ghosh P, Goswami S, Ray R, Agnes C, Arora P, Modak T, et al. Diagnosis of bacterial vaginosis in cases of abnormal vaginal discharge: Comparison of clinical and microbiological criteria. *J Infect Dev Ctries* 2011; 5 (5):353-360.
11. Barrow GI, Feltham RKA, (eds.). Cowan and Steel's Manual for identification of medical Bacteria, 3rd ed. New York: Cambridge University Press, 1993: pp 150-157.
12. Sanchez LJ, Hollingsworth B, Morris S, Sateren B W, Wurapa E, Bautista TC. Mil Med Res 2016; 3: 4.
13. Luni Y, Munim S, Qureshi R, Tareen AL. Frequency and diagnosis of bacterial vaginosis. J Coll Physicians Surg Pak 2005; 15: 270-2.
14. TAJ N, Alam USM, Sarah, Bajwa Z, Waheed A, Ehsan ullah. Bacterial Vaginosis in pregnant women and its diagnosis using Amsel's clinical criteria and Nugent's method. *JPMHS* 2014; 8 (1): 133-137.
15. Bafghi FA, Hoseizadeh A, Jafari AA, Jouzsheri NM. Frequency distribution of bacterial vaginosis in women reffered to health centres in Yazd city. *J Bio Phar Chem res* 2014; 1: 179-185.
16. Madhivanan P, Reingold L A, Krupp K, Klausner D J, Rathod DS. Epidemiologic Features of Vulvovaginal Candidiasis among Reproductive Age Women in India. *Infect Dis ObstetGynecol* 2012; 2-9.