

# Diagnosis of Bacterial Vaginosis in Females with Vaginal Discharge using Amsel's Clinical Criteria and Nugent Scoring

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## Abstract

**Background:** To determine the frequency of bacterial vaginosis in female patients with complaint of vaginal discharge and to evaluate the accuracy of Amsel's clinical criteria taking Nugent criteria as gold standard.

**Methods:** In this descriptive study female patients of a reproductive age group presenting with a complaint of abnormal vaginal discharge were included. A routine gynaecological speculum examination was performed. Vaginal samples were obtained using two vaginal swabs. Colour, consistency and odour of vaginal discharge was also noted. Amine test was performed (Whiff test) by adding 10% KOH to vaginal secretion to determine if the vaginal discharge gave off a fishy odour and wet mount was examined under microscope for presence of clue cells. Morphotypes were scored as average number. Total number of lactobacilli + *Gardenerella vaginalis* + *Mobiluncus* were quantified and scored. A score of more than 7 was considered as a positive sign for bacterial vaginosis. Nugent scoring system was considered as the gold standard for the diagnosis of BV, and accordingly sensitivity, specificity, positive predictive value and negative predictive value of Amsel's criteria were determined.

**Results:** The frequency of bacterial vaginosis was 42.16% by gram's staining. The sensitivity and specificity of Amsel's clinical criteria was 86.4% and 95.2%. Positive and negative predictive value was 85.3% and 95.6% respectively. Clue cells had the highest specificity.

**Conclusions:** Bacterial vaginosis was found in a higher proportion in symptomatic female patients with vaginal discharge. Amsel's clinical criteria was found to be useful but inferior in comparison to gram's staining for the diagnosis of bacterial vaginosis.

**Key Words:** Bacterial vaginosis, Vaginal discharge, Amsel's criteria, Gram staining, Clue cells, Whiff test,

## Introduction

Bacterial vaginosis (BV) – a poly-microbial syndrome is one of the most prevalent and least understood problem in women of reproductive age.<sup>1</sup> The frequency of 3.6-40% has been reported across different population around the world.<sup>2</sup> It is the most important cause of vaginal discharge, affecting large number of women of reproductive age group.<sup>3,4</sup> BV has emerged as a global issue in the recent years because of its association with ascending genital tract infections. The genital tract infections are associated with serious complications, such as chorioamnionitis, spontaneous abortions, preterm labour, low birth weight babies and endometritis resulting in increased susceptibility to various sexually transmitted infections including HIV.<sup>5-7</sup> Vaginal flora is dominated by the hydrogen peroxide-producing *Lactobacillus* which maintains an acidic environment in the vagina.<sup>8,9</sup> In BV the normal protective vaginal flora comprising of predominantly indigenous *Lactobacillus* is lost and gradually replaced by a mixed flora consisting of aerobic, anaerobes and microaerophilic bacterial species resulting in symptomatic and asymptomatic vaginitis.<sup>10</sup> Patients with vaginitis complain of a combination of symptoms including vaginal discharge, odor in the vaginal secretions and irritation or itching of the vagina. Vaginal discharge is characterized by color (clear, white, grey, green, yellow), consistency (thin, thick, watery or curd like) odor (foul smelling, fishy, pungent) and amount (more or less than normal) which cannot be quantified, bleeding and dyspareunia.<sup>11</sup> The number of undiagnosed patients range from 7%-72% on complaints only.<sup>12</sup> Current recommendation for diagnosis of vaginal complaints involves vaginal examination and field microscopy as the microscopic findings clear the ambiguities.<sup>13,14</sup> The current diagnostic method available for the diagnosis of BV is via assessment of clinical signs, but the clinical signs are subtle and detection of the signs is dependent on the expertise of the clinician performing the test.<sup>15</sup>

Gram stain laboratory method is the least expensive method employed for the diagnosis of BV, requires less time and is the most widely used.<sup>16</sup> The Amsel's clinical criteria and Nugent scoring system are the most commonly used methods to detect BV. Laboratory methods for the identification of BV include wet mount and gram staining which is considered as a "Gold standard" for the diagnosis of BV.<sup>15-17</sup>

### Patients and Methods

In this descriptive observational case control study a total of 332 female patients of a reproductive age group (15-42 years), married, sexually active and presenting with a complaint of abnormal vaginal discharge were included. Patients were collected from the out-patient department of Gynecology and Obstetrics at a public sector tertiary care teaching hospital of Rawalpindi Medical College. The study was conducted from Jan 2012 to Jul 2013. Exclusion criteria were female patients with any previous surgical procedures on uterus(post-delivery, post-abortion, post-operative), menstruating females or women on any antibiotic treatment and the females above the age of 42 years.

A routine gynaecological speculum examination was performed and a detailed history was obtained from the patients. Vaginal samples were obtained using two vaginal swabs from each patient and were transported to the microbiology laboratory for further processing. While collecting the swab samples from the female patients, colour, consistency and odour of vaginal discharge was also noted. One of the swabs collected from every individual was pressed briefly against an indicator paper to measure the pH range suspected to be 3.8 - 7.4 . Amine test was performed (Whiff test) by adding 10% KOH to vaginal secretion on a slide to determine if the vaginal discharge gave off a fishy odour and wet mount was examined under microscope for presence of clue cells.<sup>18</sup> Smear was air dried on the glass slide for gram staining (1000 magnification).<sup>15</sup> Morphotypes were scored as average number per oil immersion field. Total number of lactobacilli + Gardnerella vaginalis + Mobiluncus were quantified and scored. A score of more than 7 was considered as a positive sign for BV. Patients not fulfilling the minimum of three out of four Amsel diagnostic criteria were considered normal and served as control group for the study. The data were analyzed using descriptive statistics and frequency distribution in the SPSS 16 software. The present study considered Nugent scoring system as the gold standard for the

diagnosis of BV, and accordingly sensitivity, specificity, positive predictive value and negative predictive value of Amsel's criteria were determined (Table 1).

**Table 1. Nugent's scoring on Gram stain<sup>15</sup>**

Score	Lactobacillus	Gardnerella/ bacteroids	Mobiluncus
0	>30	0	0
1	5-30	<1	1-5
2	1-4	1-4	>5
3	<1	5-30	-
4	0	>30	-

### Results

Age of the patients ranged between 17-42+years with mean age of 28.01±0.29 years. Homogeneous vaginal discharge was found in 150 (45.18%) study participants. However, true and false positive cases were 62 and 88 respectively as shown in Table 2. Thus the sensitivity and specificity came out to be 75.6% and 64.8% respectively. Positive and negative predictive values were 41.3 and 89.01% respectively. Vaginal pH >4.5 was found in 190(57.22%) study participants out of which 79 were true positives and 111 were false positives (Table 3). Thus, the sensitivity and specificity for this criterion (alone) was 96.3% and 55.6% respectively. Positive and negative predictive values were 41.6 and 97.9% respectively.

**Table 2. Homogenous vaginal discharge**

Test	Disease present	Disease absent	Total
+ve	62	88	150
-ve	20	162	182
Total	82	250	332

**Table 3. Vaginal pH > 4.5**

Test	Disease present	Disease absent	Total
+ve	79	111	190
-ve	03	139	142
Total	82	250	332

Amine odour disseminated on the addition of potassium hydroxide solution to the vaginal secretions was found in 135 females (40.66%) out of which 69 were truly and 61 came out to be falsely positive (Table 4). Thus, sensitivity, specificity, PPV and NPV for Whiff test were calculated to be 84.1, 73.6, 51.1 and 93.4% respectively. Clue cells were found on field microscopy of vaginal discharge in 124(37.34%) cases

where it was subsequently proven to be true positive in 73 and false positive in 51 cases (Table 5). Thus, the sensitivity, specificity, PPV and NPV for clue cells were 89%, 79.6, 58.8 and 95.6%. Eighty two (24.69%) study participants were found to meet at least three out of four Amsel's criteria (Table 6). The sensitivity, specificity, PPV and NPV for Amsel's criteria were 86.4%, 95.2%, 85.3% and 95.6%. The Amsel criteria a clinical bedside method, is easy to perform and gives an early detection of the problem. However, the Nugent scoring system which is a laboratory method utilized for the detailed findings gives accurate results. Of the total 332 patients (table 7), those fulfilling all the relevant parameters (Homogenous discharge, pH>4.5, Amine odor and clue cells) for Amsel clinical analysis were 24.69% (n=82). Patients meeting all the scores for Nugent scoring system (Morphotypes of lactobacilli spp, Gardnerella vaginalis and Mobiluncus spp) were 42.16% (n=140). Patients with vaginal discharge but not meeting the parameters for either the Amsel or the Nugent scoring system were 57.83% (n=192).

**Table 4. Whiff test**

Test	Disease present	Disease absent	Total
+ve	69	61	135
-ve	13	184	197
Total	82	250	332

**Table 5: Presence of clue cells**

Test	Disease present	Disease absent	Total
+ve	73	51	124
-ve	09	199	208
Total	82	250	332

**Table 6. Total Amsel's criteria**

Test	Disease present	Disease absent	Total
+ve	70	11	81
-ve	12	239	251
Total	82	250	332

**Table 7. Prevalence of Bacterial Vaginosis**

No	Disease criteria	Number	%
1	Not Fulfilling	192	57.83
2	Amsel criteria	82	24.69
3	Nugent Scoring	140	42.16

## Discussion

Difficulties do arise when diagnosis is purely based only on patient symptoms and clinical findings. Anderson et al. (2004) are of the opinion that precision of vaginal symptoms refers to the degree to which an observer finds the same results when a relevant test is applied. Mostly basis of diagnosis is through symptoms that the patient shows and the clinical observations of the clinician. However, various researches have highlighted inaccuracies in clinically diagnosing a patient based on the symptoms and clinical observations, when compared, to traditional gold standards for diagnosing both symptomatic and asymptomatic patients. The choice of diagnostic test to be employed in the study requires consideration of expertise and cost.<sup>13,19</sup> Recently, some advance diagnostic methods have been developed, including the polymerase chain reaction (PCR), rapid nucleic acid hybridization test, proline amino peptidase activity and sensor arrays, for the diagnosis of bacterial vaginosis. Most of these tests are expensive and their diagnostic accuracy in terms of sensitivity and specificity do not have a clear advantage over the classical methods.<sup>20</sup> Thus, the clinical criteria by Amsel and Nugent's method based on Gram staining remain the most practical, viable and economical options for diagnosing bacterial vaginosis, especially in developing countries.<sup>21</sup> Thus, the current study was designed to evaluate the accuracy of Amsel's clinical criteria taking Nugent criteria as gold standard. In this study, bacterial vaginosis was found to be 42.16% using Nugent's criteria on Gram staining and 24.69% according to Amsel criteria. BV has a high and varied prevalence, depending on the surveyed population, varying from 4% in developed countries to 61% in the third world countries, with a mean prevalence of 14% in the developed and developing regions.<sup>22</sup> In USA prevalence of BV is 26-37% while in European countries 4-37% of BV cases were observed in general population.<sup>23</sup> National and international comparisons are hampered because of the different methodology that studies employ. A study from Rawalpindi Military Hospital found the frequency of bacterial vaginosis to be 11.3% while a similar kind of study in Railway Hospital Rawalpindi showed prevalence similar to the present study.<sup>24,25</sup> Aslam et al. (2004) found a frequency of bacterial vaginosis approximately equal to 18.7% in a small sample of pregnant women.<sup>26</sup> The sensitivity and specificity of Amsel's criteria were 86.4 and 95.2% whereas positive and negative

predictive values were calculated to be 85.3% and 95.6% respectively in the current study. Modak et al. (2010) found that sensitivity and specificity of Amsel's criteria were 66.7 and 94.7% whereas the PPV and NPV were 80 and 90%<sup>21</sup>. In the same study, the sensitivity, specificity, PPV and NPV for the characteristic vaginal discharge were 75.6%, 64.8%, 41.3% and 89.01%. Sensitivity, specificity, PPV and NPV for pH of >4.5 were 96.3%, 55.6%, 41.6 and 97.9%. In the same study whiff test was found to be 100% specific and 42% sensitive whereas clue cells were 100% sensitive but 76% specific for the diagnosis of BV<sup>21</sup>. Our findings about the sensitivity of Amsel's criteria are similar to the study conducted by Modak et al (2010). However the specificity and NPV showed higher values in the current study as compared to the study conducted by Modak et al. (2010) which can be attributed to a larger sample size.

In another study Taj.Y et al (2012) observed that Amsel criteria had a sensitivity of 77%, specificity 91%, with a positive predictive value of 97% and a negative predictive value of 53%.<sup>27</sup> Beverly et al. (2005) found that the total Amsel criteria had a sensitivity of 37% and specificity of 99% whereas vaginal pH of >4.5 had sensitivity and specificity of 83% and 69% respectively. The presence of clue cells has sensitivity of 33% and specificity of 98%. Positive Whiff test had a sensitivity and specificity of 45% and 35%. Characteristic homogenous discharge had a sensitivity and specificity of 96% and 85% respectively.<sup>28</sup> Both clinical and the gram stain criteria are acceptable methods of diagnosis of BV, however, differences are apparent when these methods are compared to each other. A study showed that sensitivity and specificity of the Nugent's score compared to the Amsel's criteria were 97% and 98%, respectively.<sup>29</sup> Schwebke et al. showed that vaginal gram stain (Nugent's score) was more sensitive than Amsel's criteria for diagnosis of BV.<sup>30</sup> While Zadeh et al. (2015) showed Amsel criteria to have high specificity and sensitivity in BV diagnosis.<sup>31</sup>

## Conclusion

1. Bacterial vaginosis was found in a significant proportion (24.69%) of females with vaginal discharge using Amsel's clinical criteria.

2. Amsel's clinical criteria were found to be useful but inferior to the Nugent Gram's staining method which showed 42.16% of correct diagnosis of bacterial vaginosis.

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