Original Article

Diagnosis of bacterial vaginosis in cases of abnormal vaginal discharge: comparison of clinical and microbiological criteria

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Abstract

Introduction: Bacterial vaginosis is a polymicrobial syndrome involving replacement of normal vaginal hydrogen peroxide producing lactobacilli by a variety of mycoplasmas and Gram-negative rods. Bacterial vaginosis has been conventionally diagnosed using Amsel criteria (a clinical method) or Nugent's score (a laboratory method with higher reproducibility). This study was undertaken to compare the diagnostic ability of the Amsel criteria with that of Nugent's score among patients presenting with abnormal vaginal discharge.
Methodology: The study was conducted at the Medical College in Kolkata, India to determine the prevalence of patients with bacterial vaginosis and their demographic profile. Subjects attending the outpatient department presenting with abnormal vaginal discharge were evaluated for the presence of bacterial vaginosis by Amsel criteria and Nugent's score.
Results: Prevalence of bacterial vaginosis was 24% by Nugent's score. In comparison, Amsel criteria had sensitivity of 66.67%, specificity of 94.74%, positive predictive value of 80% and negative predictive value of 90%. There was no perfect inter-rater agreement between the Amsel criteria and Nugent's score (Kappa = 0.58). Presence of clue cells correlated best with a positive diagnosis by Nugent's score while the amine test (whiff test) had the lowest correlation.
Conclusion: Although the Amsel criteria method is a convenient and inexpensive means of diagnosing bacterial vaginosis, it is not always reliable. Alternative reliable and inexpensive diagnostic methods that unify clinical and microbiological parameters, thus increasing sensitivity while retaining specificity, are needed.

Introduction

Vaginal discharge is an extremely distressful condition for a woman, which can result from a variety of physiological states as well as pathological conditions. Bacterial vaginosis is reported to be one of the most common causes of abnormal vaginal discharge or vaginal symptoms in women of reproductive age [1]. It remains an ill-defined syndrome of uncertain etiology with predominantly aesthetic overtones explaining the well nigh ineffective therapy of the condition. First described by Gardner and Dukes in 1955 [2], it is a polymicrobial syndrome involving replacement of normal vaginal hydrogen peroxide producing lactobacilli by a variety of bacteria and mycoplasmas, mainly *Gardnerella vaginalis*, *Mycoplasma hominis*, *Mobiluncus* species, and anaerobic Gram-negative rods belonging to the genera *Prevotella*, *Porphyromonas*, *Bacteroides* and *Peptostreptococcus* species [3]. Also detected are *Atopobium vaginae*, *Lactobacillus iners*, *Megasphaera*, *Leptotricha*, *Eggerthella* and *Dialister* [4].

This syndrome is characterized by symptoms of vaginal malodor and a slight to moderate increase of white discharge which appears homogenous, is low in viscosity, and evenly coats the vaginal mucosa. The exact pathology of the disease is uncertain. It has been hypothesized that *G. vaginalis* metabolically produces amino acids which act as a substrate for the production of volatile amines by anaerobic bacteria. These amines in turn raise the vaginal pH favoring the growth of *G. vaginalis* over lactobacilli [5]. With little or no vaginal mucosal inflammation, this

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represents a state of a disturbed eco-system rather than a true tissue infection.

The importance of bacterial vaginosis is emphasized by its association with pelvic inflammatory diseases, adverse outcome of pregnancy in the postpartum period, endometritis and cuff cellulitis [6]. Bacterial vaginosis has also been associated with infections after hysterectomy, as well as with low birth weight infants and pre-term births in affected women [7]. The complications arising out of bacterial vaginosis necessitate early diagnosis to institute prompt treatment of this polymicrobial syndrome. Bacterial vaginosis increases a woman's susceptibility to HIV infection [8]. Control of bacterial vaginosis controls sexually transmitted infections, thus contributing towards HIV/AIDS control.

Bacterial vaginosis is conventionally diagnosed using Amsel criteria. The presence of any three of the following four criteria is considered to be consistent with the presence of bacterial vaginosis: characteristic thin, homogenous vaginal discharge, vaginal pH greater than 4.5, release of a fishy amine odor on addition of 10% KOH (whiff test), and demonstration of clue cells (Figure 1) in more than 20% of the total cell population [9].

In 1991, Nugent *et al*. suggested a modification of Spiegel's method of scoring Gram-stained vaginal smears for the diagnosis of bacterial vaginosis [10]. The score, calculated by assessing the presence of large Gram-positive rods (*Lactobacillus* morphotypes), small Gram-negative/Gram-variable rods (*G. vaginalis* morphotypes), and curved Gram-variable rods (*Mobiluncus* spp. morphotypes) can range from 0 to 10 with a score of 7 to 10 being consistent with bacterial vaginosis. Compared to the Amsel criteria, the Nugent's score allows for assessment of alteration in vaginal flora as a continuum rather than a dichotomy [10].

The Amsel criteria method is dependent on clinical signs which cannot be quantified and standardized. They rely on the acumen of the clinician and suffer from subjective variation. They are neither sensitive nor specific and thus misdiagnosis and delays in treatment are common, which can place women at risk of persistent disease, discomfort and the mentioned adverse sequelae. In this respect, Nugent's score has been favored because of its superior reproducibility and sensitivity. Nevertheless, evaluation of smears is also subjective and requires an experienced slide reader.

Figure 1. Clue cells: Gram Negative bacilli in pairs and singles studding surface of squamous epithelial cell (Grams stain, 1000X).
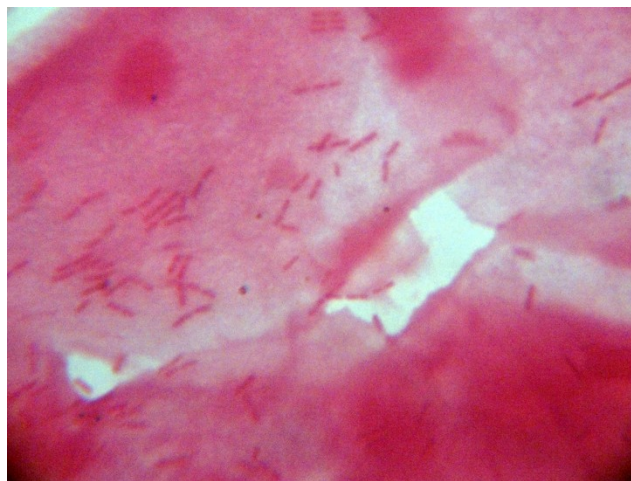
In a developing country with limited resources such as India, where highly trained skilled manual labor comes at a premium, diagnosis of bacterial vaginosis by Nugent's score would place a great strain on available resources. The Amsel criteria method requires less infrastructural and manual resources; thus clinicians would be better placed if they knew the sensitivity and specificity of Amsel criteria in relation to Nugent's score before diagnosis.

This study aimed to determine the relative prevalence of patients with bacterial vaginosis as well as their demographic profile among the patients presenting with excessive vaginal discharge attending the outpatient department of the study area, and to compare the diagnostic ability of Amsel criteria with Nugent's score in the study population.

We determined the prevalence of bacterial vaginosis among patients with abnormal vaginal discharge attending a tertiary care hospital within eastern India and evaluated the specificity and sensitivity of Amsel criteria, taking a positive Nugent's score to be the definition of bacterial vaginosis. The demographics of the population studied also provided vital insights to the prevalence of bacterial vaginosis in the various classes of society.

The present national guideline for Reproductive Tract Infection/Sexually Transmitted Infection (RTI/STI) control focuses on enhanced syndromic case management with judicious and efficient use of laboratory support. Since detection of clue cells requires the support of a microbiology lab and is difficult to perform in a field setting, this study also

investigated the potential for using only the clinical Amsel criteria as an easy-to-perform screening test for diagnosis of bacterial vaginosis before the samples are sent to a microbiology lab for further evaluation.

Methodology

The study was conducted over a four-month period (on a pre-fixed day of each week) in the Gynecology and Obstetrics Department, and the Skin and Sexually Transmitted Diseases Department of the Medical College, Kolkata, India. Laboratory investigations were performed in the Microbiology department of the Medical College, Kolkata. All procedures and protocols followed in the study had prior approval from the Institutional Ethics committee. All women with excessive vaginal discharge attending the Outpatient Department (OPD) were screened for the study. Of the 213 subjects screened, a total of 50 women were included in the study and informed consent was obtained from all participants. The study algorithm is depicted in Figure 2.

Participants were asked about their symptoms, the nature of their complaints (color and amount of discharge and presence of itching), past illness, and history of treatment before undergoing gynecological examination. Pregnant, HIV infected, and menstruating women or those who had used antibiotics and/or topical vaginal creams within seven days prior to the date of examination were specifically excluded from the study.

An un-lubricated Cusco's vaginal speculum was inserted into the vagina and characteristics of the discharge (with respect to amount, odor and type of discharge) were evaluated by an experienced clinician at the OPD, Department of Gynecology and Obstetrics. Samples of the vaginal discharge collected on dry sterile cotton wool tipped swabs (StandBio Reagents Pvt. Ltd., Kolkata, West Bengal, India) were tested for pH (pH paper, Merck, Darmstadt, Germany) and presence or absence of a fishy amine odor on addition of 10% KOH (whiff test). Swab tubes were handed over for evaluation of the Nugent's score and presence of clue cells to the department of Microbiology.

Figure 2. Roadmap of study design and methodology used.
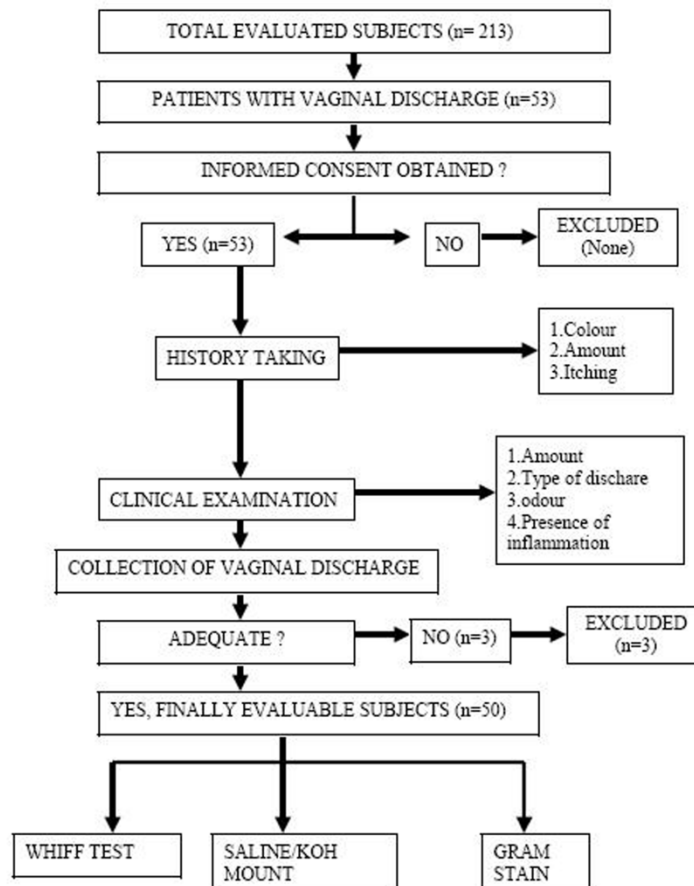
Patient samples, questionnaires, and clinician checklists were labeled with a unique identifier to ensure confidentiality and freedom from bias. The Nugent's score was evaluated only by a single experienced microbiologist to remove chances of inter-observer variation.

Operational definition of bacterial vaginosis

For the purpose of the study, the Nugent's score [10] was taken to be the gold standard. The Nugent's score was assessed for the presence of Lactobacillus, *G. vaginalis* and *Mobiluncus* spp. morphotypes (scored from 0 to 4 depending on their presence/absence as applicable). For small Gram-negative/Gram-variable rods (*G. vaginalis* morphotypes) more than 30 bacteria per oil immersion field (oif) was scored as 4; a count of 6-30 bacteria per oif scored 3; and 1-5 bacteria per oif scored 2. Less than 1 per oif scored 1 and their absence scored 0. For large Gram-positive rods (*Lactobacillus* morphotypes), the scoring was reversed, with their absence scored as 4, fewer than1 per oif scored 3; a count of 1-5 per oif scored 2; a count of 6-30 per oif scored 1; and more than 30 per oif scored 0. For curved Gram-variable rods (*Mobiluncus* sp. morphotypes), the presence of five or more bacteria was scored 2, less than 5 scored 1, and absence of bacteria was scored as 0. The sum of the 3 scores was taken and a score of 7 or more was considered the "operational definition" of bacterial vaginosis.

Description of Amsel criteria

This comprised fulfilling any three of the following four criteria: presence of homogeneous vaginal discharge, $pH > 4.5$, positive whiff test, and presence of clue cells on vaginal wet smear.

Detection of clue cells is essential to the Amsel criteria. Clue cells (epithelial cells covered with small Gram-negative/Gram-variable rods) were detected by Gram staining of the vaginal discharge by standard procedures and examination under oil immersion. Presence of clue cells in at least 20% of the oil immersion fields was considered positive by the Amsel criteria.

Data analysis

Data analysis was performed using StatCalc version 5.0.4 (AcaStat Software, Leesburg, Virginia, USA) and Microsoft Excel (Office 2000) (Microsoft Inc., Redmond, Washington, USA).

Descriptive statistics, chi-square and unpaired t-tests were used to determine the level of significant difference as applicable. Sensitivity, specificity, predictive value of a positive test and predictive value of a negative test of each criterion was individually determined. The Kappa test was used to assess the inter-rater agreement. We also computed the correlation coefficient between each criterion and the Nugent's score using the Spearman's rank correlation coefficient.

Results

A total of 50 women were enrolled in the study. Table 1 shows the demographic characteristics of the

Table 1: Demographic profile of the subjects enrolled in the study along with comparison between those with bacterial vaginosis and non-bacterial vaginosis group

Characteristics	Study subjects (n = 50)	Bacterial vaginosis (n = 12)	Non-Bacterial vaginosis group (n = 38)
Age at presentation* (in years)			
Mean ± SD	30.7 ±10.46	28.33 ± 7.90	31.13 ± 11.19
Median (range)	30 (20 - 70)	29.5 (20 - 45)	30 (20 -70)
Marital Status†			
Single	0 (0 %)	0 (0%)	0 (0%)
Currently Married	47 (94 %)	12 (100%)	35 (92.10%)
Widowed/ Divorced	3 (6 %)	0 (0%)	3 (7.89 %)
Education ‡			
Elementary	36 (72 %)	7 (58.33 %)	29 (76.31 %)
High school	9 (18 %)	4 (33.33 %)	5 (13.1 %)
Graduate	5 (10 %)	1 (8.33 %)	4 (10. 52 %)
Parity §			
0	8 (16%)	2 (16.67%)	6 (15.7 %)
1	20 (40 %)	3 (25%)	17 (44.7 %)
2 or more	22 (44 %)	7 (58.33 %)	15 (39.46 %)

* t-test between bacterial vaginosis and non-bacterial vaginosis sub-group for age at presentation p = 0.426 (t= 0.803)
† Chi-square between bacterial vaginosis and non-bacterial vaginosis sub-group for marital status p = 0.315 (chi-square with Yates correction = 1.008, df = 1)
‡ Chi-square between bacterial vaginosis and non-bacterial vaginosis sub-group for education between elementary and above elementary (high school and graduate clubbed) p = 0.226 (chi-square = 1.463, df = 1)
§ Chi-square between bacterial vaginosis and non-bacterial vaginosis sub-group for parity p = 0.443 (chi-square with Yates correction = 1.63, df = 2
Percentages relate to the percent out of the total no of cases in that column

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women enrolled. They were primarily middle-aged females (mean age 30.7 ± 10.46 years). The mean age of presentation was found to be lower for bacterial vaginosis (28.33 ± 7.90 years) than for the non bacterial vaginosis group (31.13 ± 11.19 years), although this was not statistically significant (t-test showing p = 0.426). All the patients were housewives and from a financially poor background and low socio-economic status. A few of the patients were widowed (6%) and the rest were currently married. Most had some amount of elementary education (72%); the majority of the women were parous (83.33%). The demographics of women who were part of the study were compared to the subgroup showing bacterial vaginosis as detailed in Table 1. On performing the Chi-square test between the subsets of patients with bacterial vaginosis and non-bacterial vaginosis, marital status (p = 0.315), education (p = 0.226) or parity (p =0.443) did not give a statistically significant difference.

Of the 50 vaginal swabs taken, 12 gave a positive Nugent's score, providing a prevalence rate of 24% for bacterial vaginosis in patients who complained of abnormal vaginal discharge. In contrast, Amsel criteria diagnosed 20% as suffering from bacterial vaginosis (Table 2). Thus the sensitivity of Amsel criteria was 66.67%, specificity was 94.74%, positive predictive value was 80% and negative predictive value was 90%.

The inter-rater agreement statistic (Kappa) was determined between the Amsel criteria and Nugent's score (Kappa = 0.58). Though there was no perfect agreement between the two classification systems, the agreement was better than that which can be attributed to chance.

Each of the individual components of the Amsel criteria was compared to the Nugent's score. The

Table 2: Contingency table (2 x 2) comparing the results of Amsel criteria with the Gold standard (Nugent's score)

	Amsel criteria BV positive	Amsel criteria BV negative	Total
Bacterial vaginosis*	8	4	12 (24%)
Non-Bacterial vaginosis†	2	36	38 (76%)
Total	10 (20%)	40 (80%)	50

* Nugent's score ≥ 7
† Nugent's score < 7 (among 38, score of 4-6 for 10 slides and score of < 3 for the rest 28)

sensitivity, specificity, positive predictive value and negative predictive value of each of the criteria compared to that of the Nugent's score method is given in Table 3. Presence of clue cells correlated best with a positive diagnosis by Nugent's score (rho = 0.859) while the whiff test had the lowest correlation (rho = 0.466).

Discussion

Proper diagnosis of bacterial vaginosis is challenging. In addition to scientific considerations, choosing a method for laboratory diagnosis requires consideration of complexity, cost, and the frequency of un-interpretable specimens. Nevertheless, some alternative diagnostic methods have been developed, such as the polymerase chain reaction (PCR), rapid nucleic acid hybridization test, proline amino peptidase activity [11]. More recently, several point-of-care tests based on various combinations of microbial products, presence of RNA, or more complex laboratory instrumentation such as sensor arrays, have also been introduced for the diagnosis of bacterial vaginosis [11]. However, most of these are expensive and their sensitivities and specificities do

Table 3: Diagnostic value of the Amsel criteria and each of the criterion individually

	Sensitivity (%)	Specificity (%)	Predictive value of Positive test (%)	Predictive value of Negative test (%)	Spearman's correlation coefficient*
Amsel criteria as a whole	66.67	94. 7	80	90	--
Homogenous discharge	66.67	71.05	42	87	0.6 (0.01)
pH	83.33	86.84	67	94	0.72 (0.01)
Whiff test	41.67	100	100	84	0.466 (0.02)
Presence of clue cells	100	76	57	100	0.859 (0.01)

* Rank correlation coefficient (rho) of various components of Amsel criteria in comparison to Nugent's method. (Figures in parenthesis indicate p-values for the data

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not offer a huge advantage over the classical methods [11]. Given these considerations, the Amsel and Nugent's methods remain the most practical, viable and economical options for diagnosing bacterial vaginosis, especially in developing countries. Bacterial vaginosis is often misdiagnosed using clinical criteria alone because the components are subjective and depend on the acuity of the clinician and the availability of equipment [12].

In this study, the prevalence of bacterial vaginosis among patients with the primary complaint of abnormal vaginal discharge was 24%. Using Nugent's method as the diagnostic criteria, the prevalence of bacterial vaginosis can be seen to vary considerably from study to study [13-15]. A study from southern India found the prevalence of bacterial vaginosis to be 20.5% [16], which closely matches the findings in the current investigation.

It is difficult to determine the exact prevalence of bacterial vaginosis because only one third to three quarters of the patients are symptomatic [17]. Reported prevalence also varies in different population subtypes. Prevalence in ambulatory gynecology patients has been reported to be 15% to 19%; however, in special groups the data varies (10% to 30% in pregnant patients, and 24% to 40% in patients carrying concurrent sexually transmitted diseases) [18,19]. Data from most studies suggests that women of child bearing age are more prone to developing bacterial vaginosis. In our study this might be reflected in a lower age of presentation of symptoms. The average age of the bacterial vaginosis group in this study (mean age = 28.33 ± 7.90 years) was slightly lower than that of the non suffering group (mean age = 31.13 ± 11.19 years), but the difference was not found to be statistically significant. The lack of significance might be a result of the low number of patients in the study. We also tried to determine if marital status, education, and parity brought about significant differences in the prevalence of bacterial vaginosis: no statistically significant difference was found.

The prevalence of bacterial vaginosis determined using Amsel criteria as a diagnostic tool was found to be 20%. In a similar study by Chaijareenmont *et al.*, the prevalence of bacterial vaginosis was 14.7% by Amsel criteria and 12% by Nugent's method [14]. The present study found that mutual agreement between the two diagnostic tests was lacking (kappa = 0.58), which necessitates development of a set of unified and universal diagnostic criteria to lessen the ambiguity in diagnosis.

The actual prevalence is possibly higher since at least 30% of patients with bacterial vaginosis go undetected even after a complete check up [13], which can explain the lower sensitivity of the Amsel criteria (which make use of clinical signs that cannot be standardized) compared to that of Nugent's method. This study found the sensitivity of Amsel criteria to be 66.67% considering Nugent's score as the gold standard. Difference in interpretation of data is a major drawback of Amsel criteria. In the current study, interpretation of the vaginal smears was performed by a single experienced microbiologist to eliminate the possibility of inter-observer difference; however, logistical problems prevented the use of a single clinician for evaluating the patients for interpretation of Amsel criteria. Nevertheless, the minimum possible number of clinicians was employed to evaluate the patients to minimise inter-observer variability. Amsel criteria, despite being less sensitive compared to the Nugent's set of criteria, had a high specificity (94.7%) which is in accordance with the report by Sha *et al.* [15].

The studies which compare the two sets of criteria always take one set as the operational definition of bacterial vaginosis, but basic differences continue to exist between the two sets. The variability among patients further complicates the problems. Clinicians and microbiologists are yet to agree on a set of criteria which would unite the two. It is always easy to set a number of bacteria as the normal limit beyond which a patient is declared positive. But biological variability ensures that the limit itself is changeable. Since the pathogenic microorganisms investigated in Nugent's method are themselves normal residents of the vaginal flora, what might be an acceptable level to some patients could cause extreme discomfort to others. This lack of agreement between the two methods is highlighted in the following two studies. One study showed that the sensitivity and specificity of Nugent's method were 97% and 98% respectively when Amsel criteria were taken as the definition of bacterial vaginosis [20]. In contrast, another study showed that Gram staining was more sensitive than Amsel criteria [21]. However, even when Amsel criteria have been taken as the gold standard, the sensitivity and specificity of Nugent's method was within acceptable limits. The chief drawback of Nugent's method seems to be that it turns out a higher number of false positive cases compared to the Amsel criteria method [22]. If Nugent's method is used in epidemiological surveys, it can overestimate the true prevalence and may even

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interpret normal individuals to be diseased. This possibility reiterates the need for a revised set of criteria which unifies the clinical and microbiological parameters.

The current study evaluated the predictive value of a positive test and the predictive value of a negative test of the Amsel criteria (Table 3). The predictive value of a positive test was 80% and the predictive value of a negative test was 90%. The results are in broad agreement with those of other similar studies [12,14].

In this study, each of the components of the Amsel criteria was correlated with the Nugent's score. Presence of clue cells had the best correlation with the Nugent's score, as all patients found positive by Nugent's method had demonstrable amounts of clue cells. This observation can be explained as both clue cells and Nugent's method make use of the microscope and are similar in many aspects. Though the presence of clue cells had the best correlation and was also the single most sensitive criterion, detection of clue cells involves the use of a microscope. Thus among the four criteria, detection of clue cells is the most complicated and requires the use of expensive resources, similar to Nugent's method. It is strictly not a bedside procedure. Thus, in spite of being a sensitive criterion, detection of clue cells may not be the best indicator of bacterial vaginosis on a practical basis in clinical settings, as suggested by Hilliers *et al.* [23].

The whiff test was the most specific of all the criteria (specificity = 100%). It also had the highest predictive value of a positive test. But the whiff test was not found to be very sensitive (sensitivity = 41.67%) and gave a large number of false negative cases (50%). The whiff test is dependent on the power of smell, which can vary from person to person. Though a positive whiff test is highly suggestive of bacterial vaginosis, a negative test must always be treated with caution.

Ultimately, pH seemed to be the best indicator of bacterial vaginosis, if both sensitivity and specificity are taken into consideration. It was found to be moderately sensitive and specific and had the best predictive value of a negative test. Furthermore, it is the one which could be objectively measured at the bedside.

This study suggests that although the Amsel criteria method is a convenient and inexpensive way to diagnose bacterial vaginosis, it is not very reliable. Although the Amsel criteria method has long been touted as requiring little infrastructure to detect

bacterial vaginosis, this is not always so. Detection of clue cells requires as much infrastructure and trained labour as Nugent's method, which in a developing country such as India may not always be available, especially in rural areas.

There is a great need for an inexpensive diagnostic method that is both reliable and unifies clinical and microbiological parameters to make it more sensitive while retaining its specificity. It may be beneficial to further review Amsel criteria to assign differential weights to various parameters (clue cells > pH > nature of discharge > whiff test) with evidence generated by a systematic review of related studies

Acknowledgements

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