

## Sensitivity and Specificity of Rapid Clinical Diagnostic Test for Bacterial Vaginosis and its Analytical Value

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### ABSTRACT

**Objective:** Amsel criteria and microbiological techniques are two conventional methods for the diagnosis of bacterial vaginosis. The purpose of this study was to determine the etiology of bacterial vaginosis in low socioeconomic female population of Karachi by comparing Amsel criteria, a bedside technique with standard microbiological methods.

**Study Design:** A comparative study carried out at Essa laboratory Karachi from April 2011 to March 2012.

**Material and Method:** 373 consecutive high vaginal swabs were taken from women aged 18 to 70 years received at Essa laboratory, Karachi. Diagnosis was made by bedside Amsel criteria and two swabs sent promptly to the microbiology laboratory for gram staining and culture.

**Results:** Bacterial vaginosis was determined in 373 women belonging to low socioeconomic population, history along with demographic profile of patients were taken. Overall prevalence of bacterial vaginosis calculated by microbiological method was 78% and by Amsel criteria 62%. Clinical diagnosis of bacterial vaginosis was made if three characteristics of Amsel criteria were present. Amsel criteria had a sensitivity of 77%, specificity 91%, with a positive predictive value of 97% and a negative predictive value of 53%.

By microbiologic techniques organism found in highest frequency was *Staphylococcus aureus* (46%) out of which (35%) were methicillin resistant (MRSA) as confirmed by cefoxitin disc diffusion method, *Escherichia Coli* (25%) followed by *Klebsiella pneumoniae* (16%), *Enterococcus faecalis* (6%), *Pseudomonas* species (5%) and *Proteus* species (2%).

**Conclusion:** Amsel criteria is convenient for diagnosing bacterial vaginosis at the bedside but its sensitivity is less compared to microbiological techniques which were taken as gold standard. Our study also highlights the high colonization rate of *Staphylococcus aureus* in particular multidrug resistant strains among females coming from low-socio economic background.

**Key words:** High vaginal swabs, amsel criteria, clue cells.

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### INTRODUCTION

Vaginal discharge in females is a common, miserable condition accompanied by foul odor, pruritus and other distressful symptoms.<sup>1</sup> In a developing country like Pakistan where there are severe financial constraints and poor education regarding hygiene particularly amongst our target population, diagnosis of bacterial vaginosis (BV) by laboratory techniques as a routine would place a great burden on available resources.

Amsel criteria involve fewer assets and therefore clinicians would be at a better position if they have knowledge of the application of Amsel criteria.

Reported studies on 'Amsel criteria' describe it as rapid, simple in clinical practice but it is not as sensitive and may lead to misdiagnosis because of subject variation and also depends on the physician's experience, training and education. As an alternative microbiological techniques are more reliable if performed in standard laboratories and are taken as the 'gold standard'.<sup>2</sup> The microbiologic identification is based on gram stain, culture and various biochemical tests.

BV is common in low social economic groups where the reported incidence is 20-49%.<sup>3</sup> Its reported rate is 45-55% in African American, 20-30% in Asian women and 5-15% in Caucasian women.<sup>4</sup>

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The normal vaginal flora are lactobacilli (70%) which contribute in producing low pH, restrict the growth of other harmful organisms and play a role in colonization resistance. Some bacteria like *Staphylococcus epidermidis* and diphtheroids (30% - 60%) can also be found. Organisms which can possibly become pathogenic involves gram positive bacteria like *Staphylococcus aureus* (<5%), *Group D Streptococcus* (10%-40%), alpha hemolytic and beta hemolytic *Streptococcus* (10%-25%) and gram negative organisms like *Klebsiella* and *proteus* (<10%), *Neisseria* (5-20%), *candida* (20%- 30%), *Trichomonas Vaginalis* (10-25%).<sup>5</sup>

Under certain conditions normal flora become virulent which include an alkaline pH, high glycogen content, and hormonal imbalances like increased estrogen content. BV frequently accompanies sexually transmitted diseases (STD), contraceptive devices, multiple sex-partners, and douching.<sup>6</sup> The study was considered important because lower genital tract infections have an important clinical impact such as poor outcome of pregnancy, premature delivery, endometritis, chorioamnionitis, and small for gestational age (SGA) infants.<sup>6</sup> BV has also been associated with number of complications like pelvic inflammatory disease (PID), post-hysterectomy vaginal cuff, cellulitis, infertility, post- surgical infection, and urinary tract infections.<sup>7</sup> There is a report of connection between BV and Human immunodeficiency virus.<sup>8</sup>

The aim of our study was to assess the etiology of BV among low socio-economic female population presenting with vaginal discharge. We compared Amsel criteria with microbiological techniques. Our study highlights the importance of individuals carrying these pathogenic organisms which may be multidrug resistant as a source of infection for themselves as well as the community through direct contact or via fomites.

## MATERIAL AND METHOD

The study population consisted of 373 females who presented; with complains of vaginal discharge, itching and pain from April 2011 to March 2012.

Before commencing the research, a study protocol was accepted by the hospital ethics review committee and consent was taken from all the patients prior to examination. The exclusion criteria included women who were younger than 18 years or older than 70 years, pregnant women and those presenting during their menstrual period or on antibiotics. After informed consent, data was obtained about the amount, nature, color and odor of the discharge and associated symptoms such as pain and pruritus. Sterile cotton

tipped swab was inserted in the posterior and lateral vaginal fornices at the time of speculum examination and three samples were obtained.

One swab was used to determine Amsel criteria. Amount and color of discharge was noted by visual inspection, pH was determined by observing color change of pH paper, presence of fishy odor after addition of potassium hydroxide and visualizing of clue cells on unstained microscopic examination.

A bedside diagnosis of BV was made if three of the following four Amsel criteria were met.

- (1) Homogenous grayish white vaginal discharge.
- (2) Vaginal pH >4.5.
- (3) Presence or absence of fishy amine odor on addition of 10% potassium hydroxide KOH (whiff test).
- (4) Presence of clue cells (greater than 20%) on wet mounts.<sup>9</sup>

**Microbiological Examination:** One swab was sent for smear gram staining and culture on blood agar, chocolate agar and MacConkey agar.

**Biochemical Examination:** Gram-negative organisms were further tested by the API 20 system (Biomérieux) and the color reactions were read for identification of bacteria.<sup>10</sup>

Descriptive statistics were used to summarize the data using mean and standard deviation. Diagnostic efficacy of Amsel criteria was determined by calculating sensitivity (true positives), specificity (true negatives), positive predictive values and negative predictive values. A Chi-Square test was applied for statistical analysis using SPSS software (version 16).

## RESULTS

In our results, mean age was 34.34± 8.28 years (Table 1). Among 373 women, 259 (89%) were in 18-40 years range, 27(9%) were in 41-65 years of age group. Only 2% were in the age group greater than 65 years indicating that these females are less prone to vaginal infections. This concludes that proportion of BV among age categories are not same (P=0.000, statistically significant) [Table I].

Table I: Age related frequency of bacterial vaginosis

| Range (Age in years) | Study Subjects n=373 | Bacterial Vaginosis n=291 |
|----------------------|----------------------|---------------------------|
| 18-40                | 328                  | 259                       |
| 41-65                | 36                   | 27                        |
| >65                  | 90                   | 5                         |

of 373 cases, BV was present in 291 (78%) by microbiological examination [Table II & III].

Microorganisms isolated in this study included: *Staphylococcus aureus* (46%) among which 35% were (MRSA) by cefoxitin disc diffusion test, *Escherichia coli* (25%), *Klebsiella pneumoniae* (16%), *Enterococcus faecalis* (6%), *Pseudomonas species* (5%) and *Proteus species* (2%) [Table II]

Table II: Bacterial isolates from high vaginal swabs

| Organisms                    | Bacterial Isolates | Percentage (%) |
|------------------------------|--------------------|----------------|
| <i>Staphylococcus aureus</i> | 134                | 46             |
| <i>Escherichia coli</i>      | 73                 | 25             |
| <i>Klebsiella</i>            | 46                 | 16             |
| <i>Enterococcus Faecalis</i> | 17                 | 6              |
| <i>Psuedomonas species</i>   | 15                 | 5              |
| <i>Proteus species</i>       | 6                  | 2              |

The reported organisms were found in combination with one or other organisms but these were mostly taken as normal flora as their CFU/ml was considered insignificant. We did not use any media for isolating the fungus candida or *Trichomonas*.

By Amsel criteria diagnosis of BV was made in 232 (62%) cases. [Table III]

Table III: Comparasion of amsel criteria with gram stain method

| Gram stain | Amsel criteria |          | Total |
|------------|----------------|----------|-------|
|            | positive       | negative |       |
| Positive   | 225            | 66       | 291   |
| Negative   | 7              | 75       | 82    |
| Total      | 232            | 141      | 373   |

The sensitivity of Amsel criteria was 77% (95% confidence interval [CI] =72 to 82%), specificity was 91% (95% CI = 83 to 96%) positive predictive value was 97 % ( 95% CI= 94 to 99%) while false negative value was 53 % ( 95% CI=45 to 62%).

When examining the sensitivity and specificity of individual components of Amsel's criteria, we found that Whiff test had the highest sensitivity(74%) while specificity was moderate (72%), clue cells specificity (75%) was higher than its sensitivity(66%). Vaginal discharge had good specificity (79%) among all other criteria. pH had low values of both sensitivity (61%) and specificity (60%). [Table IV]

Table IV: Sensitivity, specificity and predicive values by amsel techniques

|                         | sensitivity % | specificity % | PPV % | NPV % |
|-------------------------|---------------|---------------|-------|-------|
| Amsel criteria positive | 77            | 91            | 97    | 53    |
| Whiff test              | 74            | 72            | 89    | 49    |
| clue cell               | 66            | 75            | 89    | 43    |
| pH>4.5                  | 60            | 60            | 82    | 34    |
| Vaginal discharge       | 62            | 79            | 90    | 41    |

## DISCUSSION

BV is an infectious disease, the etiology of which is well recognized in the developed countries but this condition is under reported in our country.<sup>11</sup> Increased vaginal discharge is a common complaint among women presenting to gynaecological outpatient and prenatal clinics.<sup>5</sup> The etiology of BV is polymicrobial where normally present lactobacilli are replaced by some other bacteria such as *Gardenella vaginalis*, *Mycoplasmas*, and anaerobic gram negative rods.<sup>8</sup> Recently some rapid diagnostic techniques have been instituted such as Polymerase Chain Reaction (PCR), hybridization techniques, and test based on extra cellular protein elaboration such as proline amino peptidase activity.<sup>12</sup> Although currently, up-to-date techniques have been introduced based on RNA and sensor arrays these are very expensive and their sensitivities and specificities do not offer more benefits when compared with simple traditional methods.<sup>12,13</sup>

Previous studies have shown that BV prevalence was higher in women aged 25 years and older.<sup>11</sup> Our results revealed that BV is more prevalent in women aged 18-40 years which shows that the frequency of cases in each category of age was not same (p=0.000, statistically significant).

Traditionally the diagnosis of BV is done by assessing clinical symptoms and laboratory testing. Clinically Amsel criteria are the most commonly recognized method for the diagnosis of BV. Nugenet et al also developed a grading system for gram stain of vaginal discharge based on presence or absence of certain bacterial morphocytes (and their relative numbers), which gives more reliable evaluation assessment of BV.<sup>14</sup> In a similar study conducted in Rawalpindi in 2010, Amsel's criteria showed a frequency of 38.5% and sensitivity, specificity of 92% and 96% respectively.<sup>15</sup> In an international study conducted in USA, Amsel criteria stated a sensitivity of 74% and specificity of 94%.<sup>15</sup>

In our study Amsel criteria had a sensitivity of 77%, specificity 91%, with a positive predictive value of 97% and a negative predictive value of 53%.

In previous studies, clue cells had higher sensitivity and specificity among all four Amsel criteria in contrast to our study.<sup>16</sup> whiff test was performed by smelling the odor its results are subjective to individual variation. In our study whiff test had highest sensitivity (74%), followed by clue cells (66%), vaginal discharge (62%), and pH > 4.5 (6%).

By culture techniques, we isolated *Staphylococcus aureus* (46%) out of which (MRSA) strains were detected in 35% of cases. In another study *S.aureus*

was isolated at the rate of 36%.<sup>17</sup> Our study showed *E.coli* (25%), *Klebsiella* (16%), and *Proteus specie* (2%). In other study, *E.coli* has been reported in 5%-25% of vaginal cultures while *Klebsiella* and *Proteus* have been found in less than 10% of vaginal swabs in a study from USA.<sup>18,19</sup> These organisms along with vaginal infections may become the source of urinary tract infections.

Our study showed a very high isolation rate of MRSA. *S.aureus* is a dangerous pathogen which colonizes the vagina of healthy females where it has the potential of causing toxic shock syndrome. It is carried on skin and nose of 50-60% of individuals<sup>20</sup> and can be transmitted to other parts of the body as well as to contacts and fomites. It is more prevalent in low socio-economic population and in women with multiple childbirth, pelvic inflammatory disease, endometritis etc.

## CONCLUSION

In conclusion, standard culture methods should be adopted for diagnosis of BV with discharge. The clinical impact of this condition is important as the women carrying the organisms can have other endogenous infections as well as become the source of transmission to other contacts as is observed by the increasing isolation rate of *S.aureus* and MRSA in the community. Measures should be taken to educate public and young females of child bearing age about contact infections and hygiene.

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