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*Full Length Research Paper*

# Bacteriological study of vaginal discharge of pregnant women using Gram stain smear and culture

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**Bacterial vaginosis is caused by an imbalance of the organisms that naturally exist in the vagina. The importance of bacterial vaginosis with respect to pregnant women's health is emphasized by the association between bacterial vaginosis and adverse outcome of pregnancy. The aim of present study was to evaluate the direct smear microscopy and culture for determination of bacteria from vaginal discharge of pregnant women. In total, 240 vaginal swabs were collected from 120 pregnant women and were screened for bacterial population. For each patient one swab was used for smear preparation and Gram staining and the second swab was used for cultivation. The prepared Gram-stained smears were observed for various morphotypes. Each morphotype was quantified on a scale from 0 to 4 and weighed to yield a score of 0 to 10, as per Nugent's system. The bacteria grown in preliminary culture media were identified using standard identification tests. The majority of isolated bacteria in culture were Diphtheroid, *Lactobacillus* spp., Coagulase-negative Staphylococci and yeast. In Gram-stained smears, 78 (65%) Gram positive rods and 54 (45%) Gram positive cocci were detected. According to Nugent's criteria, 64 cases (53.33%) were classified as having normal vaginal flora, 45 (37.5%) intermediate flora and 11 cases (9.2%) having bacterial vaginosis. The prevalence of bacterial vaginosis is not very high. However we recommend the regular screening of women with Gram stain method using Nugent's criteria which is reliable, easy to perform and well suited for the routine clinical laboratory.**

**Key words:** Vaginal discharge, bacterial vaginosis, Nugent's criteria, Gram stain, culture.

## INTRODUCTION

The vaginal microflora constitutes a complicated environment, composed of varying microbiological species in variable quantities and proportions and their concentrations are indicative of the vaginal health of the individual (Donders et al., 2005). The microbial ecology subject to remarkable changes over the course of lifetime a, induced by developmental and hormonal changes (Pybus et al., 1999). In childhood, the vaginal flora contains skin commensals and bowel organisms. At menarche, the pH falls from neutral to approximately 4, and the flora becomes dominated by lactobacilli. Many other organisms may be present in lower concentrations, including anaerobic and facultative anaerobic bacteria and *Candida* spp. (Donders, 1999). In women of childbearing age this system is also dominated by *Lactobacillus* spp., a defining characteristic of which is the ability to grow in acid media and tolerate acid conditions at pH around 4.5; lactobacilli also ferment carbohydrates to produce lactic acid (Sobel, 2000). The normal vaginal bacterial flora of healthy pre-menopausal women continues to consist predominantly of *Lactobacillus* spp. These are believed to play a protective role in guarding the urogenital tract against infection by

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pathogens (Romanik and Martirosian, 2004).

Bacterial vaginosis (BV) is caused by an imbalance of the organisms (flora) that naturally exist in the vaginahttp://www.revolutionhealth.com/articles?id=hw142281. BV is among the diseases that most frequently associated with vaginitis. The other diseases are vulvovaginal candidiasis, and trichomoniasis.

Vaginitis is usually characterized by vaginal discharge, vulvar itching and irritation, or odor (Witt at al., 2002). Normally, about 95% of vaginal flora is lactobacillus bacteria. These lactobacilli help keep the vaginal pH level low and prevent overgrowth of other types of organisms. In bacterial BV, a condition characterized by a raised vaginal pH and milky discharge, the normal vaginal flora is replaced by a mixed flora of aerobic, anaerobic and microaerophilic species (Schwebke, 2000). It seems that BV is accompanied by a shift in the normal *Lactobacillus* flora to a mixed vaginal anaerobic flora including *Gardnerella vaginalis, Bacteroides* spp., and *Mobiluncus* spp. (Wilks et al., 2004). BV commonly occurs in women of childbearing age and can be especially substantial in pregnant women (Witt et al., 2002). Prevalence of 10 to 31% has been reported in various populations (Wolrath et al., 2001; Chaudry et al., 2004). Women with BV have fewer *Lactobacillus* organisms than normal and more of other types of bacteria (Koumans et al., 2002). The importance of BV with respect to women's health is emphasized by the association between BV and pelvic inflammatory diseases, adverse outcome of pregnancy, postpartum endometritis, and cuff cellulites (Donders et al., 2005; Berg, 2001). Women with BV during pregnancy, have higher risks of miscarriage, early (preterm) delivery, and uterine infection after pregnancy (Leitich et al., 2003). Centers for disease control and prevention (CDC) advise that all pregnant women with BV symptoms be screened and treated with antibiotics (CDC, 2002).

Laboratory methods for the identification of BV include wet mount, Gram stain, the "gold standard" of diagnosis, and microbiological culture. Because microscopic evaluation by wet mount or Gram stain requires special diagnostic skills not available to all practitioners, the therapy of BV is frequently empirical (Witt at al., 2002). The present study was performed with the aim to evaluate the direct smear microscopy and culture for determination of bacteria from vaginal discharge of pregnant women.

## MATERIALS AND METHODS

The study population was comprised of 120 pregnant women with gestation age ranging between 4 to 41 weeks referred to Obstetrics and Gynecology clinic of Sina hospital, Ahvaz, Iran from April to September 2009. After informed consent, a total of 240 vaginal swabs were collected from referred women. Sampling was carried out by insertion of two sterile cotton swabs for diagnosis of flora and BV, one swab was obtained for smear preparation and Gram stain and the other swab was obtained for culture. Samples were immediately transferred to the microbiology laboratory. Gram stained smears were examined under oil immersion (x 1,000) of light microscope for the following morphotypes: large Gram positive rods (*Lactobacillus morphotypes*), small Gram-variable and Gram-negative rods (Gardnerella and Bacteroides morphotypes), and curved Gram variable rods (*Mobilincus* morphotypes). Each morphotype was quantified on a scale from 0 to 4 and weighed to yield a score of 0 to 10, as per Nugent's system (Nugent et al., 1991; Delaney and Onderdonk, 2001). According to this system, the criterion for BV was a score of 7 or higher. The second swab was cultured on blood, chocolate and McConkey agars (Hi-media, Mumbai, India) for investigation of BV and normal flora. The culture plates were incubated in 37°C in presence of 5% $CO_2$ for 24-48 h. The culture plates were then examined and based on grown bacteria, the necessary biochemical tests were performed and the organisms were identified as per standard criteria (Forbes et al., 2007).

## RESULTS

The range of women' ages was 16 to 42 years with the mean of 25.9 (Figure 1). The majority of pregnant women were in age group of 21-25 years and gestational ages of 40, 39, 38, and 37. Table 1 represents the relative frequencies of the most common isolated normal flora and a few pathogenic bacteria in stained smears (no. 120) and culture (no. 120). The majority of isolated bacteria in culture were Diphtheroid, *Lactobacillus* spp., yeast, and Coagulase-negative Staphylococci. Enteric Gram-negative rods and Streptococci were isolated at lower rate. Based on the results of Gram-stained smears, 78(65%) Gram positive rods and 54 (45%) Gram positive cocci were detected. Table 2 represents the score of vaginal discharge as per Nugent's system. According to this criterion in Gram-stained smears, 64 cases (53.33%) were classified as having normal vaginal flora (a+b), 45 (37.5%) intermediate flora (c+d) and 11 cases (9.2%) BV (e+f) (Figure 2). Gram-stained smears were showed to include polymorphonuclear leukocytes, fungi and clue cells.

## DISCUSSION

Bacterial vaginosis is a very common condition characterized by alterations of the vaginal flora with acquisition of diverse communities of anaerobic and facultative bacteria and depletion of the usually dominant *Lactobacillus* flora (Sobel, 2005). Accurate diagnosis of BV is important as it is associated with adverse pregnancy outcome (Myziuk et al., 2003) . In a recent study, the investigators tried to evaluate the possible association of BV with serious reproductive complications in women and they reported a significance association of BV with infertility (Mania-Pramanik et al., 2009).

Currently the criteria as defined by Nugent et al. (1991) are considered as the standard procedure to score vaginal smears by Gram stain (Nugent et al., 1991). This method scores the smears in a standardized manner by quantification of some of the cell types present (Forsum et al., 2002). The present work confirms the findings from

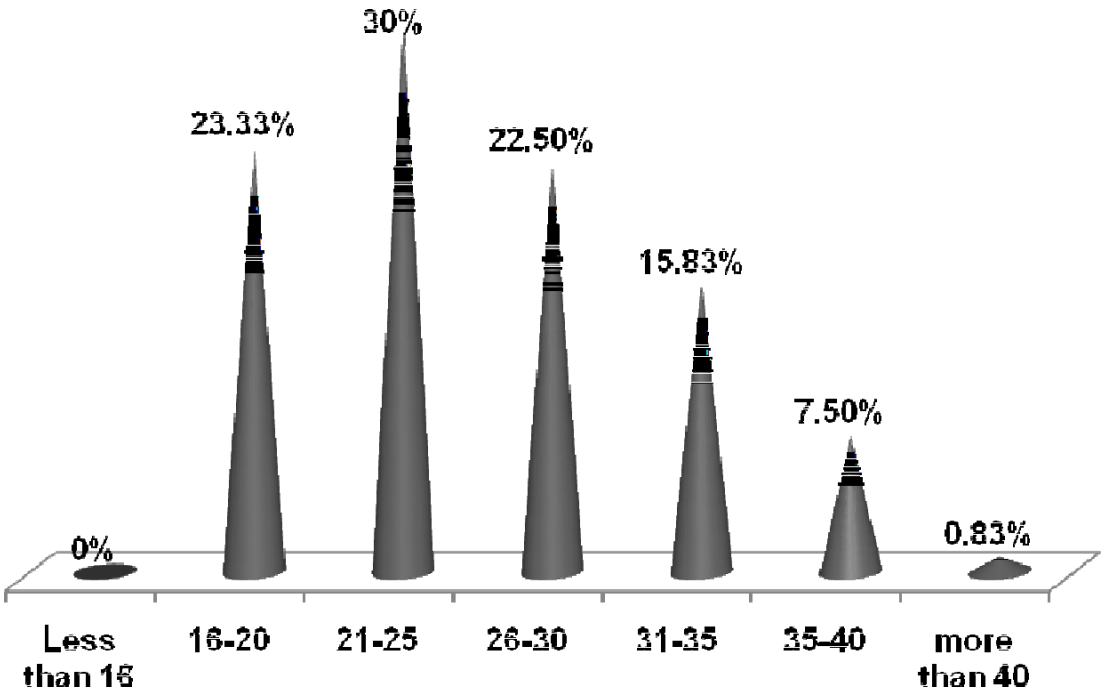


**Figure 1.** Frequency of age groups in pregnant women in this study.

**Table 1.** The relative frequency of organisms seen in Gram-stained smears and isolated from vaginal discharge cultures.

| Isolated organisms | Smear Gram stain No. (%) | Culture No. (%) |
|---|---|---|
| Gram positive rods (*Lactobacillus* spp.) | 78 (65) | 46 (38.33) |
| Curved gram variable rods (*Mobilincus*) | 22 (18.33) | - |
| Gram negative rods (*Gardnerella*) | 42 (35) | - |
| Gram positive rods (Diphtheroid) | 16 (13.33) | 48 (40) |
| Gram negative cocci (*Neisseria* spp.) | 12 (10) | 8 (6.66) |
| Enteric Gram negative rods | - | 4 (3.33) |
| *Staphylococcus aureus* | - | 18 (15) |
| Coagulase negative staphylococci | - | 43 (35.83) |
| Streptococci | 36 (30) | 11 (9.16) |
| Yeast | 36 (30) | 44 (36.66) |
| Clue cells | 18 (15) | - |

**Table 2.** Score of vaginal discharge in present study as per Nugent criteria system.

| Score | A | B | C | No. (%) |
|---|---|---|---|---|
| 0 | $4^+$ | 0 | 0 | 48 (40) |
| 2 | $3^+$ | $1^+$ | 0 | 16 (13.3) |
| 4 | $3^+$ | $3^+$ | 0 | 30 (13.3) |
| 6 | $2^+$ | $4^+$ | $4^+$ | 15 (12.5) |
| 8 | $1^+$ | $4^+$ | $4^+$ | 5 (4.2) |
| 10 | 0 | $4^+$ | $4^+$ | 6 (5) |

A: large Gram-positive rods (*Lactobacillus* morphotypes).
B: Small Gram-variable rods (*G. vaginalis* morphotypes).
C: curved Gram-variable rods (*Mobiluncus* spp. morphotypes).

previous studies demonstrating the usefulness of Nugent's criteria for determination of BV in women (Delaney and Onderdonk, 2001; Tamrakar et al., 2007; Sha et al., 2005).

We examined the vaginal flora by the objective and reproducible evaluation of Gram-stained smears and culture. To determine the prevalence of BV, we applied the strict definition given by Nugent et al. (1991). We found that in pregnant women under investigation the prevalence of BV was 9.2% with scores of 8 and 10 (Table 2). In a recent work from Iran, the prevalence of BV based on the presence of clue cells and Amsel's criteria (Amsel et al., 1983), was reported as

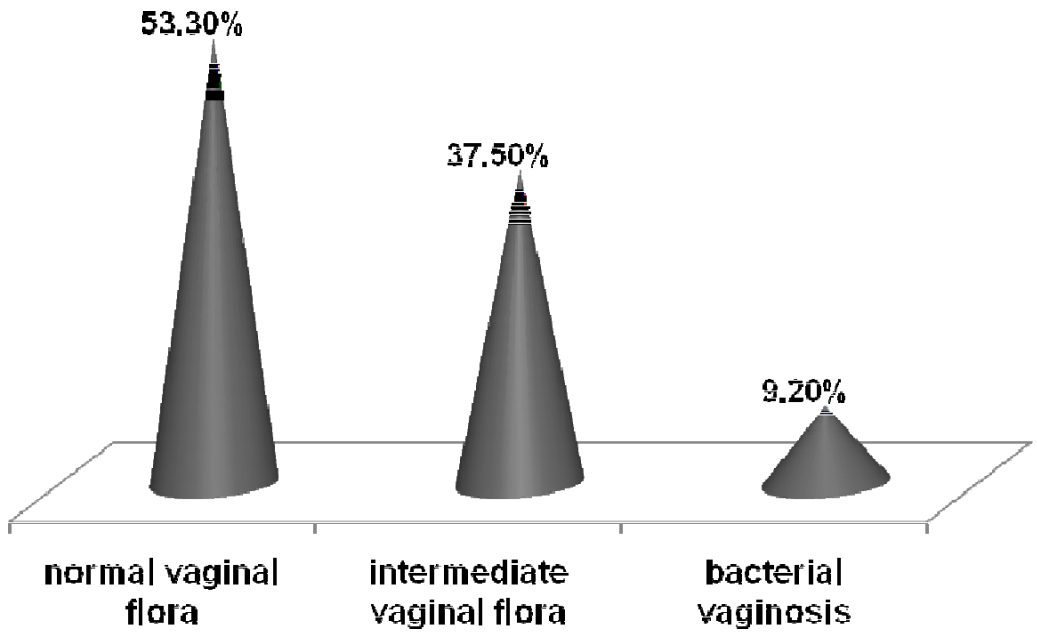


**Figure 2.** Bacteriological finding of vaginal discharge in pregnant women based on Nugent's system.

11.3% among 160 labouring women (Modares and Shafaie, 2008). Dadhwal et al. (2010) found a prevalence of 8.6% among 502 asymptomatic pregnant women. This was reported as 4.5% among 472 studied pregnant women by Gratacós et al. (1999). While their findings were close to ours, however in some other studies the reported prevalence was higher than this study. The reported BV prevalence in the study of Rizvi and Luby (2004) was 25%, in Abu Shaqra (2001) was 29.7%, and in Rouse et al. (2009) was 16.6%. The Nugent's criteria was used by these investigators as an easy and reliable diagnostic tool to determine the prevalence of BV, since the clinical criteria alone may not enough for a definite diagnosis especially in pregnant women with asymptomatic BV. In a study on 492 women, BV was diagnosed in 1.6% of women on the basis of clinical criteria, while this was 4.5% according to Gram stain (Rizvi and Luby, 2004). Lactobacilli were the most prevalent organisms in Gram- stained smears in normal women with scores of 0 and 2 and were the least or none in women with BV comprising scores 8 and 10. This finding suggests that the decrease in *Lactobacillus* colonization could be a leading cause of the BV as previously concluded (Chaudry et al., 2004; Sobel, 2005). The cultural method and data interpretation have established that the normal flora was very diverse in tested specimens, reflecting a dynamic, polymicrobial ecosystem. While the number of some morphotypes such as *Gardnerella* and *Mobilincus* was significant in stained smears, in culture we could not detect these morphotypes, probably due to sensitivity of these morphotypes and lack of special requirements in our culture media needed for their growth. The majority of grown bacteria in culture were Gram positive cocci and rods (Lactobacilli) with the least belonged to Gram negative entric rods. Since for diagnosis of BV, the culture is time-consuming compared to direct Gram stain and providing the special requirements for growth of fastidious morphotypes makes the process costly, we believe the Gram-stained smear alone, without culture, can be used to evaluate vaginal swab specimens for BV.

In conclusion, the overall results of this study which was conducted for the first time in our setting indicated that the prevalence of BV is not very high. However we recommend the regular screening of women with Gram stain method using Nugent's criteria which is reliable, easy to perform and well suited for the routine clinical laboratory. This method could be used for rapid diagnosis of bacterial BV for clinicians with minimum need for confirmation by culture to prevent treatment delay.

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