



## Review Article

# Bacterial Vaginosis

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## ABSTRACT

### Keywords

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Bacterial vaginosis (BV) is the most common cause of vaginal discharge among women in reproductive age. BV is called vaginosis and not vaginitis because it is associated with alteration in normal vaginal flora rather than due to specific infection. BV is a sexually associated condition. It is characterized by an increased vaginal pH and the replacement of vaginal lactobacilli (particularly those that produce hydrogen peroxide) with *Gardnerella vaginalis* and anaerobic Gram negative rods. BV is of special public health concern in India because of the high burden of reproductive and pregnancy related morbidity. High co-infection rates with sexually transmitted infections (STI) raise the possibility that BV may either increase susceptibility to STI or share common pathway with other STI's. BV is associated with risk for pelvic inflammatory disease (PID), postabortal PID, postoperative cuff infections after hysterectomy and abnormal cervical cytology in non-pregnant women. Pregnant female diagnosed with BV have an elevated risk of preterm labour, premature rupture of membranes, chorioamnionitis and postpartum endometritis. The patient with vaginitis must always be taken seriously. Hence this review gives the necessary details regarding this important medical condition with emphasis that this condition must never be written off as mild/ insignificant and an accurate diagnosis must always be established and it is essential to study the same in detail.

## Introduction

The term, "Bacterial vaginosis" was coined to describe abnormal bacterial flora of the vagina characterized by an overgrowth of anaerobic bacteria and *G.vaginalis* with a marked decrease in the presence of Lactobacillus. The term was chosen to replace nonspecific vaginitis, with the suffix "-osis" specifically chosen to indicate the absence of an inflammatory reaction that is the presence of neutrophils. BV is defined as

abnormal microflora of the vagina characterized by significant reduction of the dominant bacterium. Lactobacillus to extremely low levels, fewer than 10,000 col/ml of vaginal fluid and marked increase in the anaerobic bacterial population and associated with this change is an increased colonization of *G.vaginalis*.<sup>1</sup> Clinically and microscopically BV is defined as follows: vaginal pH>4.5, the liberation of amines

when vaginal discharge is mixed with 10% potassium hydroxide solution and the presence of clue cells. Important to this definition is absence of inflammatory cells.<sup>1</sup>

In the healthy vagina, *Lactobacillus* predominates whereas in BV, *Lactobacillus* is significantly reduced in number and obligate anaerobes, both gram-positive and gram negative make up the dominant flora.<sup>1</sup>

### **History of bacterial vaginosis**

1894- Doderlein described the presence of *Lactobacilli* in normal vaginal flora. *Lactobacilli* produce lactic acid which inhibits the growth of other vaginal microorganisms by maintaining a low vaginal pH and occupying an ecological niche.<sup>2</sup> 1914- Curtis associated anaerobic cocci and *Mobiluncus* with abnormal vaginal discharge. He postulated that anaerobic microorganisms were part of a complex bacterial milieu that caused not only an abnormal vaginal discharge but also postpartum endometritis.<sup>2</sup> 1921-Schroeder categorized vaginal flora using Gram stain into the least pathogenic (consisting predominantly of *Lactobacilli*), intermediate stage and most pathogenic stage that is now identified as Bacterial vaginosis (BV).<sup>2</sup> 1950-Weaver et al associated *Bacteroides* with BV and also confirmed absence of *Lactobacilli* in the syndrome.<sup>2</sup> 1955-Gardner and Dukes described a new microorganism which they called *Haemophilus vaginalis*. They believed that this agent caused vaginitis. They also described the clinical features of this syndrome that forms the basis of diagnosis today. The vaginal discharge was characterized by a grey homogenous appearance, had a pH between 5.5 and 6.0, it had a malodor, an absence of *Lactobacilli* and the presence of so-called vaginal epithelial clue cells. The discoveries of Gardner and Dukes were important in

defining the clinical disease and the association of at least one organism *H.vaginalis* with the syndrome. However, it is now apparent that a variety of anaerobic microorganisms also have a role in BV.<sup>2</sup> Confusion existed over taxonomy such that *H.vaginalis* was first reclassified as *Corynebacterium* and then classified in genus *Haemophilus*. Deoxyribonucleic homology studies were needed to ultimately classify this organism in a separate genus and the microorganism is now called *G.vaginalis* in honour of Dr. Gardner. Advancements in anaerobic microbiology in 1970s finally lead to realization that several anaerobic bacteria, as well as *G.vaginalis* were present in this disease.<sup>2</sup>

### **Microbiology of BV**

Bacterial vaginosis is not a specific, monobacterial infection, but a synergic mixture of anaerobic, microaerophilic and CO<sub>2</sub> dependent species that are present in small numbers in many normal asymptomatic women but in large numbers in vaginosis.<sup>3</sup> The normal *Lactobacillary* flora is replaced by a mixture of small bacilli normally inhibited by the *Lactobacilli*, CO<sub>2</sub> dependent *G.vaginalis* and two anaerobic gram negative groups-*Bacteroides* spp. of the melanogenicus-oralis group (principally *B.bivius* and *B.disiencs*) and curved motile rods of *Mobilincus* spp. *G.vaginalis* and *Bacteroides* spp. Are present in most cases. *Mobilincus* are curved, motile gram negative rods. They were described in vaginal discharge by Curtis. Two species have been described and named – *M.curtisii* and *M.mulieris*.<sup>3</sup> *Mycoplasma* species are also associated with BV, but their role is uncertain. The vaginal pH, normally <4.5 rises to >5.5. The lactate concentration is reduced and the amount of succinate, acetate, propionate and butyrate (all principally produced by *Bacteroides* spp.) increase. The secretions also contain volatile

amines, eg. putresine, methylamine, cadaverine etc which are products of anaerobic metabolism and cause the fishy smell.<sup>3</sup> Bacterial relationships in the pathogenesis of BV are not clear, but metabolic interactions may generate active products that cause excessive secretion, e.g pyruvate and amino acids secreted by *G.vaginalis* may be decarboxylated to amines by *Bacteroides* spp.<sup>3</sup>

Lactobacilli predominate in the normal vagina, the pH is low and the principal fatty acid product of metabolism is lactate. The vaginosis associated organisms that are present in relatively small numbers; particularly the *Bacteroides* spp. and *G.vaginalis* are inhibited in vitro by lactic acid and low pH. In susceptible women, the natural protective mechanism is lost by a combination of inhibition of the Lactobacilli, increase in pH and buffering of the lactate, and allows the proliferation of *G.vaginalis* and *Bacteroides* spp. The metabolic interactions of these synergic mixtures may then produce active metabolites which induce secretion from vaginal mucosa while endowing the discharge with its offensive character.<sup>4</sup>

Anaerobic bacteria associated with BV are *Gardnella vaginalis*, *Prevotella*, *Porphyromonas*, *Fusobacterium*, *Eubacterium*, *Propionibacterium* spp.<sup>4</sup> *Atropobium vaginae* is most recently detected organism in BV.<sup>5</sup> It has been linked to higher risk of preterm labour and recurrent bacterial vaginosis.<sup>6,7</sup> Aerobic bacteria associated with BV are *S.aureus*, Group B haemolytic streptococci and *E.coli*.<sup>4</sup>

### **Epidemiology of BV**

In a study conducted in the rural area of Shandog province in China, the prevalence of BV was 6.6%.<sup>8</sup> In another study

performed in Hamedan province, Iran, the prevalence of BV was 28.5%.<sup>9</sup> Among women referred to hospital in Vientiane, the capital of Laos, the prevalence of BV is 24.5%.<sup>10</sup> In another study conducted in the rural area of Northeast Brazil, 20% of women had BV.<sup>11</sup> In a study conducted in married rural women in Karnataka, India prevalence of BV using Nugent's criteria was reported to be 20.5%.<sup>12</sup> In another study conducted in Delhi, India highest prevalence of 38.6% was seen in urban slum followed by rural with 28.8% and urban middle class community with prevalence of 25.4%.<sup>13</sup> In a study in Mysore, India the prevalence of BV was 19%. An highest prevalence rate of 48.5% was seen in a study from Haryana, India in rural women.<sup>14</sup>

### **Risk factors and clinical features**

Approximately 50-75% of women with BV are asymptomatic. In symptomatic women the manifestations vary from increased grayish white vaginal discharge, which may have an offensive odor which is intensified after intercourse and during menstruation. Other symptoms are pruritis, lower abdominal pain, and pain during coitus, etc.<sup>12</sup>

Risk factors for acquisition of BV are Multiple sexual partners, douching, cigarette smoking, low socioeconomic stress, use of diaphragms, previous sexually transmitted diseases.<sup>15,16</sup>

In pregnancy the complications are premature rupture of membranes, post caesarean endometritis, amniotic fluid infection, vaginal cuff cellulitis and post abortal infection. In fetus the common complications seen are prematurity and intrauterine growth retardation.<sup>17</sup> BV is a risk factor for HIV acquisition and transmission. BV increases risk of HIV transmission by 2-4 fold and has been

estimated to contribute 23% to antenatal HIV seroconversion in a high prevalence population of pregnant women in Malawi.<sup>18</sup> BV is also a risk factor for HSV-2, gonorrhoea and chlamydial infections. BV is more common among female with pelvic inflammatory disease, but it is not clear if it is an independent risk factor for this disease.<sup>16</sup> The bacterial flora that characterizes BV have been recovered from endometrial and salpinges of women who have pelvic inflammatory disease (PID). BV has also been associated with endometritis and vaginal cuff cellulitis after invasive pelvic procedures like endometrial biopsy, hysterectomy, hysterosalpingography, insertion of intrauterine device, cesarean section and uterine curettage.<sup>19</sup> There are reports suggesting women suffering from BV are at greatest risk of acquiring urinary tract infection (UTI) than others. Harmanli et al conducted a study and found that 15 out of 67 women (22.4%) had both BV and UTI; whereas only 6 (9.7%) had UTI without BV. Hillebrand et al in a cross-sectional study examined 503 pregnant women from viewpoint of UTI and BV and reported that 13.6% of 140 women suffering from BV also had UTI whereas only 6.6% of 363 women without BV had UTI. He concluded that BV in pregnancy increase the risk of UTI.<sup>20</sup> Some studies have found that the presence of *Gardnerella vaginalis* on the cervix as detected on Papanicolaou smear is associated with high grade squamous intraepithelial lesion, however a causal relationship has not been proven and others have not reported this association.<sup>16</sup>

### **Pathogenesis**

The association between BV and its attendant microbes and serious infections in the female pelvis has led to understand this entity more completely to facilitate development of improved modalities for prevention and treatment.

BV is related to:

1. An increased potential for other vaginal pathogen to gain access to the upper genital tract.
2. The presence of enzymes that decrease the ability of leucocytes to reduce infection.
3. An increased level of endotoxins stimulates cytokine and prostaglandin production.

Increased vaginal levels of interleukin-1 beta, an inflammatory cytokine, among pregnant women with BV and higher levels of both cervical IL-1 beta and interleukin-8 cytokine levels among non-pregnant women with BV are previously been reported.<sup>16</sup>

Hydrogen peroxide producing Lactobacilli are important in preventing overgrowth of the anaerobes which are normally present in the vaginal flora. With the loss of Lactobacilli pH rises and massive growth of vaginal anaerobes occur. These anaerobes produce large amounts of proteolytic carboxylase enzymes, which breakdown vaginal peptides into a variety of amines that are volatile, malodorous and associated with increase vaginal transudation and squamous epithelial cell exfoliation resulting in the typical clinical features observed in patients with BV. The rise in pH also facilitates adherence of *G. vaginalis* and other organism to the exfoliating epithelial cells thereby creating the “clue cells” that are diagnostic of the disease.<sup>15</sup>

### **Diagnosis of BV:**

#### **1. Clinical composite criteria (Amsel's Criteria):**

Patients were diagnosed as having bacterial vaginosis if they fulfilled any three of the following four criteria.<sup>21</sup>

1. Thin, homogenous grayish white vaginal discharge.
2. Vaginal pH above 4.5.
3. A fishy smell on addition of 10% KOH to vaginal fluid (Whiff's test).
4. Presence of 'clue cells' on saline wet mount.

## **2. Gram Stain:**

Gram stain of vaginal fluid has been used for laboratory confirmation of bacterial vaginosis since 1965. In 1983 Spiegel modified the Gram stain criteria for BV to include those smears with the Gardnerella morphotype plus other bacteria (cocci, fusiforms and curved rods) and fewer than 5 Lactobacilli morphotype per oil immersion field.<sup>22</sup>

Nugent et al reported that the 3 bacterial morphotype that were recognized with the highest degree of reproducibility were Lactobacilli (large gram positive bacillus), Gardnerella and Bacteroides (small gram negative or gram variable rods).<sup>22</sup> The three bacterial morphotypes were used to develop a 0-10 point scoring system for diagnosis a 0-10 point scoring system for diagnosis of BV.<sup>23</sup> A score of 0-3 is normal, 4-6 is considered intermediate and 7-10 is diagnosis of BV.

## **3. Vaginal Cultures:**

Culture for *Gardenerella* and anaerobes can be done on brain heart infusion blood agar, Columbia blood agar, human blood bilayer medium with Tween 80 (HBT medium) and neomycin blood agar by semiquantitative techniques. The plates are incubated at 37°C in carbondioxide for *Gardenerella* and in anaerobic jar for anaerobes. Approximate grading can be done by grading as 1+ growth on one quadrant as 1+ to 4+ if growth is seen in all four quadrants.<sup>12</sup>

## **4. Papanicolaou smears:**

Pap smears were also used for diagnosis of BV. But recent studies suggest that Pap smears are less specific because standardized criteria for the evaluation of Pap smears have not been routinely applied.<sup>23</sup>

The Pap smear is used commonly as cytologic screening test for eradication of precancerous lesions. It has, been evaluated as a diagnostic test for BV, but the results of these studies are contradictory. Smears performed with endocervical brush are fixed in 95% ethanol and stained by Pap method. If there is filmy background of small coccobacilli, individual squamous epithelial cells with a layer of coccobacilli along the margins of the cell membrane and conspicuous absence of Lactobacilli the smear is evaluated as positive for BV.<sup>24</sup>

Schnadig et al reported a high correlation between Pap smears and Gram smears for diagnosis of BV. Davis et al reported that compared to Gram stain, cervical cytologic test results had a sensitivity of 55%, specificity of 98%, positive predictive value of 96% and negative positive value of 78%.<sup>24</sup> But recent studies suggest that Pap smears are less specific because standardized criteria for evaluation of Pap smears has not been routinely applied.

## **5. Oligonucleotide probes:**

Oligonucleotide probes have advantage of being specific and can be adjusted in sensitivity to detect either low or high concentration of bacteria. One such application had been applied to *G.vaginalis* with use of rapid, non-isotropin assay for high concentration of this microorganism. Sheiness et al reported that detection of more than 107 colony forming units of

*G.vaginalis* per milliliter of vaginal fluid was 95% sensitivity and 79% specificity for diagnosis of BV.<sup>23</sup>

### 6. Gas Liquid Chromatography:

Succinic acid, a metabolic product of anaerobic bacteria is present in more frequency and at a higher concentration among women with BV. When Lactobacilli are the predominant member of vaginal flora, lactic acid is the predominant acid present. Among women with BV, succinate, acetate and other short chain organic acids can be detected.<sup>23</sup> Spiegel et al, reported that a succinate or lactate ratio of >0.4, based on peak heights on gas chromatographic analysis of vaginal fluid was correlated with clinical diagnosis of BV.<sup>23</sup>

This method has been evaluated in several case control and cohort studies and is reported to be 54% - 89% sensitive and 80-96% specific for the diagnosis of this syndrome.<sup>23</sup> This method is probably not adaptable for wider use since it relies on laboratory equipment that is not widely available.

### 7. Proline Aminopeptidase Assay:

This test is based on the detection of enzymatic activity. Proline aminopeptidase cleaves the substitute material, proline beta naphthalamide, yielding proline and beta naphthalamide. The naphthalamide can react with many aniline dyes to form various colored complexes. It can also be combined with nitrite to form a diazo complex or it can be measured direct flurometrically. This test requires no sophisticated instruments and has greater than 80% sensitivity. Another advantage is that upto 90 specimens can be run concurrently on a single microtitre plate in 1-4 hour period in contrast to Gas liquid chromatography which requires atleast 30 minutes per specimen.<sup>25</sup>

### 8. Molecular Methods:

Many researchers are exploring a genetic basis for exploration of the complex microbial flora associated with BV. The drawback of these techniques is the complexity and cost. David N.Fredrik et al developed a series of 16SrRNA gene PCR assays for more sensitive detection of key vaginal bacteria. According to their study *Leptotrichia amnionii* / *Sneathia*, *Atopobium vaginae*, an *Eggerthella* like bacterium, *Megasphaera* species and three novel bacteria in the order Clostridiales were among the bacterial species and significantly associated with BV.<sup>26</sup>

Fluorescent in situ hybridization (FISH) confirmed that newly recognized bacteria detected by PCR corresponded to specific bacterial morphotypes visible in vaginal fluid.<sup>26</sup> Thies F et al analysed vaginal flora just within 12 hours using T-RFLP (Terminal restriction fragment length polymorphism). T-RFLP is based on the restriction endonuclease digestion of fluorescently end labeled PCR amplicates (derived from the 16SrRNA gene).

*Atopobium vaginae* and *Gardnerella vaginalis* proved to be the predominant species.<sup>27</sup> Jean Pierre Menard et al quantitatively analyzed vaginal flora. A quantitative molecular tool targeting 8 BV related microorganisms and a human gene was developed using a specific using a specific real time PCR assay and serial dilutions of plasmid suspension. The targeted microorganisms were *Gardnerella vaginalis*, *Lactobacillus* species, *M.curtisii*, *M.culieris* and *Candida albicans* as well as *Atopobium vaginae*, *mycoplasma hominis* and *Ureaplasma urealyticum*.<sup>28</sup> Estelle Pevillard et al used PCR denaturing gradient gel electrophoresis (DGGE), for the analysis of organisms associated with BV.<sup>29</sup>

**Table.1** Scoring system of Gram stained smear according to Nugent et al<sup>22</sup>

<b>Bacterial morphotypes</b>	<b>0-3 (normal)</b>	<b>4-6 (intermediate)</b>	<b>7-10 (BV)</b>
Lactobacillus	4+ to 3+	2+ to 1+	0
Gardnella	0 to 1+	2+ to 3+	>4+
Bacteroides	0 to 1+	2+ to 3+	>4+
Mobiluncus (Curved rods)	Nil	Nil	1+ to 4+
Clue cells	Nil	Nil	Present

Average number of morphotypes seen per oil immersion field:

0= Nil, 1+ = 1, 2+ = 2-4, 3+ = 5 – 30, 4+ =>30

**Other methods:**

Diagnostic cards (e.g. Femcard Quick Vue and test card) are other rapid tests for confirming the clinical suspicion of BV. These cards are particularly useful for practitioners not able to perform microscopy. One group reported these risks detected the presence of elevated vaginal pH and increase amines with sensitivity and specificity of 87 and 92% respectively, although others have reported lower values.<sup>15</sup>

**Treatment:**<sup>30</sup>

In non pregnant females:

CDC-recommended regimens (nonpregnant patients)

Metronidazole 500 mg orally twice a day for 7days or

Metronidazole gel 0.75% 1 applicator-full (5g) intravaginally once or twice a day for 5days.

.Clindamycin cream 2% 1 applicator-full (5g) intravaginally at bedtime for 7 days.

Alternative regimens (nonpregnant patients):

Tinidazole 2g orally once daily for 2 days or

Tinidazole 1g orally once daily for 5 days or

Clindamycin 300mg orally twice a day for 7 days or

Clindamycin ovules 100g intravaginally at bed time for 3 days

Multiple recurrences:

Twice weekly metronidazole gel for 4-6 months may reduce recurrences

Oral nitroimidazole followed by intravaginal boric acid and suppressive metronidazole gel.

In pregnant females:

All pregnant women with symptomatic disease should be treated with one of the following recommended regimens.

Metronidazole 500 mg orally twice a day for 7days or

Metronidazole 250 mg orally three times a day for 7days or

Clindamycin 300 mg orally twice a day for 7 days

Treatment early in pregnancy may actually be important in preventing adverse outcome.

Insufficient evidence to assess the impact of screening for BV in asymptomatic pregnant women at high risk (those who have previously delivered a premature infant)

Treatment is recommended for women with symptoms.

Therapy is not recommended for male or female sex partners of women with BV.

Treatment of asymptomatic patients with BV who to undergo surgical abortion or hysterectomy can be considered. However, data are insufficient to recommend treatment of asymptomatic patients prior to procedures other than surgical abortion or hysterectomy.<sup>30</sup>

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