Bacterial vaginosis: clinical, epidemiologic and microbiological features

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Abstract

Bacterial Vaginosis (BV) is a polymicrobial clinical syndrome, whose etiology has not been fully understood. It occurs in approximately 30% of the women in childbearing age and is the result of the shift of protective resident microorganisms as *Lactobacillus* spp. by opportunistic pathogenic bacteria such as *Gardnerella vaginalis*. Patients with BV generally present copious, thin, homogeneous, milky, foul-smelling flow. Vaginal pH is > 4.5 and microscopy reveals bacteria-covered epithelial cells, termed "*clue cells*". Around 50% of the patients are asymptomatic and the disease is associated with gynecologic complications, such as cervicitis, salpingitis, endometritis, post-operative infections and pelvic inflammatory disease; and obstetric complications, such as premature rupture of the membranes, preterm deliveries, chorioamniotitis and postpartum endometritis. Although the Nugent's method is accepted as the "gold standard" for diagnosing BV, Amsel criteria are generally used for diagnosis in clinical practice. The Papanicolaou method is a valid diagnostic option, chiefly when it yields a positive result (mean specificity 95% when compared to gold standard). *G. vaginalis* has been almost universally recovered from women with BV, plays an important role in the pathogenesis of the disease, besides it may be detected in about 50% of healthy women. According to the literature, first-line drugs for BV treatment worldwide are metronidazole and clindamycin. However, regional studies on *G. vaginalis* drug susceptibility patterns are needed faced the so fast growing antimicrobial resistance phenomenon.

Keywords: Bacterial vaginosis. Gardnerella vaginalis. Vaginal microbiota. Lactobacillus. Metronidazole.

1 Introduction

From an ecological point of view, the vagina may be considered a complex anatomical site, where several bacterial species coexist and develop complex relationships. Over 50 species of microorganisms have already been isolated from the vagina, some species occupying a predominant position, guaranteeing their survival and contributing to the prevention of infectious diseases and health maintenance (LIVENGOOD, 2009).

The vagina is sterile on birth. After a few days, when maternal estrogen raises the glycogen levels of the epithelial cells, the baby's vagina is colonized by lactobacilli from the mother. This fact underlines the existing concept that the human bacterial microbiota in this case is closely related to the maternal microorganisms (FORSUM et al., 2005). The indigenous vaginal microbiota is dominated by *Lactobacillus* mainly *L. crispatus, L. gasseri, L. jensenii* and *L. iners,* followed by *L. acidophilus, L. fermentum, L. plantarum, L. brevis, L. casei, L. vaginalis, L. delbrueckii, L. salivarius, L. reuteri,* and *L. rhamnosus,* which represent 80% to 95% of the resident bacteria at this site (CRIBBY; TAYLOR; REID, 2008; FORSUM et al., 2005). These bacteria have some properties that while enabling mucosal colonization, hamper the establishment or excessive proliferation of potentially pathogenic microorganisms. Some of these properties include: specific adhesion to the surface of epithelial cells; production of substances with antimicrobial activity, such as hydrogen peroxide (H_2O_2), organic acids and bacteriocins; and the production of other

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substances with bacterial growth-inhibiting properties (LIVENGOOD, 2009; MARTIN et al., 2008).

Protection of the vaginal mucosa depends on the specific recognition of structures on the lactobacilli surface (adhesins) and the vaginal epithelium (receptors). This adhesin-receptor interaction results in the formation of a biofilm that exerts a protective local action against colonization by undesirable microorganisms (BORIS et al., 1998; SZOKE et al., 1996).

Hydrogen peroxide, by inhibiting the growth of microorganisms which do not produce catalase, offers a great advantage to the lactobacilli. The bactericidal effect of H_2O_2 is determined by its oxidant activity, with generation of reactive oxygen species, such as the OH- radicals, which disrupt cellular DNA (MARTIN et al., 2008).

The physiologic pH of the vagina ranges from 3.8 to 4.5 (WATTS et al., 2005). By using glycogen from the vaginal epithelium as substrate, lactobacilli produce organic acids, thus keeping the vaginal pH below 4.5. This acid environment partially or fully inhibits the development of most bacteria from both, the digestive tract and the environment. This is, therefore, a very efficient mechanism of mucosal protection (HAY; FAHEY, 2002; MARTIN et al., 2008).

Besides *Lactobacillus* spp., other bacteria are frequently found in the vaginal microbiota of healthy women such as: *Streptococcus, Corynebacterium, Staphylococcus*, *Escherichia, Klebsiella, Proteus, Mycoplasma*, *Ureaplasma, Atopobium, Peptococcus, Peptostreptococcus, Clostridium, Bifidobacterium, Propionibacterium, Eubacterium, Bacteroides, Prevotella* and *Gardnerella vaginalis* (MARTIN et al., 2008). When the lactobacilli concentration decreases below a critical level, these bacteria may overgrowth becoming the dominant species in the environment as opportunistic pathogens.

2 Literature review

As bacterial vaginosis (BV) is a disease whose etiology has not been fully understood, these review is focused on microbiology, ecology, epidemiology, clinical manifestations, diagnosis and treatment of this polymicrobial clinical syndrome.

2.1 Gardnerella vaginalis and bacterial vaginosis

Bacterial vaginosis (BV), a polymicrobial syndrome characterized by an imbalance of the ordinary vaginal microbiota, results from the substitution of high concentration the H_2O_2-producing *Lactobacillus* spp. for non-dominant or exogenous bacteria (GIRALDO et al., 2007; SCHWEBKE, 2009). The condition is present in approximately 30% of the women of childbearing age, being the most common cause of vaginal flow (ALLSWORTH; PEIPERT, 2007; SRINIVASAN; FREDRICKS, 2008).

The ecologic dynamics related to the vaginal microbiota shift during BV has not been fully understood (FORSUM et al., 2005; SCHWEBKE, 2009). It is believed that, with few exceptions, all BV-associated microbial species exist in low concentrations in the vaginal ecosystem of healthy women (FORSUM et al., 2005).

Changes in some metabolic factors may affect the microbiological balance in this syndrome. Glucose from vaginal glycogen is no longer degraded into lactic acid by H_2O_2-producing lactobacilli, being transformed instead into fatty acids by anaerobic bacteria. These fatty acids increase vaginal pH over 4.5, thus creating an unfavorable milieu for lactobacilli growth, while favorable to the growth of potentially pathogenic bacteria, closing a cycle that allows for the development of BV (ESCHENBACH et al., 1989).

Amino acid degradation, through anaerobic bacteria-related enzymatic mechanisms, leads to the production of different compounds, biogenic amines or polyamines among them. Lysine and ornithine decarboxylation produces cadaverine and putrescine, respectively, while histidine degradation produces histamine. High levels of biogenic amines, such as putrescine, cadaverine and timethylamine have been found in vaginal secretions of women with BV (WOLRATH et al., 2001). These same compounds are absent from vaginal secretions of healthy women, suggesting a strong correlation between their presence in the vaginal content and BV (WOLRATH et al., 2001).

Gardnerella vaginalis is a facultative anaerobic, fastidious, β-hemolytic, oxidase-negative, catalase-negative, immobile, encapsulated, pleomorphic and Gram-variable (ranging from Gram negative to Gram weakly positive) bacterium (CATLIN, 1992; KONEMAN et al., 2008). The scientific interest in *G. vaginalis* stems from the fact that it is found in practically 100% of the women with a clinical diagnosis of BV (ESCHENBACH, 1993; HILL, 1993; SCHWEBKE, 2009), playing an important role in its pathogenesis (FERRIS et al., 2007; NYIRJESY et al., 2007; SRINIVASAN; FREDRICKS, 2008; LIVENGOOD, 2009; SCHWEBKE, 2009). The specificity of the *G. vaginalis*/BV association suggests that vaginal colonization with this microorganism is essential for disease development (SCHWEBKE, 2009). Furthermore, *G. vaginalis* has been detected in the vaginal microbiota of approximately 50% (by culture) and 70% (by molecular methods)

of clinically healthy women (LIVENGOOD, 2009; SRINIVASAN; FREDRICKS, 2008).

According to the literature, the nomenclature of BV reflects the development of microbiology being defined from a non-specific vaginitis caused by streptococci and anaerobic bacteria in 1892 to polymicrobial bacterial vaginosis, in 1984, during the first international symposium on bacterial vaginosis, held in Stockholm, Sweden (GIRALDO et al., 2007).

This clinical condition has a high and varied prevalence, depending on the surveyed population, varying from 4% in developed countries to 61% in the so called third world, with a mean prevalence of 14% considering developed and developing regions (KOUMANS et al., 2007).

Some risk factors seem to be related to BV, such as: age, ethnicity, smoking, vaginal douches, intrauterine devices (IUD), and sexual behavior (AMARAL et al., 2007; FETHERS et al., 2008; YUDIN; MONEY, 2008). BV seems to be closely related to sexual intercourse, although not defined as a sexually-transmitted disease (STD), and the explanation behind its high prevalence among sexually inactive women remains elusive (GIRALDO et al., 2007; HOLMES, 1999; MORRIS et al., 2001). Women who have never been sexually active are rarely affected. The treatment of male sexual partners has not shown beneficial effects on BV occurrence (WORKOWSKI; BERMAN, 2006).

Epidemiologic correlation has been described among BV and previous STD, high number of sexual partners, and IUD use (HOLMES, 1999; SCHWEBKE, 2009). The presence of a new sexual partner was highlighted in a prospective study undertaken in Alabama, USA, as the main risk factor for BV development (SCHWEBKE; DESMOND, 2005).

As BV is more prevalent in women at childbearing age, some authors suggest a role for sexual hormones in its pathogenesis, although far less frequently, BV may be diagnosed in children and post-menopausal women (GIRALDO et al., 2007). It is also outlined in the literature, that BV is more frequent in black women, compared to the prevalent rates in other races (YUDIN; MONEY, 2008).

2.2 Clinical relevance of bacterial vaginosis

The importance of BV is due to its gynecologic and obstetric complications. The most frequently BV-associated gynecologic conditions are cervicitis, post-operative infections, and pelvic inflammatory disease (PID). According to the literature, PID is related to a bacterial infection in the pelvic organs which results from microbial colonization in the lower genital tract towards to the upper genital tract. The disease comprises endometritis, salpingitis and oophoritis with or without tubo-ovarian abscess (LARSSON et al., 2005). Considering that in patients with BV the population levels of *G. vaginalis* are increased, it is believed that some of these microorganisms may successfully migrate upward in the ecosystem and give rise to PID (LARSSON et al., 2005).

Furthermore, BV is associated with the following obstetric conditions: premature rupture of the membranes, preterm delivery, chorioamniotitis, and postpartum endometritis (HAGGERTY et al., 2004; NESS et al., 2004; SCHWEBKE, 2009; WORKOWSKI; BERMAN, 2006). The association between preterm birth and BV has been exhaustively studied, but has not been totally understood. It is assumed that *G. vaginalis* produce endotoxins that make some women more susceptible to the production of cytokines and prostaglandins that may trigger labor (FACHINI et al., 2005; MORRIS et al., 2001). Besides, the microbial colonization towards the cervix, placenta and amniotic fluid and the protease production may be related to the membrane ruptures (FACHINI et al., 2005). It was also suggested that the production of sialidases and mucinases (mucolytic enzymes) in the vaginal environment is higher in women with BV. These substances might interfere with the local physiology favoring the pelvic inflammatory disease, infertility, chronic pelvic pain and premature birth (FACHINI et al., 2005; RIGGS; KLEBANOFF, 2004).

Although such associations have been proved, there is no evidence that large-scale BV screening and treatment of asymptomatic women at both, low risk and high risk for obstetric complications, can reduce the incidence of preterm delivery (YUDIN; MONEY, 2008). The 2008 revised United States Preventive Services Task Force (USPSTF) guidelines (NYGREN et al., 2008) concluded that: (a) the BV-preterm delivery association has been well documented; (b) there is no evidence supporting gestational BV screening for the reduction of untoward effects in asymptomatic pregnant women at low risk for preterm delivery; (c) there is no consistent evidence of any health benefit of gestational BV screening of asymptomatic women at high risk for preterm delivery. Overall, several studies have produced conflicting conclusions regarding the benefit of BV treatment in these high-risk patients (NYGREN et al., 2008; SCREENING…, 2008). On the other hand, BV seems to increase the risk of acquisition, transmission or reactivation of human immunodeficiency virus (HIV), type 2 herpes virus, and human papillomavirus (CHERPES et al., 2003; WATTS et al., 2005). The increased proliferation of microorganisms involved in the BV may induce

a decrease in the number of peroxidase-producing lactobacilli which have an antiviral effect and also prevents local activation of T-lymphocytes, resulting in susceptibility to some viral infection (GIRALDO et al., 2007; MOODLEY et al., 2002; TAHA et al., 1998).

2.3 Clinical manifestations and diagnosis

BV may be symptomatic or asymptomatic (KLEBANOFF et al., 2004). Symptomatic BV is characterized by a copious, thin, homogeneous, milky, foul-smelling vaginal flow, which is exacerbated after intercourse without condom use and menstruation. When some drops of 10% Potassium Hydroxide (KOH) are added to a vaginal secretion preparation (KOH wet mount – Whiff test), a rotten-fish odor, caused by the presence of volatile biogenic amines, such as putrescine, cadaverine, and timethylamine, can be perceived. Vaginal pH is over 4.5, and microscopic examination of vaginal flow shows clue-cells, exfoliated vaginal or ectocervical cells superficially covered with *G. vaginalis*, *Bacteroides* spp. and *Mobiluncus* spp. (SUMATI; SARITHA, 2009; WORKOWSKI; BERMAN, 2006). Around 50% of women with BV are asymptomatic (AMSEL et al., 1983; DONDERS, 1999; GIBBS, 2007; KLEBANOFF, et al., 2004; SCHWEBKE; DESMOND, 2007).

Inflammatory symptoms of the vaginal mucosa are uncommon in BV, due to the almost complete absence of polymorphonuclear neutrophyls, suggesting that the microorganisms do not invade the subepithelium (KONEMAN et al., 2008). Chemotaxis inhibition in BV is a consequence of the presence of succinic and acetic acids, final catabolites of anaerobic bacteria that inhibit monocyte and polymorphonuclear leukocyte migration. These reasons explain why the syndrome is termed "vaginosis", instead of "vaginitis" (KONEMAN et al., 2008).

Amsel criteria are generally used in clinical practice for diagnosing both, symptomatic and asymptomatic BV. At least three of the four criteria should be met: (1) copious, thin, homogeneous, milky vaginal discharge; (2) rotten-fish odor, due to the release of volatile amines, on Whiff test (3) vaginal pH > 4.5; and (4) identification of bacteria-covered epithelial cells (clue-cells) under light microscopy. Even without vaginal discharge, asymptomatic BV can be easily diagnosed when criteria 2, 3 and 4 are met (AMSEL et al., 1983; HASENACK et al., 2008; SHA et al., 2005; SIMOES et al., 2006).

Laboratory diagnosis of BV was initially described in 1983 by Spiegel, Amsel and Holmes, who standardized the microscopic examination of Gram-stained vaginal secretions. Nugent, Krohn and Hillier, in 1991, simplified the technique and their classification is now the accepted gold standard for BV diagnosis (WORKOWSKI; BERMAN, 2006). According to the Nugent method, Gram-stained smears are used for identification, classification and quantification of the following bacterial morphotypes: *Lactobacillus* spp. (Gram-positive bacilli); *G. vaginalis* and *Bacteroides* spp. (Gram-negative or Gram-variable cocobacilli) and; *Mobiluncus* spp. (curved Gram-negative bacilli). Each morphotype is quantified and scored according to a 0-10 scale, and any value equal to or greater than 7 being considered positive for BV (FIGUEIREDO, 2006; NUGENT; KROHN; HILLIER, 1991; SPIEGEL et al., 1983).

Another widely used method for diagnosing BV in clinical practice is the Papanicolaou-stained smear technique: in suggestive cases, pathologists also report the presence of clue-cells in these smears (ERIKSSON et al., 2007; HASENACK et al., 2008).

The Brazilian Ministry of Health and Cancer National Institute have issued the Brazilian Nomenclature for Cervical Diagnoses and Proposed Management, including guidelines for management of women with alterations on the Papanicolaou exam. In the microbiology chapter, the term "supracytoplasmic" bacilli (suggestive of *Gardnerella/Mobiluncus*) was standardized for the description of Papanicolaou-stained smears with the presence of clue-cells (INSTITUTO NACIONAL DO CÂNCER, 2006).

The Papanicolaou technique has 50% sensitivity and 95% specificity, on average, when compared to the gold standard (ERIKSSON et al., 2007; HASENACK et al., 2008). This means that a BV-positive Papanicolaou exam is a strong evidence that the disease is present, while the absence of supracytoplasmic bacilli does not rule out BV (LIVENGOOD, 2009). Because the Papanicolaou exam is attractive, easy to perform, and covers a large population (it is part of the national uterine cervix cancer prevention program), this method has become a practical and useful tool for BV characterization, chiefly when a positive result is obtained. It also allows for the diagnosis of asymptomatic BV (ERIKSSON et al., 2007; HASENACK et al., 2008; KARANI et al., 2007; LIVENGOOD, 2009; SCHWEBKE, 2009).

Culture is not useful for diagnosing *G. vaginalis*, since this organism may be detected in asymptomatic women with BV and in those healthy ones who are carriers of *G. vaginalis* in their vaginal microbiota (ERIKSSON et al., 2007). Furthermore, cultures for *G. vaginalis* are not specific, cumbersome, and not cost-effective enough for clinical practice. Definitive diagnosis with culture and molecular biology tools have been restricted to research (LIVENGOOD, 2009).

3 Discussion

The drugs of choice for the BV treatment, recommended by the American Centers for Disease Control and Prevention (CDC) are metronidazole and clindamycn (WORKOWSKI; BERMAN, 2006).

The established benefits of therapy for non-pregnant women are: relief of signs and symptoms of vaginal infection, reduction of the risk of infectious complications after abortion or hysterectomy, and reduction of the risk of other infections, such as HIV and other STD. All symptomatic women should be treated. The benefits of treatment of symptomatic pregnant women include the cited relief of signs and symptoms of vaginal infection and reduction of the risk of other infections, besides reduction of the risk of gestational BV-associated infectious complications (WORKOWSKI; BERMAN, 2006).

Recent studies, however, have produced conflicting evidence concerning the benefit of BV treatment in asymptomatic pregnant women, at low and high risks for premature delivery (LEITICH et al., 2003; NYGREN et al., 2008; SCREENING…, 2008). Nevertheless, several investigations indicate that treatment of high-risk pregnant women might reduce the risk for prematurity. Therefore, the CDC recommends an individualized approach to pregnant women with BV and a personal history of premature delivery (WORKOWSKI; BERMAN, 2006).

In 2006, the Brazilian National STD Program issued specific recommendations for treatment of BV: (a) for non-pregnant women, the first option includes oral metronidazole 400-600 mg, twice a day for 7 days and the second option includes oral metronidazole (2g) in a single dose, or intravaginal metronidazole 7.5% twice a day for 5 days, or oral clindamycin 300 mg, twice a day for 7 days, or intravaginal clindamycin 2%, once a day for7 days; (b) for pregnant women after the first trimester and during breastfeeding, oral metronidazole 250-400 mg, three times a day for 7 days, or clindamycin 300 mg, twice a day for 7 days (BRASIL, 2006b).

Tinidazole was the first new antibiotic approved for BV treatment in the last 20 years. This second generation of nitroimidazoles has also been approved for treatment of trichomoniasis, being the only oral agent approved for both conditions. Its half-life is twice as long as that of metronidazole, and side-effects are observed in half as many patients in comparison with the latter (LIVENGOOD, 2009).

As observed worldwide for almost all bacterial groups, antimicrobial resistance has been reported to *G. vaginalis* against metronidazole, tinidazole and clindamycin, what brings attention to the need of regional studies concerning the antimicrobial susceptibility patterns of *G. vaginalis* recovered from both healthy and diseased women (AUSTIN et al., 2005; AUSTIN; MEYN; HILLIER, 2006; FERRIS et al., 2007; LIVENGOOD et al., 2007; NAGARAJA, 2008).

According to the literature, approximately 80% of the treated patients will have another BV episode within one year of treatment. Recurrence rates thirty days after treatment completion are in excess of 20%. There is no consensus on the causes of recurrence of BV which may explain why some women, even when receiving adequate treatment, do not favorably respond to drugs effective against anaerobic bacteria (GIRALDO et al., 2007). However, some causes have been proposed: number of sexual partners, IUD, spermicides, broad-spectrum antibiotics, hygiene habits, vaginal douches, frequency of sexual intercourse, lack of vaginal immune response, and even lactobacilli contamination with bacteriophages, with the consequent death of protective microbiota (UGWUMADU; HAY; TAYLOR-ROBINSON, 1997). Add to that, Witkin and others (2007) showed that women with recurrent BV might also express altered polymorphic genes to the mannose-binding lecitin and toll-like receptor. These authors showed that lower expression levels of these proteins would enhance the proliferation of some microorganisms harmful to the vaginal ecosystem.

4 Conclusion

Prevalence studies indicate that there is a potentially large reservoir of BV infection in the population. Given the high proportion of asymptomatic cases, is likely that the prevalence of BV is under-estimated by most studies. Once questions about infection have been addressed, high risk groups could be targeted more efficiently.

In this update, we gather in a single text the scientific evidence on the subject, mainly on pathophysiology, diagnosis, risk factors and treatment of BV in pregnant and non-pregnant women. Indeed, studies on microbial ecology in the vaginal ecosystem focusing *G. vaginalis* and its virulence factors are required for a better understanding of this complex and intriguing clinical syndrome.

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