The Microbiology of Bacterial Vaginosis: A Systematic Review

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ABSTRACT

Bacterial vaginosis (BV) is a common vaginal infection with high occurrence in women of child-bearing age. The infection was first described by Gardner and Dukes in 1955 that is now referred to as BV. The normal healthy vagina is populated mainly by hydrogen-peroxide-producing lactobacilli that inhibit the growth of other vaginal flora. BV is characterized by a loss of these protective lactobacilli, an increase in vaginal pH to >4.5, and the proliferation of a variety of anaerobic species. Electronic databases including Web of Science, PubMed, Scopus, and Google Scholar were searched for observational studies such as case series, cross-sectional and cohort studies, using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guide. Data extracted included (i) patient demography (age), (ii) marital status, (iii) vaginal discharge and lower abdominal pain (iv) vaginal douching (v) use of contraceptive devices. Higher prevalence of BV is seen among women who have sex with women (WSW). In Nigeria, a prevalence rate of 17%, 17.3%, 19.0% and 25% have been reported from separate studies done in the south-east, north-east, north-west and south-west Nigeria respectively. The epidemiology of BV robustly implies that it is acquired through sexual transmission. This article has reviewed that BV is a public health infection because of the impact it has on the health, social, sexual, emotional and economic status of females, there is need for public awareness on health educational programs through different media on the impact of BV. Routine screening of especially high risk groups; (such as women of reproductive age group, women who practice vaginal douching, pregnant women, women in high institutions of learning) is recommended to avoid complications such as; (preterm birth (premature birth, low birth weight or miscarriage), sexually transmitted diseases, risk of infection following gynecological surgery, and pelvic inflammatory disease,) that could result from infections from bacterial vaginosis. The golden method for screening is by Gram's Method to stain vaginal smear. The method is simple and easy without invasion. It is cheap, sensitive, and specific.

Keywords: *Bacterial vaginosis, Epidemiology, Gardnerella vaginalis, vaginal dysbiosis, vaginal microbiota*

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INTRODUCTION

Bacterial vaginosis (BV) is a common vaginal infection with high occurrence among women of child-bearing age (Moreira *et al*., 2012). The infection was first described by Gardner and Dukes in 1955, that is now referred to as BV. The normal healthy vagina is populated mainly by hydrogen-peroxide-producing lactobacilli that inhibit the growth of other vaginal flora (Barrientos *et al*., 2020). BV is characterized by a loss of these protective lactobacilli, an increase in vaginal pH to >4.5, and the proliferation of a variety of anaerobic species (Rosca *et al*., 2020).

The etiology of BV is still debatable; it is traced to a polymicrobial condition attributed with disorder of the vaginal ecosystem. This results to partial or total destruction of commensal *Lactobacilli* spp. by over proliferation of anaerobes such as *Gardnerella vaginalis*, *Prevotella*, *Bacteroides* and *Mobilincus species* and other bacteria including *Mycoplasma* and *Ureaplasma* species (Edet *et al*., 2017). Current studies have found that the prevalence of BV among non-pregnant women to range from 15–30% and 10–23% among pregnant women (Soper, 2019). There are over 1.5 million instances reported annually in Nigeria [6]. Bacterial vaginosis normally does not cause vaginal pain or itching, but often produces abnormal vaginal discharge. This article discusses about the biology, pathogenesis, laboratory diagnosis and updated treatment guidelines for BV.

EPIDEMIOLOGY

According to the National Health and Examination Survey, the prevalence of BV is 29% in women age 14 to 49 years in the United States and 50% in African-American women (Maxwell O, Chukwu, 2019; Allsworth and Peipert, 2007). Although in another study, bacterial vaginosis was found to be commoner among women age between 19-25 years most especially single ladies in tertiary institutions (Ejike *et al*., 2019), this could be attributed to their engagement in multiple sexual partners. This is in agreement by other authors who reported a high prevalence of BV among single women less than 25 years old with multiple sexual partners' (Fredricks *et al*., 2005). It was observed in a study that religion was the only significant socio-demographic factor associated with BV, non-Muslim women were 1.9 times of having BV as compared to Muslim women (Madhivanan *et al*., 2008). Similar study was performed in Africa that supported this findings (Kapiga *et al*., 2005). Globally, the prevalence of BV varies considerably between countries, regions within countries and ethnic groups, and it can be as high as 60% in certain regions (Kenyon *et al*., 2013). This variation is because of the differences in the biological, behavioral, medical, social and economic factors (Muzny *et al*., 2016).

General population prevalence of BV is high globally, ranging from 23% to 29% across regions (Europe and Central Asia, 23%; East Asia and Pacific, 24%; Latin America and Caribbean, 24%; Middle East and North Africa, 25%; sub-Saharan Africa, 25%; North America, 27%; South Asia, 29%). Within North America, black and Hispanic women have significantly higher (33% and 31%, respectively) (Peebles *et al*., 2019). Higher prevalence of BV is seen among women who have sex with women (WSW) (Plummer *et al*., 2019). In Nigeria, a prevalence rate of 17%, 17.3%, 19.0% and 25% have been reported from separate studies done in the south-east, north-east, north-west and south-west Nigeria respectively (Asiegbu *et al*., 2018; Adesiji *et al*., 2007; Afolabi *et al*., 2016). The epidemiology of BV robustly implies that it is acquired through sexual transmission (Muzny *et al*., 2016).

PATHOGENESIS

Although the pathogenesis of BV remains unclear, alternatively, it represents a dysbiosis, an imbalance of the vaginal microbiota rather than an infection. BV is characterized by a shift from the predominance of lactobacilli spp in the vaginal microbiota to a higher concentration of other organisms (Muzny *et al*., 2016). It has been strongly demonstrated that the transmission of G. vaginalis is sexual (Muzny *et al*., 2013). G. vaginalis has been recovered from vaginal fluid in close to 100% of women with clinically diagnosed BV (Srinivasan *et al*., 2012). Other frequently identified BV-associated bacteria, recovered at variable rates in the vaginal microbiome, are the genital mycoplasmas and various strict anaerobes including species of BVAB1, BVAB2, BVAB3*, Atopobium, Leptotrichia, Megasphaera, Prevotella, and Dialister* (Nelson *et al*., 2014). *Mobiluncus mulieris*, a fastidious strict anaerobic bacteria, has long been associated with BV (Fujisaki *et al*., 2020). The assortment of strict anaerobes found in individuals with BV is heterogeneous (Muzny *et al*., 2019). Similarly, *Atopobium vaginae* were found in high titers in the vaginal fluids of most women who have BV (Riu *et al*., 2020). The precise pathogenic role of these organisms remains to be elucidated. *Mycoplasma hominis* and *Ureaplasma urealyticum* can be isolated from the vagina in many women with BV. Their role as etiologic agents of BV has not been established. The acquisition of vaginal flora is shortly after birth from maternal and environmental sources (Greenbaum *et al*., 2019). At pubertal age, owing to the production of estrogen, the vaginal flora changes to lactobacillus predominance. Estrogen enhances the deposition of glycogen in the vaginal epithelium, which in turn is used as a food source by the sacchorolytic lactobacilli. The subsequent creation of lactic acid as their metabolic end product lowers the vaginal pH to <4.5 (Tachedjian *et al*., 2017). Additionally, growth of lactobacilli increases the reduction-oxidation (redox) potential of the vagina, thus inhibiting the growth of the indigenous anaerobes (Schwebke *et al*., 2014) and appear to protect against exogenous infection. Evidence suggest that G. vaginalis is the inciting pathogen in the pathogenesis of BV. It is present in up to 95% of cases of BV (Lennard *et al*., 2018). Even though G. vaginalis is the dominant taxon in bacterial vaginosis, one study has found that pure cultures of G. vaginalis do not always cause BV, and that the organism can occur, albeit in low numbers, in healthy women (Fredricks *et al*., 2007). This has further casted doubt on the role of G. vaginalis in the pathogenesis of BV. G. vaginalis play a role in the process of biofilm development. It has the ability to adhere to host receptor sites on vaginal epithelial cells and induce the production of cytotoxic substances specific to host cells and biofilm formation (Jung *et al*., 2017). The biofilm incorporates other bacterial groups into its layers, suggesting that it may enable other anaerobes to colonize the vagina. *G. vaginalis* also produces the toxin vaginolysin, which is a member of the cholesterol-

dependent family of pore-forming toxins that lyses human red blood cells and vaginal epithelial cells (Gelber *et al*., 2008).

RISK FACTORS

Generally, prevention strategies of any disease target risk factors, therefore knowledge of risk factors of BV is important in the design of effective interventions to prevent complications. Studies have shown that there is association between BV and increasing number of male sexual partners and/or a recent change of male partner (Coudray *et al*., 2020). In another studies there is a positive association between a history of female sexual partners and risk of BV especially after treatment (Bradshaw *et al*., 2006; Bradshaw *et al*., 2005). Consistent condom use was associated with a decrease in the risk for bacterial vaginosis and associated vaginal microflora (Hutchinson *et al*., 2007). However it has also been shown that BV was higher among individuals who used condoms daily compared to those that used condoms sometimes (Coudray *et al*., 2020). This however have supported some findings that argues the inclusion of bacterial vaginosis among sexually transmitted diseases. Lack of male circumcision has been associated with a number of STIs, including genital ulcer disease (Van Howe, 2007; Rein, 2020) and HIV infection (Onywera *et al*., 2020). It is, therefore, biologically plausible that, if BV is transmitted by males, the foreskin could facilitate survival of BV organisms and render an uncircumcised male a more efficient or more prolonged transmitter of infection. BV was also diagnosed in women who did not report a history of vaginal intercourse in 2 studies (Khaparde *et al*., 2020).

Cigarette smoking has been attributed to the development BV in several studies (Plummer *et al*., 2019). Possible explanation is that cigarette smoke contains various chemical constituents like nicotine, cotinine, and benzo[a]pyrene diol epoxide (BPDE). These chemicals have been demonstrated in cervical mucus of smokers and may directly alter the vaginal microflora or may act by depleting Langerhans cells in cervical epithelium leading to local immunosuppression (Hellberg *et al*., 2000). Vaginal douching (VD) is the process of washing the vagina with water or other liquid solutions (Brotman *et al*., 2008). Various researchers have evaluated the effects of VD on the health of women. Although some studies emphasized that VD caused important health issues, others revealed no such correlation. Some studies have indicated an effect of VD on vaginal flora and on the ascension of microorganisms into the upper genital tract (Yıldırım *et al*., 2020). In the past, VD was associated with bacterial vaginosis, human immunodeficiency virus (HIV), and chlamydial infections, pelvic inflammatory disease (PID), preterm birth, low-birth-weight infants, infertility, ectopic pregnancy, cervical cancer, and AIDS (Short *et al*., 2010; Passmore *et al*., 2016; Dunkerton and McParland, 2020).

DIAGNOSTIC FEATURES

Perivaginal irritation is considerably milder than in Trichomoniasis or Candidiasis. Dysuria and dyspareunia are correspondingly rare. Affected women are usually sexually active and often complain of vaginal odor, which frequently is described as “fishy.” The discharge is often homogeneous thin grayish. About 90% of patients also notice a mild-to-moderate discharge. Abdominal discomfort is occasionally present, but it is usually mild and should prompt evaluation for coincident infections such as salpingitis (McCormack and Augenbraun, 2015). Several studies have documented a variety of clinical criteria for BV (Turovskiy *et al*., 2011; Grine *et al*., 2019; -Kahwati *et al*., 2020); the most widely adopted criteria currently used for the diagnosis of BV are those proposed by Amsel *et al*. (1983). According to these investigators, the criteria include at least three of the following four characteristics: vaginal discharge with a pH of 4.5, presence of a homogeneous discharge, a fishy volatile amine odor when the discharge is treated with a potassium hydroxide solution, and presence of squamous epithelial cells coated with bacteria (clue cells) when the discharge is examined microscopically.

Gram staining has also been widely employed as a method for evaluating vaginal health. It was first employed to detect the presence of tiny Gram-negative rods in symptomatic women (Dunkelberg, 1963). The Gram stain has been the gold-standard for the diagnosis of BV and has been used in laboratories since 1965 (Coleman and Gaydos, 2018). Gram stain results consistent with BV include Gardnerella morphotypes, including cocci, fusiforms, and curved rods, and reduced *lactobacilli* morphotypes (Coleman and Gaydos, 2018). Gram stains are more specific for BV with high interobserver and intraobserver reproducibility than are Amsel’s criteria (Forsum *et al*., 2002), however, it is time consuming. In 1991, Nugent *et al*. described a Gram stain scoring system of vaginal smears to diagnose BV (Nugent *et al*., 1991). The Nugent score is calculated by assessing for the presence of large gram-positive rods (Lactobacillus morphotypes; decrease in Lactobacillus scored as 0 to 4), small gram-variable rods (G. vaginalis morphotypes; scored as 0 to 4), and curved gram-variable rods (Mobiluncus spp. morphotypes; scored as 0 to 2) and can range from 0 to 10. A score of 7 to 10 is consistent with BV. While subjects considered “normal” had scores of 0 to 3, and scores of 4 to 6 were considered indeterminate. Women with intermediate or mixed flora often have elevated pH, clue cells, and a mild amine odor, which may be overdiagnosed as BV if Amsel’s criteria were used alone. Prior studies found that 37% to 54% of women with intermediate flora determined by the Nugent score had BV by Amsel’s criteria (Bradshaw *et al*., 2005; Taylor-Robinson *et al*., 2003). The sensitivity and specificity for Amsel's criteria have ranged from 37% to 70% and 94% to 99%, respectively, compared to the gold-standard Gram

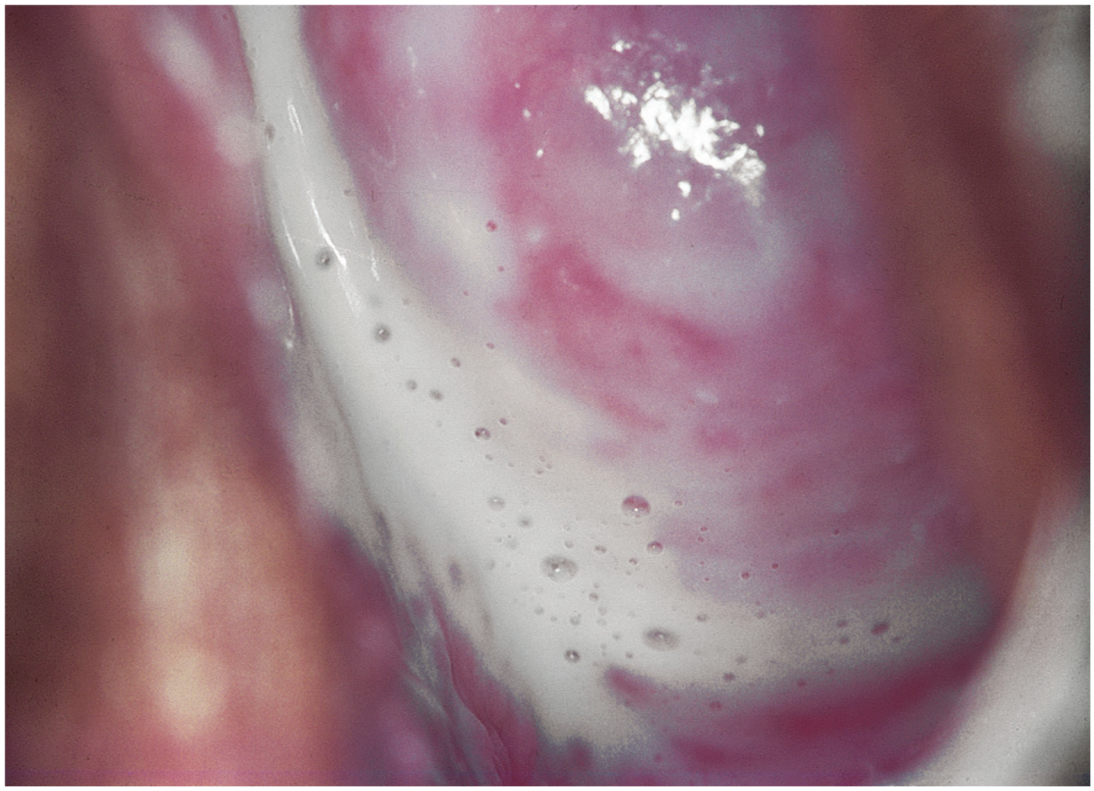

Figure 1: Bacterial vaginosis showing gray, homogeneous discharge

Table 1. Guidelines from the american college of obstetricians and gynecologists (acog): treatment options for bacterial vaginosis in nonpregnant patients

Recommended treatment regimens	Alternative treatment regimens
Metronidazole, 400mg orally twice daily for seven days **OR**	Secnidazole 2g orally in a single dose **OR**
Metronidazole gel 0.75% one full applicator (5g) intravaginally once a day for five days **OR**	Tinidazole 2g orally once daily for two days **OR** Tinidazole 1g orally once daily for five days **OR**
Clindamycin cream 2%,one full applicator (5g) intravaginally at bedtime for seven days	Clindamycin 300mg orally twice daily for seven days **OR** Clindamycin ovules, 100mg intravaginally once at bedtime for three days

stain Nugent score (Coleman and Gaydos, 2018; , Beverly *et al*., 2005), and the reproducibility is moderate. The OSOM BV Blue assay (Genzyme Diagnostics, Cambridge, MA) is a chromogenic point-of-care test (POCT) that measures sialidase levels in vaginal fluid. The sensitivity and specificity for the OSOM BV Blue assay range from 88% to 94% and 91% to 98%, respectively, compared to Nugent and Amsel's criteria[57]. The FemExam (Cooper Surgical, Shelton, CT) POCT detects metabolic products of G. vaginalis, which include amines and the activity of proline aminopeptidase, and measures vaginal pH. It consists of two plastic cards; one card is for pH measurement and the presence of trimethylamine, and the second card is for proline aminopeptidase measurement. The combined sensitivity of cards 1 and 2 is 91% and the specificity is 61% compared with the Nugent score. It is very fast (2 min), objective, and easily performed (West *et al*., 2003) (Figure 1).

Owing to the limitations of microscopy and other POCTs for BV diagnosis, using molecular technique have been employed for diagnosis of BV. Molecular diagnostics has several advantages over POCTs and microscopy-based tests because they are objective, are able to detect fastidious bacteria, enable quantitation, and are ideal for self-collected vaginal swabs. These assays are based on the detection of specific bacterial nucleic acids (Table 1).

TREATMENT

The up-to-date guidelines from the American College of

Obstetricians and Gynecologists (ACOG), in January 2020, recommend the treatment of symptomatic BV to restore the vaginal microbiome to a healthy state and reduce a woman's risk of acquiring and transmitting other STIs, The guidelines has been summarized in Table 1 above (Chavoustie *et al*., 2020). The limitation to this guidelines is that, it does not include treatment of asymptomatic BV, some prospective data show that treating asymptomatic BV may also reduce the incidence of STIs (Schwebke and Desmond, 2007). ACOG include Metronidazole, Clindamycin, Secnidazole, and Tinidazole; however, the choice of treatment should be individualized based on patient preference, cost, convenience, adherence, ease of use, and history of response or adverse reactions to previous treatments. Also recommended in the guidelines for BV treatment include alcohol use, sexual activity, contraceptive use, and tampon use (Chavoustie *et al*., 2020). Abstaining from alcohol during treatment and for 24 hours after completion of oral metronidazole treatment or 72 hours after oral Tinidazole treatment is currently recommended by the drug manufacturers. Patients should refrain from sexual activity during BV treatment unless condoms are used. Tampons should also be avoided during treatment with intravaginal products.

CONCLUSION AND RECOMMENDA-TIONS

This article has reviewed that BV is a public health infection because of the impact it has on the health, social, sexual, emotional and economic status of females, there is need for public awareness on health educational programs through different media on the impact of BV. Routine screening of especially high risk groups; (such as women of reproductive age group, women who practice vaginal douching, pregnant women, women in high institutions of learning) is recommended to avoid complications such as; (preterm birth (premature birth, low birth weight or miscarriage), sexually transmitted diseases, risk of infection following gynecological surgery, and pelvic inflammatory disease,) that could result from infections from bacterial vaginosis. The golden method for screening is by Gram's method to stain vaginal smear. The method is simple and easy without invasion. It is cheap, sensitive, and specific.

Competing interests

Authors have declared that no competing interests exist.

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