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Interpreting the Epidemiology and Natural History of Bacterial Vaginosis: Are We Still Confused?

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Abstract

Bacterial vaginosis (BV) is a common cause of vaginitis and increases women's risk of pelvic inflammatory disease, adverse pregnancy outcomes, and risk of STD/HIV acquisition. The etiology of BV is unclear, though it is believed to involve loss of vaginal hydrogen peroxide-producing lactobacilli and acquisition of complex bacterial communities that include many fastidious BV-associated bacteria (BVAB) that have recently been detected using PCR methods. Treatment failure (persistence) is common, and may be facilitated by unprotected sex. Potential contributions to BV and BV persistence include (1) sexual partners as a reservoir for BVAB; (2) specific sexual practices, including male partners' condom use; and (3) the composition of the vaginal microbiota involved in BV. Specific BVAB in the Clostridiales order may predict BV persistence when detected pre-treatment, and have been detected in men whose female partners have BV. BVAB may be associated with unprotected sexual behavior and failure of BV to resolve in women, supporting the hypothesis that BVAB colonization of male genitalia may serve as a reservoir for re-infection of female partners. Moreover, specific sexual practices may favor vaginal colonization with certain BVAB that have been associated with persistence. This review provides background on BV, and discusses the epidemiologic and microbiologic data to support a role for acquisition of BVAB and how this process might differ among subsets of women.

Introduction

During the *Anaerobe 2010* meeting, a session entitled "Vaginal Microbiota: The Complex Anaerobic Environment of Bacterial Vaginosis" reviewed current knowledge about the complex anaerobic environment of bacterial vaginosis (BV), a common cause of vaginal symptoms and risk for numerous adverse health outcomes.

Clinical and Epidemiologic Features of BV

In women of reproductive age, a vaginal environment that is quantitatively dominated by hydrogen peroxide (H_2O_2)-producing *Lactobacillus* species typically has a pH considered to be normal (<4.7), and has consistently been associated with healthy pregnancy outcomes, lack of abnormal vaginal symptoms, and reduced risk for acquiring several sexually transmitted pathogens, including HIV. The most commonly isolated *Lactobacillus* species associated with this healthy environment are *L. crispatus* and *L. jensenii.* Some

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Lactobacillus species that predominate in the healthy vagina, including *L. jensenii*, reduce gonococcal adherence to and invasion of human endometrial epithelial cells in a cell culture model,[1] providing insight into the clinical observation that BV, with concomitant loss of H_2O_2-producing lactobacilli, is a risk for acquisition of gonorrhea.[2, 3] In HIV-infected women, higher levels of vaginal lactobacilli have been associated with reduction in the quantity of HIV shed in genital secretions.[4] These lactobacilli are autoinhibited by high levels of H_2O_2, so that the levels of H_2O_2-generating lactobacilli are self-regulated in the vagina. In addition to the direct antimicrobial effects of lactic acid and the consequent acidic pH these bacteria engender, the natural defense mechanisms of the vagina include production of endogenous defensins (including HNP1–3), secretory leukocyte protease inhibitor (SLPI), cytokines, and endocervical mucous.

BV represents a condition in which the normal protective lactobacilli are replaced by high quantities of commensal anaerobes, resulting in symptomatic vaginitis in many women. BV is the most prevalent form of vaginal infection in women of reproductive age, affecting 8% to 23%, and is the most common etiology of vaginal symptoms prompting women to seek medical care. [5] Of 3,739 women enrolled during 2001–2004 in a nationally representative sample of the U.S. civilian non-institutionalized population, almost one in three (29.2%; 95% C.I. 27.2–31.3) had BV by Gram stain of vaginal fluid.[6, 7] BV occurs more frequently among women who report sex with other women, and those who report new or a higher number of male sex partners.[8] Other risks include douching,[9] intrauterine device, [10] Black race,[11] hormonal contraception,[12] smoking,[13] menses,[14] and chronic stress.[15]

Symptomatic BV, which accounts for approximately 60% of all cases, typically causes abnormal vaginal discharge that is increased in amount and often malodorous. Why some women develop symptoms and others do not is not known. The discharge results in part from degradation of the normal vaginal mucin gel, which is efficiently performed by mucin-degrading enzymes produced by BV-associated bacteria (BVAB) (particularly Gram-negative anaerobes).[16] The odor, usually described as “fishy,” is derived from volatilization of the amines produced by the metabolism of anaerobic bacteria that characterize this disorder. The profound increase in anaerobe concentrations is characterized by enhanced production of sialidase (which degrades IgA), glycosidase, volatile amines, and a characteristic cytokine profile including elevated levels of IL-1B and IL-10, and reduced levels of IL-8 and SLPI.

In clinical practice, BV is typically diagnosed using the Amsel criteria, which include the presence of at least three of four findings: vaginal pH greater than 4.5, homogeneous vaginal discharge on examination, detection of fishy odor on addition of potassium hydroxide to vaginal fluid (positive “whiff test”), and presence of significant clue cells (defined as >20% of the total vaginal epithelial cells seen on 100× magnification on saline microscopy).[17] Other point-of-care diagnostic tests take advantage of immediate methods of detecting either high concentrations of *Gardnerella vaginalis*, a variety of the amines that are prominent, including sialidase, trimethylamine, and prolineaminopeptidase, or some combination of amines and abnormal pH.[18] Despite the ease of use of these tests, clinicians unfortunately do not regularly pursue a specific diagnosis of vulvovaginal complaints, and rely (usually inappropriately) on syndromic management to direct treatment. Moreover, accuracy of the Amsel criteria in clinical practice is likely limited by clinicians’ lack of or limited skill in using microscopy to detect clue cells and rule out other important findings, including trichomonads and yeast forms.

BV may also be diagnosed using a score applied to Gram stains of vaginal fluid, the Nugent criteria, which quantifies the number of lactobacilli relative to BV-associated bacterial morphotypes to create a scale of flora abnormality ranging from normal (score = 0–3)

through intermediate (score = 4–6) to frank BV (score = 7–10).[7] The Nugent score is widely regarded as the gold standard for the diagnosis of BV in research studies. Most recently, targeted qualitative and quantitative polymerase chain reaction (PCR) assays for detection of various BVAB have been studied; this approach may offer some utility in the future, but has not been widely validated for diagnosis of BV in large, diverse populations of women and is costly.[19, 20] Moreover, quantitative PCR has not yet been well studied for its ability to differentiate between women who have intermediate flora vs. frank BV as determined by the Nugent score; women with intermediate flora by Nugent score may have relatively high quantities of the BVAB *G. vaginalis* and *Atopobium vaginae* as determined by quantitative PCR (qPCR).[20] The single commercially available panel using PCR offers detection of *G. vaginalis*, *Bacteroides fragilis*, *Mobiluncus mulieris*, and *Mobilincus curtisii*, and thus detects only a small proportion of the bacterial species that characterize BV. The optimal molecular methodology or criteria, both qualitatively and quantitatively, to serve as the next generation diagnostic modality has not been determined.

While antibiotics with broad activity against most anaerobic bacteria are effective in relieving symptoms of BV, relief is often short-lived, and the majority of women with BV will experience a recurrence in the next several months. Metronidazole and clindamycin are the mainstays of therapy. Published studies have consistently reported resolution of BV in 71% to 89% or more of women one month after administration of these regimens.[21] Intravaginal therapies have had efficacy similar to that of oral metronidazole regimens with fewer side-effects. More perplexing is the high rate of early recurrence (30% at three months, 50% at six months) reflecting early relapse and possibly late reinfection, for which successful management has not been forthcoming. Although each symptomatic episode usually responds rapidly to conventional antibiotic treatment, rapid recurrence is frequently inevitable unless it is suppressed with ongoing antibiotic therapy (biweekly vaginal metronidazole gel).[22]

BV is associated with serious sequelae related to the upper genital tract, increasing the risk of preterm delivery, first trimester miscarriage in women undergoing in vitro fertilization, amniotic fluid infections, chorioamnionitis, postpartum and postabortal endometritis, and postabortal pelvic inflammatory disease (PID).[23] In non-pregnant women, BV increases risk of post-hysterectomy infections and PID.[24] BV itself may cause endocervical inflammation that manifests as mucopurulent cervicitis.[25] Among HIV-infected women, the quantity of HIV shed in vaginal secretions from those with BV was increased nearly 6-fold increase relative to those without BV.[26] BV probably enhances women's likelihood of sexual acquisition of HIV, possibly through inducing reversible changes in the cervical or other mucosal immune environment.[27, 28] The exact means by which BV effects adverse reproductive tract sequelae is not clear. Possible explanations include loss of antimicrobial compounds produced by lactobacilli, destruction of mucin gel coating the vaginal/cervical epithelium via inhibition of glycosidase-producing anaerobes, degradation of SLPI, induction of a cervical pro-inflammatory environment, and alteration in the immune cell environment of the cervix.

The Microbiology of BV and Role of Recently Defined BV-Associated Bacteria

Critically, the initial event leading to the shift of the anaerobic predominance that characterizes BV is unknown, though data suggest that sex likely contributes—at least in some women. BV occurs more frequently among women who report new or higher numbers of male sex partners, is common and highly concordant among female sex partners, and rarely occurs before sexual debut, patterns that invoke the epidemiology of a typical

sexually transmitted infection (STI). Limited data also suggest that male condoms and circumcision may prevent BV and its recurrence.[29, 30]

Conventional cultivation of flora from vaginal fluid of women with BV typically yields a spectrum of primarily anaerobic commensals: *G. vaginalis*, *Prevotella* species, anaerobic gram-positive cocci, *Mobiluncus* species, *Ureaplasma urealyticum*, and *Mycoplasma hominis*.[31] More recently, molecular techniques that bypass cultivation requirements have been employed to greatly expand the microbiologic spectrum of BV.[32] In addition to confirming the presence of previously described cultivatable BVAB, these studies have detected *A. vaginae*, *L. iners*, *Eggerthella*, *Megasphaera*, *Leptotrichia*, *Dialister*, *Bifidobacterium*, *Slackia*, and bacteria related to *Arthrobacter*, *Caulobacter*, and *Butyrivibrio*. They have also detected several newly described bacteria in the *Clostridiales* order that are currently designated BVAB1, BVAB2, and BVAB3.

The consensus findings of an NIH-sponsored working group tasked with considering how future research in vaginal microbiota should consider the new data on microbiota have recently been published.[33] Key recommendations were that several features could be used to encourage inclusion of appropriate target participants and to improve the generalizability and reliability of vaginal findings. The group recommended continued use of Nugent score as a gold standard, despite limitations, and use of modified Amsel criteria. The group proposed a framework for defining the vaginal microbiota based on these criteria, and recognizing the dynamic nature of vaginal microbiota, emphasized the value of daily, prospective collection (including self-collection) of vaginal samples for characterization of the microbiota. The group agreed that normal vaginal microbiota could be characterized by the presence or absence of specific bacteria, but that further work was needed to define the performance characteristics of assays that would do this. For example, different BVAB are likely to have different predictive values for detection of BV, and combining some of these bacteria might enhance these values. *G. vaginalis*, which appears to be ubiquitous in women with BV and characterizes the BV-associated biofilm in studies that have specifically looked for this bacterium, is also present in the vagina of up to 70% of women with normal microbiota.[19] Other BVAB, including *A. vaginae*, BVAB1, BVAB3, and *Megasphaera* sp. type 2, are highly specific for BV, each with a negative predictive value for BV >92% when used as a single assay and, in the case of BVAB-2 and *Megasphaera* sp. type 2, >95% when used in combination.[19] However, the group acknowledged that approaches using bacterium-specific assays to diagnose BV require validation in larger and more diverse groups of women, as the studies to date have been small or have focused on epidemiologically distinct groups with limited data collected on subjects. An additional objective of such studies should be to consider whether quantitative thresholds of BVAB—for example, with quantitative PCR—might offer additional enhancements to diagnostic performance.

The role of antibiotic resistance among BVAB in promoting treatment failure is uncertain. Clindamycin-resistant bacteria have been reported among women treated with vaginal clindamycin, although this was not associated with reduced cure rates.[34] Although *Mobiluncus* is often resistant to metronidazole, early studies indicated that women with *Mobiluncus* who were treated with metronidazole had the same rate of cure as women not colonized by *Mobiluncus*.[35] In a recent prospective study assessing response to vaginal metronidazole gel among 131 women, incidence of persistent BV at 30 days was 26%, and significantly higher in women with baseline detection of BVAB1 (risk ratio (95% C.I.) 2.0 (1.1–4.0), BVAB2 (risk ratio (95% C.I.) 8.7 (2.5–∞), or BVAB3 (risk ratio (95% C.I.) 3.1 (1.7–5.8)), or of *Peptoniphilus lacrimalis* (risk ratio (95% C.I.) 3.5 (1.6–15.5)) or *Megasphaera* sp. type 2 (risk ratio (95% C.I.) 3.4 (1.4–5.5)).[36] Detection of these bacteria at test-of-cure was associated with persistence, while post-treatment sexual activity was not. Taken together, these findings suggest that anaerobic Gram-negative rods have a more

central role in the etiology of BV than *Gardnerella, Mobiluncus*, or the genital mycoplasmas.

Evidence supports a role for male partners in BV. BV is more frequent among women who report new or higher number of male sex partners.[8] Real time PCR assays targeting the 16S rRNA gene of 10 vaginal bacterial species were applied to 20 women (14 with and 6 without BV) and to urine, urethral swab, and coronal sulcus swab of their male partners.[37] *G. vaginalis, A. vaginae*, BVAB1 and *Megasphaera* sp. type 1 were detected in the partners of women with BV, with highest quantities in coronal sulcus swab samples; concentrations of these bacteria were low or undetectable in specimens from women without BV and from their male partners. Using broad range PCR, BVAB (*Leptotrichia, Sneathia, Prevotella, Gemella, Veillonella, Atopobium* spp) were detected in first-catch urine in 20 men; information on sex partners was not reported.[38] However, a study that obtained urethral swabs for *G. vaginalis* culture from men attending an STD Clinic found no difference in culture positivity whether BV was present in female partners or not, though report of recent condom use was associated with significantly lower culture positivity.[39] A number of studies suggest that condom use prevents BV. In one, women whose partners consistently used condoms had a fivefold reduction in BV persistence and recurrence.[29] In another, women reporting less frequent condom use were less likely to sustain normal vaginal flora.[8] Wives of men who participated in a randomized trial of circumcision to prevent HIV acquisition in Uganda were followed to assess effects on vaginal infections.[30] Among women without BV at enrollment, BV at follow up was significantly less common in wives of men who had been circumcised compared to wives of men who had not (prevalence risk ratio (PRR) 0.80; 95% CI 0.65–0.97). In women with BV at enrollment, persistent BV at one year was significantly lower in the circumcision arm than control arm women (PRR 0.83; 95% CI, 0.72–0.96).[30] Assessment of the microbiota pre- and post-circumcision among 12 of the male participants revealed not only that several anaerobic bacterial families were detected in the subprepuceal space, but that they were significantly decreased in quantity and species diversity after circumcision.[40]

Despite these intriguing data, several placebo-controlled trials have demonstrated that treatment of the male partner(s) does not improve the clinical outcome of treatment of BV or reduce recurrence.[21] The discrepancy between data suggesting sexual acquisition of BV, and the lack of benefit of treating the male partner, remains puzzling, but does not rule out a role for sexual transmission. Part of the issue may be that the selection or dosing of the antibiotics used in the trials done to date was not appropriate or adequate for eradicating a potential reservoir for BVAB in men.

High BV prevalence (27% to 52%) in women who have sex with women, and concordance of BV status in female couples, also support a role for sexual transmission. Sexual practices that transmit vaginal fluid between women increase BV risk.[41] Gardner failed to causally implicate *G. vaginalis* after inoculating it into the vagina in 13 healthy women, as only one developed BV.[42] However, 11 of 15 women developed BV when inoculated with vaginal fluid of women with BV,[42] suggesting transmissible factors. The practice of receptive oral sex has also been suggested as a risk for BV or inability to maintain normal vaginal flora.[43] Marrazzo and colleagues assessed results of bacterium-specific PCR assays by participants' characteristics in a diverse group of women.[44] Of 171 women with BV, women reporting intercourse with men were significantly more likely to have *Megasphaera* sp. type 2 and *L. crispatus* relative to those who reported no recent intercourse with men. Moreover, the likelihood of detecting *Megasphaera* sp. type 2 increased with report of higher number of male partners in the prior 3 months. Women reporting sex with women only were significantly more likely to have *Megasphaera*. However, self-reported race also was significant, with Black women more likely to have *Megasphaera* sp. types 1 and 2, *M.*

mulieris, TM7-BVAB and *P. lacrimalis* detected. Some of these associations were modified when interactions between race and reported sexual practices were considered, suggesting that vaginal communities of *Megasphaera* sp. types 1 and 2, which are highly specific for BV, are associated with recent sexual behavior and host characteristics that track with race. These two *Megasphaera*-like species are phylogenetically distinct and only distantly related to previously known cultivated *Megasphaera* species. These data support roles for both exogenous (sexual acquisition) and endogenous (host) factors in determining the composition of the microbiota in BV.

Conclusion & future directions

Despite considerable research effort and recent advances, BV remains an enigmatic condition. Efforts to link BV to a single cultivated bacterial pathogen, such as *G. vaginalis*, have been unconvincing. Molecular tools have achieved some inroads. For one, they have revealed the complex microbiology of BV, expanding the spectrum even farther than was possible with cultivation-based approaches. However, it is possible that even with the more advanced technology, we are still missing key species. Some of the newly defined BVAB do have very high specificity for BV, suggesting that they may play some critical role, but they do not appear to function as credible single causal pathogens. Numerous key questions still remain. What are the critical steps in the causal pathway to developing BV? Why are some women symptomatic with the alterations of vaginal microbiota associated with BV while others are not? What accounts for the high recurrence rate? Are all BVABs susceptible to antibiotics used to treat BV? Is biofilm formation critical to pathogenesis in BV? Finally, and critically, are some communities or species of BVAB more pathogenic – for example, more strongly associated with adverse sequelae, such as preterm delivery—than others?

BV is most likely a heterogeneous syndrome caused by different communities of vaginal bacteria, similar to what occurs in conditions such as periodontitis linked to changes in oral microbial communities.[45] In this sense, BV could represent a dysbiotic condition caused not by a single pathogen but by a change in microbial composition and community structure. A woman's individual risk for a acquiring a particular etiologic vaginal bacterial community might depend on specific practices, such as unprotected vaginal or oral sex. Future studies of BV will need to emphasize frequent prospective sampling of the vaginal microbiota, with careful attention to concurrent sexual and hygienic practices and ultimately, innate characteristics of host immunity. The characterization of extra-vaginal reservoirs for BVAB, including the rectum and oropharynx, should also shed considerable light on the routes by which these bacteria establish dominance in BV.

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