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REVIEW

An Updated Conceptual Model on the Pathogenesis of Bacterial Vaginosis

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Bacterial vaginosis (BV) is the most common cause of vaginal discharge. It is associated with an increased risk of preterm delivery, pelvic inflammatory disease, and an increased risk of acquisition of sexually transmitted infections including human immunodeficiency virus (HIV). The epidemiology of BV supports sexual transmission. However, its etiology remains unknown. At the center of the debate is whether BV is caused by a primary pathogen or a polymicrobial consortium of microorganisms that are sexually transmitted. We previously published a conceptual model hypothesizing that BV is initiated by sexual transmission of *Gardnerella vaginalis*. Critics of this model have iterated that *G. vaginalis* is found in virginal women and in sexually active women with a normal vaginal microbiota. In addition, colonization does not always lead to BV. However, recent advances in BV pathogenesis research have determined the existence of 13 different species within the genus *Gardnerella*. It may be that healthy women are colonized by nonpathogenic *Gardnerella* species, whereas virulent strains are involved in BV development. Based on our results from a recent prospective study, in addition to an extensive literature review, we present an updated conceptual model for the pathogenesis of BV that centers on the roles of virulent strains of *G. vaginalis*, as well as *Prevotella bivia* and *Atopobium vaginae*.

Keywords. Bacterial vaginosis; biofilm; *Gardnerella vaginalis*; *Prevotella bivia*; *Atopobium vaginae*.

Bacterial vaginosis (BV), the most common cause of vaginal discharge [1], is a vaginal dysbiosis characterized by loss of lactic acid–producing lactobacilli and proliferation of facultative and strict anaerobes [2]. It is associated with multiple adverse outcomes, including an increased risk of preterm birth, as well as acquisition of human immunodeficiency virus (HIV) and other sexually transmitted pathogens [2]. BV is diagnosed clinically, using the Amsel criteria, and microbiologically, using the Nugent score [2]. Epidemiological data strongly suggest that BV is a sexually transmitted infection (STI) with an incubation period of around 4 days, similar to that of other bacterial causes of STIs, such as *Neisseria gonorrhoeae* [3]. The most significant risk factor for incident BV is a new sex partner [4], whereas that for recurrent BV is a regular sex partner [5].

Despite >60 years of research, the etiology of BV remains unknown. *Gardnerella vaginalis*, present in 95%–100% of BV cases [6], was originally thought to be the primary BV pathogen [7]. In vitro, it is more virulent (ie, it has a greater ability to harm a host) than other BV-associated bacteria, adhering in larger aggregates to vaginal epithelial cells and exhibiting greater cytotoxic activity through production of a pore-forming toxin (vaginolysin) [8–10]. However, *G. vaginalis* has been found in virginal women [11] and in sexually active women with a normal vaginal microbiota [12]. In addition, colonization with *G. vaginalis* does not always lead to BV [13], suggesting that *G. vaginalis* alone may be necessary but not sufficient for BV development.

Recent advances in BV pathogenesis research have suggested distinct roles for *G. vaginalis* subgroups [14]. Earlier genomic studies of *G. vaginalis* revealed that it consists of 4 nonrecombining groups/clades of organisms with distinct gene pools and genomic properties that may confer distinct ecological or pathological properties [15]. The presence of multiple *G. vaginalis* clades has since been found to have a positive association with BV (ie, clades 1 and 2), whereas the presence of a single clade (ie, clade 4) has been negatively linked with BV [16]. More recently, Vaneechoutte et al analyzed 81 full-genome sequences of *G. vaginalis* by performing digital DNA-DNA hybridization and measuring the average nucleotide identity and demonstrated the existence of at least 13 different species within the genus *Gardnerella* [17]. While these *Gardnerella* species may be closely related genetically, only a few may be involved in disease (ie, BV) [17]. These new genomic data put into perspective previous criticisms regarding *G. vaginalis* colonization in sexually active women with normal vaginal microbiota as evidence against its central role in BV etiology [13]. It may be that healthy women are colonized by *Gardnerella* species with low

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virulence potential and that other virulent strains are involved in BV development. Future research should include study of potential sexual transmission of the different *Gardnerella* strains.

Recent data now suggest a potentially important synergistic relationship between *G. vaginalis, Prevotella bivia*, and *Atopobium vaginae* in BV pathogenesis [18, 19]. Gilbert et al recently showed in a mouse model that coinfection with *G. vaginalis* and *P. bivia* recapitulates several features of BV [19]. In a prospective study of women who have sex with women, we found that the mean relative abundance of *P. bivia* and *G. vaginalis* became sequentially higher 4 days (for *P. bivia*) and 3 days (for *G. vaginalis*) prior to the development of incident BV, compared with findings for women who maintained a normal vaginal microbiota; the mean relative abundance of *A. vaginae* became significantly higher on the day of incident BV in this study [18]. *P. bivia* has been found in high concentrations in women with BV [20] and in women with preterm birth [21]. *A. vaginae* is highly specific for BV and rarely occurs in the absence of *G. vaginalis* [22].

To put these new data into context, we performed an updated literature review on BV pathogenesis research, focusing on more-recent data on *G. vaginalis* [8, 14, 16, 17, 23, 24], as well as interactions among *G. vaginalis*, *P. bivia*, and *A. vaginae* [18, 19, 25–27]. We revised our prior hypothetical model [28], which mainly centered on *G. vaginalis*, and present an updated conceptual model (Figure 1) that includes these additional BV-associated bacteria as potential key pathogens in the development of BV.

PROPOSED STEPS IN BV DEVELOPMENT

Sexual Transmission of Virulent Strain(s) of *G. vaginalis* Displace Vaginal Lactobacilli and Initiate BV Biofilm Formation on the Vaginal Epithelium

A notable feature of BV is the appearance of a polymicrobial biofilm on vaginal epithelial cells. Presence of this biofilm creates a favorable environment for anaerobic bacteria, owing to the presence of an oxygen gradient within the biofilm [29]. The BV biofilm has been found to contain abundant *G. vaginalis*, fewer *A. vaginae*, *Lactobacillus* species, and other bacterial species [30]. Desquamation of vaginal epithelial cells coated with BV biofilm results in clue cells, which can be seen on wet-mount analysis of vaginal fluid specimens. Polymicrobial biofilms, such as the BV biofilm, incorporate secondary bacteria after an initial colonizer species adheres to the surface; a synergetic relationship between these species allows the bacterial biofilm to prosper and mature [31].

Initial adhesion to the vaginal epithelium is a crucial step in BV development [30] and the first step in BV biofilm formation [32]. We have previously proposed that BV development is triggered by sexual transmission of *G. vaginalis*, which displaces healthy vaginal lactobacilli, such as *L. crispatus*, and initiates BV

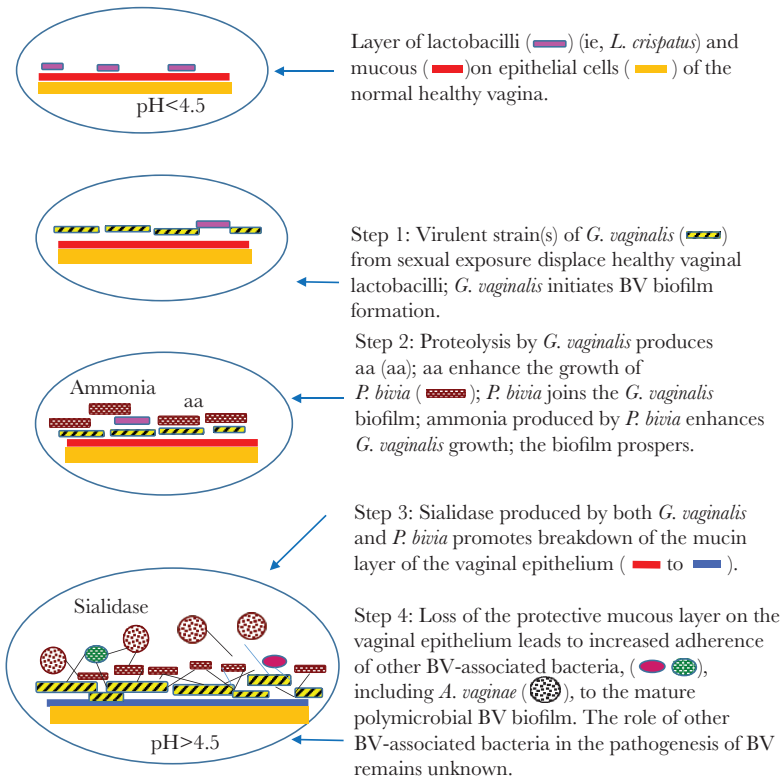

Figure 1. Updated conceptual model for the pathogenesis of bacterial vaginosis (BV). This model is a schematic representation and is not to scale. aa, amino acids; *A. vaginae, Atopobium vaginae*; *G. vaginalis, Gardnerella vaginalis*; *L. crispatus, Lactobacillus crispatus*; *P. bivia, Prevotella bivia.*

biofilm formation on the vaginal epithelium [28]. As a microaerophilic and facultative anaerobe, *G. vaginalis*, unlike other strict BV-associated bacteria, can tolerate the high redox potential of a lactobacillus-dominated vaginal microbiota [28]. Similar to facultative anaerobes involved in dental plaque biofilms [33], virulent strain(s) of *G. vaginalis*, perhaps at a certain threshold [34], may lower the redox potential in the vaginal microbiota, leading to a marked decrease in lactobacilli and an increase in other strict anaerobic BV-associated bacteria (eg, *P. bivia* and *A. vaginae*) [28]. These other BV-associated bacteria, acquired from maternal and environmental sources, may normally be present in very low concentrations [35]. Non-BV strains of *G. vaginalis* are less likely to displace vaginal lactobacilli from monolayers of HeLa cells in vitro than strains of *G. vaginalis* from women with BV [23].

During lactobacillus displacement, *G. vaginalis* adheres to vaginal epithelial cells in large numbers and triggers BV biofilm growth [9, 23]. In a study by Patterson et al, strains of *G. vaginalis* obtained from women with BV were found to form significantly thicker biofilms on tissue-culture–treated polystyrene, compared with other common BV-associated bacteria (*P. bivia*, *A. vaginae*, *Mobiluncus mulieris*, *Fusobacterium nucleatum*, *Veillonella* species, *Peptostreptococcus* species, and *Peptoniphilus* species) [9]. Correspondingly, in a study of 30 BV-associated bacteria, using an in vitro biofilm formation model, Alves et al also found that BV strains of *G. vaginalis* had the greatest propensity to form biofilms [8]. Interestingly, in this study, many of the other BV-associated bacteria also had a tendency to grow as biofilms, particularly *Mycoplasma hominis*, *Staphylococcus hominis*, *Brevibacterium mcbrellneri*, and *Enterococcus faecalis*. However, when tested for initial adhesion to HeLa cells, these species had a significantly lower ability to adhere than *G. vaginalis*. This suggests that, when in contact with human cell lines, smaller numbers of BV-associated bacteria are not sufficient to induce biofilm formation.

G. vaginalis biofilms tolerate H_2O_2 and lactic acid (substances produced by vaginal lactobacilli that reduce the vaginal pH to <4.5 and prevent colonization by pathogenic anaerobes) better than planktonic (free-floating) cells [36]. This protective mode of growth allows bacteria embedded in the BV biofilm to survive in harsh environments. Castro et al have recently shown that *G. vaginalis* biofilm cells alter their gene expression profiles significantly, which may contribute to antimicrobial resistance and BV biofilm persistence, promoting the recurring and chronic nature of BV [24].

Synergy Between Virulent Strain(s) of *G. vaginalis* and *P. bivia* Occurs on the Vaginal Epithelium

After virulent strain(s) of *G. vaginalis* displace vaginal lactobacilli, adhere to the vaginal epithelium in high numbers, and initiate BV biofilm formation, we propose that key interactions between *G. vaginalis* and *P. bivia* promote the next step in BV development. It is during this time that *P. bivia* joins *G. vaginalis* in the lower layers of the BV biofilm. *P. bivia* is a common constituent of the human vaginal microbiota during BV [37], as are other *Prevotella* species, and has been found in infected amniotic fluid specimens, as well as in uterine and placental tissue specimens from women undergoing preterm birth and other pregnancy complications [38]. *G. vaginalis* and *P. bivia* have a well-established symbiotic relationship in vitro [39], and interactions between these bacteria may be the next key step in BV pathogenesis. Proteolysis by *G. vaginalis* produces amino acids [40] that enhance *P. bivia* growth [39], and ammonia produced by *P. bivia* stimulates *G. vaginalis* growth in vitro [39]. It has also been shown that *P. bivia* enhances biofilm formation initiated by *G. vaginalis* [26]. If this mutualistic relationship exists in vivo, it could account for the presence of both of these bacteria in high concentrations during BV [27].

While no in vivo studies have examined the incorporation of *P. bivia* in the BV biofilm along with *G. vaginalis*, we have found that *P. bivia* coaggregates with *G. vaginalis* and can incorporate multispecies BV biofilms in vitro [26]. Future research should explore whether *P. bivia* incorporates into the BV biofilm in vivo. To determine the presence of *P. bivia* in the BV biofilm in vivo, a specific probe for *P. bivia*, such as a peptide nucleic acid probe, would need to be developed.

Vaginal Sialidase, Produced by BV-Associated Bacteria, Including *G. vaginalis* and *P. bivia*, Promotes Breakdown of the Mucin Layer on the Vaginal Epithelium

We next propose that vaginal sialidase, produced by several BV-associated bacteria, promotes breakdown of the protective mucus layer on the vaginal epithelium and leads to enhanced susceptibility to ascending infection in the female genital tract. Vaginal sialidase is highly correlated with BV [41] and preterm birth [42] and has been implicated in multiple aspects of host-pathogen interactions, including mucosal barrier degradation (which likely contributes to the characteristically thin consistency of vaginal fluid during BV), bacterial attachment, and release of carbon sources to facilitate bacterial growth [19]. Sialidase activity also has immunomodulatory consequences for receptors on host cells.

G. vaginalis and *P. bivia* are 2 BV-associated bacteria that produce sialidase in vivo [41]. Other *Prevotella* species (*Prevotella oralis* and *Prevotella loescheii*) and *B. fragilis* also produced sialidase in this study, but in much smaller quantities. In contrast, other BV-associated bacteria, including *Mobiluncus* species, *Peptostreptococcus* species, and *M. hominis*, did not produce detectable sialidase.

Gilbert et al have recently shown in their mouse model that both *G. vaginalis* and *P. bivia* produce measurable levels of vaginal sialidase, with *G. vaginalis* producing higher levels than *P. bivia* [19]. In this model, *G. vaginalis* but not *P. bivia* also induced vaginal epithelial cell exfoliation. Furthermore,

G. vaginalis enhanced the invasive potential of *P. bivia* and facilitated its ascension into mouse uteri. Interestingly, neither *G. vaginalis* nor *P. bivia* induced a purulent inflammatory response. The authors concluded that this study provides strong evidence that *G. vaginalis* and *P. bivia* are both direct contributors to the pathogenesis of BV, as well as its associated symptoms and outcomes [19]. However, they did note several limitations to their study, which included the use of single strains of *G. vaginalis* and *P. bivia*, as well as the inherent limitations of murine models in BV pathogenesis research (ie, mice do not have dominant *Lactobacillus* microbiomes in the vagina as do many women; thus, the mouse model may not accurately reflect all aspects of human vaginal physiology that accompany a shift away from a lactobacillus-dominant vaginal microbiota). Nevertheless, the findings by Gilbert et al provide an important framework for advancing BV pathogenesis research, particularly that involving *G. vaginalis* and *P. bivia*, as well as other BV-associated bacteria.

Loss of the Protective Mucous Layer on the Vaginal Epithelium Leads to Increased Adherence of Other BV-Associated Bacteria, Including *A. vaginae*, to the Mature Polymicrobial BV Biofilm

We next propose that loss of the protective mucous layer on the vaginal epithelium leads to increased adherence of other BV-associated bacteria, including *A. vaginae* [43], and to the formation of a mature, polymicrobial BV biofilm. There is considerable evidence supporting the involvement of *A. vaginae* in the pathogenesis of BV. As previously mentioned, *A. vaginae* is highly specific for BV and rarely occurs in the absence of *G. vaginalis* [22]. *A. vaginae* has been found in BV biofilms in vivo, although in smaller numbers than *G. vaginalis* [30]. Similar to *G. vaginalis*, *A. vaginae* has also been found in a dispersed form that is nonadherent [25]. In a study of 120 women participating in a clinical trial in Rwanda, *A. vaginae* and *G. vaginalis* were visualized by fluorescence in situ hybridization in 54.1% and 82.0% of vaginal specimens containing BV biofilms, respectively [25]. *A. vaginae* was accompanied by *G. vaginalis* in 99.5% of the samples in this study. The odds of having a Nugent score >4 were increased for vaginal samples with dispersed *G. vaginalis* and/or *A. vaginae* present (odds ratio [OR], 4.5; 95% confidence interval [CI], 2–10.3). The odds of having a high Nugent score were even higher when a combination of adherent *G. vaginalis* and dispersed *A. vaginae* were present (OR, 75.6; 95% CI, 13.3–429.5) and highest when both bacteria were found in the BV biofilm (OR, 119; 95% CI, 39.9–360.8). Only rarely (ie, in only 2 of the vaginal samples in the Rwanda study [25]) has *A. vaginae* been identified in BV biofilms in the absence of *G. vaginalis*. Women with *G. vaginalis* and *A. vaginae* have higher rates of recurrent BV than women with *G. vaginalis* alone [22]. In addition, it has been demonstrated that a combination of a *G. vaginalis* DNA level $\geq 10^9$ copies/mL and an *A. vaginae* DNA level $\geq 10^8$ copies/mL is the best diagnostic definition of BV [44].

A. vaginae has been found to stimulate a stronger immune response from vaginal epithelial cells than *G. vaginalis*, leading to localized cytokine and β-defensin production [45]. Thus, it may be a potent component of the host response to BV. This host immune response may contribute to the symptoms (ie, vaginal discharge and odor) of BV, as well as to the adverse outcomes (ie, preterm birth and pelvic inflammatory disease), as increased vaginal levels of inflammatory cytokines and neutrophils are predictive of both [46]. Vaginal odor in particular is also linked to increase levels of vaginal biogenic amines, including putrescine, cadaverine, and trimethylamine, which are produced by multiple BV-associated bacteria and may mitigate the acidic barrier that favors vaginal lactobacilli [47].

The presence of *A. vaginae* in the BV biofilm may also have an impact on BV treatment outcomes [25]. *A. vaginae* is highly resistant to metronidazole but susceptible to clindamycin (3 of 3 clinical isolates tested in one study) [48]. In a study of 0.75% topical metronidazole gel applied by women once daily for 5 days, a high pretreatment concentration of *A. vaginae* was associated with partial or complete BV treatment failure at 4 weeks after treatment [49]. However, this study was small, and quantitative polymerase chain reaction analysis of other BV-associated bacteria before and after treatment was not performed. If *A. vaginae* is integral to the pathogenesis of BV, one would expect superior cure rates by using clindamycin as compared to metronidazole. However, metronidazole and clindamycin were found to have similar short-term efficacy in a review of BV clinical trials [50]. It is important to note that the quality of trials included in that review varied widely, which could have biased comparisons of efficacy between drugs. Although the authors included a sensitivity analysis in their study, they acknowledged that making direct comparisons of treatment efficacy between metronidazole and clindamycin was difficult. In addition, *A. vaginae* is not present in all women with recurrent BV [22]. Thus, the role of *A. vaginae* in BV pathogenesis remains controversial and should be further investigated.

DISCUSSION

Understanding the etiology of BV is essential for improvements in diagnosis, treatment, and prevention of this extremely common clinical condition. We have proposed an updated conceptual model highlighting the potential key roles of virulent strains of *G. vaginalis*, *P. bivia*, and *A. vaginae* in BV pathogenesis. We propose that this model of BV pathogenesis is the same regardless of how BV is diagnosed (eg, by the Amsel criteria, Nugent score, or molecular diagnostic assay) or whether patients perceive themselves to be symptomatic (of note, patients' perception of their vaginal symptoms varies greatly and does not necessarily correlate with signs of BV [3]). Nevertheless, it is essential to note that other BV-associated bacteria may play a role

in BV pathogenesis but remain less well characterized and are not included in our current model; this should be an active area of research moving forward. Along these lines, the ecological interactions between *G. vaginalis* and 15 other BV-associated bacteria were recently analyzed by Castro et al, using a dual-species biofilm model [26]. Interestingly, this study revealed distinct biofilm structures between each bacterial consortium, leading to at least 3 unique dual-species biofilm morphotypes. *A. vaginae*, *Corynebacterium tuscaniense*, *Mobiluncus mulieris*, *P. bivia*, and *Streptococcus anginosus* were the bacterial species that coaggregated the most with *G. vaginalis* in vitro (coaggregation is a virulence factor and a common mechanism for survival of bacteria in nature). However, surprisingly, transcriptomic findings indicated that *Enterococcus faecalis* and *Actinomyces neuii* had the highest impact on enhancing key *G. vaginalis* genes associated with BV development, namely *vly* (which encodes the pore-forming toxin vaginolysin), *sld* (which encodes sialidase), and *HMPREF0424_0821* (which is thought to be associated with biofilm formation metabolism). Interestingly, *P. bivia* also enhanced *vly*, while *A. vaginae* enhanced *HMPREF0424_0821*. The authors suggested that perhaps not all BV-associated bacteria contribute to the enhancement of BV pathogenesis by influencing *G. vaginalis* virulence. Nevertheless, these results suggest that "social networking" between *G. vaginalis* and an array of BV-associated bacteria can occur. Additional research focusing on these complex interactions between bacteria during BV is needed.

If *G. vaginalis*, *P. bivia*, and *A. vaginae* are found to be central to the pathogenesis of BV, future studies should focus on the design of molecular diagnostic assays targeting all 3 of these bacteria. Additional research should also focus on interventions that modify or block the synergistic relationship between these bacteria, particularly the synergistic relationship between *G. vaginalis* and *P. bivia*. In addition, clinical trials evaluating the treatment of *P. bivia* or *A. vaginae* colonization in women without BV should be conducted. Of note, we are currently conducting a clinical trial of treatment of *G. vaginalis* colonization with amoxicillin among women without BV (clinical trials registration NCT03211156 [J. R. S., principal investigator]), with results forthcoming.

Notes

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Potential conflicts of interest. C. A. M. is a consultant for Lupin Pharmaceuticals, BioFire Diagnostics, and Cepheid and has also received honoraria from Roche Diagnostics. J. R. S. has been a consultant for and received research support from Hologic/GenProbe, BD Diagnostics, Cepheid, Quidel, Symbiomix, StarPharma, Lupin Pharmaceuticals, and Toltec. All other authors: No reported conflicts.

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