Vaginal Biofilms and Bacterial Vaginosis: Of Mice and Women

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(See the major article by Hymes et al on pages 1491–7.)

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“What is bacterial vaginosis, anyway? Why can’t we treat it very well? What does a biofilm have to do with it? I thought those were involved in prosthetic device infections! Isn’t there an animal model for this condition that can help us answer these questions, whose importance I personally doubt, as there is for any respectable disease?”

In this issue of the Journal, Hymes and colleagues present data that speak to several of these questions, which, despite the interlocutor’s skepticism above, seriously affect women’s (and men’s, as it turns out) health. These investigators report a novel finding that might explain why the vaginal biofilm associated with bacterial vaginosis appears to be so tenacious, and how it might be disrupted.

Most investigators would agree that a vaginal microbiome dominated by specific species of Lactobacillus—namely, Lactobacillus crispatus and Lactobacillus jensenii—optimizes the likelihood of several desirable outcomes. These outcomes include healthy pregnancy and delivery, absence of upper genital tract infection, lack of abnormal vaginal symptoms, and reduced risk for acquiring several sexually transmitted pathogens, including human immunodeficiency virus. When these species of Lactobacillus are replaced by a diverse mix of copious numbers of anaerobic bacteria, the vaginal pH increases (above the optimal range, which is ≤4.5), and bacterial vaginosis results, if this disruption proceeds to the point where clinical or Gram stain criteria are fulfilled. Among the most important adverse outcomes that have consistently been linked to bacterial vaginosis are elevated risk of HIV acquisition [1], preterm delivery [2], and pelvic inflammatory disease [3]. In a recent, prospective cohort study of HIV-serodiscordant heterosexual couples in which the female partner had HIV infection at enrollment, bacterial vaginosis that occurred during follow-up was associated with an increased risk of HIV acquisition among the male sex partners of these women [4].

It would seem intuitively simple to treat the anaerobic abundance that defines bacterial vaginosis, but this approach has proved to be difficult. In fact, relief from antibiotic therapy of bacterial vaginosis—metronidazole or clindamycin, given topically or orally—is often short-lived, and the overwhelming majority of affected women are subject to recurrence in the next several months unless ongoing antibiotic therapy (typically, biweekly vaginal metronidazole gel) is used as a suppressive approach [5]. Most investigators believe that in addition to a reduction in the bacterial vaginosis–associated bacterial concentrations, adequate repletion of the desirable Lactobacillus species must be established and sustained to fully “heal” the vaginal microenvironment. Critically, the initial event leading to the shift of the anaerobic predominance that characterizes bacterial vaginosis is unknown, although data suggest that sexual activity likely contributes—at least in some women [6].

Numerous studies conducted over the past 3 decades have substantiated the association of Gardnerella vaginalis with bacterial vaginosis. However, with use of more-sensitive detection methods, including bacterium-specific polymerase chain reaction, G. vaginalis can be detected in women who have no signs of bacterial vaginosis or, indeed, any other vaginal infection. In fact, G. vaginalis is estimated to colonize approximately 50%–70% of women whose vaginal fluid is characterized as normal on the basis of the Nugent score [7]. These observations have led some to suggest that G. vaginalis may act synergistically with other anaerobic bacteria to cause bacterial vaginosis [8].

The role of biofilms in potentiating the tenacity of bacterial vaginosis has emerged...
rather recently, and the reports are relatively scant. In 2005, Swidsinski and colleagues reported that a highly adherent biofilm dominated by dense clusters of *G. vaginalis* and, to a lesser extent, *Atopobium* species characterized women with bacterial vaginosis [9]. Subsequently, these investigators demonstrated that this biofilm persisted when women did not respond to standard treatment for bacterial vaginosis with metronidazole, suggesting that the biofilm itself may provide an anatomic haven protecting these bacteria from the microbicidal effects of the antibiotic [10]. This notion has gained support in recent years with fascinating work demonstrating the advantages that biofilms can provide to bacteria attempting to establish a foothold in a hostile environment. For example, in biofilms formed by *Pseudomonas aeruginosa*, the bacteria actively respond to the nutrient-limited environment inside the biofilm with antibiotic tolerance, in a mechanism elegantly outlined by Nguyen and colleagues [11]. In an excellent review of the shifting paradigms of biofilms, Monds discusses in depth the complexity that determines microbial survival and dynamic movement within biofilms, noting that the bacteria ending up at the bottom (the attachment surface) of the microcolony face constraints on growth and are thus forced to adapt in complex ways in order to survive: “not even a bacterium wants the bottom bunk” [12]. Of related interest, we have demonstrated that among women without bacterial vaginosis, detection of *G. vaginalis* in the oral cavity—a site where biofilm formation has been extensively studied—predicts subsequent development of bacterial vaginosis [13]. Could the biofilm of the oral cavity also sustain this bacterium and others that have the potential to promote bacterial vaginosis? Or are women whose behaviors or innate immunity are more likely to “permit” formation of vaginal biofilms with *G. vaginalis* more likely to support oral reservoirs of this bacterium, as well?

The study by Hymes et al adds to this growing body of knowledge by showing that not only is *G. vaginalis* present in the biofilm of bacterial vaginosis, but its extracellular DNA is critical to maintaining the infrastructure of that very biofilm. Moreover, deconstructing that infrastructure could offer a clue to a better chance at bacterial vaginosis cure. How might one actually disrupt a biofilm in an anatomic niche like the vagina? One could envision an approach that would need to be different from that for the oral cavity, which is highly adapted to encountering many types of substances in the daily ingestion of sustenance. Presumably the repertoire of the vagina in this regard is somewhat more limited, and one would ideally like to avoid transmission of any potentially harmful substance into the endocervical canal. Again, Hymes et al developed and tested the novel idea that a DNase might effectively destroy the very extracellular DNA that helps to maintain the biofilm of bacterial vaginosis. The authors present evidence that, when DNase is used in combination with an antianaerobic antibiotic, DNase actually freed *G. vaginalis* from the biofilm, presumably making these bacteria susceptible to the action of the antibiotic. In accompanying work, the authors demonstrated a >10-fold inhibition of *G. vaginalis* colonization by DNase, concluding that this agent might synergize with traditional antibiotics, like metronidazole or clindamycin, to eradicate the infection and enhance treatment response and duration. Among the novel approaches used by Hymes et al was use of the checkerboard assay to circumvent the inability to estimate a traditional measure of bacterial killing (eg, the minimum inhibitory concentration).

There are obvious limitations to the approach used by Hymes and colleagues, as they note in their thoughtful discussion. First, synergy between the antibiotic studied (metronidazole) and DNase can be inferred, but not necessarily proven, with these methods; this is a challenge already acknowledged by researchers studying biofilms in the setting of cystic fibrosis [14]. Second, the murine model of bacterial vaginosis presents as many questions as it begins to address: can a bacterial vaginosis–associated biofilm be established as a chronic process in the animal model? The authors were not able to establish a chronic biofilm in this study. Why not? Does the native murine vaginal microenvironment have some innate resistance to establishing a *G. vaginalis* biofilm or, equally intriguing, does bacterial vaginosis itself offer resistance? What constitutes the native murine vaginal microbiota? Further attempts to refine and understand this model could provide critical insights into the pathogenesis of bacterial vaginosis in humans.

Finally, the report of a new animal model for bacterial vaginosis is exciting, even with the caveats noted above. The lack of an animal model for bacterial vaginosis has posed a formidable challenge to progress on understanding the pathogenesis of and optimizing treatment for bacterial vaginosis. Using a new murine model, Hymes et al offer the possibility of ushering in a new era of understanding and—we can hope—even respect for this underappreciated and poorly understood condition that has serious consequences for women’s sexual and reproductive health.

**Note**

**Potential conflicts of interest.** Author certifies no potential conflicts of interest.

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**References**