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Understanding vaginal microbiome complexity from an ecological perspective

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Abstract

The various microbiota normally associated with the human body have an important influence on human development, physiology, immunity, and nutrition. This is certainly true for the vagina wherein communities of mutualistic bacteria constitute the first line of defense for the host by excluding invasive, nonindigenous organisms that may cause disease. In recent years much has been learned about the bacterial species composition of these communities and how they differ between individuals of different ages and ethnicities. A deeper understanding of their origins and the interrelationships of constituent species is needed to understand how and why they change over time or in response to changes in the host environment. Moreover, there are few unifying theories to explain the ecological dynamics of vaginal ecosystems as they respond to disturbances caused by menses and human activities such as intercourse, douching, and other habits and practices. This fundamental knowledge is needed to diagnose and assess risk to disease. Here we summarize what is known about the species composition, structure, and function of bacterial communities in the human vagina and the applicability of ecological models of community structure and function to understanding the dynamics of this and other ecosystems that comprise the human microbiome.

Keywords

vagina; microbiome; microbial community; bacterial vaginosis

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Introduction

Bacterial communities in the human vagina are thought to have a critical role in protecting the host against infectious disease. In reproductive age women, it is thought they do so through the production of lactic acid resulting in a low pH environment that restricts the growth of pathogens and other opportunistic organisms.^{1, 2} Thus, maintaining high numbers of lactic acid bacteria is a hallmark of healthy conditions. Although there are marked differences in the species composition and rank-abundances of populations in vaginal bacterial communities^{3, 4} among women, it appears that all are probably dominated by homofermentative lactic acid bacteria.⁵ This suggests the ecological function of various vaginal communities in reproductive age women – creating a low pH environment through the production of organic acids – is conserved despite differences in the bacterial species present.

The vaginal microbiota throughout a woman's lifespan

The vaginal microbial ecosystem undergoes significant structural changes at various stages in a woman's life that are directly linked to the level of estrogen in the body.⁶ Initial colonization occurs at birth, when the infant is first exposed to her mother's vaginal tract if delivered vaginally, or by the skin bacteria of persons handling the infant in the case of a Caesarian-section delivery.⁷ While little is known about the importance of this initial colonization event, it is believed to establish the gut, skin and vaginal microbiota, and in the weeks and months following birth, these differentiate into communities distinct to each habitat.⁷⁻⁹ During the first 2-4 weeks following birth, maternal estrogen mediates thickening of the vaginal epithelium and the production of glycogen that is fermented by indigenous bacteria resulting in a lowering of the vaginal pH. This is transitory however, as subsequent metabolism of maternal estrogen is accompanied by thinning of the vaginal mucosa, a reduction of the level of glycogen, and a concomitant increase in vaginal pH.⁶

During childhood (Tanner stage 1¹⁰), the pH of the vagina is nearly neutral and cultivation-dependent methods have shown it to be colonized by diverse assemblages of aerobic, strictly anaerobic, and enteric species of bacteria.¹¹⁻¹⁴ Between the ages of 8 and 13 years of age, pubertal changes in the vulva and vagina occur that are induced by adrenal and gonadal maturation. During the maturation process, follicular development causes estrogen production to rise, and once again this is accompanied by a thickening of the vaginal epithelium and intracellular production of glycogen.⁶ These new environmental conditions select for microorganisms capable of fermenting glycogen to lactic acid and the concomitant acidification of the vaginal environment that is characteristic of reproductive age women.^{6, 11} Remarkably, the shifts in microbial community composition that occur during this transition have seldom been studied. Using cultivation-dependent methods, Alvarez-Olmos *et al.*¹³ found that the vaginal microbiota of many adolescent girls (14-18 y) resembled those of adult women with bacterial vaginosis. Yamamoto *et al.*¹⁵ assessed the vaginal microbiota of adolescent girls (13-18 y) using cultivation-independent methods and observed that the bacterial communities were comparable to those found in adults but remarked that this may not be the case for premenarcheal or perimenarcheal girls.

When vaginal epithelial cells are sloughed in reproductive age women the glycogen present is then presumably metabolized by bacterial populations to produce organic acids; however, as widely cited as this mechanism is it is backed by very little evidence collected from *in vitro* analyses.¹⁶ The resulting low pH (4.0-4.5) of the vagina creates an environment that restricts or precludes the growth of many pathogenic organisms. However, with the onset of menopause, estrogen levels again decrease, and menstruation ceases. This is accompanied by atrophy of the vaginal epithelium and reduced cervico-vaginal secretions.⁶ In most menopausal women the vaginal microbiota is thought to shift from populations of lactic acid

producing bacteria to an assortment of species that include strictly anaerobic and enteric bacteria.^{17, 18} The dynamic nature of this ecosystem underscores the importance of resolving its microbial constituents at different stages of human development and the prominent influence of estrogen levels in the host on the vaginal environment.

Community performance in reproductive age women: lactic acid production

In general, the presence of high numbers of lactic acid bacteria in the vagina is often equated with “healthy” and low numbers, or absence thereof, as being “abnormal.”¹⁹⁻²¹ This has historically focused attention on members of the genus *Lactobacillus* as keystone species because of their well-known ability to produce lactic acid through the fermentation of sugars. This view originates from the earliest studies of the vaginal microbiota over a century ago, when Professor Albert Döderlein first reported culturing the bacteria from vaginal secretions. He found they produced lactic acid, which in turn inhibited growth of pathogens both *in vitro* and *in vivo*.²² “Döderlein’s bacillus” was later classified in 1928 as *Lactobacillus acidophilus*.²³ Several decades later in the 1980s, it was determined that *L. acidophilus* was not a single species, but rather a group of closely related, obligately homofermentative species collectively known as the *Lactobacillus acidophilus* complex.²⁴ Because species within this complex are difficult to distinguish phenotypically or biochemically,²⁵ they were differentiated on the basis of DNA homology.^{26, 27} All of the *Lactobacillus* spp. found to be prevalent in the vagina today are members of this complex.

Beyond Lactobacillus: findings from cultivation-dependent and -independent studies

Following Döderlein’s discovery of what later came to be known as *Lactobacillus*, cultivation-dependent studies eventually revealed that a diverse array of facultative and strictly anaerobic bacteria, and sometimes the yeast *Candida*, can be present in the healthy vagina but typically in much lower numbers.²⁸⁻³⁰ Furthermore several species of *Lactobacillus* belonging to the *Lactobacillus acidophilus* complex were identified, including *L. jensenii*, *L. casei*, *L. gasseri*, *L. crispatus*, *L. plantarum*, *L. fermentum*, *L. cellobiosus*, *L. brevis*, *L. minutus*, and *L. salivarius*.³¹⁻³⁴ It is interesting to note that *L. minutus* was reclassified in 1992 as a member of a new genus, *Atopobium*, and subsequently renamed *A. minutum*.³⁵ Given the uncertainty and controversy surrounding the potential role of *Atopobium* species in bacterial vaginosis,³⁶⁻³⁸ future studies might seek to better understand the traits that distinguish these genera and the species within them.

Efforts to characterize vaginal microbial communities using cultivation methods undoubtedly led to significant improvements in understanding the role of microbes in vaginal health, but they were limited due to the inherent biases in cultivation methods. Today it is well known that most host-associated and environmental microbes resist cultivation in the laboratory using traditional techniques.³⁹ Undoubtedly cultivation of microorganisms is fundamental to understanding their physiology and phenotypic characteristics, and it remains a very useful tool in studies of microbial ecology. Promising developments in cultivation of fastidious bacteria using state-of-the-art techniques⁴⁰⁻⁴³ are likely to enable the cultivation of many previously inaccessible microbes. However, studies aimed at assessing fine-scale variation in host-associated microbial communities within and among individuals or exploring ecological relationships within these communities require methods that provide detailed information about microbial diversity while also being cost-effective and scalable to high-throughput sample processing. In response to this need, cultivation-independent methods have in recent years become the standard approach to characterizing the diversity of microbes residing in and on the human body.⁴⁴⁻⁴⁸

Major advances in DNA sequencing technology over the last decade have fundamentally changed the way we assess microbial community structure and composition. For

investigations of bacterial diversity, these methods commonly utilize 16S rRNA gene sequences as a means to compare and classify taxa. This approach circumvents the need for cultivation by analyzing DNA sequences extracted directly from samples. Typically, partial 16S rRNA gene sequences are amplified using primers that anneal to highly conserved sequences in the gene, and the resulting amplicons are sequenced. Phylogenetic analyses of the sequences allows for classification of phylotypes and determination of the numerically dominant taxa in a community. Other methods that rely on other conserved genes (cpn60⁴⁹, rpoC, uvrB or recA⁵⁰) have also been developed but are not as widely used.

Vaginal microbial community types

Using these methods numerous studies have been done to characterize the vaginal microbial communities of healthy, asymptomatic, reproductive age women.^{3-5, 37, 51-55} Although these studies have relied on various analytical methodologies and study designs – sampling different regions of the vagina, women from various ethnic backgrounds, different geographical locations of populations, sampling times in relation to the menstrual cycle, and so on – their findings consistently demonstrate that vaginal bacterial community composition differs both within and between women, and several types of communities are known to exist. Together, these findings paint a much more complicated picture of the vaginal microbiota than had been considered in the past.

Previous studies performed in our laboratory have shown that several distinct kinds of vaginal communities with markedly different species composition occur in white, black, Hispanic and Asian women in North America^{3, 4} and Japanese women in Tokyo, Japan.⁵ Since vaginal bacterial communities differ in species composition they are likely to differ in how they respond to disturbances. Conceptually this is important since vaginal communities continually experience various kinds of chronic and acute disturbances caused by human behaviors such as the use of antibiotics, hormonal contraceptives and other methods of birth control, sexual intercourse, vaginal lubricants, douching and many others. In addition, the structure and composition of vaginal microbial communities are known to be influenced by natural changes in normal healthy women including aging,^{17, 56} time in the menstrual cycle,⁵⁷ menstruation,⁵⁸⁻⁶¹ pregnancy,⁶² and stress.⁶³⁻⁶⁵

In a previous study we analyzed 144 vaginal samples from healthy Caucasian and black women in North America.⁴ The results showed that 80% of the women had microorganisms phylogenetically related to *Lactobacillus iners*, *L. crispatus*, *L. jensenii*, or *L. gasseri* as a numerically dominant member of the vaginal microbiota. Overall, *L. iners* was the most common species of *Lactobacillus* in women of both ethnic groups having been recovered in 66% of the women sampled, and other groups have reported this organism as being highly common in the vaginas of reproductive age women as well.^{51, 52, 54, 66-76} Surprisingly *L. iners* was only first described in 1999⁷⁷ as it does not grow on the media typically used to isolate and enumerate *Lactobacillus*, so it was absent from earlier cultivation-dependent studies of the vaginal microbiota. The remainder of communities in our study contained a relatively low proportion of lactobacilli, exhibited greater species evenness and included high numbers of clones most closely related to *Atopobium* and genera of the order *Clostridiales*, including *Megasphaera*, *Dialister*, *Anaerococcus*, *Fingoldia*, *Peptostreptococcus*, and *Eubacterium*. Additionally, 20-30% of the clones from these communities were from novel clades in the phylum *Firmicutes*. Comparable results were obtained in a recent study of healthy, reproductive age Japanese women.⁵ The findings of these studies indicate there are a limited number of different kinds of vaginal microbial communities in asymptomatic, apparently healthy women. Moreover, from studies of adolescent women (13-15 y),¹⁵ it appears that these communities are established in puberty and may reside in women until menopause.

Recently, we completed a more detailed and expansive study to characterize vaginal microbiota using high-throughput methods based on pyrosequencing of barcoded 16S rRNA genes.⁴ The subjects were a cohort of 396 North American asymptomatic, reproductive age women equally representing four ethnic backgrounds (Asian, white, black, and Hispanic). We found a total 282 phylotypes among these women. Their vaginal bacterial communities were characterized into five groups, four of which were dominated by *Lactobacillus iners*, *L. crispatus*, *L. gasseri*, or *L. jensenii*, and the fifth which had lower proportions of lactic acid bacteria and higher proportions of strict and facultative anaerobes. The latter community type accounted for about 25% of the women sampled, a notable finding considering the prevailing view that high numbers of *Lactobacillus* are necessary for a healthy vaginal tract. Furthermore we observed high bacterial species diversity in all vaginal communities, even those in which the phylotype abundance distribution was highly skewed toward one or very few numerically dominant phylotypes.

An important finding from these studies is that the distribution of community types varies significantly among women from different ethnic backgrounds (Fig. 1). For example, in the study by Ravel *et al.*⁴ vaginal bacterial communities dominated by *Lactobacillus* spp. were found in 80.2% and 89.7% of Asian and white women, respectively, but just 59.6% and 61.9% of black and Hispanic women, respectively. On the other hand, occurrence of communities with low proportions or no detectable *Lactobacillus* species community type were elevated in Hispanic (38.1%) and black (40.4%) women compared to Asian (19.8%) and white (10.3%) women. These findings are in accordance with results obtained by Zhou *et al.*,^{3, 5, 53} who assessed the vaginal bacterial communities of white, black and Japanese women. Moreover, vaginal pH was found to differ among ethnic groups as well (Table 1), with the overall median vaginal pH of black (4.7 ± 1.04) and Hispanic (5.0 ± 0.74) women being slightly elevated over what is typically considered to be healthy (4.0-4.5). Vaginal pH was elevated (5.3 ± 0.6) among women of all racial groups in the “diversity group,” and it was above 4.5 for the community types dominated by *L. gasseri* (5.0 ± 0.7) and *L. jensenii* (4.7 ± 0.4). *L. crispatus* and *L. iners*-dominated community types had median pH values of 4.0 ± 0.3 and 4.4 ± 0.6 , respectively.⁴

A rose by any other name

There is compelling evidence to suggest *Lactobacillus* spp., the production of lactic acid, and the resulting low pH are important for preventing the proliferation of nonindigenous organisms in the vagina.^{1, 28, 78-81} However, these observations have been over-interpreted and through faulty logic have led to the assertion and common wisdom that *Lactobacillus* spp. must be present for health to be maintained. This has been extended and some claim that women whose vaginal communities are depauperate of *Lactobacillus* spp. are somehow abnormal. Unfortunately, this fallacy is the premise of the commonly used Nugent criteria used for the diagnosis of bacterial vaginosis wherein the degree of “healthiness” is in part assessed by scoring the abundance of *Lactobacillus* morphotypes, ignoring the possibility that their ecological function could be supplanted by bacteria with other morphotypes. It might be more reasonable to postulate that a key ecological function of vaginal communities, namely the production of lactic acid, might be accomplished by a variety of taxa capable of homolactic and heterolactic fermentation of substrates. *Atopobium*, *Streptococcus*, *Staphylococcus*, *Megasphaera* and *Leptotrichia* are among the genera found in the vagina that possess this capability in addition to the better known *Lactobacillus*. If the maintenance of a low environmental pH is indeed a key function of the vaginal microbial community then perhaps it may be more appropriate to consider ‘lactic acid bacteria’ *in toto* to be members of the same ecological guild⁸² because they use the same resource pool to accomplish the same ecological function. If this is the case, then the prevailing view that species of *Lactobacillus* are both necessary and sufficient for maintaining health, may well

be overly simplistic because functionally equivalent species may in fact ‘substitute’ for species of *Lactobacillus*.

Microbial ecology of the vaginal microbiota

Vaginal bacterial communities reside in an ecosystem that is strongly influenced by characteristics of the host, local environment, and constituent populations (Fig. 2). The human microbiome is often referred to as a commensal relationship in which one member derives benefit from the other member without providing benefit or causing harm in return. This is almost certainly not the case for the vaginal microbiome wherein the bacteria are entirely dependent on the host for nutrients, and in turn the bacterial communities play a role in protecting against disease-causing organisms. Consequently, it should be viewed as a mutualism in which an understanding of community composition, function, and dynamics requires that the vagina be viewed as an ecosystem and not simply the sum of its parts. For example, it is now clear that bacterial communities in the vaginas of reproductive age women are reasonably complex and include diverse species from several bacterial lineages. Hence, although vaginal bacterial communities are dominated by lactic acid producing bacteria, they coexist and interact with a wide array of other bacterial species by competition for space and resources. Through metabolic activities these populations modify their environment in ways that either facilitate or preclude colonization by other species through resource competition, predation by bacteriophage and the production of antimicrobial substances. Likewise the host influences the composition of communities by determining the quantity and composition of vaginal transudates that constitute an important source of nutrients for resident bacterial populations. Moreover, it is likely though not proven that vaginal mucus and epithelial cell receptors play an important role in colonization by certain bacterial species. Further, innate and local immune systems may work to exclude or select community members (reviewed by Linhares *et al.*²).

The available evidence suggests vaginal communities are in a state of dynamic equilibrium, in which short term fluctuations (at least in reproductive age women) occur in response to changes driven by hormonal changes that are a part of normal menstrual cycles (reviewed by Farage and Sobel⁸³). It is unknown whether any stage in a menstrual cycle should be thought of as a “disturbed” state and therefore more susceptible to invasion. However, it is known that many normal human activities are associated with destabilization of vaginal communities. For example, frequent sexual intercourse, having multiple sex partners, and frequent episodes of receptive oral sex⁸⁴⁻⁸⁷ cause destabilization of vaginal microbial communities, as do douching⁸⁸ and use of spermicides.⁸⁹⁻⁹² Each of these has the potential to destabilize vaginal bacterial communities and increase their invasibility. Here we discuss an ecological model as it may apply to the vaginal microbiota and their stability. First we must emphasize that a relevant and meaningful ecological theory for the vaginal microbiome is currently not feasible until additional research on community dynamics has been done. However, while the model presented here is conceptual in nature it does provide a useful framework for evaluating the possible importance and roles of community members and guiding future studies in this field.

Drivers and Passengers

The strong linkage between high numbers of lactic acid bacteria and a “healthy” vaginal microbial community is consistent with Walker’s Driver-Passenger model of community structure and function.⁹³ Under this model, species of lactic acid bacteria would be considered ‘drivers’ that strongly influence the function or structure of the ecosystem by producing lactic acid and maintaining a low pH. The environment thus created would be a strong determinant of community species composition and activity because they would all have to flourish or at least tolerate an environmental pH of 4.0-4.5. The non-lactic acid

bacteria would be considered ‘passengers’ that are typically present at lower numerical abundance, may have little influence on the ecology of the system, and might be lost from the community or change over time without markedly affecting community function. Vaginal communities seem to conform to this model at least from a numerical perspective since the rank abundance of species is highly skewed and lactic acid bacteria often outnumber others by two orders of magnitude, with diverse kinds of organisms present in lower numbers.

The occurrence of functional redundancy among ‘drivers’ (i.e., multiple species of lactic acid bacteria) may impact the stability and resilience of a community in the face of disturbances that lead to changes in community structure or function. Such disturbances will differ in magnitude, frequency and source. Those disturbances that are small in scale or infrequent may not affect communities that have functional redundancy in driver species, because if one driver is disadvantaged or lost, another can compensate and thereby maintain community function. If, however, the disturbance is overwhelming or the community is driven by a single species, it may be more susceptible to change in function. This change in function could be harmful if it is necessary for health, or it can provide opportunities for colonization by pathogens.

Resilience and stability

Ecological theory and empirical data indicate that communities are not equally resilient or equally stable. The stability (or resistance) of a community reflects its capacity to resist change in structure or function in response to a disturbance event⁹⁴ (Fig. 3), while resilience reflects the ability of a community to recover from a disturbance and return to a ‘quasi-stable’ equilibrium state⁹⁴⁻⁹⁶ (Fig. 4). The resistance of a community is reflected in the magnitude of change that can occur without having an impact on community function, whereas resilience is a measure of disturbance frequency or intensity that alters community function. Both the resistance and resilience of communities are largely determined by the ability of ecological networks of indigenous species to tolerate stresses and disturbance events, the physical, chemical and metabolic interactions among the species present, and the degree of functional redundancy present. A disturbance event is an environmental change that causes shifts in population densities, the gain or loss of species, and concomitant changes to community function.⁹⁷ The response of any community to one or more disturbance events (or to a disturbance regime characterized by a distribution of disturbance sizes, frequencies, intensities, and timing) is determined by the attributes of component species.⁹⁸ Disturbed communities may or may not return to their previous state.⁹⁷ The ability of an ecosystem to buffer against perturbations and resist species invasions is dependent on the redundancy of species that have important stabilizing roles as well as their ability to differentially respond to perturbations. This ‘insurance hypothesis’ posits that increasing diversity increases the odds that at least some species will respond differentially to variable conditions and perturbations, and that greater diversity increases the odds that an ecosystem has functional redundancy by containing species that are capable of functionally replacing important species.⁹⁴

Events that destabilize microbial communities are not equal in terms of intensity or duration. Communities with low resistance and resilience may be disturbed by a single intense event of short duration, or multiple events of moderate to low intensity. This can result in transitory changes to the structure of these communities rendering them more susceptible to invasion by species that are not indigenous to the human vagina including transient species of fecal origin and opportunistic pathogens. In contrast, robust vaginal communities will exhibit stability in the face of more frequent events of low to moderate intensity and retain ecological functions that are characteristic of healthy communities. Given this, we postulate that stability and resilience of vaginal bacterial communities is likely to vary widely since

the species composition and structure of these communities differs among women and this in turn may account for differences in the susceptibility of individuals to urogenital infectious diseases. Nonetheless, this hypothesis has yet to be empirically tested. To date, most studies of vaginal microbiology have relied on cross-sectional study designs in which individuals are sampled at one time point or over regular intervals of a few weeks or months.^{57, 99-101} As a result, very little is known about the dynamics of vaginal microbiota over short time-scales and in response to various host-associated perturbations.

Susceptibility to invasion by nonindigenous species

As discussed above, acidification of the vaginal milieu via lactic acid production is probably important to preventing the invasion of vaginal communities by nonindigenous organisms. It has been suggested that hydrogen peroxide may also help prevent such invasions since some vaginal species of *Lactobacillus* produce H₂O₂ in vitro.^{34, 85, 102-106} However recent studies have shown this is not likely the case *in vivo* since dissolved oxygen levels in the vagina are exceptionally low.^{1, 2, 107, 108} Other mechanisms such as production of bacteriocins have also been suggested⁸⁰ but their importance has yet to be documented.

The establishment of a pathogen in a community closely mirrors the process of exotic species invasion in plant and animal communities. One concept from invasive species research that applies most readily is the relationship between disturbance and invasion. A review by Didham *et al.* (2005)¹⁰⁹ examined whether invasive species were drivers of change in community composition or passengers that took advantage of a disturbance that had changed the community's structure. The implication for medical microbiology is that without disturbance a pathogen may be able to invade some community types but not others. The second concept is the role of redundancy in driver species to maintain function and prevent invasion. Understanding the extent of functional redundancy and the contribution of specific community members to a healthy vaginal microbiota could help define risk factors for infection.

Ecologists have long known that the biological communities of disturbed ecosystems are more susceptible to invasion by non-indigenous, 'weedy' species.⁹⁸ This is likely true for the bacterial communities of the human vagina too. If the proliferation of these invasive species proceeds unchecked, it could lead to clinically significant symptoms and disease. If the resilience of a vaginal community is low, then transitory changes to the structure of these communities may occur more readily in response to disturbances and these disturbed communities may be more susceptible to invasion by species that are not indigenous to the human vagina. These might include transient species of fecal origin and opportunistic pathogens.⁹⁸ Moreover, we speculate that the disturbed state may itself constitute the clinical syndrome of BV where there is a reduction in the presence of lactic acid bacteria.

The intimate relations of women and their vaginal microbiota

Evidence that host specific characteristics influence the species composition and dynamics of microbial communities that colonize the vagina is accruing, though direct evidence is lacking. As described previously, recent studies have shown that the vaginal communities of women can be classified into several types based on similarities in bacterial community composition.³⁻⁵ This can be viewed in two ways. One is that vaginal communities can show marked differences in the composition and rank abundance of species present,^{55, 110, 111} and these differences may be potentially important in terms of ecosystem resilience and resistance to infectious agents. On the other hand, it also demonstrates that the differences among women are apparently not boundless, and therefore colonization of the host and vaginal community composition are probably not random events. Said plainly, there appear to be host factors that facilitate or select for bacterial species with particular characteristics.

These might be linked to the presence of epithelial cell surface receptors, variation in the composition or amount of vaginal secretions, the host immune system, or other factors (reviewed in Linhares *et al.*² and Wira *et al.*¹¹²).

The notion that there is selection for particular bacterial species by host-determined characteristics is also supported by the observation that a rather limited number of different *Lactobacillus* species are found to dominate vaginal communities. Recent reports^{3-5, 15, 51, 53} suggest only four species of *Lactobacillus*, namely, *L. crispatus*, *L. jensenii*, *L. gasseri* and *L. iners*, are found as dominant members of vaginal communities. This is surprising given the plethora of different species of lactobacilli that are recognized²⁶ and suggests that species of lactobacilli found in the vagina possess characteristics that allow them to compete and be successful under the environmental conditions of the vagina. If there is selection within an individual for species (or strains of species) that possess a suite of specific characteristics, then this could have important implications for efforts to develop prebiotics and probiotics for maintaining or re-establishing normal, healthy vaginal communities, because ideally they would be tailored to reflect differences in the species composition of an individual's normal community.

Bacterial vaginosis: an enigma of women's health

Bacterial vaginosis (BV) is the most frequently cited cause of vaginal discharge and malodor and the most common vaginal disorder of reproductive age women, resulting in millions of health care visits annually in the United States alone.^{113, 114} Moreover, in non-pregnant women BV is associated with serious adverse sequelae including infertility,¹¹⁵ endometritis,¹¹⁶ and pelvic inflammatory disease,¹¹⁷ as well as an increased risk of acquiring HIV, *Neisseria gonorrhoeae*, and other sexually transmitted diseases.¹¹⁸⁻¹²² During pregnancy, BV is associated with several adverse outcomes including preterm delivery of low birth weight infants,¹²³ spontaneous abortion,^{124, 125} premature rupture of membranes,¹²⁶ preterm birth,¹²⁶ amniotic fluid infections,¹²⁷ postpartum endometritis,¹²⁵ and endometritis following Caesarian section.¹²⁸ The exact etiology of BV remains elusive, but different pathogenicity models have been proposed involving either the depletion or displacement of lactobacilli in the development of BV (Fig. 5).³⁶ The prevalence of BV among women varies widely and depends on the subject population. It is present in 10% to 20% of white, non-Hispanic women and 30%-50% of African-American women, and it may occur in up to 85% of sex workers in Africa.^{113, 129-131} It has a prevalence of 5-26% in pregnant women worldwide.¹¹⁰

Despite over a century of work, attempts to find a single causative agent have failed. Since Duke and Gardner implicated *Gardnerella vaginalis* as a causative agent of BV (1955),¹³² numerous efforts have been made to associate BV with the presence of certain bacteria in hopes of identifying an infectious agent. In recent years investigators have used cultivation-independent methods to continue the search for organisms that might cause BV. Fredricks *et al.*¹³³ used broad-range PCR and sequencing of 16S rRNA genes to find that women with BV had a relatively high prevalence abundance of bacteria such as *Atopobium vaginae*, *Leptotrichia amnionii*, *Sneathia sanguinegens*, *Porphyromonas asaccharolytica*, *G. vaginalis*, and novel members of the Clostridiales referred to as BV-associated bacteria (BVAB). Moreover, in a study by Ferris *et al.* broad range PCR assays were also used to characterize the vaginal microbiota before and after metronidazole treatment to find that the diversity of anaerobic bacterial types of BV flora was shifted to a predominantly *L. iners* flora in cured patients and that unresponsive patients had the highest concentrations of *A. vaginae*.⁷¹ While these studies corroborate the co-occurrence of BV with so-called BV-associated bacteria it is unclear if these bacterial species are causally related to the symptoms of BV or whether the association is contingent on the criteria used to diagnose BV. Studies in our lab

and others have demonstrated these very same suspected agents and other closely related bacteria are found in asymptomatic women though perhaps at somewhat lower number (Fig. 6). This argues that these organisms might not be ‘infectious agents’ in the strict sense, but when present in high number they might elicit some or all of the symptoms classically associated with bacterial vaginosis. That said, it is risky to deduce that certain organisms cause BV simply because they are abundant when symptoms are present.

Others have pursued the question of whether BV is a sexually transmitted disease. Evidence supporting this notion comes from the observed concordance of BV status in monogamous lesbian couples ranges up to 95%.^{30, 134, 135} Also, women with BV have more sex partners and an earlier age of sexual debut than women without BV,^{84, 86} and an association between receptive oral sex and BV has been suggested.⁸⁴ However, BV has been diagnosed in asymptomatic virginal women,¹³⁶⁻¹³⁸ and this begs the question of what, if any, role sexual behaviors may have in the acquisition of BV. On balance the data available support the hypothesis that BV is not an infectious disease.

Is bacterial vaginosis being mischaracterized as a disease?

Over the years, the definition of BV and the diagnostic criteria commonly used have been conflated, and they remain mired in controversy. The Amsel test, which is often used for the clinical diagnosis of BV, is based on four criteria: (a) a vaginal pH of >4.5, (b) the presence of clue cells, (c) a fishy odor upon addition of 10% KOH to vaginal discharge, and (d) a white, thin, homogenous vaginal discharge.¹³⁹ The diagnosis of BV is made if at least three of these criteria are confirmed. The gold-standard for the diagnosis of BV in research and laboratory settings has been the Nugent score.¹⁴⁰ This diagnostic test is a scored scale based on (1) the presence of Gram-positive rods (*Lactobacillus* morphotypes) (2) the presence of Gram-variable rods and cocci (*Gardnerella vaginalis*, *Prevotella*, *Porphyromonas*, and peptostreptococci morphotypes) and (3) the presence of curved Gram-variable rods (*Mobiluncus* spp. morphotypes). In a formal sense, an obvious potential problem is the logic of the Nugent score premise that high numbers of *Lactobacillus* spp. define “health,” and this imposes a bias against normal vaginal microbial communities that lack appreciable numbers of lactobacilli, yet maintain a low pH. The Amsel test, on the other hand, may lack sensitivity due to the subjectivity of the clinician’s interpretation. Reports comparing the two diagnostic measures arrive at opposing conclusions,^{141, 142} which has led many to suspect the accuracy of these tests.

Two fallacies permeate thinking about the diagnosis and treatment of BV. The first is that BV is an infectious disease. This seems to arise from classical thinking about infectious diseases in the framework of Koch’s postulates, wherein a single species is both necessary and sufficient to cause infection. Although this is certainly true for a number of pathogens, it may be inadequate as an explanation for other diseases caused by mixtures of organisms. These so-called polymicrobial infections may not be infections in the strict sense of the word, wherein a nonindigenous organism invades a community. Instead they may be caused by indigenous populations that are typically rare but become abundant due to changes to ecologically important characteristics modulated by the host (e.g., nutrient levels) or disturbances that alter the competitive dynamics of bacterial populations. In other words, the incidence of infectious disease may often depend not only on the competitiveness of an invasive species (i.e., the infectious agent) but also on the ecological dynamics of the habitat (i.e., anatomical site). To us it seems there is much to be gained from borrowing and testing ecological theory that has been developed over decades by plant and animal ecologists who have studied invasive species in a wide variety of circumstances.

A second fallacy is directly tied to the notion that the vaginas of normal healthy women are populated by high numbers of *Lactobacillus* spp. This statement is accurate so far as it goes.

However, the converse statement – that women whose vaginal communities have few or no *Lactobacillus* spp. – are abnormal is unsupported by data. We postulate that because of this logical fallacy, BV is often over-diagnosed. This could partly account for the reported high incidence of so-called ‘asymptomatic’ BV in reproductive age women,^{143, 144} and also explain a proportion of BV treatment failures and apparent recurrences of BV in women. Acknowledging that not all vaginal communities of healthy women are dominated by *Lactobacillus* spp. would also be in accordance with the observation that the vaginal communities of post-menopausal women (not taking hormone replacement therapy) often lack appreciable numbers of *Lactobacillus* spp., yet these individuals do not exhibit other untoward symptoms. We suspect that the causes and cures of BV will continue to be enigmatic until it is recognized that while ‘normal and healthy’ can be equated with high numbers of lactobacilli, the converse statement (“unhealthy” is equated with low numbers of or no lactobacilli) is not necessarily true. We must be vigilant and realize that for a significant proportion of women ‘normal and healthy’ can also occur in the absence of appreciable numbers of *Lactobacillus* spp.

Conclusion

Multiple kinds of normal vaginal microbial communities are found in healthy women. The data provide strong evidence that more species than lactobacilli can dominate the vaginal microbial ecosystem of healthy women. The community function of maintaining low pH is highly conserved among women despite the difference in their vaginal microbial community composition and structure. These communities are postulated to provide different levels of protection against disease and infection, and their ability to offer protection may be lessened if the communities are disrupted. We propose that all vaginal microbial communities are not equally resilient and their stabilities differ in the face of disturbances. Moreover, differences in the resilience of various vaginal microbial communities may account for the differential susceptibility of races to HIV, BV and other urogenital infectious diseases. Obviously our knowledge of the factors that affect and control the vaginal microbiota is still incomplete and increasingly we should view the vagina as a microbial ecosystem so that we can better understand the full range of factors that affect risk to disease.

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References

1. O#x2019;Hanlon DE, Moench TR, Cone RA. In vaginal fluid, bacteria associated with bacterial vaginosis can be suppressed with lactic acid but not hydrogen peroxide. *BMC Infectious Diseases*. 2011; 11:200. [PubMed: 21771337]
2. Linhares IM, Summers PR, Larsen B, Giraldo PC, Witkin SS. Contemporary perspectives on vaginal pH and lactobacilli. *Am J Obstet Gynecol*. 2010; 203:1.e1–1.e5. [PubMed: 20609700]
3. Zhou X, Hansmann MA, Davis CC, et al. The vaginal bacterial communities of Japanese women resemble those of women in other racial groups. *FEMS Immunology and Medical Microbiology*. 2010; 58:169–181. [PubMed: 19912342]
4. Ravel J, Gajer P, Abdo Z, et al. Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci U S A*. 2011; 108(Suppl 1):4680–4687. [PubMed: 20534435]

5. Zhou X, Brown CJ, Abdo Z, et al. Differences in the composition of vaginal microbial communities found in healthy Caucasian and black women. *The ISME Journal*. 2007; 1:121–133. [PubMed: 18043622]
6. Farage M, Maibach H. Lifetime changes in the vulva and vagina. *Archives of Gynecology and Obstetrics*. 2006; 273:195–202. [PubMed: 16208476]
7. Dominguez-Bello MG, Costello EK, Contreras M, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U S A*. 2010; 107:11971–11975. [PubMed: 20566857]
8. Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO. Development of the human infant intestinal microbiota. *PLoS Biol*. 2007; 5:e177. [PubMed: 17594176]
9. Koenig JE, Spor A, Scalfone N, et al. Colloquium Paper: Succession of microbial consortia in the developing infant gut microbiome. *Proc Natl Acad Sci U S A*. 2011; 108:4578–4585. [PubMed: 20668239]
10. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. *Archives of Disease in Childhood*. 1969; 44:291–303. [PubMed: 5785179]
11. Hammerschlag MR, Alpert S, Rosner I, et al. Microbiology of the vagina in children: normal and potentially pathogenic organisms. *Pediatrics*. 1978; 62:57–62. [PubMed: 98750]
12. Hammerschlag MR, Alpert S, Onderdonk AB, et al. Anaerobic microflora of the vagina in children. *Am J Obstet Gynecol*. 1978; 131:853–856. [PubMed: 686083]
13. Alvarez-Olmos MI, Barousse MM, Rajan L, et al. Vaginal lactobacilli in Adolescents. *Sex Transm Dis*. 2004; 31:393–400. [PubMed: 15215693]
14. Randjelovi G, Koci B, Stojanovi M, Misi M. Bacteriological findings of the vulvar swab specimens from girls with vulvovaginitis. *Facta Universitatis: Medicine and Biology*. 2005; 12:159–163.
15. Yamamoto T, Zhou X, Williams CJ, Hochwalt A, Forney LJ. Bacterial populations in the vaginas of healthy adolescent women. *Journal of Pediatric and Adolescent Gynecology*. 2009; 22:11–18. [PubMed: 19232297]
16. Wylie JG, Henderson A. Identity and glycogen-fermenting ability of lactobacilli isolated from the vagina of pregnant women. *J Med Microbiol*. 1969; 2:363–366. [PubMed: 4996481]
17. Larsen B, Goplerud CP, Petzold CR, Ohm-Smith MJ, Galask RP. Effect of estrogen treatment on the genital tract flora of postmenopausal women. *Obstet Gynecol*. 1982; 60:20–24. [PubMed: 6283447]
18. Ginkel PD, Soper DE, Bump RC, Dalton HP. Vaginal flora in postmenopausal women: the effect of estrogen replacement. *Infectious Diseases in Obstetrics and Gynecology*. 1993; 1:94–97. [PubMed: 18475325]
19. Priestley CJ, Jones BM, Dhar J, Goodwin L. What is normal vaginal flora? *Genitourinary Medicine*. 1997; 73:23–28. [PubMed: 9155551]
20. Pybus V, Onderdonk AB. Evidence for a commensal, symbiotic relationship between *Gardnerella vaginalis* and *Prevotella bivia* involving ammonia: potential significance for bacterial vaginosis. *The Journal of Infectious Diseases*. 1997; 175:406–413. [PubMed: 9203662]
21. Donders GGG. Definition and classification of abnormal vaginal flora. *Best Practice & Research Clinical Obstetrics & Gynaecology*. 2007; 21:355–373. [PubMed: 17434799]
22. Döderlein A. Das scheidensekret und seine bedeutung für puerperalfieber. *Zentralblatt für Bakteriologie*. 1892; 11:699.
23. Thomas S. Döderlein's bacillus: *Lactobacillus acidophilus*. *The Journal of Infectious Diseases*. 1928; 43:218–227.
24. Lauer E, Helming C, Kandler O. Heterogeneity of the species *Lactobacillus acidophilus* (Moro) Hansen and Møcquot as revealed by biochemical characteristics and DNA-DNA hybridization. *Zentbl Bakteriol Microbiol Hyg Abt*. 1980; 1:150–168.
25. Johnson JL, Phelps CF, Cummins CS, London J, Gasser F. Taxonomy of the *Lactobacillus acidophilus* group. *International Journal of Systematic and Evolutionary Microbiology*. 1980; 30:53–68.
26. Schleifer KH, Ludwig W. Phylogeny of the Genus *Lactobacillus* and Related Genera. *Systematic and Applied Microbiology*. 1995; 18:461–467.

27. Du Plessis EM, Dicks LM. Evaluation of random amplified polymorphic DNA (RAPD)-PCR as a method to differentiate *Lactobacillus acidophilus*, *Lactobacillus crispatus*, *Lactobacillus amylovorus*, *Lactobacillus gallinarum*, *Lactobacillus gasseri*, and *Lactobacillus johnsonii*. *Current Microbiology*. 1995; 31:114–118. [PubMed: 7606186]
28. Redondo-Lopez V, Cook RL, Sobel JD. Emerging role of lactobacilli in the control and maintenance of the vaginal bacterial microflora. *Reviews of Infectious Diseases*. 1990; 12:856–872. [PubMed: 2237129]
29. Larsen B, Monif GR. Understanding the bacterial flora of the female genital tract. *Clinical Infectious Diseases*. 2001; 32:e69–77. [PubMed: 11181139]
30. Marrazzo JM, Koutsky LA, Eschenbach DA, Agnew K, Stine K, Hillier SL. Characterization of vaginal flora and bacterial vaginosis in women who have sex with women. *The Journal of Infectious Diseases*. 2002; 185:1307–1313. [PubMed: 12001048]
31. Rogosa M, Sharpe ME. Species differentiation of human vaginal lactobacilli. *J Gen Microbiol*. 1960; 23:197–201. [PubMed: 14438406]
32. Levison ME, Corman LC, Carrington ER, Kaye D. Quantitative microflora of the vagina. *Am J Obstet Gynecol*. 1977; 127:80–85. [PubMed: 831488]
33. Reid G, McGroarty JA, Tomeczek L, Bruce AW. Identification and plasmid profiles of *Lactobacillus* species from the vagina of 100 healthy women. *FEMS Immunology and Medical Microbiology*. 1996; 15:23–26. [PubMed: 8871112]
34. Antonio MA, Hawes SE, Hillier SL. The identification of vaginal *Lactobacillus* species and the demographic and microbiologic characteristics of women colonized by these species. *The Journal of Infectious Diseases*. 1999; 180:1950–1956. [PubMed: 10558952]
35. Collins MD, Wallbanks S. Comparative sequence analyses of the 16S rRNA genes of *Lactobacillus minutus*, *Lactobacillus rimae* and *Streptococcus parvulus*: proposal for the creation of a new genus *Atopobium*. *FEMS Microbiology Letters*. 1992; 74:235–240. [PubMed: 1382033]
36. Srinivasan S, Fredricks DN. The human vaginal bacterial biota and bacterial vaginosis. *Interdisciplinary Perspectives on Infectious Diseases*. 2008; 750479
37. Verhelst R, Verstraelen H, Claeys G, et al. Cloning of 16S rRNA genes amplified from normal and disturbed vaginal microflora suggests a strong association between *Atopobium vaginae*, *Gardnerella vaginalis* and bacterial vaginosis. *BMC Microbiology*. 2004; 4:16. [PubMed: 15102329]
38. Verstraelen H, Verhelst R, Claeys G, Temmerman M, Vaneechoutte M. Culture-independent analysis of vaginal microflora: The unrecognized association of *Atopobium vaginae* with bacterial vaginosis. *Am J Obstet Gynecol*. 2004; 191:1130–1132. [PubMed: 15507931]
39. Bakken LR. Separation and purification of bacteria from soil. *Applied and Environmental Microbiology*. 1985; 49:1482–1487. [PubMed: 16346816]
40. Connon SA, Giovannoni SJ. High-Throughput Methods for Culturing Microorganisms in Very-Low-Nutrient Media Yield Diverse New Marine Isolates. *Applied and Environmental Microbiology*. 2002; 68:3878–3885. [PubMed: 12147485]
41. Stingl U, Tripp HJ, Giovannoni SJ. Improvements of high-throughput culturing yielded novel SAR11 strains and other abundant marine bacteria from the Oregon coast and the Bermuda Atlantic Time Series study site. *The ISME journal*. 2007; 1:361–371. [PubMed: 18043647]
42. Goodman AL, Kallstrom G, Faith JJ, et al. Extensive personal human gut microbiota culture collections characterized and manipulated in gnotobiotic mice. *Proc Natl Acad Sci U S A*. 2011; 108:6252–6257. [PubMed: 21436049]
43. Park J, Kerner A, Burns MA, Lin XN. Microdroplet-enabled highly parallel co-cultivation of microbial communities. *PLoS ONE*. 2011; 6:e17019. [PubMed: 21364881]
44. Hugenholtz P, Goebel BM, Pace NR. Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *J Bacteriol*. 1998; 180:4765–4774. [PubMed: 9733676]
45. Dekio I. Detection of potentially novel bacterial components of the human skin microbiota using culture-independent molecular profiling. *J Med Microbiol*. 2005; 54:1231–1238. [PubMed: 16278439]
46. Eckburg PB. Diversity of the Human Intestinal Microbial Flora. *Science*. 2005; 308:1635–1638. [PubMed: 15831718]

47. Bik EM, Eckburg PB, Gill SR, et al. Molecular analysis of the bacterial microbiota in the human stomach. *Proc Natl Acad Sci U S A*. 2006; 103:732–737. [PubMed: 16407106]
48. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. *Nature*. 2007; 449:804–810. [PubMed: 17943116]
49. Schellenberg J, Links MG, Hill JE, et al. Pyrosequencing of the chaperonin-60 universal target as a tool for determining microbial community composition. *Applied and Environmental Microbiology*. 2009; 75:2889–2898. [PubMed: 19270139]
50. van der Lelie D, Lesaulnier C, McCorkle S, Geets J, Taghavi S, Dunn J. Use of Single-Point Genome Signature Tags as a Universal Tagging Method for Microbial Genome Surveys. *Applied and Environmental Microbiology*. 2006; 72:2092–2101. [PubMed: 16517658]
51. Tärnberg M, Jakobsson T, Jonasson J, Forsum U. Identification of randomly selected colonies of lactobacilli from normal vaginal fluid by pyrosequencing of the 16S rDNA variable V1 and V3 regions. *APMIS*. 2002; 110:802–810. [PubMed: 12588421]
52. Burton JP, Cadieux PA, Reid G. Improved understanding of the bacterial vaginal microbiota of women before and after probiotic instillation. *Applied and Environmental Microbiology*. 2003; 69:97–101. [PubMed: 12513982]
53. Zhou X, Bent SJ, Schneider MG, Davis CC, Islam MR, Forney LJ. Characterization of vaginal microbial communities in adult healthy women using cultivation-independent methods. *Microbiology*. 2004; 150:2565–2573. [PubMed: 15289553]
54. Hill JE, Goh SH, Money DM, et al. Characterization of vaginal microflora of healthy, nonpregnant women by chaperonin-60 sequence-based methods. *Am J Obstet Gynecol*. 2005; 193:682–692. [PubMed: 16150261]
55. Hyman RW, Fukushima M, Diamond L, Kumm J, Giudice LC, Davis RW. Microbes on the human vaginal epithelium. *Proc Natl Acad Sci U S A*. 2005; 102:7952–7957. [PubMed: 15911771]
56. Cauci S, Driussi S, De Santo D, et al. Prevalence of bacterial vaginosis and vaginal flora changes in peri- and postmenopausal women. *J Clin Microbiol*. 2002; 40:2147–2152. [PubMed: 12037079]
57. Eschenbach DA, Thwin SS, Patton DL, et al. Influence of the normal menstrual cycle on vaginal tissue, discharge, and microflora. *Clinical Infectious Diseases*. 2000; 30:901–907. [PubMed: 10852812]
58. Smith CB, Noble V, Bensch R, Ahlin PA, Jacobson JA, Latham RH. Bacterial flora of the vagina during the menstrual cycle: findings in users of tampons, napkins, and sea sponges. *Ann Intern Med*. 1982; 96:948–951. [PubMed: 7046549]
59. Onderdonk AB, Zamarchi GR, Rodriguez ML, Hirsch ML, Muñoz A, Kass EH. Qualitative assessment of vaginal microflora during use of tampons of various compositions. *Applied and Environmental Microbiology*. 1987; 53:2779–2784. [PubMed: 3435143]
60. Onderdonk AB, Zamarchi GR, Rodriguez ML, Hirsch ML, Muñoz A, Kass EH. Quantitative assessment of vaginal microflora during use of tampons of various compositions. *Applied and Environmental Microbiology*. 1987; 53:2774–2778. [PubMed: 3435142]
61. Shiraishi T, Fukuda K, Morotomi N, et al. Influence of menstruation on the microbiota of healthy women's labia minora as analyzed using a 16S rRNA gene-based clone library method. *Japanese Journal of Infectious Diseases*. 2011; 64:76–80. [PubMed: 21266747]
62. Verstraelen H, Verhelst R, Claeys G, De Backer E, Temmerman M, Vaneechoutte M. Longitudinal analysis of the vaginal microflora in pregnancy suggests that *L. crispatus* promotes the stability of the normal vaginal microflora and that *L. gasseri* and/or *L. iners* are more conducive to the occurrence of abnormal vaginal microflora. *BMC Microbiology*. 2009; 9:116. [PubMed: 19490622]
63. Culhane J. Exposure to chronic stress and ethnic differences in rates of bacterial vaginosis among pregnant women. *Am J Obstet Gynecol*. 2002; 187:1272–1276. [PubMed: 12439519]
64. Nansel TR, Riggs MA, Yu K-F, Andrews WW, Schwebke JR, Klebanoff MA. The association of psychosocial stress and bacterial vaginosis in a longitudinal cohort. *Am J Obstet Gynecol*. 2006; 194:381–386. [PubMed: 16458633]
65. Culhane JF, Rauh VA, Goldenberg RL. Stress, bacterial vaginosis, and the role of immune processes. *Current Infectious Disease Reports*. 2006; 8:459–464. [PubMed: 17064639]

66. Vásquez A, Jakobsson T, Ahrné S, Forsum U, Molin G. Vaginal *Lactobacillus* flora of healthy Swedish women. *J Clin Microbiol.* 2002; 40:2746–2749. [PubMed: 12149323]
67. Verhelst R, Verstraelen H, Claey s G, et al. Comparison between Gram stain and culture for the characterization of vaginal microflora: definition of a distinct grade that resembles grade I microflora and revised categorization of grade I microflora. *BMC Microbiology.* 2005; 5:61. [PubMed: 16225680]
68. Anukam KC, Osazuwa EO, Ahonkhai I, Reid G. *Lactobacillus* vaginal microbiota of women attending a reproductive health care service in Benin city, Nigeria. *Sex Transm Dis.* 2006; 33:59. [PubMed: 16385223]
69. Tamrakar R, Yamada T, Furuta I, et al. Association between *Lactobacillus* species and bacterial vaginosis-related bacteria, and bacterial vaginosis scores in pregnant Japanese women. *BMC Infectious Diseases.* 2007; 7:128. [PubMed: 17986357]
70. De Backer E, Verhelst R, Verstraelen H, et al. Quantitative determination by real-time PCR of four vaginal *Lactobacillus* species, *Gardnerella vaginalis* and *Atopobium vaginae* indicates an inverse relationship between *L. gasseri* and *L. iners*. *BMC Microbiology.* 2007; 7:115. [PubMed: 18093311]
71. Ferris MJ, Norori J, Zozaya-Hinchliffe M, Martin DH. Cultivation-independent analysis of changes in bacterial vaginosis flora following metronidazole treatment. *J Clin Microbiol.* 2007; 45:1016–1018. [PubMed: 17202272]
72. Thies FL, König W, König B. Rapid characterization of the normal and disturbed vaginal microbiota by application of 16S rRNA gene terminal RFLP fingerprinting. *J Med Microbiol.* 2007; 56:755–761. [PubMed: 17510259]
73. Nam H, Whang K, Lee Y. Analysis of vaginal lactic acid producing bacteria in healthy women. *Journal of Microbiology.* 2007; 45:515–520.
74. Alqumber MA, Burton JP, Devenish C, Tagg JR. A species-specific PCR for *Lactobacillus iners* demonstrates a relative specificity of this species for vaginal colonization. *Microbial Ecology in Health and Disease.* 2008; 20:135–139.
75. Shi Y, Chen L, Tong J, Xu C. Preliminary characterization of vaginal microbiota in healthy Chinese women using cultivation-independent methods. *The Journal of Obstetrics and Gynaecology Research.* 2009; 35:525–532. [PubMed: 19527394]
76. Zozaya-Hinchliffe M, Lillis R, Martin DH, Ferris MJ. Quantitative PCR Assessments of Bacterial Species In Women With And Without Bacterial Vaginosis. *J Clin Microbiol.* 2010; 48:1812–1819. [PubMed: 20305015]
77. Falsen E, Pascual C, Sjöden B, Ohlén M, Collins MD. Phenotypic and phylogenetic characterization of a novel *Lactobacillus* species from human sources: description of *Lactobacillus iners* sp. nov. *International Journal of Systematic Bacteriology.* 1999; 49:217–221. [PubMed: 10028266]
78. Boskey ER, Telsch KM, Whaley KJ, Moench TR, Cone RA. Acid production by vaginal flora in vitro is consistent with the rate and extent of vaginal acidification. *Infect Immun.* 1999; 67:5170–5175. [PubMed: 10496892]
79. Boskey ER, Cone RA, Whaley KJ, Moench TR. Origins of vaginal acidity: high D/L lactate ratio is consistent with bacteria being the primary source. *Hum Reprod.* 2001; 16:1809–1813. [PubMed: 11527880]
80. Aroutcheva A, Gariti D, Simon M, et al. Defense factors of vaginal lactobacilli. *Am J Obstet Gynecol.* 2001; 185:375–379. [PubMed: 11518895]
81. Valore EV, Park CH, Igreti SL, Ganz T. Antimicrobial components of vaginal fluid. *Am J Obstet Gynecol.* 2002; 187:561–568. [PubMed: 12237628]
82. Jaks i FM. Abuse and misuse of the term ‘guild’ in ecological studies. *Oikos.* 1981:397–400.
83. Farage MA, Miller KW, Sobel JD. Dynamics of the Vaginal Ecosystem - Hormonal Influences. *Infectious Diseases: Research and Treatment.* 2010; 3:1–15.
84. Schwebke JR, Richey CM, Weiss HL. Correlation of behaviors with microbiological changes in vaginal flora. *The Journal of Infectious Diseases.* 1999; 180:1632–1636. [PubMed: 10515826]

85. Vallor AC, Antonio MA, Hawes SE, Hillier SL. Factors associated with acquisition of, or persistent colonization by, vaginal lactobacilli: role of hydrogen peroxide production. *The Journal of Infectious Diseases*. 2001; 184:1431–1436. [PubMed: 11709785]
86. Schwebke JR, Desmond RA, Oh MK. Predictors of Bacterial Vaginosis in Adolescent Women Who Douche. *Sex Transm Dis*. 2004; 31:433–436. [PubMed: 15215700]
87. Beigi RH, Wiesenfeld HC, Hillier SL, Straw T, Krohn MA. Factors associated with absence of H₂O₂-producing *Lactobacillus* among women with bacterial vaginosis. *The Journal of Infectious Diseases*. 2005; 191:924–929. [PubMed: 15717268]
88. Onderdonk AB, Delaney ML, Hinkson PL, DuBois AM. Quantitative and qualitative effects of douche preparations on vaginal microflora. *Obstetrics & Gynecology*. 1992; 80:333–338. [PubMed: 1495688]
89. Klebanoff SJ. Effects of the spermicidal agent nonoxynol-9 on vaginal microbial flora. *The Journal of Infectious Diseases*. 1992; 165:19–25. [PubMed: 1309374]
90. McGroarty JA, Tomczek L, Pond DG, Reid G, Bruce AW. Hydrogen peroxide production by *Lactobacillus* species: correlation with susceptibility to the spermicidal compound nonoxynol-9. *The Journal of Infectious Diseases*. 1992; 165:1142–1144. [PubMed: 1316413]
91. Rosenstein IJ, Stafford MK, Kitchen VS, Ward H, Weber JN, Taylor-Robinson D. Effect on normal vaginal flora of three intravaginal microbicidal agents potentially active against human immunodeficiency virus type 1. *The Journal of Infectious Diseases*. 1998; 177:1386–1390. [PubMed: 9593030]
92. Gupta K, Hillier SL, Hooton TM, Roberts PL, Stamm WE. Effects of contraceptive method on the vaginal microbial flora: a prospective evaluation. *The Journal of Infectious Diseases*. 2000; 181:595–601. [PubMed: 10669343]
93. Walker B. Conserving biological diversity through ecosystem resilience. *Conservation Biology*. 1995; 9:747–752.
94. McCann KS. The diversity-stability debate. *Nature*. 2000; 405:228–233. [PubMed: 10821283]
95. Pimm SL. The complexity and stability of ecosystems. *Nature*. 1984; 307:321–326.
96. Gunderson LH. Ecological resilience--in theory and application. *Annual Review of Ecology and Systematics*. 2000:425–439.
97. White PS, Jentsch A. The search for generality in studies of disturbance and ecosystem dynamics. *Progress in Botany*. 2001; 62:399–450.
98. Hobbs RJ, Huenneke LF. Disturbance, diversity, and invasion: implications for conservation. *Conservation Biology*. 1992; 6:324–337.
99. Wilks M, Tabaqchali S. Quantitative bacteriology of the vaginal flora during the menstrual cycle. *J Med Microbiol*. 1987; 24:241–245. [PubMed: 3669056]
100. Coolen MJL, Post E, Davis CC, Forney LJ. Characterization of microbial communities found in the human vagina by analysis of terminal restriction fragment length polymorphisms of 16S rRNA genes. *Applied and Environmental Microbiology*. 2005; 71:8729–8737. [PubMed: 16332868]
101. Srinivasan S, Liu C, Mitchell CM, et al. Temporal variability of human vaginal bacteria and relationship with bacterial vaginosis. *PLoS ONE*. 2010; 5:e10197. [PubMed: 20419168]
102. Eschenbach DA, Davick PR, Williams BL, et al. Prevalence of hydrogen peroxide-producing *Lactobacillus* species in normal women and women with bacterial vaginosis. *J Clin Microbiol*. 1989; 27:251–256. [PubMed: 2915019]
103. Hawes SE, Hillier SL, Benedetti J, et al. Hydrogen peroxide-producing lactobacilli and acquisition of vaginal infections. *The Journal of Infectious Diseases*. 1996; 174:1058–1063. [PubMed: 8896509]
104. Rosenstein IJ, Fontaine EA, Morgan DJ, Sheehan M, Lamont RF, Taylor-Robinson D. Relationship between hydrogen peroxide-producing strains of lactobacilli and vaginosis-associated bacterial species in pregnant women. *European Journal of Clinical Microbiology & Infectious Diseases*. 1997; 16:517–522.
105. Al-Mushrif S, Jones BM. A study of the prevalence of hydrogen peroxide generating lactobacilli in bacterial vaginosis: the determination of H₂O₂ concentrations generated, in vitro, by isolated

- strains and the levels found in vaginal secretions of women with and without infection. *Journal of Obstetrics and Gynaecology*. 1998; 18:63–67. [PubMed: 15512007]
106. Wilks M, Wiggins R, Whiley A, et al. Identification and H₂O₂ Production of Vaginal Lactobacilli from Pregnant Women at High Risk of Preterm Birth and Relation with Outcome. *J Clin Microbiol*. 2004; 42:713–717. [PubMed: 14766841]
 107. Hill DR, Brunner ME, Schmitz DC, et al. In vivo assessment of human vaginal oxygen and carbon dioxide levels during and post menses. *Journal of Applied Physiology*. 2005; 99:1582–1591. [PubMed: 15932958]
 108. O#x2019;Hanlon DE, Lanier BR, Moench TR, Cone RA. Cervicovaginal fluid and semen block the microbicidal activity of hydrogen peroxide produced by vaginal lactobacilli. *BMC Infectious Diseases*. 2010; 10:120. [PubMed: 20482854]
 109. Didham RK, Tylanakis JM, Hutchison MA, Ewers RM, Gemmell NJ. Are invasive species the drivers of ecological change? *Trends in Ecology & Evolution*. 2005; 20:470–474. [PubMed: 16701420]
 110. Goldenberg RL, Klebanoff MA, Nugent R, Krohn MA, Hillier S, Andrews WW, Vaginal Infections and Prematurity Study Group. Bacterial colonization of the vagina during pregnancy in four ethnic groups. *Am J Obstet Gynecol*. 1996; 174:1618–1621. [PubMed: 9065140]
 111. Royce RA, Jackson TP, Thorp JM, Hillier SL, Rabe LK, Pastore LM, Savitz DA. Race/Ethnicity, Vaginal Flora Patterns, and pH During Pregnancy. *Sex Transm Dis*. 1999; 26:96–102. [PubMed: 10029984]
 112. Wira CR, Fahey JV, Sentman CL, Pioli PA, Shen L. Innate and adaptive immunity in female genital tract: cellular responses and interactions. *Immunol Rev*. 2005; 206:306–335. [PubMed: 16048557]
 113. Sobel JD. What#x2019;s new in bacterial vaginosis and trichomoniasis? *Infect Dis Clin North Am*. 2005; 19:387–406. [PubMed: 15963878]
 114. Koumans EH, Sternberg M, Bruce C, et al. The Prevalence of Bacterial Vaginosis in the United States, 2001–2004; Associations With Symptoms, Sexual Behaviors, and Reproductive Health. *Sex Transm Dis*. 2007; 34:864–869. [PubMed: 17621244]
 115. Sweet RL. Role of bacterial vaginosis in pelvic inflammatory disease. *Clinical Infectious Diseases*. 1995; 20(Suppl 2):S271–5. [PubMed: 7548573]
 116. Haggerty CL, Hillier SL, Bass DC, Ness RB, investigators PIDEaCHs. Bacterial vaginosis and anaerobic bacteria are associated with endometritis. *Clinical Infectious Diseases*. 2004; 39:990–995. [PubMed: 15472851]
 117. Wiesenfeld HC, Hillier SL, Krohn MA, et al. Lower genital tract infection and endometritis: insight into subclinical pelvic inflammatory disease. *Obstet Gynecol*. 2002; 100:456–463. [PubMed: 12220764]
 118. Taha TE, Hoover DR, Dallabetta GA, et al. Bacterial vaginosis and disturbances of vaginal flora: association with increased acquisition of HIV. *AIDS*. 1998; 12:1699–1706. [PubMed: 9764791]
 119. Hillier SL. The vaginal microbial ecosystem and resistance to HIV. *AIDS Res Hum Retroviruses*. 1998; 14(Suppl 1):S17–21. [PubMed: 9581879]
 120. Martin HL, Richardson BA, Nyange PM, et al. Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. *J Infect Dis*. 1999; 180:1863–1868. [PubMed: 10558942]
 121. Schmid G, Markowitz L, Joesoef R, Koumans E. Bacterial vaginosis and HIV infection. *Sexually Transmitted Infections*. 2000; 76:3–4. [PubMed: 10817059]
 122. Wiesenfeld HC, Hillier SL, Krohn MA, Landers DV, Sweet RL. Bacterial vaginosis is a strong predictor of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infection. *Clinical Infectious Diseases*. 2003; 36:663–668. [PubMed: 12594649]
 123. Hillier SL, Nugent RP, Eschenbach DA, et al. The Vaginal Infections and Prematurity Study Group. Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. *N Engl J Med*. 1995; 333:1737–1742. [PubMed: 7491137]
 124. Ralph SG, Rutherford AJ, Wilson JD. Influence of bacterial vaginosis on conception and miscarriage in the first trimester: cohort study. *BMJ*. 1999; 319:220–223. [PubMed: 10417083]

125. Leitich H, Bodneradler B, Brunbauer M, Kaider A, Egarter C, Husslein P. Bacterial vaginosis as a risk factor for preterm delivery: A meta-analysis. *Am J Obstet Gynecol.* 2003; 189:139–147. [PubMed: 12861153]
126. Hay PE. Bacterial vaginosis and miscarriage. *Curr Opin Infect Dis.* 2004; 17:41–44. [PubMed: 15090889]
127. Silver HM, Sperling RS, St Clair PJ, Gibbs RS. Evidence relating bacterial vaginosis to intraamniotic infection. *Am J Obstet Gynecol.* 1989; 161:808–812. [PubMed: 2782365]
128. Watts DH, Krohn MA, Hillier SL, Eschenbach DA. Bacterial vaginosis as a risk factor for post-cesarean endometritis. *Obstet Gynecol.* 1990; 75:52–58. [PubMed: 2296423]
129. Ledru S, Nicolas M, Mohamed F, Georges S, Jules BA, Jean Paul C. Etiologic study of genitourinary infections in women of childbearing age in Bobo-Dioulasso, Burkina Faso, 1992. *Sex Transm Dis.* 1996; 23:151. [PubMed: 8919743]
130. Morris M, Nicoll A, Simms I, Wilson J, Catchpole M. Bacterial vaginosis: a public health review. *Br J Obstet Gynaecol.* 2001; 108:439–450.
131. Newton ER, Piper JM, Shain RN, Perdue ST, Peairs W. Predictors of the vaginal microflora. *Am J Obstet Gynecol.* 2001; 184:845–53. discussion 853-5. [PubMed: 11303191]
132. Gardner HL, Dukes CD. *Haemophilus vaginalis* vaginitis: a newly defined specific infection previously classified non-specific vaginitis. *Am J Obstet Gynecol.* 1955; 69:962–976. [PubMed: 14361525]
133. Fredricks DN, Fiedler TL, Marrazzo JM. Molecular identification of bacteria associated with bacterial vaginosis. *The New England Journal of Medicine.* 2005; 353:1899–1911. [PubMed: 16267321]
134. Marrazzo JM, Thomas KK, Fiedler TL, Ringwood K, Fredricks DN. Relationship of specific vaginal bacteria and bacterial vaginosis treatment failure in women who have sex with women. *Ann Intern Med.* 2008; 149:20–28. [PubMed: 18591634]
135. Marrazzo JM, Antonio M, Agnew K, Hillier SL. Distribution of genital *Lactobacillus* strains shared by female sex partners. *The Journal of Infectious Diseases.* 2009; 199:680–683. [PubMed: 19199538]
136. Yen S, Shafer MA, Moncada J, Campbell CJ, Flinn SD, Boyer CB. Bacterial vaginosis in sexually experienced and non-sexually experienced young women entering the military. *Obstet Gynecol.* 2003; 102:927–933. [PubMed: 14672465]
137. Jones FR, Miller G, Gadea N, et al. Prevalence of bacterial vaginosis among young women in low-income populations of coastal Peru. *International Journal of STD & AIDS.* 2007; 18:188–192. [PubMed: 17362553]
138. Vaca M, Guadalupe I, Erazo S, et al. High prevalence of bacterial vaginosis in adolescent girls in a tropical area of Ecuador. *Br J Obstet Gynaecol.* 2009; 117:225–228.
139. Amsel R, Totten PA, Spiegel CA, Chen KCS, Eschenbach D, Holmes KK. Nonspecific vaginitis: Diagnostic criteria and microbial and epidemiologic associations. *The American Journal of Medicine.* 1983; 74:14–22. [PubMed: 6600371]
140. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of Gram stain interpretation. *J Clin Microbiol.* 1991; 29:297–301. [PubMed: 1706728]
141. Chaijareenont K, Sirimai K, Boriboonhirunsarn D, Kiriwat O. Accuracy of Nugent's score and each Amsel's criteria in the diagnosis of bacterial vaginosis. *Journal of the Medical Association of Thailand.* 2004; 87:1270–1274. [PubMed: 15825698]
142. Sha BE, Chen HY, Wang QJ, Zariffard MR, Cohen MH, Spear GT. Utility of Amsel criteria, Nugent score, and quantitative PCR for *Gardnerella vaginalis*, *Mycoplasma hominis*, and *Lactobacillus* spp. for diagnosis of bacterial vaginosis in human immunodeficiency virus-infected women. *J Clin Microbiol.* 2005; 43:4607–4612. [PubMed: 16145114]
143. Eschenbach DA, Hillier S, Critchlow C, Stevens C, DeRouen T, Holmes KK. Diagnosis and clinical manifestations of bacterial vaginosis. *Am J Obstet Gynecol.* 1988; 158:819–828. [PubMed: 3259075]
144. Sobel JD. Bacterial vaginosis. *Annu Rev Med.* 2000; 51:349–356. [PubMed: 10774469]

145. Fredricks DN, Fiedler TL, Thomas KK, Oakley BB, Marrazzo JM. Targeted PCR for detection of vaginal bacteria associated with bacterial vaginosis. *J Clin Microbiol.* 2007; 45:3270–3276. [PubMed: 17687006]

Figure 1.

Representation of vaginal bacterial community groups within four ethnic groups of women. The number of women from each ethnic group is in parentheses. The roman numerals indicate the five common vaginal bacterial community groups described by Ravel *et al.*⁴ Community groups I, II, III and V are predominated by *Lactobacillus crispatus*, *L. gasseri*, *L. iners* and *L. jensenii*, respectively, while community group IV contains a diverse assemblage of facultative and strictly anaerobic bacteria. Percent values are the percentages of women in each ethnic group whose vaginal bacterial community clustered with a particular community group. (reproduced from data in reference 4)

Figure 2.

The vaginal ecosystem and bacterial communities therein are strongly influenced by characteristics of the host, local environment, and constituent populations.

Figure 3.

The resistance or ‘quasi-stability’ of a community reflects its capacity to resist change in structure in response to a disturbance event. Ecosystem disturbances can occur at varying intensities and frequencies or durations, indicated here on the y-axis (magnitude or intensity) and x-axis (frequency or duration), respectively. Panels (A) and (B) represent two communities with different levels of resistance. The lighter portion in the bottom left-hand portion of each space represents an ecosystem’s quasi-stable state in which changes may occur to the community structure without pushing it into a ‘disturbed’ state. The darker portion in the upper right represents the disturbed ecosystem. Circles surrounded by dashed lines with an “*I*” inside represent various initial states of an ecosystem, and circles with solid lines and “*f*” inside represent the final state following a disturbance event. Some disturbances may push the ecosystem to another point within its quasi-stable space (e.g., f_1 in [A]; f_1 and f_2 in [B]) whereas some disturbances may be great enough to push the community into a ‘disturbed’ state (e.g., f_2 and f_3 in [A]; f_3 in [B]). Communities that differ in species composition are likely to have different degrees of resistance. In our example, communities A and B experience the same disturbances, but in (A) disturbance events 2 and 3 push the community into a disturbed state whereas in (B) only disturbance event 3 is strong enough to disturb the ecosystem from its quasi-stable state.

Figure 4.

Resilience is the ability of a community to return to a quasi-stable state following a disturbance event. Different communities, particularly if they differ in species composition, are presumed to possess different degrees of resilience. The x-axis and y-axis are the same as in Figure 3. Panels (A) and (B) represent two communities with different levels of resilience. Circles surrounded by dashed lines with an “i” inside represent various initial states of an ecosystem, dashed circles with “d” represent intermediate disturbed states, and circles with solid lines and “f” inside represent the final state following the disturbance. Dashed arrows indicate disturbance events (these are the same location, direction and magnitude in [A] and [B]), and the solid arrows indicate the community rebounding toward its quasi-stable state. The thickness of the solid line represents the relative degree of resilience. In this case, the resilience of community A is sufficient to restore quasi-stability in disturbance event 1 but not 2, whereas the resilience of community B is sufficient to recover from both disturbance events.

Figure 5.

Possible models for the pathogenesis of bacterial vaginosis (BV). Following an ecological insult or disturbance, dysbiosis may result when there is a change in the total abundance of microorganisms. This could result in a relative decrease in lactobacilli or a relative increase in facultative and anaerobic bacteria. Both scenarios may elicit a host response that eventually results in BV. This schematic is modified from one presented by Srinivasan and Fredricks³⁶ wherein the two models were referred to as the ‘*Lactobacillus* depletion model’ and ‘primary pathogen model.’

Figure 6.

A phylogenetic tree showing the relationship of selected phylotypes from vaginal communities of healthy Caucasian and black women⁵ (marked by triangles), type strains from the RDP database (unmarked) and three BV-associated bacteria (BVAB) (marked with arrows; sequences deposited by Fredricks *et al.*¹⁴⁵). The phylogenetic tree was constructed using a neighbor-joining algorithm, with *Mycoplasma* spp. serving as the out-group. Bootstrap values (from 500 replicates) greater than 50% are shown at the branch points, and the bar indicates 10% sequence divergence.

Table 1
Median pH of of vaginal community groups in women of different ethnicities

Ethnic groups	Community groups ^a											
	I (L.crispatus)			II (L.gasserii)			III (L.iners)			IV (Diversity group)		
	Subjects	Median pH ±MAD ^c	^b	Subjects	Median pH ±MAD ^c	^b	Subjects	Median pH ±MAD ^c	^b	Subjects	Median pH ±MAD ^c	^b
Asian	24	4.4 ± 0.52		5	4.4 ± 0.44		41	4.0 ± 0.0		19	5.5 ± 0.44	
White	44	4.0 ± 0.0		8	4.7 ± 0.44		26	4.3 ± 0.30		10	5.5 ±0.74	
Black	23	4.0 ± 0.0		5	5.0 ± 0.0		33	4.0 ± 0.0		42	5.3 ± 0.44	
Hispanic	14	4.0 ± 0.0		7	4.7 ± 0.22		35	4.4 ± 0.59		37	5.3 ± 0.44	
										4	5.0 ± 0.59	
										96	4.4 ± 0.59	
										97	4.2 ± 0.30	
										104	4.7 ± 1.04	
										97	5.0 ± 0.74	

^aCommunity groups are defined as in Ravel et al. (ref. 4).

^bTotal number of subjects within a community group.

^cMedian Absolute Deviation