The Vaginal Microbiome: II. Vaginal Dysbiotic Conditions

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Objective: This series of articles, titled The Vaginal Microbiome (VMB), written on behalf of the International Society for the Study of Vulvovaginal Disease, aims to summarize the recent findings and understanding of the vaginal bacterial microbiota, mainly regarding areas relevant to clinicians specializing in vulvovaginal disorders.

Materials and Methods: A search of PubMed database was performed, using the search terms “vaginal microbiome” with “dysbiosis,” “bacterial vaginosis,” “cytolytic vaginosis,” “desquamative inflammatory vaginitis,” and “aerobic vaginitis.” Full article texts were reviewed. Reference lists were screened for additional articles.

Results: The second article in this series focuses on vaginal dysbiotic conditions. Dysbiosis is a term describing imbalances in bacterial communities. Given that lactobacillus-dominated microbiota are thought to be the most optimal, vaginal dysbiosis is usually considered as lactobacilli-depleted VMB. Bacterial vaginosis (BV), the most common vaginal dysbiotic condition, is a polymicrobial disorder, considered the leading cause for vaginal discharge in women worldwide. In addition, we review the VMB in other vaginal conditions associated with lactobacilli depletion: desquamative inflammatory vaginitis and aerobic vaginitis. We also discuss the controversies in the diagnosis of cytolytic vaginosis, related with lactobacilli overgrowth.

Conclusions: Bacterial vaginosis displays complex microbiology. The heterogeneity and diversity within the genus Gardnerella may impact the progression of BV. Bacterial biofilms may contribute to the etiology and persistence of BV, and various bacteria may affect its clinical presentation and pathogenicity. Lack of lactobacilli is not always accompanied by an overgrowth of anaerobes.

Key Words: vaginal microbiome, vaginal dysbiosis, bacterial vaginosis, cytolytic vaginosis, desquamative inflammatory vaginitis, aerobic vaginitis

Moreover, the concept of what is a “normal” VMB is still controversial,2,3 as was discussed in part I. Alternatively, vaginal dysbiosis is often characterized as a VMB not dominated by lactobacilli.4 Dysbiotic, lactobacilli-depleted BV, has been associated with increased susceptibility to sexually transmitted infections (STIs), including HIV (see part III), and increased risk of pregnancy complications (see part IV). Nevertheless, the concept that absence of lactobacilli necessarily represents VMB imbalance may be incorrect or inadequate, as lactobacilli are often absent in asymptomatic women, who are not at increased risk for complications, such as in the cases of prepubertal girls and postmenopausal women.

Knowledge about different types of dysbiosis and their relationship to urogenital and reproductive disorders has increased in recent years by applying molecular techniques. This part of the VMB review discusses the complexity of the VMB in bacterial vaginosis (BV), which is the most common and most studied vaginal dysbiosis. It also discusses the diagnoses of desquamative inflammatory vaginitis (DIV) and aerobic vaginitis (AV), which are associated with lactobacilli depletion and dysbiotic VMB, demonstrating the complexity of defining whether dysbiosis associated with vaginal inflammatory conditions is a cause or a result. We also review the controversial diagnosis of cytolytic vaginosis (CyV), which is another form of vaginal dysbiosis that contradicts the accepted concept of lactobacilli depletion.

BACTERIAL VAGINOSIS

The Microbiome Characteristics of BV

Bacterial vaginosis (BV) is a polymicrobial disorder, which is considered the most common cause of vaginal discharge,2 affecting millions of reproductive-aged women worldwide. Bacterial vaginosis is associated with multiple adverse gynecologic and obstetrics consequences, including an increased risk of preterm birth, pelvic inflammatory disease, endometritis, cervical intraepithelial neoplasia (CIN), and acquisition of STIs including HIV and human papillomavirus (HPV).6

The diagnosis of BV is complicated by the lack of consensus on its definition,7 the natural difference of the VMB in women of diverse racial backgrounds, and its polymicrobial etiology.8 Symptomatic BV can be described as a syndrome based on the presence of clinical features (discharge and/or malodor) interpreted by vaginal fluid features (i.e., Amsel criteria) or gram stain (the Nugent score), without a specific etiologic agent defined. Bacterial vaginosis is not characterized by inflammation on microscopy, with relative absence of polymorphonuclear cells on wet mount, and was therefore termed “vaginosis” and not “vaginitis.”9 Despite decades of research, the etiology of BV remains unknown; it is a form of vaginal dysbiosis, marked by depletion of lactobacilli and proliferation of various gram-negative and/or anaerobic bacteria.10,11

The epidemiology of BV strongly suggests that it may be acquired via sexual transmission,12,11 and it is associated with various risk factors, including a new sexual partner,12 douching,14 and smoking.15 Although usually treatable with antibiotics, recurrence is a major problem, with relapse rates greater than 50% within 12 months of treatment.16

Bacterial vaginosis is diagnosed clinically in 1 of 2 ways: Amsel criteria or Nugent score. Amsel criteria comprises a set of

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4 criteria including: (1) vaginal discharge, (2) fishy odor with or without the addition of 10% KOH (whiff test), (3) elevated pH >4.5, and (4) the presence of clue cells (epithelial cells studded with adherent bacteria) on microscopy, with 3 of 4 criteria are required to make a positive diagnosis. The Nugent score is based on gram-stained vaginal smear, with high numbers of lactobacilli species indicative of health, and their depletion coupled with increased numbers of small and/or curved gram variable rods being indicative of BV.

More than half of the women with diagnosable BV have no clear symptoms. Patients’ perception of their vaginal symptoms varies significantly and does not necessarily correlate with signs of BV. Some women do not report symptoms; nevertheless, discharge is noted on examination by a clinician, and diagnostic criteria are present, highlighting that many women with BV may consider their discharge to be normal. These women may be asymptomatic despite the presence of BV. This may contribute to the debate regarding the definition of “normal” VMB, as was discussed in part I.

Microbiology of BV

Despite multiple molecular and genomic studies, there is no consensus on the group of bacterial species that may directly cause BV. Multiple studies using both deep sequencing methods and species-level taxonomic classification have described the diversity of microbial communities in BV. In a study published in 2005, Fredricks et al. described bacterial communities in samples of vaginal fluid from 27 subjects with BV (defined according to Amsel criteria) and 46 without the condition, using combination of broad-range PCR amplification of 16S rDNA with clone analysis and bacterium-specific PCR assay of 16S rDNA (see part I for technical details). Among subjects without BV, 1–6 bacterial species (mean = 3.3) were found. Lactobacillus species were the dominant bacteria detected, particularly L. crispatus and L. iners. In addition, most bacterial 16S rDNA sequences in subjects without BV closely matched known bacteria. Nevertheless, analysis from vaginal fluid of subjects with BV showed a high level of species diversity, with a mean of 12.6 bacteria (range = 9–17), and newly recognized bacteria were present in 60% of BV samples. Bacteria that were frequently detected in women with BV included Gardnerella vaginalis, Atopobium vaginae, Megapathera types, Leptotrichia amnionis, Sneathia sanguinegens, Porphyromonas asaccharolytica, a bacterium related to Eggerthella hongkongensis, and bacteria related to Prevotella genus. Thirty-five unique bacterial species were identified in women with BV, 16 of which were newly characterized, including fastidious bacteria termed BV-associated bacterium 1–3 (BVAB1–3), which were subsequently found as highly specific for BV. Lactobacillus crispatus was not detected in subjects with BV, whereas L. iners was detected in most subjects. In addition, G. vaginalis was detected in all BV samples; however, it was also found in 59% of subjects without BV.

In a subsequent study, broad-range 16S rRNA gene PCR and pyrosequencing (a method of DNA sequencing that detects light emitted during the sequential addition of nucleotides during the synthesis of a complementary strand of DNA) were performed on vaginal swabs from 220 women with and without BV, diagnosed separately by both Amsel criteria and Gram stain (Nugent score). In accordance with the previous findings, women with BV had diverse, heterogeneous vaginal bacterial communities, which were usually not dominated by a single bacteria, showing increased species richness and diversity. No bacterium was present in all women with BV; however, G. vaginalis was present in 80% of women with BV. A. vaginae in 92%, L. iners in 86%, and Eggerthella species in 85%. In the absence of BV (by Gram stain), vaginal bacterial communities were mostly dominated by either L. crispatus or L. iners. Lactobacillus jensenii and L. gasseri were present in 65% and 34% of women, respectively. Of note, although women with high levels of L. crispatus did not have BV, women with high levels of L. iners could be either BV negative or positive. Hypothesizing that bacterial community subtypes may be shaped by synergistic or antagonistic relationships among individual BV-associated bacteria, the researchers examined bacterial co-occurrence. They found that lactobacilli were strongly correlated with each other, as were several subgroups among BV-associated bacteria. Strong negative correlations were found between most lactobacilli and the bacteria associated with BV. These correlations suggest metabolic or other dependencies; bacteria that are negatively correlated may compete for similar nutrients or change the environment in ways that inhibit growth of each other. Lactobacillus crispatus had strong positive correlations with Lactobacillus jensenii and G. vaginalis but was negatively correlated with L. iners.

Role of G. vaginalis in BV

Gardnerella vaginalis, present in 95%–100% of BV cases, was originally thought to be the primary BV pathogen. In vitro, G. vaginalis possesses various virulence factors, adheres in large aggregates to vaginal epithelial cells, exhibits a significant cytotoxic activity, and produces a biofilm matrix (see hereinafter). However, G. vaginalis is found in many women without BV in lower abundances. Whole genome sequence analysis experiments were conducted in 81 Gardnerella strains by Vanechouette et al., who pointed out the existence of at least 13 groups, distinct enough to be classified as separate species, within the taxon formerly known as G. vaginalis. Distinct genomic properties may present different pathological features (i.e., cytotoxicity, adhesion to epithelial cells, biofilm formation, sialidase production, and antibiotic susceptibility), as some subgroup(s) or species have been found to have an association with BV, whereas others have not. Therefore, it is possible that women who are colonized by Gardnerella species or clades with low virulence potential do not develop BV, whereas acquisition of virulent strains results in BV. Another explanation suggests that G. vaginalis alone may be necessary but not sufficient for BV development. In an article published in 1955 by Gardner and Dukes, isolated G. vaginalis were introduced into the vaginas of 13 healthy women, which resulted in the development of BV in one of them. However, when vaginal fluid obtained from subjects with BV was inoculated into the vaginas of 15 healthy women, 11 developed BV. These observations suggest that whole vaginal fluid is a much more successful inoculum for the transmission of BV than is pure G. vaginalis, indicating that synergy between G. vaginalis and other bacteria may be important in BV development. Such potentially significant synergistic relationships between G. vaginalis and other bacteria, the researchers have been reported in BV pathogenesis.

The ecological interactions between G. vaginalis and other BV-associated bacteria were analyzed by Castro et al. in a dual-species biofilm model. This study revealed distinct biofilm structures between each bacterial consortium, leading to at least 3 unique dual-species biofilm morphotypes. Furthermore, their findings seem to indicate that Enterococcus faecalis and Actinomyces naesii had a higher impact on the enhancement of G. vaginalis virulence, whereas the other tested species had a lower or no impact. This study proposed that not all BV-associated bacteria contribute to the enhancement of BV pathogenesis by influencing G. vaginalis virulence.

Biofilm in BV

Another notable feature of BV is the presence of a polymicrobial biofilm on vaginal epithelial cells. A biofilm is a structured
community of microorganisms in a self-produced extracellular matrix, adherent to the surface of epithelial cells. The BV biofilm has been found to contain abundant *G. vaginalis* and *A. vaginae*.13

Shedding of vaginal epithelial cells coated with BV biofilm presents as clue cells. After the initial colonizing species adhere to the surface, the BV polymicrobial biofilm may incorporate additional bacteria; a synergetic relationship between these bacteria within the biofilm allows the biofilm’s growth and maturation.30

Within the biofilm, gradients of pH, nutrients, and oxygen can be found.31 *Gardnerella vaginalis* biofilms can adhere to epithelial cells and provide protective features, such as tolerance to H₂O₂ and lactic acid produced by lactobacilli, inhibition of elimination by the immune system, and antimicrobial resistance, promoting the recurring and chronic nature of BV.32

Little is known about the exact mechanisms of biofilm formation in BV: the genes responsible, communication strategies (quorum sensing, metabolic communication), and genetic exchanges between biofilm-associated bacteria. It is not clear whether all bacteria found in the BV biofilm have a pathogenic role or are simply a consequence of biofilm formation.30

In a model suggested recently by Muzny et al.,22 it was proposed that BV development is triggered by sexual transmission of virulent strains of *G. vaginalis*, which displaces healthy vaginal lactobacilli, and initiates BV biofilm formation on the vaginal epithelium. *Gardnerella vaginalis* can tolerate the high oxidation-reduction (redox) potential of a *Lactobacillus*-dominated vaginal microbiota. These bacteria may lower the redox potential in the vagina, remarkably reducing lactobacilli, resulting in an increase in other strict anaerobic BV-associated bacteria, such as *P. bivia*, which is normally present in low concentrations. This results in the production of metabolites facilitating bacterial growth. Subsequently, vaginal sialidase and other enzymes, produced by *G. vaginalis* and *P. bivia*, promote breakdown of the mucous layer of the vaginal epithelium. The loss of the protective mucous layer leads to increased adherence of secondary colonizers to the mature, polymicrobial BV biofilm. One of these secondary colonizers is *A. vaginae*, an obligate anaerobic species, that, unlike *G. vaginalis*, is usually not present in the health-related VMB.31

Alternatively, it was suggested that infection by polymicrobial biofilms containing *G. vaginalis* between sex partners may contribute to BV formation, with increasing evidence of colonization by "vaginal" bacteria and clue cells in the male reproductive tract.32,34

**Different Bacteria, Different BV Symptoms, and Different Pathogenic Potential**

Associations between certain bacteria and BV symptoms were reported. These findings may account for discrepancies often observed between Arnsel and Nugent diagnostic criteria among women with BV, as well as between symptomatic and asymptomatic women. In a study investigating associations of Arnsel criteria with bacterial tax, it was described that *Eggerthella* species and *Leptotrichia amnionii* were the only BV-associated bacteria that were positively associated with all 4 Arnsel criteria.29

*Lactobacillus crispatus* was the only *Lactobacillus* species associated with low pH, negative whiff test, absence of clue cells, and normal vaginal discharge. In contrast, women with high *L. iners* levels can have either low or high pH. The fishy amine odor is attributed to polyamines such as putrescine, cadaverine, and trimethylamine.35,36

Several bacteria including *Prevotella* species, *BVAB1* and *B. parvum*, *Megasphaera* and *Meganin*s were associated with a positive whiff test.22 *Gardnerella vaginalis* and *A. vaginae* were each associated with 3 criteria: *G. vaginalis* was not associated with abnormal vaginal discharge, whereas *A. vaginae* was not associated with amine odor. *Lactobacillus iners* was not associated with any of Arnsel clinical criteria for BV. The difference in bacteria composition in BV not only may differ among racial and ethnic groups, but also varies within women over time.36

**Longitudinal Changes in BV**

In a prospective, longitudinal study, Ravel et al.38 evaluated the spectrum of events that occur in vaginal microbial communities over 2 menstrual cycles, among women with symptomatic BV, asymptomatic BV, and healthy subjects. Bacterial community dynamics in women who had symptomatic and asymptomatic BV seemed to be highly personalized, with some women experiencing shifts in VMB composition while others having stable microbiota, depleted of *Lactobacillus* species. The VMB of healthy women was consistently dominated by *Lactobacillus* species or *Bifidobacterium* but was not always stable in terms of the dominant species of *Lactobacillus* present. In most women, the treatment of BV reduced the proportion of anaerobes and increased the relative proportions of *Lactobacillus* species (mainly *L. iners*). However, this effect was short-lived, and in most individuals, the VMB returned to its pretreatment state within 2–4 weeks.38

**Bacterial Vaginosis Associations With Demographics**

A systematic review describing the global epidemiology of BV showed that BV prevalence varies by ethnic group and within countries.39 This has been most extensively studied and documented in the United States,39 showing that BV prevalence was highest in African American and lowest in non-Hispanic Whites and Asians, with Hispanics women having an intermediate prevalence.38,39 Bacterial vaginosis prevalence tended to be highest in sub-Saharan Africa and lowest in Asia, Australasia, and western Europe.39 Nevertheless, there were populations with high and low BV prevalence in all these regions.

Among those with a clinical diagnosis of BV, African American women were more likely colonized by *Anaerococcus tetras* and *BVAB1*, whereas White women were more likely colonized by *M. hominis*, *D. microaerophilus*, and *Gemella* species.30 Tanzanian women with BV had a high abundance of *P. bivia*.41 Although these limited data suggest that BV-VMB may vary between subpopulations, it is important to note that studies in sub-Saharan Africa differ considerably in terms of experimental techniques used, and therefore, direct comparisons are limited.42

**Summary**

Understanding the etiology of BV has important implications for improvements in diagnosis, treatment, and prevention of this common clinical condition. For example, in recent years, several highly sensitive and specific PCR assays, which use various combinations of bacteria, became available for the diagnosis of BV in symptomatic women, possibly replacing the currently used tests...
A vaginitis, and noninfectious conditions, such as lichen planus. The bacteria gain terrain. Most often, isolated bacteria from patients in the colonic milieu is adverse to lactobacilli and, consequently, other bacteria. The overgrowth of lactobacilli is associated with hyperacidity and low pH. In CV as its name implies, there is lysis of epithelial cells, presenting with numerous bare nuclei and debris cytoplasm, which is generally assumed to be because of overacidification.

The etiology is unknown, but it was suggested that hormonal factors, mainly progesterone, play a role, as it is more often encountered during pregnancy, the luteal phase, and in perimenopause. Despite the predominance of lactobacilli in these conditions, in some nonpregnant women, symptoms of itching, burning, irritation, dyspareunia, dysuria, and white cheesy vaginal discharge may indicate an unhealthy state. The symptoms may be explained by excessive production of H₂O₂ and/or low pH (≤3.8). It is unknown whether these entities are part of a continuum or not.

In microbiological terms, CV is characterized by low diversity and dominance of L. crispatus and a near absence of Faecalibacterium species. In cases with CV, a lesser diversity of Lactobacillus species was found compared with women without the condition. Lactobacillus crispatus was found in both groups but demonstrated enhanced acid-producing capability in the CV group.

Desquamative Inflammatory Vaginitis and Aerobic Vaginitis

Desquamative Inflammatory Vaginitis (DIV) is an uncommon vaginitis, associated with symptoms of copious vaginal discharge, burning, irritation, and dyspareunia. Physical examination features may include cervical and vaginal erythema, introital erythema, spotted hemorrhages, erosions of the vaginal and cervical mucosa, and purulent discharge. The vaginal discharge of women with DIV is characterized by the dominance of parabasal/basal cells, increased number of leukocytes (ratio leukocytes: epithelial cells >1:1), and often, mixed microbiota with dominance of cocci (usually Streptococcus species). Differential diagnosis includes trichomoniasis, severe vaginal atrophy (literally, “atrophic vaginitis”), Streptococcus Group A vaginitis, and noninfectious conditions, such as lichen planus.

The dysbiosis associated with DIV presents an unclear primary or secondary relationship with the DIV condition per se. The unanswered question is whether inflammation, tissue erosion, and subsequent exposure of deep layers of epithelial cells are triggered by specific bacteria or, alternatively, whether the inflammatory milieu is adverse to lactobacilli and, consequently, other bacteria gain terrain. Most often, isolated bacteria from patients with DIV have been Streptococcus agalactiae, Escherichia coli, and Staphylococcus aureus.

Others have described a spectrum of vaginal discharge changes, based on wet mount microscopy, which include varying degrees of inflammation and presence of parabasal cells, as well as replacement of dominant lactobacilli microbiota by other bacilli or cocci. This spectrum of conditions was named “aerobic vaginitis” (as “opposite” of the anaerobic counterpart, BV), with the severe forms of AV corresponding to DIV.

Moderate/severe AV prevalence in nonpregnant women has been reported to range between 2.0% and 25.8%, mostly ranging between 7% and 13%. The huge differences in terms of prevalence may be due to geographical or ethnic factors, similar to BV. The prevalence is systematically lower in pregnant women.

The bacteria most often isolated in AV are Streptococcus species, S. aureus, S. epidermidis, S. anginosus, E. coli, and E. faecalis. During pregnancy, the same bacteria were found, but E. coli was most frequently identified. This study may, however, be biased, as the population studied was more than 35 weeks of gestation and AV/DIV is considered a risk factor for preterm labor. More recent studies, using next-generation sequencing, confirmed these findings and showed that the prevalence of anaerobic species typically associated with BV, such as G. vaginalis, A. vaginae, Prevotella species, and Sneathia species, are also prevalent in women with AV.

Cytolytic Vaginosis

Cytolytic vaginosis (CyV) and lactobacillosis are diagnoses not accepted by all authors. These are characterized by an excessive number of lactobacilli with or without associated cytolyis—“cytolytic vaginosis” or “lactobacillosis.” The overgrowth of lactobacilli has been assumed that the normal status of a healthy woman is a homogenous entity can lead to better strategies to prevent disease and the full picture of the lactobacilli-depleted forms of dysbiosis is still incomplete. Nevertheless, it is already clear that acknowledging that “dysbiosis” includes more than BV and that BV itself is not a homogenous entity can lead to better strategies to prevent disease and complications in the future. This can also explain contradictory results in the past, for example, in the attempts to reduce preterm labor by treating BV or dysbiosis.

On the opposite spectrum of dysbiosis are the cases of excessive lactobacilli, which challenge the concept that lactobacilli are always beneficial, and confirm that more is not always better.

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