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The Vaginal Microbiome: III. The Vaginal Microbiome in Various Urogenital Disorders

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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 "*Candida*," "vaginitis," "urinary microbiome," "recurrent urinary tract infections," "sexually transmitted infections," "human immunodeficiency virus," "human papillomavirus," "non-specific vaginitis," "vulvodynia," and "vulvovaginal symptoms." Full article texts were reviewed. Reference lists were screened for additional articles. The third article in this series describes VMB in various urogenital disorders.

Results: Variable patterns of the VMB are found in patients with vulvovaginal candidiasis, challenging the idea of a protective role of lactobacilli. Highly similar strains of health-associated commensal bacteria are shared in both the bladder and vagina of the same individual and may provide protection against urinary tract infections. Dysbiotic VMB increases the risk of urinary tract infection. Loss of vaginal lactic acid–producing bacteria combined with elevated pH, increase the risk for sexually transmitted infections, although the exact protective mechanisms of the VMB against sexually transmitted infections are still unknown.

Conclusions: The VMB may constitute a biological barrier to pathogenic microorganisms. When the predominance of lactobacilli community is disrupted, there is an increased risk for the acquisition of various vaginal pathogens. Longitudinal studies are needed to describe the association between the host, bacterial, and fungal components of the VMB.

Key Words: vaginal microbiome, *Candida*, urinary microbiome, recurrent urinary tract infections, sexually transmitted infections, vulvodynia

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Urogenital symptoms, such as dysuria, discharge, odor, and itching, are major health problems worldwide, often related to microbial pathogenicity or imbalance. Better understanding of the factors that affect the composition and dynamics of the vaginal microbial communities in health and disease may allow development of useful diagnosis, prevention, and treatment strategies. Over the past decades, research provided insights into the role of microbial communities inhabiting the vagina. The focus of most of these studies remains the bacterial portion of the vaginal microbiota. Nevertheless, in clinical practice, causes of vaginitis and vulvovaginal complaints are often fungal (i.e., candidiasis), viral (i.e., human papillomavirus [HPV] and herpes), or protozoal (i.e., trichomoniasis).

Most of our knowledge regarding fungi in women's genital tract comes from culture-based studies and characterization of single organisms.[1] The vaginal mycobiome (defined as the fungal communities within the microbiome) is an evolving field of study, as genomics tools are increasingly used.[1] An improved understanding of the role fungal communities have in human health and disease, as well as the interactions between *Candida* species, bacteria, and other microorganisms in the vagina, is fundamental to our characterization of the vaginal microbiome (VMB).

Microbiome characterization using molecular techniques also expanded our knowledge regarding the urinary bladder, which was considered a sterile organ until recently. In this review, we discuss the urine microbiome and the mechanisms by which VMB can impact the pathogenesis of urinary tract infection (UTI).

The idea that a dysbiotic VMB participates in transmission of HIV, other sexually transmitted infections (STIs; trichomonal, gonococcal, and chlamydial infections) and its potential role in HPV infection, is also described. Finally, we discuss the putative role of the VMB in vulvodynia.

THE VAGINAL MICROBIOME AND CANDIDA VAGINITIS

The mycobiome is defined as the fungal communities within the microbiome, and despite their relatively large biomass, they represent a small percentage of genes. The study of the vaginal mycobiome lags behind that of bacterial microbiome, with the first study applying next-generation sequencing published only in 2013,[1,2] and reference databases are currently insufficient. In addition, most studies focus on bacterial-fungal interactions, mainly regarding *Candida albicans*.

Candida Albicans in the Vagina—a Commensal with Pathogenic Properties

Candida albicans is the most common fungus within the human mycobiome. It has been identified in the vagina in approximately 20%–30% of asymptomatic women with culture methods and in up to 65% with molecular methods.[2] When symptomatic, it is referred to as vulvovaginal candidiasis (or candidosis) and is caused by *C. albicans* in 80%–92% of cases.[3]

Specific features of the different species of the *Candida* genus (*Candida* species) and, in particular, *C. albicans*, may explain

its evolutionary success within the human host. First, it can switch from an oval-shaped blastoconidia (nonvirulent, budded cells) form to virulent and invasive branched hyphae. Commensalism takes place mostly in the blastoconidia shape, where *Candida* species can evade the epithelial recognition system.[4]

The delicate balance between commensalism and opportunistic infection is maintained by the responses of the innate immune system.[5] Clearance of *C. albicans* from the host tissue relies significantly upon the phagocytosis of the fungal pathogen by innate immune cells (i.e., macrophages, neutrophils, and dendritic cells).[5] Most of these reactions are mediated by the interaction between pattern recognition receptors expressed on the surface of innate immune cells and pathogen associated molecular patterns (PAMPs) found in the fungal cell wall.[5] Multiple families of receptors were recognized, which identify distinct pathogen associated molecular patterns. Innate immune cells interact differently with the blastoconidia and hyphal morphologies of *Candida*.[6] When transformed to hyphae, the interaction of *C. albicans* with vaginal epithelial cells results in the production of potent neutrophil chemoattractants, neutrophil recruitment, and symptomatic disease.[7] These morphologic changes have been described as a response to the hosts immune status and the microbiome and include quorum sensing mechanisms.[8,9] Quorum sensing is a microbial reaction based on the ability to detect and respond to cell population density by gene regulation. It has been shown to affect virulence and biofilm formation (see part II) and is considered a type of communication between different types of microbes.[4,9,10]

In addition, *C. albicans* has been shown to change its cell wall in response to specific environmental signals.[11] The growth of *C. albicans* in lactate, which is the predominant carbon source at the vaginal mucosa (due to the presence of lactobacilli), promotes the suppression of β-glucans in the fungal cell wall. This, in turn, reduces innate immune recognition and, therefore, possibly contributes to the reduced neutrophil phagocytosis rates.[12,13] Conversely, acidic environments promote β-glucan exposure on the cell wall, leading to hyperactivation of innate immune responses and significant neutrophil influx.[14] Acid-adapted fungal cells are more resistant to neutrophil killing.[15]

In addition to widely recognized pathogenesis, *C. albicans* has been recently hypothesized to have potential health benefits for the human host. This includes *C. albicans*–mediated inhibition of *Escherichia coli* migration from the rectum to the bladder, protecting from UTIs,[16] and a role of *C. albicans* in the development of the mucosal immune response in the human gut.[9]

The Vaginal Microbiome in Vulvovaginal Candidiasis

Vulvovaginal candidiasis (VVC) involves host and fungal factors (for a review, see Willems et al.[17]). Nevertheless, it has been suggested that pathogenicity of *Candida* is related to resident bacterial communities and that bacteria modulate its virulence directly by altering gene expression.[9] Polymicrobial infections often present clinically more severe and frequently demonstrate increased resistance to antimicrobial treatment or incomplete recovery.[18] Communication between yeasts and bacteria occurs via signaling molecules (like quorum sensing, see hereinabove), metabolites, and toxins, which are also able to modulate the human immune response and affect treatment.[18] Such molecules can be markers for disease and potentially offer new treatment modalities.[19]

Vaginal microbiome, and specifically lactobacilli, are thought to inhibit *Candida* from becoming pathogenic by the production of lactic acid, bacteriocins, hydrogen peroxide, and biosurfactants.[20] Early experiments led to the hypothesis that vaginal bacteria do not prevent colonization of *Candida* but rather prevent its proliferation.[1] This hypothesis was supported by studies showing that metabolites (short-chain fatty acids and lactate) produced by *Lactobacillus* species inhibit the blastoconidia-to-hyphae structural switch in *C. albicans*.[1] This suggested protective role of lactobacilli in VVC pathogenesis was challenged, as it was reported that all patterns of lactobacilli colonization (reduced, elevated, and unchanged) coexist with VVC.[3,21–25]

Several studies have found reduced lactobacilli colonization rates or variation in the species, with dominance of *L. iners* and reduction of *L. crispatus*.[3,22] A study compared the VMB of 4 groups (1) healthy controls, (2) women with bacterial vaginosis (BV), (3) women with *Chlamydia trachomatis* infection, and (4) those with *C. albicans* VVC.[3] The *Lactobacillus* species level analysis showed that VMB of healthy controls was dominated by *L. crispatus* (61% of the total lactobacilli sequences), with a significant reduction in this species abundance in VVC (33.4%, $p = .006$). *Candida albicans* VVC–infected women had lower lactobacilli proportion overall compared with healthy controls (57% compared with 79%, $p < .001$), a higher *L. gasseri* compared with controls (9.7%, $p = .005$), and a relative increase in *Gardnerella*, *Prevotella*, *Megasphera*, *Roseburia*, and *Atopobium*.

Similarly, a recent study showed that women with *L. iners*–dominant microbiomes (defined as 50% relative abundance or greater) were more likely to harbor *Candida* species than women with *L. crispatus*–dominated microbiomes.[22]

In contrast to studies associating lactobacillus deficiency and VVC, a culture-based study reported that lactobacilli-dominated microbiome was associated with greater risk for VVC.[23] This may be explained by the previously discussed finding that lactic acid suppresses immune responses to *C. albicans*.[13] A major limitation of this study, which was not confirmed by recent molecular-based studies, is the absence of data regarding the identity of the dominating lactobacilli.

Liu et al.[21] evaluated the vaginal microbial community in patients with VVC, BV, and mixed infection of VVC/BV and found highly variable patterns of the VMB in VVC patients. Although control and BV communities had typical patterns, the VMB of VVC was complex. The mixed BV/VVC infection group showed a unique pattern, with a relatively higher abundance of lactobacilli than the BV group and a higher abundance of *Prevotella*, *Gardnerella*, and *Atopobium* than the normal control. In contrast, the VVC-only group could not be described by any single profile, ranging from a community structure similar to the normal control (predominance of *Lactobacillus* species) to BV-like community structures (abundance of *Gardnerella* and *Atopobium*). Treatment of VVC resulted in inconsistent changes of the VMB.

Other studies did not provide evidence for the existence of altered or unusual VMB communities in women with VVC compared with women without it.[24,25] In a study comparing microbiome composition of women with and without recurrent VVC, no significant differences were found between the VMB of women in the 2 groups.[24] Moreover, no novel bacteria were found in the communities of women with recurrent VVC, and the vaginal communities of 90% of women in both groups were dominated by species of lactobacilli.

Swidsinski et al.[26] investigated the histopathology of VVC using fluorescent-in-situ hybridization (FISH) probes specific for fungi and bacteria. Their findings suggested that *Candida* species colonization and infection occur in polymicrobial environments, with possible bacteria-yeast interactions involved in tissue invasion. They indicated a *Candida* promoting role for *Gardnerella* as well as for lactobacilli. *Candida* infection occurred even more often with lactobacilli than *Gardnerella*, especially with high concentrations or density of lactobacilli within the vaginal epithelium (both for *L. iners* and *L. crispatus*).

Lower numbers of lactobacilli as a causative factor in *Candida* species vaginitis after antibiotic therapy have also been suggested. Nevertheless, not all studies have supported the link between VVC and antibiotic usage,[1] and evidence is lacking

regarding an association between reduction or loss of function of lactobacilli or other bacteria during antibiotic treatment.[27]

The Vaginal Microbiome in Recurrent Vulvovaginal Candidiasis

Recurrent VVC is defined as three or more episodes of VVC per year, with a lifetime prevalence of 8%.[28] The currently predominant hypothesis for recurrent VVC pathogenesis is based on the human host's own dysregulated immunsystem, resulting in inflammation, and only in a small proportion, upon a deficient immune response.[24] As for the possible role of VMB in recurrent VVC, published culture-based and murine studies provided conflicting results. Both nonspecific patterns in the VMB, as well as low density of lactobacilli, were reported.[24,29] Future treatment options under investigation are immunotherapy and vaccination.[30]

New Diagnostic Options for Vulvovaginal Candidiasis

With the commercial availability of nucleic acid amplification tests, the standard diagnostic tests (culture with Sabouraud agar) for *Candida* species are challenged. Molecular tests are far more sensitive, result in a short turnaround time, and enable the possibilities for self-testing and the diagnosis of coinfections.[31,32] Disadvantages of molecular tests include false-positive results for patients with a low probability for infection, identification of asymptomatic *Candida* that does not require treatment, and higher costs compared with microscopy. Cost effectiveness studies are ongoing.[33]

Summary

Vulvovaginal candidiasis is a multifactorial condition, with a predominant abnormal immune response of the host. Undoubtedly, fungal and bacterial communities have the potential to impact each other through physical interactions, metabolites production, chemical interactions, etc. Despite the uncertain effect of lactobacilli in vivo, a majority of in vitro and animal studies have shown that *Lactobacilli* species exerts an inhibitory effect on the growth, morphological transition, virulence, and biofilm formation of *C. albicans.*[17] The metabolites of *Lactobacilli* species were shown to have antifungal properties. However, only specific lactobacilli strains can produce these metabolites in quantities required for antifungal activity, which possibly explains the failure of vaginal lactobacilli to suppress *Candida* colonization. For example, it has been reported that physiological levels of lactic acid may be too low to inhibit *Candida* species overgrowth, and therefore, the role of lactic acid for the control of its overgrowth is not clear.[34] Lactic acid at physiological concentrations may, however, increase the efficacy of azoles against *C. albicans* and at higher concentrations against *C. glabrata.*[35] A relative overrepresentation of *L. iners* and a relative underrepresentation of hydrogen-peroxide producing lactobacilli have been reported in many studies in women with VVC. Discrepancies among studies evaluating correlation between VMB and VVC may be due to different patients' geography, age and symptoms, as well as approaches used for sample collection, processing, and identification. More longitudinal studies are needed to describe the association between the host, bacterial, and fungal components of the VMB to enable insight into the potential interactions and antifungal mechanisms of vaginal lactobacilli, to understand their role in VVC.

VAGINAL MICROBIOME, URINARY MICROBIOME, AND RECURRENT UTIs

The Urinary Microbiome Research

Traditionally, the study of urinary bacterial communities mainly included standard urine cultures. This had significant limitations for detection of the full spectrum of urinary bacterial species due to both the existence of unculturable (fastidious) microorganisms and to the absence of representation of mucosal organisms in urine specimens. Microbiome characterization using 16S rRNA sequencing and enhanced or expanded quantitative urine culture have led to rapid progress in the urinary microbiome (UMB) knowledge.

The human bladder, assumed for decades to be a sterile organ, has been recently identified as a site of permanent microbial colonization.[36] These findings generated new questions, such as what role do microbes colonizing the bladder play in urinary tract syndromes and infections, and are they a cause or effect of these conditions?

Study design and methodology have been substantially heterogeneous, which leave important knowledge gaps in understanding the UMB-VMB relationship. Reported variability in periurethral microbiota may be attributed to inconsistent specimen collection techniques, contamination from the vulva/vagina, sexual activity, menstruation, and recent use of hygiene products.

Urinary Microbiome in Urinary Disorders

Lactobacillus and *Streptococcus* have been the most frequently reported colonizing microbes in the UMB. Both genera are lactic acid–producing bacteria, allowing a protective role against pathogens.[37,38] Less frequently identified UMB bacteria, include *Alloscardovia*, *Burkholderia*, *Jonquetella*, *Klebsiella*, *Saccharofermentans*, *Rhodanobacter*, and *Veillonella*.

Although the literature on the UMB suggests both acute cystitis and interstitial cystitis are related to urinary dysbiotic conditions, the nature of the dysbiotic microbiome differs between the 2 conditions. Moreover, there is also evidence that patients with refractory urge incontinence and coexistent recurrent UTI (rUTI) have a diverse UMB, suggesting that persistent bladder colonization might augment the pathology of this chronic condition.[39]

The Associations Between the Urinary and Vaginal Microbiomes

Alterations and variations in the VMB may influence urinary tract health, playing a key role in the pathogenesis of acute and rUTIs. The VMB has been demonstrated to be altered not only in women with rUTIs but also at the time of acute UTI and after treatment of a single infection, even in women without a history of rUTI.[40–42] Reproductive-aged women with rUTI show VMB depleted of H_2O_2-producing lactobacilli and increased rates of colonization with *E. coli.* Among lactobacilli, vaginal colonization with *L. crispatus* or *L. jensenii* significantly inhibits colonization with *E. coli* compared with other *Lactobacillus* species.[43,44]

Rates of UTI rise in postmenopausal women. Estrogen diminution decreases the relative amounts of lactobacilli in parallel to thinning of the urogenital epithelium due to hormonal-based atrophy (see part I). Postmenopausal women receiving systemic or topical vaginal hormone therapy retain vaginal lactobacilli and show a lower rate of rUTI.[45,46]

Mechanistically, uropathogens, such as *E. coli*, can colonize the vaginal introitus and the periurethra. The *E. coli* UTI is characterized by a series of colonization events, starting in the gastrointestinal tract, followed by establishment at the vaginal introitus and urethral meatus, and eventually reaching the bladder with the potential risk of further extension to the kidneys and even sepsis. Longitudinal examinations and sampling have shown that introital and urethral colonization precedes UTI symptoms.[47,48] In addition, rUTI can be triggered by transient exposure to vaginal bacteria, most notably *G. vaginalis*, which is not traditionally considered uropathogenic. This transient urinary tract exposure to vaginal bacteria has been defined as "covert pathogenesis," as the exposure to *G. vaginalis* seems to induce *E. coli* emergence into urine, increasing its pathogenicity.[49] Conversely, exposure to *L.*

crispatus or heat-killed *G. vaginalis* does not induce *E. coli* emergence.[41] Considering these findings, *G. vaginalis* should be itself considered a potential cause of urinary tract pathology. Individuals from whom *G. vaginalis* is isolated in urine samples are more likely to have a history of rUTI or recurrent pyelonephritis.[49]

Microbial sharing between the vaginal and bladder microbiota not only is limited to uropathogens but also includes commensal bacteria, such as *L. iners* and *L. crispatus.*[50] This means that some bacteria normally reside in both the bladder and vagina and could provide protection against UTIs, suggesting that the microbes of these adjacent niches could be considered a single urogenital microbiota.

In the BV patient, the bladder becomes colonized by bacteria that are commonly found vaginally.[51] A VMB characteristic of BV is typically observed during a disease-free period among women prone to UTI.[52] Detailed evaluation of the immunologic consequences of vaginal dysbiosis of BV indicates that UTI-prone women show reduced vascular endothelial growth factor, platelet derived growth factor-bb, which are first-line factors in tissue repair, and monocyte chemoattractant protein-1, a chemoattractant for monocytes and dendritic cells in the vaginal mucosa, suggesting an altered vaginal immune response. Mechanistically, a dysbiotic VMB increases the risk of UTI not only on a microbiological basis but also by the immunologic status that diminishes innate resistance to infections by uropathogens.

The Relationships Between Sexual Intercourse, UTIs, and Contraception

A number of older, epidemiologic studies have shown a strong relationship between UTI and vaginal intercourse.[53] Anecdotal reports, as well as one prospective study, have suggested that uropathogens may be transmitted between sex partners.[53] It is postulated that vaginal intercourse facilitates the transfer of potential uropathogens to the vagina or enhance the entrance of potential bacteria into the urethral meatus from the vagina.[54] It was also found that the level of vaginal colonization with *E. coli* after intercourse was statistically significant.[55] Although the causal role of sexual transmission in BV is recognized[56] (see part II), there are no data correlating UTI, vaginal dysbiosis, and sexual transmission.

Several contraceptive methods may influence the VMB-UTI relationship, and independent associations between UTI risk and contraceptive method were described.[53] Topical spermicidal products, containing compounds such as nonoxynol-9, affect vaginal lactobacilli colonization, due to direct toxicity, while simultaneously increasing *E. coli* colonization.[42] Limited available data suggest that oral contraceptives do not affect UTI risk.[57,58] The effects of intrauterine device on the VMB are overall conflicting.[59] Condom use was found to reduce the risk of a second UTI caused by a different uropathogen, but not by the same species,[60] supporting the options of both a sexual transmission and the existence of an internal reservoir.

In summary, there are multiple mechanisms by which vaginal bacteria can affect the pathogenesis of UTI. Vaginal bacteria may cause UTI themselves or can act as "covert pathogens" facilitating the pathogenesis of another organism. It is often the goal of clinicians who evaluate urine specimens to focus mainly on gram-negative enteric organisms, but current research suggests that in the future, a broader concern for a wider range of organisms will be warranted. Rather, full knowledge of their role is desirable as well as a deep understanding of the possible role of vaginal intervention, whether to increase lactobacilli colonization (see part V), enhance innate immunologic efficacy, eliminate uropathogen reservoir, or treat associated vaginal conditions, on the improvement of UTIs outcome or prevention of rUTIs. A question yet to be answered conclusively is whether strategies that enhance beneficial microbial colonization in the vagina will also result in beneficial colonization of the uroepithelium and may, in turn, will have a clinically beneficial effect on UTI risk.

THE VAGINAL MICROBIOME AND STIs

A dysbiotic vaginal microbiota characterized by low lactobacilli quantity is a risk factor for STIs.[61–63] The National Institutes of Health Longitudinal Study of "Vaginal Flora" has identified a 1.5- to 2-fold increased risk for incident trichomonal, gonococcal, and/or chlamydial infection with dysbiosis (i.e., intermediate and high Nugent scores; see part II).[64] The loss of vaginal lactic acid–producing bacteria combined with elevated pH and local cytokine production were major factors increasing STI risk, although the exact protective mechanisms of the normal VMB against STIs remain unknown.[65,66] Consequently, treatment of vaginal dysbiosis is proposed to be protective against STIs and their sequelae. For example, twice weekly prophylactic metronidazole treatment of women with asymptomatic BV results in a significantly longer time to the development of STIs (driven by a significant difference in the number of chlamydial infections), compared with women not receiving treatment.[67]

The Relationship of Vaginal Microbiome to HIV and Herpes Simplex Virus

Several studies have analyzed whether vaginal dysbiosis could influence the acquisition of HIV infection. Cross-sectional studies have revealed that HIV is frequently correlated with BV and independent of behavioral variables associated with BV and HIV, whereas HIV infection is lower in women with vaginal eubiosis.[68,69] Likewise, HIV seroprevalence significantly increases in pregnant women with severe BV compared with women with lactobacilli-dominated VMB.[70,71] A prospective cohort study showed that HIV-1–seronegative women demonstrated different risks for HIV acquisition based on the presence or absence of vaginal lactobacilli. This was proposed to be based on differences in the proportion of non-H_2O_2–producing lactobacilli and H_2O_2-producing lactobacilli, with the latter being more beneficial in HIV prevention.[62,72]

Mechanistically, nonlactobacillus-dominated VMB is associated with increased recruitment of mucosal immune cells compared with *L. crispatus*–dominant VMB,[73] leading to inflammation and increased level of activated $CD4^+$ HIV target cells. To note, the 2 isoforms of lactic acid might differ in their effects, as D-lactic acid but not L-lactic acid levels have been inversely associated with the ability of HIV to transverse cervicovaginal mucus. *Lactobacillus crispatus* produces both isoforms whereas *L. iners* produces only L-lactic acid.[74–76] Similarly, proinflammatory chemokines and cytokines, such as interleukin 1β (IL-1β), IP-10, MIP-1α, MIP-1β, and IL-8, are higher in women who seroconvert to HIV compared with HIV-uninfected women. These immune soluble factors, being chemotactic for inflammatory cells, increase the number of potential HIV target cells.[77,78] Furthermore, a dysbiotic VMB has been associated with the presence of an HIV-inducing factor (HIF) in vaginal secretions,[79] which increases HIV-1 replication in T cells and monocytes by activating AP-1 and NF-κB.[80] Especially, *Mycoplasma hominis* has been significantly associated with HIF.[81] Conversely, a significant increase in IL-1RA, an anti-inflammatory cytokine, has been observed in vaginal and cervical epithelial cell lines treated with lactic acid,[82] highlighting a novel anti-inflammatory mechanism by which lactic acid may impact HIV susceptibility.

Other studies found an association between lactobacilli absence and increased risk of HIV-1 infection as well as herpes simplex virus (HSV) 2 seroconversion.[61,62] Concomitant genital HSV-2 infection increase the transmission of HIV[83] and, in turn,

is associated with a dysbiotic VMB.[84] It is suggested that HSV-2 reactivation disrupts the epithelial barrier and recruits activated CD4 cells, which are target cells for HIV infection, into the lesion.

Bacterial vaginosis increases the rate of HSV-2 seroconversion,[61,85,86] although it is not possible to determine the direction of the causality, meaning that the relationship between BV and HSV-2 infection may be bidirectional.[87] Herpes simplex virus 2 reactivation induces an immune response in the genital tract, leading to changes in microbiota composition.[88] In turn, BV facilitates genital shedding of HSV-2.[89]

The Relationship of Vaginal Microbiome to HPV Infection and Disease Progression

Alteration of the VMB is considered a cofactor to HPV infection, which is often detected in concomitance with *G. vaginalis*, *U. urealyticum*, *C. albicans*, *Bacteroides* species, and overgrowth of anaerobes.[90] Incidence and prevalence of HPV are associated with intermediate vaginal microbiota (i.e., by Nugent score; see part II) and overt BV, whereas the predominance of *Lactobacillus* species is significantly associated with a negative HPV test.[90,91]

Vaginal microbiome with low abundances of lactobacilli or with *L. iners* dominance (corresponding to community state types [see part I] IV and III, respectively) are associated with higher HPV prevalence compared with *L. crispatus*–dominated VMB (community state type I). This association is confirmed both when restricted to high-risk HPV or any HPV genotype.[92]

A key factor in counteracting HPV infection is the ability of lactobacilli to lower pH by lactic acid production. This different ability corresponds to the experimental findings that *L. crispatus*–dominated VMB has a lower risk for HPV, squamous intraepithelial lesions, and cancer, whereas *L. iners*–dominated VMB has a higher risk.[92]

Some other bacterial species, such as *G. vaginalis* and *Atopobium vaginae*, have been proposed as molecular markers for possible HPV infection, as both are involved in biofilm formation (see part II) and may contribute to viral persistence by favoring mechanisms of immune escape.[93–95] In addition, exogenous bacteria, such as the oncogenic *Fusobacterium*, could contribute to cervical intraepithelial neoplasia.[96,97] *Fusobacterium* is an opportunist pathogen, part of the normal oral and the gut microbiota,[98] involved in inflammatory diseases in both mouth (periodontitis) and gut (inflammatory bowel disease).[99] *Fusobacterium* enhances the levels of IL-4,[100] creating an immunosuppressive microenvironment characterized by anti-inflammatory cytokines. This immunosuppression plays a key role in blocking cellular immunity and favoring HPV-driven progression toward cervical cancer.[101,102] The relation of the VMB and HPV infection/cervical cancer is discussed in detail in part IV.

In summary, the VMB may constitute an important biological barrier to pathogenic microorganisms. When the predominance of lactobacilli community is disrupted, reduced, and replaced by different anaerobes, there is an increased risk for the acquisition of STIs. Understanding the role of the altered VMB in the acquisition of STIs may help develop new preventive strategies.

THE VAGINAL MICROBIOME, NONSPECIFIC SYMPTOMS, AND VULVODYNIA

Up to 30% of women with vaginal symptoms are not assigned a diagnosis after standard diagnostic assessment, including pH testing, microscopy, and cultures.[103] A potential idiopatic disease category into which these patients may fall is the diagnosis of vulvodynia.[104] Progress in identifying organisms that were unculturable with molecular methods may help to clarify the true diagnoses in these patients. In addition, altered inflammatory response, associated with the VMB and discovered by molecular methods, may ultimately be linked to vulvodynia by VMB-mediated activation of nociceptive neurons.[105–107] Aiming to identify possible etiologies for bothersome vulvovaginal symptoms of pain and vaginitis, Mitchell et al.[108] evaluated microbial and immunologic characteristics of women with "idiopathic vaginitis" (defined as moderate-severe vaginal discharge, itching, and irritation without a diagnosis) and vulvodynia and compared them with healthy women. Although *G. vaginalis* was less prevalent in women with vulvodynia (7% vs 31% in controls and 25% in idiopathic vaginitis, $p = .03$), there was no significant difference between the groups. A number of small case-control studies report conflicting results when analyzing the VMB status in vulvodynia. Jayaram et al.[109] found no differences in vaginal or vestibular microbiota in women with vulvodynia and controls, whereas Murina et al.[110] found higher bacterial complexity in the vestibular samples compared with vaginal samples, in cases and controls, and differences in the dominant bacteria between patients with provoked vestibulodynia (PVD) and controls (*Lactobacillus*, *Gardnerella*, and *Atopobium* in PVD patients and *Lactobacillus*, *Gardnerella*, *and Bifidobacterium* in the control group). In addition, the researchers found that *L. gasseri* was dominant only in women with PVD, showing a significant correlation with burning/pain intensity and dyspareunia severity. Other, small, case-control studies found associations between vulvodynia and *Streptococcus*, *L. iners*, BV, and *Candida*, as well as with an overall lower number of lactobacilli in the vagina.[109,111–113] In a substantially larger case-control study, Bedford et al.[114] reported no differences in the VMB profile of women with vulvodynia and those without it.

A history of yeast infections is strongly associated with vulvodynia, but because of the vast majority of these infections are self-reported or not confirmed by culture, it is still unknown whether these are true or only perceived yeast infections.[115] Bedford et al.[114] found a strong association between vulvodynia and a history of more frequent yeast infections, among women with a low alpha diversity (the diversity of microbiome profile within a sample). Similarly, the association between prior yeast infections and vulvodynia was mainly confined to women with *L. crispatus and L. iners* dominance.

Overall, no specific microbiome pattern has been consistently recognized in women with vulvodynia. In line with the hypothesis of a multifactorial etiology of this pain syndrome, specific microbiomes may contribute to specific vulnerability. The study of the microbiome in women with vulvodynia carries inherent bias and difficulty to control for various factors, specifically the frequency of sexual intercourse, as sporadic or no intercourse is associated with a less diverse microbiome.[116] Proof of causality is, however, lacking, and these changes might just as well be the result of vulvodynia and the resulting changes in behavior or use of medications, such as antibiotics and hormones.

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