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Microbiome and Cervical Cancer

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Abstract
Persistent infection with some types of mucosal human papillomavirus (HPV) is the etiological factor for the development of cervical cancer and its precursor lesions. Besides, several cofactors are known to play a role in cervical disease onset and progression either by favoring or by preventing HPV infection and persistence. The microbiome of a healthy female genital tract is characterized by the presence of 1 or few varieties of lactobacilli. However, high-throughput studies addressing the bacterial diversity and abundance in the female genital tract have shown that several factors, including hormonal levels, hygiene habits, and sexually transmitted diseases may disrupt the natural balance, favoring the outgrowth of some groups of bacteria, which in turn may favor some pathological states. Recently, the vaginal microbiome has emerged as a new variable that could greatly influence the natural history of HPV infections and their clinical impact. In this context, changes in the vaginal microbiome have been detected in women infected with HPV and women with HPV-associated lesions and cancer. However, the role of specific bacteria groups in the development/progression or prevention/regression of HPV-associated pathologies is not well understood. In this review we summarize the current knowledge concerning changes in vaginal microbiome and cervical disease. We discuss the potential functional interplay between specific bacterial groups and HPV infection outcomes.

Introduction

Cervical cancer (CC) represents the fourth most frequent malignant neoplasia among women worldwide and it is a serious public health problem. In 2018, approximately 570,000 new cases and 311,000 deaths occurred, and most of them were in developing countries [1–4]. Persistent infection with human papillomavirus (HPV) types of high-oncogenic-risk HPV (hrHPV) is the main factor for the development of CC, and it has been found in 99.7% of CC samples [5]. Infection with hrHPV is highly prevalent in sexually active women. However, the occurrence of CC precursor lesions is relatively low [6, 7]. In fact, approximately 90% of infections by hrHPV are transient and regress spontaneously [8]. A woman's risk of acquiring an infection by any type of HPV during her

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lifetime is approximately 80%, whereas her risk of developing CC is 0.6% [9].

Infection with hrHPV is necessary but not sufficient for CC development, and additional factors are involved in disease establishment, progression, or regression. Some of these factors, such as the viral type involved, are virus related, and others, such as individual immunity, smoking, parity, use of hormonal contraceptives, and sexual behavior, are host related [8].

Recent studies have assessed the potential relationship between the vaginal microbiome (VMB) and gynecological cancer [10]. The VMB composition may influence the local immune response and could be involved in cervical oncogenesis and HPV clearance. The VMB with a predominance of certain species of lactobacilli may have a protective role against opportunistic infections and it may represent a new therapeutic target [11]. This review will address the likely relationship between VMB composition and CC development.

Vaginal Microbiome

The human body possesses trillions of microorganisms that coexist with one another and interact with the host [12, 13]. The concept of microbiome was first used by Lederberg and McCray [14] to designate a set of commensal, symbiotic, or pathogenic microorganisms, which share the same living space and develop a complex interaction with certain human tissues.

The first large study to address the diversity of microorganisms present in the different organs of the human body was the Human Microbiome Project (HMP), which started in 2008. In this study the microbiome composition of different parts of the body, including the lower genital tract, of 242 healthy people was analyzed [15].

In the intestine, a great diversity of microorganisms is associated with a healthy environment. However, in the healthy female genital tract there is usually just 1 or few varieties of lactobacilli [16, 17]. After studying the vaginal flora of 396 women of different ethnicities, 5 community state types (CST) of the VMB were identified [18]. CST I, II, III, and V are comprised of a low microbial diversity dominated by *Lactobacillus* (*L*) *crispatus*, *L. gasseri*, *L. iners*, and *L. jensenii*, respectively. In contrast, CST IV is formed by a reduction of lactobacilli and a great diversity of bacterial vaginosis (BV)-related bacteria, which are primarily anaerobic bacteria. The most frequently detected bacteria are *Gardnerella vaginalis*, *Megasphaera*, *Sneathia*, and *Prevotella* species. The VMB composition is dynamic and there is a frequent transition from one microbiome to another in the same woman throughout her life, mainly from CST III to IV [19, 20].

The VMB composition is influenced by various factors, such as ethnicity, hormonal alterations, sexual activity, and hygiene habits, as well as lactation, diabetes mellitus, stress, and dietary factors [20–22]. Some studies have shown that the VMB differs in women of different ethnicities. These data are important given that the environments dominated by BV-related bacteria are more associated with sexually transmitted infections [23, 24]. Other studies have demonstrated that African-American and Hispanic women exhibit a VMB in which bacteria other than *Lactobacillus* spp. predominate [23, 25, 26]. BV can affect over 50% of the women in sub-Saharan Africa [27–29]. It has become the most prevalent alteration in women of reproductive age. Such studies have implies that differences in VMB in women of different races may partly explain the differing incidence rates of BV and sexually transmitted infections among diverse ethnicities. Such diversity may be related to the genetic differences among races including a few mitochondrial DNA haplotypes. This reveals the importance of genetic factors in determining the microbiome of individuals, making them more or less prone to diseases [26].

The sex hormones interfere with VMB composition, regulating the release of proinflammatory cytokines, chemokines, and AMP contributing towards the selection of vaginal microbial species. Estrogen, in particular, has been implicated in the passage to a lactobacillus-rich microbiome during puberty and inversely poor during menopause [30].

Estrogen in the vaginal epithelium leads to its maturation and proliferation, as well as to the accumulation of glycogen, which is needed for a lactobacillus-rich environment. After menopause, the decline in estrogen production is accompanied by a decrease in lactobacilli and a predominance of anaerobes [21, 30]. The lactobacilli are believed to directly metabolize glycogen to form the lactic acid responsible for acidification of the environment and maintenance of the lactobacilli. This concept was abandoned following identification of the α-amylase enzyme in mature vaginal epithelium which is under estrogen action. It was demonstrated that this enzyme catabolizes glycogen generating simple sugars such as maltose, maltotriose, maltotetraose, and α-dextrins, which favor the formation of lactobacilli colonies [31].

During the reproductive age, it has been observed that a healthy vaginal microbiota amplifies the fluctuation of local immune responses in synchronicity with the hor-

monal alterations of the menstrual cycle [32]. Variations in the vaginal flora composition during the menstrual cycle as a consequence of variations in estrogen and progesterone levels have been demonstrated [20]. In the phases in which estrogen declines as in menopause, a decrease in lactobacilli is observed, which can be reverted with estrogen replacement therapy [21].

The use of hormonal contraceptives has also been shown to influence the vaginal flora composition, reducing the incidence, prevalence and recurrence of BV episodes [33]. Additionally, sexual activity is a factor that seems to contribute to a reduction of the lactobacillus population, favoring bacterial diversity [34]. Interestingly, vaginal douching increases the risk of BV, thereby demonstrating the influence of douching in the vaginal ecosystem [35].

Nicotine and its main metabolite, i.e., cotinine, are found in the cervical mucus of women and in the semen of men who smoke [36]. It has been shown that, in hrHPV-infected cells, tobacco smoke induces an increase in oncogene E6 transcription, leading to a decrease in p53 activity and levels [37], which may facilitate the development of squamous cell carcinoma [38, 39]. It is worth noting that CST IV was increased in women who smoked [40].

BV (CST IV) is characterized by changes in the vaginal flora, including reduced lactobacilli and a high bacterial diversity [41]. BV has been associated with inflammatory pelvic disease, a higher risk of miscarriage, preterm labor, and a greater risk of acquiring sexually transmitted diseases, including HIV [9]. The diagnosis can be made based on clinical criteria, following the Amsel classification. According to this, a BV diagnosis requires fulfillment of 3 of the following 4 criteria: fluid or bullous white discharge, presence of clue cells established directly under a microscope or through microscopic examination of a Gram-stained smear from genital discharge, a vaginal pH over 4.5, and a positive amine test (fishy odor produced by the addition of 2 drops of 10% potassium hydroxide to the vaginal discharge) [26, 42]. Another common diagnostic method is the microscopic classification, which analyzes different types of bacterial morphologies by Gram staining of *Lactobacillus* spp., *Gardnerella* spp., and *Mobiluncus* spp. If the stained discharge receives a score from 0 to 3, the vaginal flora is normal; if the score is 4–6, it is intermediate; and if it is 7–10 it is characterized BV [26, 43].

Even though we recognize the merit of such classifications, they are subjective and may lead to overdiagnosis of BV in a significant number of asymptomatic women. Studies utilizing molecular techniques, like PCR, for detection were able to identify other BV-related agents, such as *Atopobium vaginae*, *Clostridiales*, and *Megasphaera* species [26]. Next-generation sequencing techniques enabled the identification of a larger number of bacteria because they amplified the gene fragments of pathogens [44].

Microbiome Composition and Cervicovaginal Microenvironment

In humans, the cervicovaginal microbiome interacts with the local microenvironment, maintaining tissue homeostasis [45]. When this balance is broken, leading to a condition known as dysbiosis, several pathological processes, including epithelial barrier breach, abnormal cellular proliferation, genome instability, angiogenesis, chronic inflammation, and metabolic dysregulation can be triggered [17].

Microbiome and Host Defenses

Several defense mechanisms act to protect the female genital tract against infectious agents. These include the mucosal epithelial barrier, mucus, lactic acid secretion, and the immune response. From this perspective, the vaginal mucosa is a barrier that provides protection against invading pathogens as a consequence of the interaction among its epithelial cells, the immune system, and colonizing microorganisms [46, 47].

The major protective mechanism associated with lactobacilli is their ability to produce lactic acid and maintain a local pH lower than 4.5, which is harmful to most pathogens, as well as to produce bacteriocins, which inhibit or eliminate pathogens transmitted sexually [48]. Besides, lactobacilli may form microcolonies which adhere to the epithelial cells, preventing the adhesion of pathogens and their ability to trigger host defenses [49]. A healthy vaginal microbiota has also been associated with an increased expression of defensins, which are vaginal antimicrobial peptides (AMP) that prevent binding of specific pathogen proteins to female genital tract cells. Thus, AMP levels were found to be decreased in women with BV [50, 51]. The expression of other types of AMP, such as the secretory leukocyte protease inhibitor (SLPI), is thought to be correlated with BV-related bacteria [52]. Increased concentrations of SLPI in were reported in healthy women [53], while women with BV demonstrated lower concentrations [54].

Moreover, a possible mechanism for vaginal dysbiosis is an increase in the production of proinflammatory cytokines and chemokines associated with an increase in pathogenic microbial diversity, which contributes to the additional recruitment of immune cells and amplification of the inflammatory response [30].

Microbiome, Immune Response, and Cervical Alterations

BV is associated with the induction of local inflammation [25, 55]. One important factor is lactic acid depletion and, consequently, the reduction of its anti-inflammatory effects. It was shown that lactic acid induced an anti-inflammatory condition and inhibited inflammation elicited by toll-like receptor (TLR) agonists. Besides that, lactic acid triggers the interleukin (IL)-1 pathway through production of its antagonist IL-1 receptor antagonist (IL-1Ra) [56]. In contrast, treatment with a vaginal microbiota metabolite mixture corresponding to BV increased the TLR-induced production of proinflammatory cytokines such as TNF-α and decreased the production of RANTES and interferon-γ-induced protein 10 (IP-10) [57].

It is suggested that BV-related inflammation is mainly due to high proinflammatory cytokines levels and not to cervical immune cell recruitment. A study comparing women with CC or dysplasia and women without neoplasia showed that an environment with non-*Lactobacillus* dominance was characterized by proinflammatory (IL-36γ), chemotactic (IP10, MIP1β, and RANTES), hematopoietic (FLT3 ligand), and adaptive immune (IL-2, IL-4, and soluble CD40 ligand) cytokines, therefore, correlating dysbiosis, inflammation, and CC [25].

A cohort study including asymptomatic young South African women showed that cervicovaginal environment modifications, including alterations in vaginal acidity and the cytokine profile, can be associated with the local microbial pattern and that high-diversity bacterial communities without lactobacillus dominance is associated with higher levels of proinflammatory cytokines. The group of women in which the bacterial community was composed of a high diversity of species (*Sneathia sanguinigens*, *S. amnii*, *Mobiluncus mulieris*, *Prevotella amnii*, *Aerococcus*, and *Fusobacterium*) presented higher proinflammatory genital cytokine levels. Induction of IL-1α, IL-1β, and IL-8 by these bacteria has also been demonstrated in vitro [58]. In addition, an important increase in IL-1β and TNF-α levels in persistent abnormal vaginal microbiota samples has been demonstrated in vivo [59].

It was postulated that Gram-negative bacterial membrane lipopolysaccharide may be sensed by cervical antigen-presenting cells (APC) inducing TLR4 signaling, NF-kB activation, and the production of proinflammatory cytokines and T-cell chemoattractants. In women with a high *Prevotella* abundance, a marked response to lipopolysaccharide, IFN-γ, and IL-1β was observed, probably reflecting an immune response against Gram-negative bacteria. CST IV APC also showed a higher expression of CD80, ICAM-1, and MHC II, contributing to T-cell priming and effector function [58].

On the other hand, cervicovaginal environment modifications may act together with HPV infection, contributing since the early stages of CC and creating, for instance, a local immunosuppression state [60]. Some studies have suggested that certain cervicovaginal microbiota species can modulate the local inflammatory immune response, possibly promoting the expression of immunosuppressive cytokines, and that abnormal vaginal microbiota is related to HPV infection and persistence [61].

Audirac-Chalifour et al. [60] conducted a study to analyze the cervical microbiome and cytokine profiles in various stages of CC. They proposed that, after hrHPV infection of the cervical epithelium, the microbiome composition switches from *L. crispatus* to *L. iners*. As the infection progresses to a squamous intraepithelial lesion (SIL), there is an increase in microbiota diversity marked by *Sneathia* and *Fusobacterium* spp. In CC, *Fusobacterium necrophorum* was also present, increasing the microbiome diversity. In this suggested model, HPV infection is responsible for creating an immunosuppressive microenvironment (through IL-10 expression and macrophage type 2 induction) that is enhanced by TGFβ-1 derived from microbiota, creating positive feedback between microbiota and cytokine profile.

CST dominated by *Fusobacterium* spp. are associated with an immunosuppressive microenvironment, characterized by higher levels of IL-4 and TGFβ-1 and by a shift from a Th1 to a Th2 immune response. In addition, there is direct interference on the E-cadherin/β-catenin signaling pathway on cervical HPV-transformed cells [60]. In the gut, however, *F. nucleatum* has been shown to exert a proinflammatory and tumorigenic effect [62].

Microbiome, Oxidative Stress, and Cervical Alterations

Oxidative stress, which reflects an imbalance of the intracellular redox state due to an excess of reactive oxygen species (ROS) production over to the antioxidant system may also be a consequence of dysbiosis [63–65]. ROS, in-

cluding the superoxide radical anion ($O_2^{\bullet -}$), the hydroxyl radical ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2), can play a beneficial role as well as a harmful role in biological systems. These species can have important functions in regulation of cellular signaling pathways, such apoptosis and immune defense against infectious agents. However, the accumulation of ROS can lead to oxidative damage on cellular structures and biomolecules, such as proteins, lipids, and DNA [65, 66].

Therefore, oxidative stress has been linked to the promotion and progression of several types of tumors, including CC [64, 67]. ROS and reactive nitrogen species play a significant role in HPV-mediated cervical carcinogenesis, since the accumulation of these species can increase the levels of DNA damage, which may allow HPV genome integration and subsequently cell transformation [68]. Viral DNA integration into the host genome frequently promotes disruption of the early viral gene E2, which inhibits E6 and E7 oncogenes expression. The result is an uncontrolled expression of HPV E6/E7 proteins that leads to increased cellular proliferation and decreased apoptosis [9, 20, 69].

Although there are studies that associate oxidative stress [64, 67] and VMB [11, 20, 70, 71] with CC, the association between oxidative stress and the VMB is under discussion in the literature. Chen et al. [72] observed that the levels of H_2O_2 in women with BV were almost 10 times higher than those in healthy patients, which suggests that oxidative stress was formed in these patients. In contrast, Piyathilake et al. [73], analyzing women with HPV-positive CIN, hypothesized that the cervicovaginal microbiome could induce oxidative DNA damage. However, no association was observed between microbiome diversity and oxidative DNA damage, as measured by the presence of 8-hydroxy-2′-deoxyguanosine (8-OHdG), a biomarker of oxidative stress-induced DNA damage.

Vaginal health has been attributed to the presence of *Lactobacillus* H_2O_2-producing species/strains, since some epidemiological studies have reported a possible protective effect of H_2O_2 against BV and other transmitted infections, such as HIV-1 [74–80]. Besides *Lactobacillus* spp. (except *Lactobacillus iners*), leukocytes and endothelial and cervical transformed cells, among others, can also generate hydrogen peroxide. One of the mechanisms of H_2O_2 production is dismutation of $O_2^{\bullet -}$ (produced by NADPH oxidase) into H_2O_2, which can occur spontaneously, optimally at pH 4.8, or catalyzed by SOD enzymes (superoxide dismutase) [80, 81].

Moreover, myeloperoxidase (MPO), an enzyme expressed by polymorphonuclear neutrophils and macrophages, is responsible for the reaction of halides (e.g., Cl^-) and H_2O_2, producing hypochlorous acid (HOCl), a type of ROS involved in oxidative burst when an innate immune response is required. Thus, the antimicrobial role of H_2O_2-producing lactobacilli has been demonstrated to be efficiently higher in the presence of peroxidase and halides [77, 81–83]. Consequently, it is thought that HOCl is a powerful antimicrobial compound produced by the H_2O_2/peroxidase/halide system present in phagocytes and tissue fluids [83, 84]. Interestingly, Klebanoff et al. [77] reported increased concentrations of peroxidase in the vaginal fluids of almost all of the patients analyzed (and sufficient HOCl production). Additionally, the authors not only observed the antimicrobial effect of H_2O_2-producing lactobacilli but also proposed an antitumoral effect of these bacteria.

Krüger and Bauer [83] suggested that the H_2O_2 produced by *Lactobacillus* spp., per se, is not favorable for vaginal epithelial cells, since it induces nonselective apoptosis in transformed and nontransformed cells. On the other hand, the addition of peroxidase allows nontransformed cell protection against the apoptosis mediated by lactobacillus-derived H_2O_2. Considering that HOCl reacts with $O_2^{\bullet -}$ derived from transformed cells, leading to $OH^{\bullet}$ production that induces apoptosis [82], it was proposed that there is an association between the presence of H_2O_2-producing lactobacilli in VMB and the increased MPO activity, which causes clearance of HPV-infected cells and induces transformed cell apoptosis [84, 85].

However, there is no consensus on the protective role of H_2O_2 on the cervicovaginal microbiome. Some researchers consider that it is improbable that H_2O_2 could have an antimicrobial effect on VMB, since the cervicovaginal environment is hypoxic most of time and lactobacilli require large amounts of O_2 to form hydrogen peroxide [80, 86]. In accordance with this argument, H_2O_2 produced by lactobacilli was not detected under hypoxic conditions in cervicovaginal fluid [87] and supernatant of *Lactobacillus* strains [88]. Moreover, H_2O_2 generated by lactobacilli, in physiological concentrations, did not inactivate HSV-2 (herpes simplex virus type 2) or any BV-associated bacteria analyzed in vitro, even when MPO was supplemented [87]. In the same study, it was also observed that cervicovaginal fluid and semen blocked the antimicrobial activity of H_2O_2.

Under aerobic conditions, which is used by most studies that observe antimicrobial effects of H_2O_2-producing lactobacilli, hydrogen peroxide production rather than lactic acid production is favored. However, when lactobacilli are growing in the absence of oxygen, fermentation can be preferred [80, 86, 89]. Thus, lactic acid was found

to be produced at higher concentrations under hypoxic conditions [86, 90]. Additionally, lactic acid can inactivate BV-associated bacteria and *Chlamydia trachomatis*, which were not observed in the presence of H_2O_2 [91, 92]. Furthermore, H_2O_2 could represent a benefic characteristic of lactobacilli or it could be just a marker for lactobacilli strains that protect the cervicovaginal environment for other beneficial causes [80, 86].

Further studies are essential to understand the role of oxidative stress on the VMB and how it could contribute to the development and progression of cervical lesions and cancer.

Protective Mechanisms Associated with Lactobacillus

Lactobacillus spp. predominates in low pH environments. Vaginal acidity prevents colonization by anaerobes, maintains the cervical epithelial barrier through production of bacteriocins, and acts against mucin degradation, keeping away opportunistic infections [21]. As mentioned before, lactic acid production inhibits the growth of several anaerobic agents associated with sexually transmitted diseases that may contribute to the progression of cervical lesions when associated with hrHPV persistence [93]. Infection by *C. trachomatis* seems to increase the risk of hrHPV infection of CC through an inflammatory response, which increases ROS production and free radicals [94].

Di Pietro et al. [95] evaluated simultaneous cervical infection by *C. trachomatis* and HPV and the varieties of associated cervical microbiomes. Women with both infections exhibited a greater bacterial diversity primarily related to the presence of anaerobes such as *G. vaginalis*, *A. vaginae*, and, in reduced numbers, *Lactobacillus*, thus associating dysbiosis with the occurrence of infections. On the other hand, healthy women showed a dominance of *Lactobacillus*, with anaerobic bacteria representing <2% of the cervical flora. Likewise, women infected by *C. trachomatis* alone had a diverse cervical flora but poor levels of *Lactobacillus*. *L. iners* was more frequently detected in coinfected women in comparison with healthy ones. Furthermore, it was found that the flora composition of HPV-positive and *Chlamydia*-negative women did not differ significantly from that of healthy women. Still, in this study *L. gasseri* was not identified in HPV-positive women, supporting the findings of other authors who related the presence of *L. gasseri* to the clearance of HPV infection [96, 97].

Species of lactobacilli can produce 2 lactic acid isomers, i.e., L- and D-lactic acid. The latter exerts more protective action against vaginal dysbiosis [21]. Besides producing lactic acid, species of *Lactobacillus* produce peptides with an antimicrobial action, such as bacteriocins and biosurfactants. *L. iners* can synthesize only L-lactic acid and cannot produce hydrogen peroxide, which also exhibits an inhibitory action against bacterial growth [21, 98]. Moreover, *L. iners* is able to produce inerolysin, a pore-forming cytotoxin, similar to the vaginolysin protein secreted by *Gardnerella* spp., which forms pores in the vaginal epithelium, favoring infections [98]. Thus, an *L. crispatus*-dominated VMB is related to maintenance of protective mucosal surface layer integrity and poses a smaller risk of opportunistic bacterial and viral urogenital infections, including by HPV. On the other hand, a VMB dominated by *L. iners* is associated with a greater risk of viral infections and development of precursor lesions and CC [21].

Microbiome, Genital Infection, and Cancer

Several studies have correlated the different VMB with HPV infection, different grades of CIN, and CC. Mitra et al. [70] evaluated a group of 169 women referred for colposcopy and found an increased bacterial diversity coupled with diminished lactobacilli associated with the severity of the cytological lesion. CST IV was present in 40% of the women with CC and only in 10% of those with a normal cytology. On the other hand, CST I was present in 50% of the cytological examinations and only in 20% of the CC cases; low levels of *L. jensenii* were related to severe lesions [70].

Some dysbiotic bacterial communities are known to cause immune dysregulation favoring a tumor-promoting microenvironment [99, 100]. It is known that HPV is necessary but not sufficient to cause CC. In most infected women the immune response is able to control the infection and prevent high-grade lesions and tumors [101]. Among the cofactors in CC development, the VMB may play an important role [44, 102].

BV was associated with higher HPV infection rates, suggesting that an increase in the diversity of vaginal bacteria together with a reduction of lactobacilli may contribute to the persistence of HPV infection [44]. *G. vaginalis* is capable of secreting the enzyme sialidase, which degrades vaginal mucus by cleaving its glycoproteins. One of these proteins is mucin, which provides a physical barrier to the surface of the vaginal mucus [21]. Further-

more, the bacteria present in CST IV are also capable of producing butyric acid, which can regulate histone acetylation. The epigenetic regulation promoted by the metabolite favors reactivation of the latent HIV-1 virus, indicating a potential involvement of VMB in AIDS progression [103]. Still, women with CST IV exhibit an increase in the production of proinflammatory cytokines and in the recruitment of CD4+ and CCR5+ cells activated to the vaginal mucus, favoring the acquisition of HIV [28].

Kwasniewski et al. [44] assessed the vaginal flora of 250 women, including 70 healthy controls, 95 women with low-grade SIL and HPV positivity, and 85 women with high-grade SIL and HPV positivity. In the control group, high levels of *L. crispatus*, *L. iners*, and *L. taiwanensis* and an absence of *G. vaginalis* and *L. acidophilus* were detected. In the low-grade-SIL group, *L. crispatus* was less frequent than in the control group and *L. acidophilus* and *L. iners* predominated. On the other hand, in the high-grade-SIL group, *G. vaginalis* and *L. acidophilus* were increased, while the frequencies of *L. iners*, *L. crispatus*, and *L. taiwanensis* were lower than in the control group. These results show a possible relationship between the VMB, HPV infection, and CIN development. A microbiome dominated by *G. vaginalis* and poor in *L. iners*, *L. crispatus*, and *L. taiwanensis* may be a cofactor for HPV persistence, CIN development, and CC [44].

Di Paola et al. [104] evaluated the VBM of women positive for HPV DNA, and after 1 year of treatment they reassessed them with new viral genotyping. Interestingly, the study classified CST IV into 2 categories, i.e., CST IV-BV and CST IV-AV. CST IV-BV was composed, predominantly, of anaerobic bacteria such as *Gardnerella*, *Prevotella*, *Atopobium*, *Sneathia*, and (scarce) *Lactobacillus* species. On the other hand, the CST IV-AV group was composed of aerobic and anaerobic bacteria, such as *Pseudomonas*, *Brevibacterium*, *Peptostreptococcus*, *Enterococcus*, *Streptococcus*, *Propionibacterium*, *Bifidobacterium*, and *Shigella* species. In the women with persistent HPV infection, the dominant VMB was formed by anaerobes (CST IV-BV) and scarce lactobacilli, and in the women with a cleared HPV infection there was a prevalence of CST IV-AV. The controls had a dominance of lactobacilli [104]. These data correlate flora deficient in lactobacilli with a greater risk of HPV infection and the presence of BV with a smaller chance of clearance of the viral infection [104, 105]. Hence, different lactobacilli play distinctive roles. A VMB dominated by *L. crispatus* is associated with a lower risk of infection by HPV, CIN, and CC, whereas a VBM dominated by *L. iners* is associated with a higher risk of being infected by such agents [106].

Cervical precancer lesions that regress, compared to those that progress to cancer, harbor a different immune microenvironment [107]. Specific bacteria, such as *Gardnerella*, and a rise in microbiological diversity may be used as biomarkers of cervical changes to identify women with a high risk of developing persistent HPV infection, CIN, and cancer [108].

Are Probiotics a Therapeutic Option for HPV?

According to the World Health Organization, probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [109]. The species of *Bifidobacterium*, *Lactobacillus*, and *Streptococcus* are capable of altering the host's microbiome, improving the immune response and the inflammatory state [110].

Probiotics containing species of lactobacilli have been used in the treatment of urogenital infections to improve the vaginal flora. The mechanism of action would involve vaginal acidification, prevention of bacterial adhesion, and synergistic action with the host's immune system [110]. Although there are no conclusive data on their efficacy, probiotics seem to be an alternative complementary treatment of BV and sexually transmitted diseases given that they do not induce inflammation, promote resistance, or have adverse effects [111].

The effects of probiotics on cytological alterations of the uterine cervix and on HPV infection were evaluated by Verhoeven et al. [112] in 51 individuals. Twenty-four women received the Yakult® probiotic, which contains *L. paracasei* strain Shirota, daily, and 27 women made up the control group. At baseline all women had a positive PCR for HPV. After 3 months, HPV had cleared in 25% of the women who ingested the probiotic against 7.7% of the control group. After 6 months, the clearance rates were 29.2 and 19.2% in the probiotic and control groups, respectively. Still, in the same study, the HPV-infection-related cytological abnormality clearance was twice as high in the probiotic group than in the control group [112].

Palma et al. [113] assessed 117 women with BV or fungus infection (with cytological changes or presence of HPV/PCR). Women were divided in 2 groups and received a vaginal capsule of *L. rhamnosus* BMX 54 following treatment of the infections. Group 1 ($n = 60$) received probiotics for 3 months and group 2 ($n = 57$) received

probiotics for 6 months. After 3 and 6 months, samples were collected for cytology, colposcopy, and bacterioscopy, as well as for investigation of fungi and vaginosis. After 9 months, analyses of HPV/PCR were included. The results indicated that the clearance of cytological changes was 2-fold in group 2, which used the probiotic for a longer period. Clearance of the HPV infection was also greater in group 2 (i.e., 31.2% vs. 11.6% in group 1), which also had lower relapse rates of vaginal infection [113]. Additional longitudinal studies are needed to understand whether CC and lesions outcomes are associated with the constitution of the VMB.

Conclusion

Studies conducted during the last decade have evidenced the variable and complex composition of the VMB. Persistent HPV infection with hrHPV types is the main risk factor for the development of almost all CC and a significant proportion of vaginal and vulvar malignancies. Recent data suggest that the presence and abundance of some bacterial species may prevent HPV infection and contribute to virus clearance reducing the risk of development of cancer precursor lesions at these anatomic sites. Conversely, other bacterial types may promote the pathological state. Therefore, understanding the impact of the VMB composition and its alterations (dysbiosis) on HPV infection/persistence may contribute to a better prediction of the outcomes of infections by this virus. Moreover, clear identification of the bacterial components associated with HPV-caused pathologies may have a clinical relevance and provide an opportunity for alternative therapeutic strategies. Presently available technologies allow the rapid high-throughput analysis of VMB. These tools should be applied in longitudinal studies to determine the involvement of specific bacterial species in the establishment, prevention, progression, or regression of HPV-related cervical, vaginal, and vulvar pathologies.

Conflict of Interest Statement

The authors have no conflict of interests to declare.

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Author Contributions

L.T. conceived, organized, and revised this review. C.P.C., M.L.S., and R.A.L.N. gathered information from the literature and prepared this paper. N.P.C.L. assisted in the discussion for this review. All of the authors read and approved the final version of this paper.

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