Review

Vaginal microbiota and human papillomavirus: a systematic review

Mortaki et al. Vaginal microbiota and human papillomavirus: a systematic review

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Abstract
Background: Accumulating evidence indicates the potential correlation between the vaginal microbioma and the acquisition and persistence of Human Papillomavirus (HPV) infection. This study has as intention to demonstrate the potential relationship through a systematic review of the current literature.
Methods: A search was conducted on the following medical databases: PubMed and Scopus. Nineteen studies were meeting our inclusion criteria and, finally, incorporated, in the present review.
Results: A total of 12,204 patients and their demographic characteristics were studied. Commercially available DNA tests and PCR were used for the detection of different HPV subtypes, while the identification of the microbiomes was performed through specific diagnostic methods and PCR assay. The most frequently encountered species were classified based on their protective or detrimental impact on the progression of HPV infection. The beneficial role of some types of Lactobacillus (Lactobacillus gasseri, Lactobacillus jensenii, Lactobacillus crispatus) is generally supported. On the other hand, high microbial diversity and specific microorganisms such as Sneathia, Anaerococcus tetradius, Peptostreptococcus, Fusobacterium and Gardnerella vaginalis were found to be implicated with higher frequency and severity of disease, potentially resulting in pre-cancerous and cancerous cervical lesions.
Conclusion: The role of vaginal microbiota seems to play a -yet not fully understood- role concerning the susceptibility to HPV infection and its natural history.
Keywords: microbiome; microbiota; human papillomavirus; HPV; *Sneathia*; *Lactobacillus gasseri, Lactobacillus jensenii, Lactobacillus crispatus*

Introduction

Sexually transmitted Diseases (STDs) are among the most frequent infectious diseases worldwide and are defined as those which include transmission of infectious organisms between sex partners. According to the Centers for Disease Control and Prevention (CDC), in the United States, almost 19 million cases are reported as infected each year by more than 20 different STDs (1).

Human Papillomavirus (HPV) represents ~~is~~ one of the most frequent causes of STDs in women around the world (2). More than 200 different HPV genotypes have been reported and generally classified into two groups including high and low risk, which is based on the potential cancer risk of developing cancer. To that end, in about 99% of all cervical malignancies one or more of the HPV types classified as high risk (16, 18, 31, 35, 39, 45, 51, 52, 56, 58, 59) are identified. Additionally, high risk types have been reported to play an important role in other malignancies such as anal, oropharyngeal, vulvar, and penile. The genotype distribution as well as the genome of each HPV are considered critical for disease prevention, prognosis, and treatment (3, 4).

The viral types of HPV can be easily transmitted from one person to another via skin and mucous membranes, which makes the possibility of infection from HPV ~~is~~ relatively common. Nevertheless, the majority of the infections are subclinical and transient as they are suppressed by a robust immune system (5). Therefore, even though, the incidence of HPV infection is common throughout life (>80% in sexually active people), the incidence of HPV- related diseases is relatively lower (6).

Cervical cytology and HPV tests are widely used for cervical cancer screening and thus early detection of the underlying disease. Co-infections by multiple HPV types are likely to occur in approximately more than 30% of HPV patients (2). Information regarding HPV co-infections can be an essential part of determining treatment options and therapy. Molecular diagnostic systems are capable of detecting more than 40 distinctive HPV types, particularly those correlated to high grade dysplasia (HSIL). The human microbiome refers to the sum of microorganisms that may reside in various parts of the human body (eukaryotic, archae, bacteria and viruses), their genetic information and how they interact with the host environment (7). We now have a lot of data regarding the mapping of microbiota in several sites of human body, especially the gut, due to the development in sequencing technology. During the last years, there is emerging evidence that vaginal microbiota may play a crucial role in HPV carcinogenesis (8) and is related to protection against dysbiosis and HPV infection (9, 10). In healthy women of reproductive age, vaginal pH is primarily regulated by lactic acid producing bacteria such as *Lactobacillus* species. In women, whose vaginal microbiota is not lactobacilli-dominated, anti-bacterial defensive mechanisms are decreased (11). Alterations in vaginal microbiota, such as bacterial vaginosis and vaginal infections, are usually correlated to respective changes in vaginal pH. In that setting a decrease in vaginal pH has been related with decreased risk of infections, such as *Chlamydia trachomatis*, trichomoniasis, and urinary tract infections.In the vaginal environment, five major community state types are recognized by Ravel et al (CST I-V), who studied the vaginal microbiota of 396 asymptomatic women and characterized the found species in five groups based on their genes. In a healthy environment, the following microorganisms are recognized; CST I, II, III, V and are dominated by *Lactobacillus crispatus, L. gasseri, L. iners and L. jensenii*. respectively. On the contrary, CST IV is characterized by depletion of lactobacilli and increased diversity of anaerobic bacteria, such as *Atopobium* (12). Nevertheless, a lot are yet to be answered referring to the relationship between the vaginal microbiota and how it correlates with the HPV natural history.

The aim of the present study was to present the current knowledge and to evaluate the correlation between microbiome and human papillomavirus.

Methods

Data Sources
An extensive systematic search was performed in both PubMed and Scopus. All databases were searched up to February 25, 2019. The search strategy used in both databases included the combination of the key words: (microbiome OR microbiota) AND (HPV OR human papillomavirus). The references of relevant articles were also hand-searched, for additional studies.

Study selection criteria
Studies reporting data on the association of microbiota and human papillomavirus were included in this systematic review. Abstracts in scientific conferences, editorials, reviews as well as animal studies were not included in the study. Studies published in languages other than English, Dutch, German, Greek, Italian or Spanish were not taken into consideration.

Selected Studies
A total of 78 and 291 articles were retrieved during searching PubMed and Scopus respectively. From those studies, 16 studies were identified as eligible for inclusion in our review. No additional studies were identified through hand-searching of references. The included studies are graphically presented in Fig. 1 (flow diagram).

Techniques
From the eligible articles, the techniques that were used for HPV detection and microbiomes' identification were DNA tests, Sequencing and PCR amplification.

HPV Detection
HPV detection and genotyping was performed either with commercially available DNA tests, such as Roche Linear Array HPV Genotyping Test and Digene Hybrid Capture II DNA Test, or through an assay of PCR using specific primers (MY09/MY11, GP5+/GP6+, etc). A range of 15-49 HPV types was identified, including predominantly high risk HPV types with or without low risk subtypes (13, 14).

Vaginal microbiota
For the detection of vaginal microbiota, the initial assessment included diagnostic methods such as microscopic evaluation, Gram stain test, microbiological cultures and measurement of vaginal pH. Amsel criteria were used for diagnosing bacterial vaginosis (15). PCR amplification of V1-V5 hypervariable region of the 16S rRNA genes was used in 12 studies. PCR results were analyzed in accordance with databases such as BLAST, QIIME and Illumina MiSeq in order to identify specific species (Table 1).

Results
Study characteristics
Sixteen studies, including a total of 12,204 patients, have been published in the literature with regard to the association between vaginal microbiota and HPV infection (16-34).

According to the searched literature, participants have been categorized based on several epidemiologic criteria which included age, race/ ethnicity, educational status, marital status, time of first intercourse, lifetime number of sexual partners, number of live births, use of hormonal contraception or antibiotics/probiotics, prevalence of smoking, alcohol use, menstrual phase.

Concerning specific population characteristics, the correlation between HPV infection and vaginal microbiota has been studied in HIV infected patients (24, 28), while Lee et al studied a twin cohort, as well as their siblings and mothers (18). A total of 824 individuals were reported to be female sex workers (17, 29). Moreover, Kero et al enrolled 329

asymptomatic, pregnant women in the third trimester of their pregnancy and they studied the potential impact of vaginal microbiota on the outcome of known HPV infection during a 72-month follow-up (31). Brotman et al, conducted a study of a total 32 patients, in which they assessed 937 self-collected samples before and after vaginal douching cessation (22).

The demographic details of the population under study are shown in Table 2.

Types of vaginal microbiota

The detected microbiotas in these studies included several types of microorganisms; *L.iners* classified as CSTIII (13 studies, 72,22%), *L.crispatus* classified as CSTI (8 studies, 44,44%), CSTIV-B which represents anaerobic microbiomes combined with reduced Lactobacillus (5 studies, 27,77%), *Megasphaera* , *G.vaginalis* and *L.jensenii* which is classified as CSTV (4 studies, 22,22%), *Sneathia*, *L.gasseri* classified as CSTII as well as CSTIV-A which represents *Peptoniphilus, Anaerococcus, Corynebacterium, Finegoldia* and *Prevotella* (3 studies, 16,66 %), *Prevotella, L.vaginalis, Phylum protebacteria, L.reuteri* and other members of Pseudomonas family (2 studies, 12,5%) and in one study each (6,25%) *Dialister, L.formicalis, Fusobacterium, L.gallinarum* and *L.salivarus* (found only among South African women).

Vaginal microbiota association with HPV and CIN

Interestingly, it was found that women with HPV infection had a higher diversity and a lower proportion of *Lactobacillus*. A lower prevalence of *L.iners* and *L.crispatus* was also observed. [20]. Dols et al also speak about a shift of the composition of the vaginal lactobacilli in HPV (+) women as well as a significantly reduced prevalence of *Lactobacillus crispatus*. Other common microorganisms among HPV (+) patients were *L.gasseri* and *Gardnerella vaginalis* (17).

Additionally, patients who ended up with a cervical intraepithelial neoplasia (CIN) had also a high diversity of their vaginal microbiota (25) and they were usually colonised by *Sneathia*, while regarding women with invasive cervical cancer (ICC), *Fusobacterium* was the most common type of microorganism (26). Interestingly, Piyathilake et al (27) has found an abundance of *Lactobacillus* and *L.reuteri* specifically in women with CIN II.

On the other hand, in HPV(-) women, *L.crispatus*/CSTI and *L. gasseri*/ CSTII were the most common species (26). *L.crispatus* was related by Reimers et al (26) and Borgdoff et al (19) to decreased prevalence of oncogenic HPV types, while Darend identified prevalent high risk HPV infectionscamong women with a decreased population of *Lactobacillus* and an increased abundance of anaerobes, particularly of the genera *Prevotella* and *Leptotrichia* (22).

Vaginal microbiota and HPV remission

The relationship between vaginal microbiota and HPV remission was highlighted by Brotman et al (28). CSTIII was the one with the fastest remission, while on the other hand, CSTIV-B was the one with the slowest. This was also confirmed by Di Paola et al, who considered CSTIV-B to be a risk factor for HPV persistence (32).

Transition to HPV and severity of infection

Brotman et al compared the community state types (CSTs) among women HPV (-) who later turned to HPV (+) and report that CSTIV-A was related to higher transition to HPV (+) status than CSTI.

Concerning the severity of the HPV infection, Mitra et al present CSTIV and CSTV to be associated with high severity when CSTI is associated with low. CSTIII is related, by Piyathilake et al, to high severity CIN lesions (25, 27).

Vaginal microbiota, HIV and HPV infections
Five of the above mentioned studies have shown an association between vaginal microbiota, HPV and HIV infection. *Lcrispatus* was found to have an advantageous effect on the HPV infection evolvement in both HIV- infected and not infected women and in general *L.crispatus* was found to be a protective factor against HIV, high risk HPV and Herpes Simplex type 2 as well, as it was found in high abundance in uninfected women while on the contrary infected women had a reduced prevalence. In both HIV and HPV infections, a comparable shift in the composition of the *Lactobacillus* flora was identified.

Vaginal microbiota, ethnicityand HPV infection
Another factor that affects strongly the vaginal microbiota seems to be the ethnicity. The studies included in our review refer to women of all ethnicities: European/Caucasian, Asian, Latin-American and African. It has been found that Afro-Caribbean women have a 4 times higher possibility to suffer from a vaginal dysbiosis or high microbiota diversity, this meaning that the most common type of microbiota among them is CST IV (in comparison with European/Caucasian and African women). However, the prevalence of HPV and the rate of more severe lesions is not accordingly high (35).

Microbiological markers of HPV infection
It is remarkable, that among all the microbiota, Fusobacteria, including *Sneathia*, were identified as a possible microbiological marker correlated to HPV infection, as it was proved by Lee et al (29). However, the relation between HPV infection and its coexistence with other types of vaginal microbiota appears to be either protective or facilitating to the HPV infection. In addition, the evolution of HPV infection is in direct correlation with the species or genus of the vaginal microbiota which is mainly present at the vaginal environment. Specifically, some types of *Lactobacillus* including *L.gasseri, L.jensenii* and *L.crispatus* seem to protect from HPV infection while on the contrary other microorganisms, especially *Sneathia., Anaerococcus tetradius, Peptostreptococcus, Fusobacterium, Gardnerella vaginalis* and *L.iners*, often together with a low abundance of the other types of *Lactobacillus* and other factors such as smoking and lack of barrier contraception or low estrogen levels not only lead to elevated rates of HPV infection, but also to higher disease severity and lower HPV remission. This leads to the conclusion that some of the microbiota species may be used as a disease marker or even as a therapeutic mean against HPV. The effect of the vaginal microbiota on the evolution of an HPV infection is described in Table 3.

Discussion
A systematic review of the literature was conducted with the intent to examine if molecular vaginal microbiota composition patterns can be related to HPV infection and intraepithelial lesions. Our study indicates a significant correlation among vaginal microbiota and HPV infection on the setting that some certain microbial species play a protective role against the infection while some others predispose to either the progression or the remission of the disease.

Alterations in vaginal microbiota have been associated with a variety of complications either obstetric or gynecology associated, such as tubal factor infertility, spontaneous abortion, intrauterine fetal demise, premature rupture of membranes, pre-term labor and delivery, intrauterine growth restriction, endometritis, postpartum infection, chorioamnionitis, ectopic pregnancy and pelvic inflammatory disease (35). To that end, some of the aforementioned conditions may cause chronic and severe congenital tract infections which have been associated with vaginal flora alterations (36). Despite the advances in molecular elucidation of the vaginal microbiota, the exact pathophysiologic pathway has not yet clearly identified.

Microbiota analysis of HPV positive patients and patients with CIN revealed significant alternations compared to that of those without the aforementioned diseases. Certain microbiotic species found in the vagina have been associated with increased risk of infection from HPV and can serve as a critical predictive and prognostic marker for the early detection of those pathologies. In that setting, a significant proportion of *Lactobacillus* species have been documented to be protective against HPV whereas Sneathia species can negatively affect the evolution of HPV. This is in accordance with respective studies in the field which showed that decrease in *Lactobacillus* species in vaginal flora, had significant

impact on eubiosis and led to increase in concentration of pathogenic anaerobic bacteria such as *Gardnerella* and *Sneathia* (37). Furthermore, remission and severity of HPV infection were additionally influenced by the presence of certain microbiotic species at the vaginal environment. Moreover, it strongly seems that dysbiosis protection and HPV infection are related to each other, even though the pathophysiology of such event is not yet fully understood. Hence, strong suspicion has been formulated that the associated mechanisms, not only encompass the patient's defensive functions but also her past immunological response against HPV (38). The potential impact of dysbiosis on immunological system could explain the susceptibility of those women to HIV infections (39).

The association between ethnicity and HPV with regards to the vaginal microbiota has not yet been clearly determined. Our study also revealed that Afro-Caribbean ethnicity was associated with alterations in vaginal flora. HPV incidence was not significantly different in Afro-Caribbean women with dysbiosis. On the contrary, according to a recent meta-analysis, Africa presented higher rates of HPV infection compared to other ethnicities (40). Nonetheless, research on those populations is still limited and further studies are needed so as to elucidate the association among dysbiosis and HPV infection in African populations. Another factor that should be addressed is that not only flora alterations are responsible for increased susceptibility to HPV infections, but also has the presence of cancerous and precancerous cervical disorders (8). More specifically, vaginal dysbiosis has been related to cause more rapid progression in more advanced disease stages (25).

Several limitations can be found in such a newly studied field. First and foremost, the number of studies and of patients included is limited, which however, underlines the innovation of this approach. As far as our chosen search strategy, it could be considered restricted due to the exclusion of abstracts, review articles, conference papers, editorials, animal studies, as well as commentaries. The retrospective nature of the available studies could also be highlighted. Prospective randomized controlled trials are necessary to clarify the possible correlation of vaginal microbiota with HPV and the related pathologies.

Conclusion

Rapidly emerged data suggest a potential association between vaginal microbioma and HPV infection. Specifically, (i) highly diverse vaginal flora, (ii) identification of specific species such as *Sneathia*, (iii) low concentration of *Lactobacillus* and the subsequent vaginal dysbiosis were found to affect the incidence, persistence and severity of HPV infection. The aforementioned parameters should be subjected to further investigation via multicentre trials in order to be established as valid independent risk factors.

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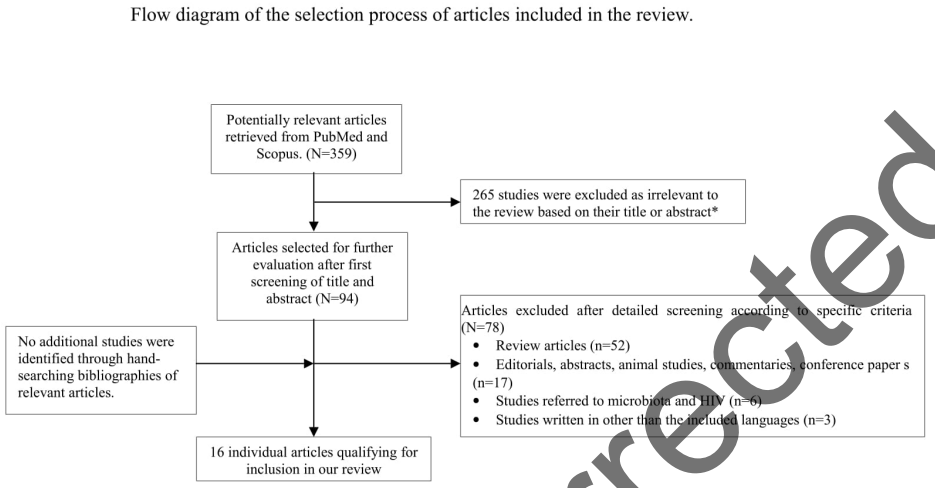

Fig. 1: Flow diagram of the selection process of articles included in the review.

Table 1. Summary of the techniques applied in the included studies.

First author, year [ref]	Patients included	HPV identification method	Detection method of microbioma
Dols et al, 2012 [16]	100	INNO-LiPA® method	microarray technology
Rodriguez-Cerdeira, 2012 [17]	208	Digene Hybrid Capture® II DNA Test hrHPV probe B cocktail	microscopically, microbiological culture
Clarke, 2012 [18]	9,165	Hybrid Capture® Tube test PCR: MY09/MY11 L1 primer with AmpliTaq Gold polymerase	vaginal pH measurement with pHydrion strip Detection of C.trachomatis using a C.trachomatis PCR-DEJA assay
Gao, 2013 [19]	70	PCR: 1) MY09/MY11 and then 2)GP5+/GP6+ primers	V2-V3 region of the 16S rRNA genes amplified by PCR BLAST database searches to identify bacterial species
Lee, 2013 [20]	68	PCR: 1) MY09/MY11 2) GP5+/GP6+ primers	V2-V3 region of the 16S rRNA genes amplified by PCR Data analyzed using QIIME
Borgdorff, 2014 [21]	174	cervical cytology	microarray containing 461 DNA hybridization probes targeting microorganisms and 164 positive (16S conserved regions) and negative controls

Brotman, 2014 [22]	32	Roche Linear Array® HPV Genotyping Test	pyrosequencing V1-V2 hypervariable regions of 16S rRNA genes using primers barcoded 27F and 338R
Oh, 2015 [23]	120	Digene Hybrid Capture® II DNA Test	V1-V3 region of the 16S rRNA genes amplified by PCR Data analyzed using EzTaxon-e and BLASTn diversity indices calculated using Mothur program
Dareng, 2015 [24]	278	Roche Linear Array® HPV Genotyping Test	V4 region of the 16S rRNA genes amplified by PCR (primers: 515F, 806R) Data analyzed using Illumina® MiSeq
Mitra, 2015 [25]	169	Abbott RealTime High Risk hrHPV® assay on Abbott M2000® platform	V1-V2 region of the 16S rRNA genes amplified by PCR Data analyzed in Mothur program using Illumina® MiSeq
Audirac-Chalifair, 2016 [26]	32	HPV testing technique: Seegene Anyplex™ II HPV HR Detection assay NGS technique: 16S rRNA gene regions: V3–V4	high throughput sequencing of 16S rDNA amplicons and classification in community state types (CST)
Piyathiyake, 2016 [27]	430	Roche Linear Array® HPV Genotyping Test	V4 region of the 16S rRNA genes amplified by PCR Data analyzed using Illumina® MiSeq

Reimers, 2016 [28]	64	PCR: MY09/MY11/HMB01 L1 primer	V1-V2 region of the 16S rRNA genes amplified by PCR (primers 27F,338R) Data analyzed using QIIME
Menon, 2016 [29]	616	Riatol HPV Test	microscopically, candida:10% KOH method bacterial vaginosis: Nugent criteria
Adebamowo, 2017 [30]	194	SPF25/LiPA10 for hrHPV genotypes	V3-V4 region of the 16S rRNA genes amplified by PCR Data analyzed using Illumina® MiSeq
Kero, 2017 [31]	329	PCR: 1) MY09/MY11 2)GP5+/GP6+ primers, genotyping with Multimetrix® kit	Hay/Ison criteria used for defining bacterial vaginosis as grade I, II or III
Di Paola, 2017 [32]	72	Hybrid Capture 2 assay, followed by genotyping by amplifying the target DNA with PGMY09/11 and HLA primers	V3-V5 region of the 16S rRNA genes amplified by PCR, diversity measured using OTUs, Quantitative Real Time PCR of sialidase-encoding gene from Gardenella vaginalis
Shannon, 2017 [33]	59	Roche Linear Array® HPV Genotyping Test	V3-V4 region of the 16S rRNA genes amplified by PCR Data analyzed using Illumina® MiSeq, coinfection diagnostics: microscopically, Nugent criteria, nuclei acid amplification test, HerpeSelect gG-1 ang gG-2 ELISA

Di Pietro, 2018 [34]	35	cervical cytology	V3-V4 region of the 16S rRNA genes amplified by PCR, Data analysed using Illumina® MiSeq

Abbreviations: hrHPV = high risk HPV, PCR = polymerase chain reaction, ELISA = Enzyme-linked Immunosorbent Assay, OTU = operational taxonomic unit, HLA = human leukocyte antigen, rRNA= ribosomal RNA, QIIME = Quantitative Insights Into Microbial Ecology, BLASTn = Basic Local Alignment Search Tool of Nucleotide-nucleotide, NGS = Next-generation sequencing.

Table 2. Demographics of the populations in the included studies.

First author, year [ref]	Patients included	Mean age (in yrs)	Ethnicity/race (%)	Specific population characteristics
Rodriguez-Cerdeira, 2012 [17]	208	27	Spanish	female sex workers
Clarke, 2012 [18]	9,165	41.8	Costa Rica	NM
Gao, 2013 [19]	70	HPV (+): 37.8 HPV (-): 37	Chinese	NM
Lee, 2013 [20]	68	siblings:43 mothers:65	Korean	twins and their families
Brotman, 2014 [22]	32†	37	African American (50) Caucasian (40)	vaginal douching cessation study
Oh, 2015 [23]	120*	range: 18-65	Korean	NM
Dareng, 2015 [24]	278	HPV (+): 34.2 HPV (-): 37.9	Nigerian	included pts with HIV
Mitra, 2015 [25]	169	31	Caucasian (83)	NM
Piyathiyake, 2016 [27]	430	26.1	non-Hispanic black(53)	NM
Menon, 2016 [29]	616	28	Western Kenyan	female sex workers

Reimers, 2016 [28]	64	32.1	African American	included pts with HIV
Adebamowo, 2017 [30]	194	38	Nigerian	NM
Kero, 2017 [31]	329	NM	Turkish	pregnant, asymptomatic women
Di Paola, 2017 [32]	55§	range: 26-64	Italian/Caucasian	NM
Shannon, 2017 [33]	59	HPV (+):33 HPV (-):37.5	African/Caribbean	NM
Di Pietro, 2018 [34]	35	healthy: 34.7 C.trachomatis: 28 HPV + C.trachomatis: 29.3 HPV: 35.8	Italian	NM

Abbreviations: yrs = yrs, NM = not mentioned, HPV = human papillomavirus, HIV = human immunodeficiency virus, pts = patients,

* 70 with CIN versus 50 control.

† Has been received 937 samples.

§ 55 HPV (+) versus 17 control.

Table 3. Protective and burdening parameters of vaginal microbiota regarding HPV infection/persistence.

PROTECTIVE	BURDENING
L.gasseri L.jensenii L.crispatus Barrier contraception Estrogen levels → protection for dysbiosis	Sneathia spp. Reduction of Lactobacillus Anaerococcus tetradius Peptostreptococcus Fusobacterium Gardnerella vaginae L.iners + unclassified lactobacillus CST IV Vaginal dysbiosis

Abbreviations: CST = community state type