# What influence does vaginal microbiota have on *Human Papillomavirus* infection?

## *Que influência a microbiota vaginal tem sobre a infecção por Papilomavírus humano?*

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**ABSTRACT**

**Introduction:** The human body is colonized by trillions of microbial cells, called the microbiota. The microbiome is defined as microbial cells and their genomes. Vaginal microbiota, especially lactic acid bacteria (mainly *Lactobacillus* sp.), seem to play a critical role in the prevention of various urogenital diseases such as bacterial vaginosis, fungal infections, sexually transmitted diseases, urinary tract infections, and human papillomaviridae (HPV) infections. Changes in the vaginal microbiome contribute to the development of precancerous cervical lesions. **Objective:** To evaluate studies associating the vaginal microbiota with HPV, including the risk and persistence of infection and evolution to squamous intraepithelial lesions of the cervix. **Methods:** A systematic review was conducted based on articles published between September 2011 and September 2019, using the following keyword combinations: "HPV [All Fields] AND "microbiota" [MeSH Terms] OR "microbiota" [All Fields] OR "microbiome" [All Fields])." The keyword search was performed in the MEDLINE, Latin American, and Caribbean Literature in Health Sciences (Lilacs), Cochrane Library, Highwire Stanford, and Embase databases. **Results:** In total, 239 original articles published between 2011 and 2019 were found in the researched databases on microbiome/microbiota and HPV. After exclusion, only six articles remained. **Conclusion:** There is a relationship between HPV and the cervicovaginal microbiota, but the mechanism of this influence cannot be specified.

**Keywords:** cervix uteri; microbiota; cervical intraepithelial neoplasia.

**RESUMO**

**Introdução:** O corpo humano é colonizado por trilhões de células microbianas, denominadas microbiota. Microbioma é definida como células microbianas e seus genomas. A microbiota vaginal, especialmente as bactérias produtoras de ácido lático (principalmente *Lactobacillus* sp.), parece desempenhar um papel crítico na prevenção de várias doenças urogenitais, como a vaginose bacteriana, infecções fúngicas, doenças sexualmente transmissíveis, infecções do trato urinário e infecção pelo *Papilomavírus humano* (HPV). As alterações no microbioma vaginal parecem contribuir para o desenvolvimento de lesões cervicais pré-cancerosas. **Objetivo:** Avaliar estudos que associem a microbiota vaginal ao risco de infecção por HPV, sua persistência e evolução para lesões escamosas intraepiteliais do colo do útero. **Métodos:** Esta é uma revisão sistemática desenvolvida com base em artigos publicados entre setembro de 2011 e setembro de 2019, usando as seguintes combinações de palavras-chave: "HPV" [Todos os Campos] AND ("microbiota" [Termos MeSH] OU "microbiota" [Todos os Campos] OU "microbioma" [All Fields]) nas bases de dados MEDLINE, Latin American and Caribean Health Sciences Literature (Lilacs), Cochrane Library, Highwire Stanford e Embase. **Resultados:** Entre 2011 e 2019, foram encontrados 239 artigos originais nas bases de dados pesquisadas sobre microbioma/microbiota e HPV. Desse total, após o uso dos critérios de exclusão, restaram apenas seis artigos. **Conclusão:** Existe uma relação entre o HPV e a microbiota cervicovaginal, mas não foi possível especificar qual mecanismo está envolvido.

**Palavras-chave:** colo do útero; microbiota; neoplasia intraepitelial cervical.

## INTRODUCTION

The human body is colonized by trillions of microbial cells, referred to as microbiota. Microbial cells and their genomes are defined as the microbiome. The scientific community has already established that there are considerable differences in the microbiota between individuals, body regions, and even over time in the same individual[1-3].

Efforts such as the Human Microbiome Project are attempting to elucidate the variations found in healthy adult microbial communities. It is currently uncertain whether these differences, especially those observed in many states of illnesses, will be a consequential or a contributing factor. Defining a healthy microbial state, however, is a critical step in determining the associations between the variations and diseases[1,4,5].

Vaginal microbiota, especially lactic acid bacteria (mainly *Lactobacillus* sp.)[6], seem to play a critical role in the prevention of various urogenital diseases, including bacterial vaginosis, fungal infections, sexually transmitted diseases, urinary tract infections, and *Human papillomaviridae* (HPV) infections[7-9].

HPV infections are very prevalent and are often sexually transmitted[10]. More than 200 genotypes have already been identified and classified into two main groups: high and low oncogenic risk[11-13]. The infection can be latent without associated lesions, a productive infection with visible lesions, or, in the middle of this spectrum, an infection that sometimes integrates into the host cell genome to cause subclinical lesions, detectable only by magnification methods such as colposcopy and microscopy[6,14-16]. With the persistence of oncogenic HPV, the expression of viral oncoproteins results in cellular transformation and morphological disorders identified as high-grade squamous intraepithelial lesions (HSIL). Untreated HSIL can evolve into invasive diseases. These HPV-mediated cellular changes are

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morphologically similar to the squamous epithelia of the lower anogenital tract in both genders[6,17].

The Lower Anogenital Squamous Terminology Project suggests unified histopathological nomenclature with a single set of diagnostic terms for all pre-invasive squamous lesions. The HPV-related squamous lesions of the lower anogenital tract are either low-grade squamous intraepithelial lesion (LSIL) or HSIL. Eventually, a tumor marker ($p16^{ink4a}$) is necessary to determine the final diagnosis[18,19].

Many changes in the vaginal microbiome have been shown to contribute to the development of pre-cancerous HPV-associated cervical lesions[6]. The chronic cervical inflammation due to cervicovaginal pathogens may be linked to persistent HPV infection[14-16]. The immunosuppressive action of bacterial vaginosis may also facilitate infection and progression to lesions[17-19]. At the same time, as a significant proportion of bacterial species has been deemed protective, vaginal dysbiosis could negatively affect the evolution of HPV infection[3,7,16].

## OBJECTIVE

The objective of this systematic review was to evaluate studies associating vaginal microbiota and HPV regarding the risk of infection, persistence of infection, and evolution to squamous intraepithelial lesions of the cervix.

## METHODS

To carry out this systematic review, an extensive search was conducted for articles published between September 2011 and August 2019 in the MEDLINE, Latin American, and Caribbean Literature in Health Sciences (Lilacs), Cochrane Library, Highwire Stanford, and Embase databases. A search in the PROSPERO database for any previous systematic review on the subject yielded no records.

The search was performed using the following keyword combinations: "HPV [All Fields] AND ("microbiota" [MeSH Terms] OR "microbiota" [All Fields] OR "microbiome"[All Fields])." The listed word combinations were entered in the databases above.

Articles were selected using eligibility criteria. Priority was given to original articles published in English, Portuguese, and Spanish that studied the relationship between HPV and vaginal microbes. Review articles excluded were those with different themes from the proposed objective, in languages other than those mentioned above, or published before 2011.

Initially, studies were selected from the title. A detailed analysis of the abstracts was then carried out to restrict the selection to those that met the inclusion criteria. All selected articles were found in the search, as shown in Figure 1.

## RESULTS

In total, 239 original articles published between 2011 and 2019 were found in the databases dealing with microbiome/microbiota and HPV. After exclusion, only six articles remained (Table 1).

## DISCUSSION

The vaginal microbiome has been considered an important defense against infections. Studies among women of different ethnicities, including Caucasian, African American, Hispanic, and Asian reported that most vaginal communities could be defined by the presence of a dominant species of *L. iners, L. crispatus, L. gasseri*, or *L. jensenii*[20].

The contributions of the vaginal microbiome to genital health have not yet been disclosed[20]. To investigate, the microbiome composition of 396 American women of different ethnicities was characterized by 16S rRNA gene pyrosequencing[20]. The communities were grouped into five groups in the dominant order: *Lactobacillus iners*, *L. crispatus*, *L. gasseri*, and *L. jensenii*, respectively, while the fifth had a lower proportion of lactic acid bacteria and a higher proportion of non-lactic acid organisms[20].

Another study assessed the association of HPV and cervical microbiota in 87 samples collected from several black women of reproductive age and HIV-negative status. Only 23 women (26.4%) had cervical microbiota dominated by a single species of *Lactobacillus* (*L. crispatus*, *L. jensenii*, and *L. iners*). Most women had diverse cervical microbiota, consisting mainly of bacteria associated with bacterial vaginosis (BV)[21].

Events leading to disturbances in the local microbiota could be associated with HPV-induced carcinogenesis. One study evaluated the vaginal microbiome of 250 women by sequencing the 16S ribosomal DNA using a high-performance assay. Analysis of the bacterial classes revealed that the cervixes of healthy women were characterized by *Lactobacillus crispatus, Lactobacillus iners,* and *Lactobacillus taiwanensis*; however, *Gardnerella vaginalis* and *Lactobacillus acidophilus* were absent[5]. The study results indicated that the development of HPV-induced cancer is associated with high diversity in the vaginal microbiome, which is involved in the control of viral persistence and, therefore, is indicative of disease prognosis[22,23].

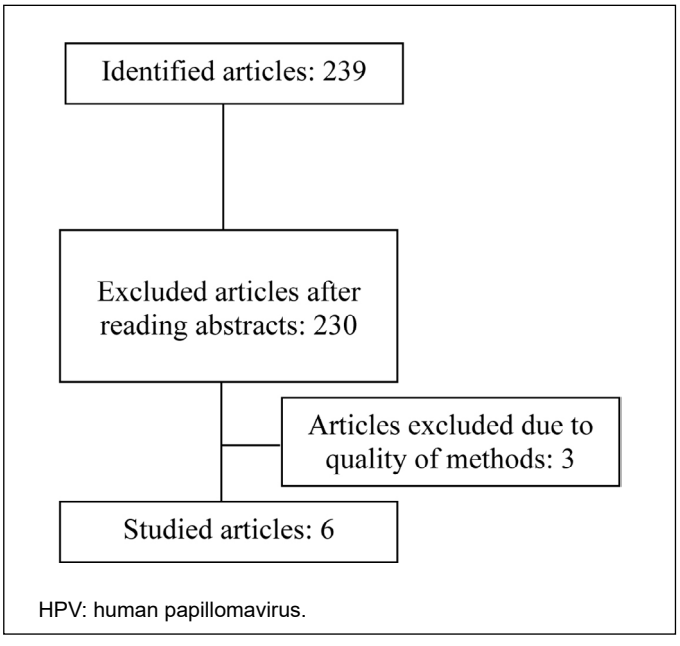


**Figure 1 –** Flowchart of the selection of articles studying microbiome/microbiota and HPV in the databases between 2011 and 2019.

**Table 1 –** Studies published between 2011 and 2019 on the microbiome and its relationship with HPV.

| Study | Year of publication | Study design | Women (n) | Analysis method | Results |
|---|---|---|---|---|---|
| Ravel et al.[20] | 2011 | Non-randomized study group | 396 | Pyrosequencing. | It showed alterations in the microbiota and its relationship with genital tract pathologies. |
| Onywera et al.[21] | 2019 | Non-randomized study group | 87 | Illumination sequencing of the hypervariable regions V3-V4 of the 16S rRNA bacterial gene. | Women with HR-HPV had a significantly higher relative abundance of Aerococcaceae, Pseudomonadaceae, and Bifidobacteriaceae compared to those with LR-HPV or without HPV infection. |
| Zhou et al.[22] | 2019 | Non-randomized study group | 62 | Amplification of the 16S rRNA gene and IlluminaMiSeq sequencing. | The diversity of the vaginal microbiome was more significant. The composition was different with LR-HPV infection. |
| Kwasniewski et al.[23] | 2018 | Randomized study group | 250 | Cervical microbiome swabs were collected from 250 women. 16S ribosomal DNA was sequenced using a high-performance assay. | The development of HPV-induced cancer is associated with high diversity of the vaginal microbiota, which is involved in controlling viral persistence. |
| Klein et al.[24] | 2019 | Non-randomized study group | 144 | Amplification of the hypervariable region V4 of the 16S rRNA gene, followed by deep sequencing. | There was a greater bacterial richness in patients with high-grade squamous intraepithelial lesions. |
| Mortaki et al.[25] | 2019 | Meta-analysis, systematic review | 1,218 | 16S rRNA analysis was used to characterize vaginal microbiome, including microarray and polymerase chain reaction/gel electrophoresis with denaturing gradient. | There is greater microbiome diversity and lower abundance of lactobacilli among HPV-positive women. |

HR: high risk; LR: low risk; HPV: human papillomavirus.

There is an intimate relationship between HPV and genital cancer, as well as other pathologies. More recently, the close relationship between the local microbiota and the installation and development of HPV has been added to this large study pool[24].

To investigate the role of the bacterial microbiome in cervical intraepithelial lesions, samples were taken from the cervical lesions of 144 women. The hypervariable V4 region of the 16S rRNA gene was amplified and sequenced in depth, revealing greater bacterial richness in patients with HSIL than in those without lesions. The most abundant bacteria associated with HSIL were *Mycoplasmas*, *Pseudomonas*, and *Staphylococcus*[25].

It is still unclear why some HPV infections resolve clinically, while others persist, causing squamous intraepithelial lesions and cervical cancer. Several risk factors for HPV infection, including smoking, sexual and reproductive factors, and immunosuppression, such as an infection by HIV, have been identified. Women with a specific vaginal microbiota composition may be more likely to acquire HPV or to show a faster progression of dysplasia, thereby requiring more attention[24].

# CONCLUSION

This systematic review determined that there exists a relationship between HPV and the cervicovaginal microbiome; however, it was not possible to specify the route by which such regulation occurs. Well-designed research is still needed to unravel this relationship, just as there is a need to discover whether such a relationship of HPV happens in other body microbiomes.

## Participation of each author

Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work: José Eleutério Jr., Rafael Pereira de Vasconcelos, Marina Mara Sousa de Oliveira and Renata Mírian Nunes Eleutério.

Drafting the work or revising it critically for important intellectual content: José Eleutério Jr., Paulo César Giraldo and Ana Katherine Silveira Gonçalves

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## Conflict of interests

The authors declare that there is no conflict of interests.

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