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Review

Aerobic Vaginitis: is *Enterococcus faecalis* Another Risk Factor in the Progression of Cervical Intraepithelial Neoplasia to Cervical Cancer—Literature Review

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Abstract

Objective: The aim of our review article is to show *Enterococcus faecalis* (*E. faecalis*) as a risk factor of cervical cancer. **Mechanism**: Aerobic vaginitis (AV) is the absence of a balance of vaginal flora containing aerobic and intestinal pathogens, varying degrees of vaginal inflammation and immature epithelial cells. The causes of AV responsible for inflammatory changes are: *E. faecalis*, *Esherichia coli*, *group B streptococcus* and *Staphylococcus aureus*. The pathogenic effect of aerobic microorganisms such as *E. faecalis* shows that it causes spontaneous abortion, premature birth, puerperal sepsis, abscesses, and urinary tract infections. **Findings in Brief**: AV caused by *E. faecalis* is more common in low-grade and high-grade cervical intraepithelial neoplasia (CIN) than in women with a normal Pap test and is thought to contribute to the progression of cervical cancer. *E. faecalis* produces hydrogen superoxide which causes chromosomal instability in intestinal epithelial cell infection, which is considered a key factor in the carcinogenic process. The presence of the Human papilloma virus (HPV) 16 gene and genome in *E. faecalis* in cervical cancer biopsy material leads to a specific link that may be a risk factor in the progression of CIN toward cancer. The presence of HPV in this bacteria can lead to persistent HPV infection, CIN development and progression to cervical cancer. **Conclusions**: Increased vaginal pH, lactobacilli deficiency, and the ability of *E. faecalis* to contain HPV-16 in episomal form in *E. faecalis*-induced AV could be key promoters of persistence and proliferation of cervical HPV as a risk factor in development of CIN and cervical cancer.

Keywords: aerobic vaginitis; *E. faecalis*; HPV-16; cervical intraepithelial neoplasia

1. Introduction

Aerobic vaginitis (AV) is a lack of balance of the vaginal flora and was first described in 2002 by Donders *et al.* [1] and is characterized by abnormal vaginal flora containing aerobic and intestinal pathogens, varying degrees of vaginal inflammation and immature epithelial cells [2].

The prevalence of AV varies from 5 to 10.5% in symptomatic non-pregnant women and 4 to 8% during pregnancy [2], although some studies report an incidence of 2.9 to 23.7% and an increased risk for sexually transmitted diseases [3,4]. Donders *et al.* [2] found moderate or severe AV in 11% of women at a routine gynecological examination in Kampala, Uganda. Vieira-Bapista *et al.* [5] found AV in 7.4% Portuguese women during a routine Pap test. Fan *et al.* [6] found AV in 23.7% women. Most studies across Europe, Asia, and Africa report a frequency about 7% to 13% of women who are not pregnant [2].

The causes of AV that are responsible for inflammatory changes are: *E. faecalis*, *Escherichia coli*, *group B streptococcus and Staphylococcus aureus* [3–5]. The most common isolated pathogen of AV is *E. faecalis* in 32% [7]. The pathogenic effect of aerobic microorganisms such as *E. faecalis* has been shown to cause spontaneous abortion, premature birth, puerperal sepsis, abscesses, and urinary tract infections [6].

AV in pregnancy is associated with pregnancy complications, partially increases the risk of preterm birth, premature rupture of membranes and increases the risk of postpartum complications [3,8,9], is more common in cervical intraepithelial neoplasia (CIN) than in women with an orderly Pap test and is thought to contribute to the progression of cervical cancer [10–12].

A study of cervical-vaginal flora variations in Human papilloma virus (HPV)-positive women conducted in Peru in 2017 found a higher incidence of *E. faecalis* in high-risk HPV-positive women compared to low-risk HPV-positive women [13]. The presence of the HPV-16 gene and genome in *E. faecalis* in cervical cancer biopsy material, as well as the ability for HPV-16 genes to be translated and transcribed in these bacteria, and that the HPV gene can form viral particles in these bacteria leads to certain links that may be a risk factor in the progression of cervical lesions to cancer [11,12]. *E. faecalis* positive AV is very often unrecognized or ignored and may be the reason for a neglected diagnosis.

E. faecalis isolated from cervical cancer biopsy is considered as the microbiological flora of the vagina and cervix

that may cause vaginal and cervical infection [12]. If AV is undiagnosed or neglected and the woman is HPV positive, there is a justified possibility of long-term infection due to the presence of HPV in *E. faecalis*. The presence of HPV in this bacteria can lead to persistent HPV infection, CIN development and progression to cervical cancer.

This review describes the vaginal ecosystem and the role of *E. faecalis* in vaginal infection, and its role as a possible risk factor together with high risk HPV in the development of cervical intraepithelial neoplasia and cervical cancer.

2. Vaginal Ecosystem

The ecosystem of the vagina is a complex and dynamic system consisting of: multilayered squamous epithelium, vaginal transudate and endogenous flora of various microorganisms.

2.1 Vaginal Microflora

The vaginal microflora is not a static population of microorganisms but is in a dynamic state in which the number and type of microorganisms continuously change with the changes that occur in the vaginal environment. The common and dominant microflora of the vagina consists of bacteria of the genus Lactobacillus, designated as Döderlein bacilli. There are over 170 species of lactobacilli that vary considerably in size and shape [14,15]. During pregnancy, the number of lactobacilli increases, the number of anaerobic bacteria decreases. This physiological increase in lactobacilli from early pregnancy to birth enables the protection of the fetus from pathogenic microorganisms [16].

During life, a heterogeneous biofilm is formed on the vaginal mucosa [17]. More than 50 microorganisms with a predominance of anaerobic versus aerobic participate in the formation of normal vaginal microflora [18,19]. A number of microorganisms that form the normal vaginal microflora also participate in the formation of the normal microflora of the gastrointestinal tract, oral and nasal cavities such as *Lactobacillus sp.*, *Actinomyces sp.*, *Streptococcus mutans* and saprophytic treponema [20]. In addition to the presence of lactobacilli, vaginal secretions taken from healthy women by cultivation show an increase in other microorganisms: *Staphylococcus spp.*, *Streptococcus agalactiae*, *Enterococcus spp.*, Gram-positive anaerobic cocci, and other anaerobic bacteria, diphtheroids, other potentially pathogenic bacteria [14,16,19]

2.2 Vaginal Ecosystem Control Mechanisms

2.2.1 Acidic Environment (Lactic Acid Production)

Vaginal environment is a special ecosystem that has its own self-protection—an acidic environment that prevents the colonization of the vagina by pathogenic bacteria. The acidic environment in the vagina is essential for maintaining a healthy vaginal condition and this pH value has been shown to have microbicidal activity for many sexually transmitted diseases including Human immunodeficiency virus (HIV) [16,21]. Lactic acid is the primary product responsible for the acidity of the vagina. The role of lactobacilli in maintaining the low pH value of the vagina is undoubted, but their role in the self-protection of the vaginal ecosystem is much more complex because they participate in other mechanisms of vaginal self-protection. The importance of low pH of vaginal secretions is in slowing down the growth and reproduction of a larger number of pathogenic bacteria [22,23].

In 1997 Cailloette *et al* gave new importance to determining the pH value of the vagina, by taking the pH of vaginal secretions as a marker and suggesting that elevation of vaginal pH in the range of 5.0–6.0 should be associated with the presence of pathogens or decreased estradiol [24]. pH values below 4.2 virtually rule out bacterial vaginosis, but pH values above 6.0 strongly indicate genital tract infection and are more useful for assessing the condition than determining the type of secretion because they necessitate confirmatory microbiological tests [25].

2.2.2 Peroxidase System

The production of hydrogen peroxide by some species of bacteria of the genus Lactobacillus is one of the mechanisms that helps regulate the vaginal environment and is a major factor in the homeostasis of the vaginal ecosystem [15]. This mechanism is based on bacterial antagonism [26]. Hydrogen peroxide producing lactobacilli can be considered as a representative of non-specific antimicrobial defense of the vaginal ecosystem because the formed Hydrogen peroxide (H_2O_2) has a toxic effect on microorganisms from the vaginal environment, especially those that do not have catalase enzyme [18,21,24].

2.2.3 Production of Bacteriocin-Like Substances

Bacteriocins are low molecular weight peptides that are synthesized by bacteria of one species with a strong inhibitory effect on the growth of bacteria of another species [27]. There are numerous papers that show that a large number of lactobacilli synthesize these substances, inhibiting the growth of a large number of gram-positive, gram-negative bacteria and fungi [28]. Scientists have shown that certain substances belonging to bacteriocins-like substances show *in vitro* activity against the uropathogenic *Escherichia coli* and *E. faecalis* [20,29].

34 species of lactobacilli were found to adhere better and more strongly to the intestinal, vaginal and uroepithelium than most pathogens [19]. Only in 1995, it was determined that 15 species of lactobacilli produce a biosurfactant that adheres to the epithelial cells of the mucosa and prevents the initial adhesion of *E. faecalis* by 70% [26,29]. Lactobacilli interfere with pathogenic microorganisms in at least two ways. First, they competitively exclude genitourinary pathogens from receptors present on the surface of the genitourinary epithelium. Second, lactobacilli co-

aggregate with most uropathogenic bacteria, releasing antibacterial components such as hydrogen peroxide, lactic acid, and bacteriocin-like substances that inhibit pathogen growth [17].

New bacteriocin-like substances produced by vaginal *Lactobacillus salivarius subsp. salivarius* with activity against *E. faecalis*, *Enterococcus faecium* and *Niseriae gonorrhoeae*, have a role in the prevention of urogenital infections [30].

Lactobacillus acidophilus produces lactic acid that inhibits the growth of the uropathogenic *Escherichia coli* [31]. Thus, thanks to their antagonistic and adherent abilities, microorganisms of the genus Lactobacillus play a major role in maintaining the positive balance of this ecosystem and preventing the occurrence of infection [29,32].

2.2.4 Competition in Adherence

The precondition for the colonization of the vagina by bacteria is that they adhere to the epithelial cells of the vaginal wall. Bacterial colonization of the vagina involves the adhesion of bacteria to the membranes of vaginal cells [33,34]. Adhering to vaginal epithelial cells, lactobacilli, as the dominant vaginal flora, prevent the binding of other bacteria. It is believed that lactobacilli for vaginal epithelial cells adhere with the help of proteins, carbohydrates, and possibly with the help of lipoteichoic acid [26,31]. In the case of lactobacilli, the phenomenon of self-aggregation has been described, which implies the colonization of lactobacilli in the form of a bacterial film on the vaginal epithelium, due to which colonization by pathogenic microorganisms cannot occur [20,26]. Thus, thanks to their antagonistic and adherent abilities, bacteria of the genus Lactobacillus play a major role in maintaining the positive balance of this ecosystem and preventing the occurrence of infection [35].

The formation of the vaginal microflora - biofilm depends on many factors that exert selective pressure on the vaginal mucosa during life [15,17]. These factors can be exogenous or endogenous. They can lead to the creation of disorders in the vaginal microflora and imbalance between different organisms. This imbalance can be manifested by a change in the normal vaginal environment and the emergence of vaginal infection or vaginitis [17].

3. *E. faeclis* Positive AV

3.1 Prevalence

Data on the prevalence of AV in the general population are very scarce, the frequency of *E. faecalis* in healthy women in 6% was found by Jahić in her 2007 doctoral dissertation [36], while research in Spain shows a frequency of 4% *E. faecalis* in healthy pregnant women [22].

3.2 Etiology

E. faecalis is a gram-positive microorganism, round or oval, immobile, without capsules 0.8–1.2 pm in diameter. It is divided in one plane and can be seen in Gram-stained preparations on a microscopic field in the form of individual oval cocci that form pairs, shorter chains and groups [37]. Until 1984, it was classified in group D of streptococci according to the Lancefield classification of streptococci. However, in 1984, it was classified into the genus Enterococcus using the DNA-DNA hybridization method. With this classification, *S. faecalis* was renamed *E. faecalis* [38] and *E. faecalis* and *E. faecium* are the most important for human pathology. From the material for microbiological examination in human infections, *E. faecalis* is isolated in 80% and *E. faecium* in 20% [34,39,40].

E. faecalis can be isolated from various samples taken from humans. It is regularly isolated from the stool sample where it makes up the normal microbiological flora of the stool in amounts of 10^8/g and from healthy skin where it is found as a contaminant [41]. However, it can be isolated from sputum, nasal swabs, urine, blood, cerebrospinal fluid, wound swabs, vaginal and cervical swabs, conjunctival swabs, and various punctures [37]. It grows on enriched nutrient media such as blood agar, brain-heart agar, bile-esculin agar, KF streptococcal agar at a temperature of 37 °C, however, it grows at a temperature of 10 to 45 °C. It is extremely tolerant to high temperatures, salt concentrations and changes in the pH of the environment [37,38,42].

3.3 Pathogenesis

Enterococci normally colonize the gastrointestinal tract of humans. It is found in relatively large quantities in the faeces. It can be transferred from the gastrointestinal tract in different ways to other places. It can contaminate healthy skin and can colonize the vaginal mucosa and anterior urethra of women and men [38]. However, if it enters the systemic circulation it can lead to various diseases [43]. Colonization of the mucous membrane is possible with a large number of factors such as: natural microflora, their pH and natural cleansing [44]. Immunoglobulin A (IgA) is significantly reduced in the vaginal mucosa during colonization by various bacteria [44,45]. It is likely that *E. faecalis* in these places is exposed to other factors by the organism such as age, immune status, hormonal status, influence of various antibiotics, etc. [37]. The basic mechanisms of pathogenicity of this microorganism are enabled: ability to colonize mucous membranes second, to produce pathological changes in the host by its toxic activity by inducing an inflammatory process and to avoid the host's immune defense mechanisms [43].

Colonization of the vaginal mucosa with *E. faecalis* is also enabled by the reduced number or disappearance of lactobacilli, natural colonizers of the vaginal mucosa [38]. These microorganisms can prevent the colonization of the vaginal mucosa with *E. faecalis* and other microorganisms in various ways. The use of broad-spectrum antibiotics or spermicidal ointments leads to a reduction in the number or destruction of lactobacilli and thus allows the growth of the

vaginal mucosa with *E. faecalis* and/or other bacteria [39]. Colonization of the mucosa with *E. faecalis* is conditioned by its natural as well as acquired resistance to various antibiotics and disinfectants. Therapy with drugs that have a limited effect on *E. faecalis* is one of the main predisposing factors for the occurrence of colonization and infection with these conditionally pathogenic bacteria. Therapy with metronidazole and cephalosporins to which *E. faecalis* is resistant causes overgrowth and, ultimately, bacteraemia and infection with this pathogen [6,46].

E. faecalis produces cytolysin (hemolysin) which facilitates infection by its cytolytic action on cells [40]. It hemolyzes the erythrocytes of humans, sheep, rabbits, and cattle. Hemolysin also lyses some gram-positive microorganisms [37,47]. The aggregative substance participates in the adhesion of *E. faecalis* to cells of the urogenital tract and renal epithelium [26,48].

Different strains of *E. faecalis* differ in virulence factors. Those strains isolated in patients with endocarditis, sepsis, or urogenital infections usually have all or almost all of these virulence factors (hemolysin/cytolysin, aggregative, gelatinase), while strains isolated from the gut usually do not have them [37,38]. This suggests that some mechanisms present in the host organism also play a role in enhancing the virulence of *E. faecalis*. Many strains of *E. faecalis* produce superoxide and hydrogen peroxide [49]. A comparison of those isolated from bacteraemia and those that are normal commensals in human intestines showed that strains isolated from the blood produce superoxide far more often (60%) [47,50]. H_2O_2, a small, uncharged molecule capable of freely diffusing across the cell membrane, can activate several signaling pathways, including the epidermal growth factor receptor (EGFR) pathway. The EGFR signaling pathway is a chief regulator of cell proliferation in various cell types, including epithelial, endothelial, and fibroblastic cells. Abnormal transactivation of the EGFR has been described in the development and prognosis of malignancies. Hydrogen-peroxide is known to cause chromosomal instability in intestinal epithelial cell infection, which is a key factor in the carcinogenic process [50].

Virulent properties of *E. faecalis* include adhesion to host tissue, ability to invade and abscess formation, resistance to antibacterial agents, modulation of host defense capacity, and secretion of cytolysin and other toxic products [38].

Propagation of *E. faecalis* from the vaginal mucosa to the uterus, fallopian tubes and ovaries can lead to inflammation of these organs. This microorganism can cause pelvic abscesses and bacteraemia as complications after caesarean section, endometritis or salpingitis [51]. Also, *E. faecalis* can propagate to the urethra and ascendantly lead to inflammation of the urethra, bladder, and renal pelvis [9,45]. Vaginitis caused by *E. faecalis* in HPV-16 positive women, if left untreated, can persist for a very long time and thus provide conditions for persistent HPV infection, which are the prerequisites for the development of precancerous cervical neoplasia [12]. Recently, its frequent presence in cervical cancer, oral cancer and colon cancer has been increasingly reported [13,52,53].

4. *E. Faecalis* Positive AV and HPV in the Vaginal Flora

HPV infection is a necessary factor in the development of cervical precancerous neoplasia and cervical cancer [52], and the presence of inflammation increases the risk of cancer [54]. In recent years, an increasing number of studies have stated that abnormal vaginal flora plays an important role in the development of CIN [13,14]. Very little is known about the role of *E. faecalis* positive AV and precancerous lesions. Plisko [11] recently investigated the association of abnormal vaginal flora including AV and histological findings of CIN and found that AV was significantly more common in subjects with high lesions (CIN II) than low lesions (CIN I). She also states that in women with cervical pathology there is a significantly higher pH value of the vaginal environment associated with CIN change, as well as the association of severe AV with HPV-induced lesion [55,56]. Reducing the number of lactobacilli in vaginal secretion reduces the ability to defend and change the pH value of the vaginal environment, which favors the development of bacterial inflammation. *E. faecalis* positive AV leads to a change in vaginal pH above 5, and elevated vaginal pH in HPV-positive women may be associated with [56].

In a letter to the author, Donders and Viera Bapista [57] comment on the work of Jucar Maria and the strong association between bacterial vaginosis and HPV infection and find that AV is a higher predictor of CIN lesions in 889 Portuguese women than bacterial vaginosis [58]. AV has elevated vaginal pH and decreased lactobacilli, which are key promoters of HPV proliferation in the cervix [58,59]. It has been proven by biochemical methods that in AV there is a decrease in the vaginal succinate content, which is an increase in interleukin-6, in contrast to the condition in developed bacterial vaginosis [57].

New evidence suggests that the vaginal microflora plays an important role in cervical carcinogenesis [60]. Epidemiological studies have revealed an association between different lactobacilli-free vaginal microflora and HPV infection [61]. Reports from three studies involving women with cervical dysplasia and cancer showed lactobacilli deficiency and a significant increase in the diversity of vaginal microflora in women with CIN and cervical cancer compared to healthy women [60]. A significant increase in vaginal pH was also found, which was associated with the severity of CIN and a decrease in the number of lactobacilli [62].

Microbial pathogens cause tumorigenesis in a high percentage of about 20% of cancers [63,64]. Microbial dysbiosis is thought to contribute to epithelial barrier damage as well as reprogram immune and metabolic signaling, in-

cluding other factors such as inflammation, regulation of cell proliferation and apoptosis, promoting genome instability, and affecting cell stability [65].

Based on the genome and whole transcript sequencing studies examined in the Cancer Genome Atlas (TCGA) for microbial readings, unique microbial signatures in tissue and blood have been found in many cancer types and therefore an oncological diagnostic tool based on microbiome and it becomes clear that there is a link between different types of cancer and specific microflora [63].

Specific bacteria or dysbiotic bacteria cause damage to the epithelial barrier, immune dysregulation and genotoxicity, and create a tumor permissive microenvironment [65].

There are three main mechanisms by which a microbiome can trigger the growth and development of cancer:

(1) Causing Deoxyribonucleic acid (DNA) damage and promoting mutagenesis,

(2) Initiation of oncogenic signals,

(3) Impaired immune response system [63].

It is believed that microbial dysbiosis can promote tumorigenesis in organs such as the skin, oral cavity and genital system. The reason for such induced carcinogenesis is found in the stimulated altered host response to dysbiotic microflora [66]. Studies in mouse models of colon and liver cancer have shown that antibiotic treatment and bacterial-free status leads to a significant reduction in the number of cancers. Thus, a study in 2019 showed that transplantation of fecal material in patients with colorectal cancer into bacteria-free mice caused lesions and epigenetic changes characteristic of the development of malignancy [60]. This report is strong evidence of the effects of dysbiotic intestinal flora that promote tumor in several malignancies, especially colorectal cancer, but similar to gut and microbial dysbiosis can promote tumorigenesis and other organs inhabited by microorganisms such as lungs, skin, oral cavity and urogenital system [66,67]. Furthermore, the carcinogenic potential of intestinal bacteria leads to an increase in IL-6 and tumor necrosis factor (TNF), activation of signal transducers and transcriptional activators 3 (STAT3), and activation of IL-17-IL-23 pathways. Together, all of these factors can contribute to tumor development and progression by promoting inflammation. Members of the urogenital flora, especially associated with genital dysbiosis, certainly use similar biological mechanisms that can contribute to the development and progression of gynecological cancers [60].

In a mice study of AV in 2021, Shazadi [68] stated that *E. faecalis* is the cause of histological changes in cells in the form of cell damage, increased cell thickness and peeling. In his research on the pH value of the vagina, Jahic states that under the influence of *E. faecalis* there is an increase in the pH of the vaginal environment above 4 with a decrease in the number of lactobacilli [36].

Vieira-Baptista also finds a link between abnormal AV type vaginal flora and cervical precancerous neoplasia where he cites a significantly higher association between AV but not bacterial vaginosis with abnormal Pap test cytology compared to normal cervical cytology [52,57]. Severe forms of AV with HPV-induced precancerous cervical neoplasia could play a common and crucial role in the progression of the neoplasia to invasive cancer. Indeed, one of the critical mechanisms of HPV carcinogenesis is inflammation, which shows an increase in inflammatory interleukins in the progression of CIN [69].

A dominant pathogen in AV such as *E. faecalis* may reduce the protective effect of lactobacilli causing inflammation as well as an increase in interleukin (IL)-6, IL-8 and TNF, increasing the risk of HPV-16 infection resulting in CIN and cervical cancer [69–71]. In the study of cervical cancer, the presence of genes and genomes (except E1) of human papilloma virus (HPV) type 16 was found in bacteria such as *E. faecalis* and *Staphylococcus aureus* from cervical cancer biopsy [12]. High-risk HPV DNA is usually integrated into high-grade squamous intraepithelial lesions (CIN III) and invasive cervical cancer [71]. The DNA integration profile of the virus is different in cervical cancer depending on the type of virus; for HPV-16, episomal and integrated forms may coexist in cancer cells [59].

It is unknown why some women develop persistent cervical HPV infection. So far, it has been shown that the reduction of lactobacilli combined with the diversity of the vaginal microflora is associated with HPV persistence [72]. HPV infection leads to loss of lactobacilli, destroying the biological barrier of the local vaginal immune microenvironment, promoting abnormal adhesion of HPV in the vagina, causing local microecological imbalance in the vagina and destroying local immune functions of the cervix while increasing adhesion, invasion and colonization. This leads to a vicious circle in the vaginal environment and further development of HPV infection, which causes a lesion of the cervix.

Whether long-term infection with *E. faecalis* may be a risk factor that supports persistent HPV-16 infection (*E. faecalis* may contain HPV-16 in episomal form) is still an open question with some indications in favor of this observation. Modulation of the microflora in terms of intensive AV treatment can encourage the elimination of *E. faecalis* and thus the removal of HPV and reverse the natural history of HPV infection. An understanding of the functional properties of *E. faecalis* positive AV and HPV is needed to supplement what we already know about their interaction, and its impact on the development of CIN. Association of E. faecalis with different forms of cancer through reviewed studies was shown in Table 1 (Ref. [12,36,50,73]).

5. Treatment of *E. Faecalis* Positive AV

Recommended treatment for AV includes a combination of therapies such as: antibacterial (antiseptic and antibiotic), hormonal, nonsteroidal anti-inflammatory drugs and/or probiotics, which may be administered as local or systemic therapy [2].

Table 1. Association of *E. faecalis* with cancer through reviewed studies.

Carcinoma	Reference	Number of samples	*E. faecalis* detected
Precancerous lesions of cervical carcinoma	Jahic M [36]	$N_{ASCUS} = 15$	In ASCUS *E. faecalis* was detected in 7/15 (46.7 %)
		$N_{LSIL} = 12$	in LSIL in 5/12 (41.7%)
		$N_{HSIL} = 2$	in HSIL 1/2 (50%) cases
Cervical carcinoma	Ma Zhenghai, *et al.* [12]	N = 14 (cervical cancer biopsies)	*E. faecalis* was HPV-16 positive in 12/14 (85.7%)
Oral cavity	Boonanantanasarn, *et al.* [50]	$N_{case} = 20$	In oral cavity samples 20/20 (100%)
		$N_{control} = 20$	In control samples 5/20 (25%) ($p < 0.0001$)
Colorectal	Khodaverdi, *et al.* [73]	$N_{case} = 40$	In colorectal cancer tissue 34/40 (85%)
		$N_{case} = 40$	In control tissue 25/40 (62.5%) ($p = 0.005$)

There is no generally accepted clinical strategy for the treatment of *E. faecalis* positive AV. Han and co-workers suggested that therapy be based on microscopic or microbiological findings using a local antibiotic for an infectious agent, a local steroid to reduce inflammation, and estrogen to treat atrophy [46].

E. faecalis positive AV can be treated with ampicillin, a combination of ampicillin and aminoglycosides such as gentamicin or spectinomycin [46]. In her work on the treatment of *E. faecalis* and *Escherichia coli* positive AV, Jahić 2009 found a successful elimination of symptoms and causes in 72%, using nitrofurantoin 500 mg once daily for 6 days of application [74].

Sangeetha *et al.* [7] found about 40% of isolated Enterococci resistant to penicillin and ampicillin, while only 10% showed resistance to aminoglycosides. In her research in 2007, Jahić found the highest sensitivity of *E. faecalis* to vancomycin in 100%, nitrofurantoin 94.23%, doxycycline 90.38%, ampicillin 86.65% and gentamycin 88.46% [36].

It is very important to know that AV cannot be treated with metronidazole as bacterial vaginosis is treated, because it will not cure but will lead to increased infection, due to the natural resistance of *E. faecalis* to this drug [46]. If it is a pregnancy, it is better to opt for clindamycin. Fluoroquinolones such as ciprofloxacin and ofloxacin can be used in the treatment of AV because they have little effect on the normal vaginal flora leading to rapid recovery from AV [17,46].

In a large number of women with severe atrophy (microscopic examination of a large number of parabasal cells — more than 10%), topical application of estrogen is very useful in postmenopausal or premenopausal women [24].

It has recently been noticed that probiotics (Lactobacillus acidophilus) can improve the condition of vaginal flora after AV targeted antibiotic therapy [27,35]. Also some studies state that vaginal probiotics can be valuable for preserving and restoring the vaginal microflora. The direct use of vaginal probiotics such as Lactobasillus may reduce the rate of HPV infection and thus contribute to a new approach to establishing homeostasis of the vaginal mucosa [31,35].

Topical application of corticosteroids can be used when inflammation (over 20 leukocytes per epithelial cell) predominates with antibiotic therapy against an isolated microorganism [2].

Substantial treatment should result in the establishment of homeostasis of the vaginal environment where Lactobacilli play a key role. In essence, this could mean several steps towards establishing this balance that depend on several factors. Some of them are: type of HPV cause, type of cause of inflammation, degree of inflammatory change, duration of inflammation, hormonal status, immune status of the woman and stress component.

6. Conclusions

The presence of *E. faecalis* positive AV has been associated with Sexually transmitted diseases (STD)s and an increased risk of abnormal Pap tests in patients infected with HPV type 16.

Several mechanisms may explain the pathogenesis and pathology of *E. faecalis* positive AV, including decreased lactobacilli protection, increased inflammatory cytokine response, the presence of opportunistic or pathogenic bacteria, and increased risk for cervical cytological abnormalities in high-risk HPV-infected women.

Increased vaginal pH, lactobacilli deficiency, and the ability of *E. faecalis* to contain HPV-16 in episomal form in *E. faecalis*-induced AV could be key promoters of cervical persistence and proliferation of cervical HPV as a risk factor in development of CIN and cervical cancer.

The ability of *E. faecalis* to carry and contain HPV-16 opens up a field of risk factors for CIN and cervical cancer.

7. Limitation

Although this paper shows a link between *E. faecalis* positive AV and HPV infection, it is important to note that most papers that were used and cited offer little data. Therefore, further research with more data is needed.

Author Contributions

MJ and AC designed the research study. MJ and AC performed the research. MJ and AC analyzed the data. All authors contributed to editorial changes in the manuscript. All authors have read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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