Chapter 5

Bacterial Vaginosis and Sexually Transmitted Diseases: Relationship and Management

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Additional information is available at the end of the chapter

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Abstract

In the last few decades, bacterial vaginosis (BV) has become an emerging pathology; its relationship with pregnancy, pelvic inflammatory disease (PID), infertility, preterm delivery, and neonatal small for gestational age are well established. BV substantially changes vaginal microbiome and these modifications could facilitate sexually transmitted infections (STIs). Several studies have reported an association between abnormal vaginal microbiota, in particular, BV and depletion of lactobacilli species, and increased risk of sexually transmitted infections (STIs) acquisition. Immunologic, enzymatic, and metabolic mechanisms could operate independently or in combination to enhance STIs' transmission. Several studies have pointed out this association: vaginal microbiome modifications in BV could predispose to sexually transmitted diseases (STDs). Considering the high social impact of BV together with its relationship with STDs, it seems to be "crucial" to restore vaginal microbiome in childbearing age women in order to reduce STIs acquisition. Some experimental clinical data seem to confirm this observation: vaginal microbiome restoration by probiotics/synbiotics seems to improve not only STIs' acquisition but also STDs' pathology progression. Restoring vaginal microbiome could represent an international, innovative, and less-expensive gold standard to counteract STDs' spread and acquisition.

Keywords: bacterial vaginosis (BV), sexually transmitted diseases (STDs), vaginal microbiome, probiotics/synbiotics, *Lactobacillus*

1. Introduction

The infections of the human reproductive system include sexually transmitted diseases (STDs) that are defined as "infection that spreads primarily through person-to-person," and non-STDs that are "endogenous infections of the genital organs such as "bacterial vaginosis" (BV) [1].

Both STDs and non-STDs are a major concern for public health system worldwide [1].

Sexually transmitted diseases (STDs) are the most frequently "unrelieved" diseases in the United States [2]; its prevalence is high all over the world, especially in the United States where about 12 million new cases occur each year [2].

Surprisingly, STDs represent a public health problem also in the developing countries, being the second cause of health loss in childbearing women [2].

STDs have a lot of health-related consequences between women, adolescent, and children, particularly in ethnic/racial minority group: In U.S. a lot of women (more than a million) experienced an episode of pelvic inflammatory disease (PID) per year: considering that PID represents an important health consequences of STDs and that 15% of the infertile U.S. women experienced a tubal inflammation related to PID, it seems obvious to ascribe to STDs as playing a leading role in women health [2].

Also, the relationship between untreated STDs and pregnancy are well known: neonatal pneumonia, neonatal ophthalmia, mental and physical developmental disabilities, and fetal death related to syphilis are the more impacting consequences of untreated STDs [2].

Unprotected sexual encounters and having multiple sexual partners, together with higher biologic aptness, are the leading causes of STDs in adolescents between 10 and 19 years, which seems to be the higher risk category among all age groups [2].

Bacterial vaginosis (BV) is a clinical syndrome resulting from the replacement of normal hydrogen peroxide-producing lactobacillus species in the vagina with high concentration of anaerobic bacteria such as *Gardnerella vaginalis* and *Mycoplasma hominis* [3].

BV is the most prevalent form of vaginal infection among women of reproductive age, affecting 8–23%, and is the most common etiology of vaginal symptoms prompting women to seek medical care [4].

It does not appear to be a sexually transmitted disease, although it has been associated with having multiple sex partners [3].

It is the most common cause of vaginal discharge or malodor and is commonly encountered in the context of STDs [3].

Since the relationship between BV and STDs is not well established, the aim of this chapter will be to describe the role of BV in determining STDs and to point out how BV management could improve STDs' epidemiology and prevalence.

2. Vaginal microbiome

There are approximately 10 times as many microbes associated with a human as there are human cells in the body [4]: despite recognition of the importance of the interactions between the host human body and the bacteria it supports, there remain many unanswered questions

regarding how the microbial environment varies with and among individuals in healthy and diseased states [5, 6].

Historically, bacteria have been identified using Gram stain or culture-based techniques but, unfortunately, as few as 20% of bacteria closely associated with the human body can be cultivated via culture-based techniques [6].

Culture-based methods may therefore underestimate the diversity of microbiome [5, 6].

During the past decade there has been an explosion of interest in molecular-based, culture-independent techniques to study the microbiome [7–14].

Molecular-based techniques involve analysis of 16S ribosomal RNA (rRNA), DNA hybridization, or fingerprinting and next-generation sequencing [7–14].

The National Institute of Health, recognizing the potential of molecular techniques to further understand human bacterial communities, initiated the Human Microbiome Project (HMP) in 2007 [15].

The HMP targeted the genitourinary system because it has been well established for more than a century that bacteria are present within the vagina and that an imbalance within this microbial environment may be associated with disease [16–19].

Research has demonstrated that alterations in the vaginal microbiome affect susceptibility to gynecologic infections, including cervicovaginitis, postoperative infections, and human immunodeficiency virus (HIV) infection, but also that many of the differences in the vaginal microbiome may represent normal variation and may not necessarily indicate disease [20–24].

However, despite evidence from both culture-dependent and independent methods supporting the dynamic nature of the vaginal microbiome, both methods suggest that the microbiome is relatively stable through periods of hormonal fluctuation, such as puberty or the menstrual cycle [20–24].

It can be partially confirmed by the old statement of Albert Doderlein (1892): by using culture method he found that vaginal microbiome of healthy women possess a predominance of Gram-positive rods named “Doderlein lactobacillus” from his name [25].

More than 120 years later, it is universally accepted that lactobacilli are the predominant species of the human microbioma and that they have a key role in maintaining an acidic environment able to protect women against virus and bacteria responsible for opportunistic infections and STDs [26].

It was remarkable to observe that humans species are the only one with this relative abundance of lactobacilli in vagina (more than 70%), while other mammals present not more than 1% of lactobacilli in the vaginal microbiota [26] (**Figure 1**).

To date, most comparative studies in mammals find that hosts with similar lifestyles and evolutionary histories harbor similar microbiomes at a given body site, both in the bacteria taxa they contain and the functions they provide to hosts [27, 28].

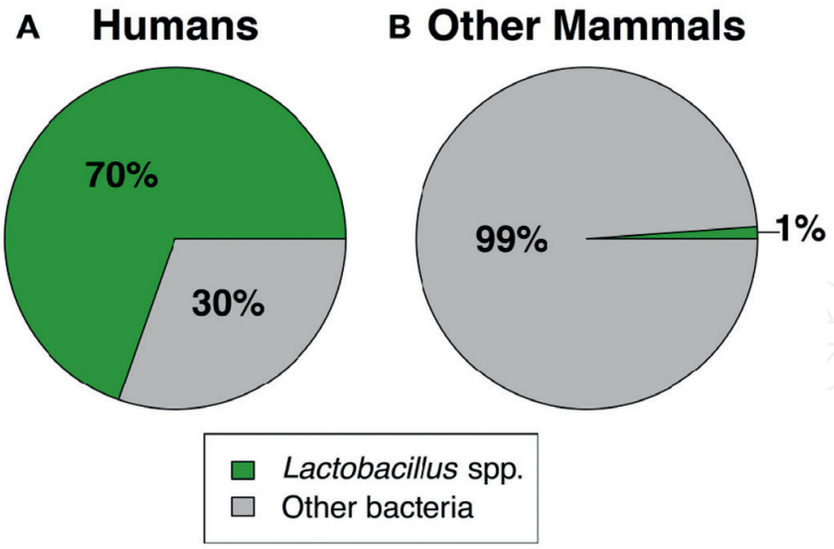

Figure 1. Mean vaginal relative abundance of *Lactobacillus* spp. vs other bacteria in humans (A) and nonhumans (B) [26].

One important exception to this pattern is the vaginal microbiome, where humans exhibit striking differences in community composition compared to other mammals; specifically, the human vaginal microbiome is dominated by *Lactobacillus* spp. [29, 30].

These lactobacilli process glycogen and it breaks down products to produce lactic acid, leading to an exceptional low vaginal pH of < 4.5 [26].

Lactobacilli dominance and low pH of the human vaginal microbiome are hypothesized to benefit women by reducing disease risk [31].

Furthermore, the loss of lactobacilli dominance is linked to bacterial vaginosis (BV), which is associated with an overgrowth of anaerobic bacteria, relatively high vaginal pH (>4.5) infertility, preterm birth, maternal infections, and increased risk of STDs [21, 30–33].

To date, four hypotheses have been proposed to explain the uniqueness of the human vaginal microbiome relative to other mammals: two mechanistic explanation and two evolutionary explanation: the first "mechanistic hypothesis" considers that the differences in vaginal microbioma between human and nonhuman mammals are related to the differences in reproductive physiology: a typical 28 days ovarian cycle of the humans is quite different from other mammals [34].

This 28 days ovarian cycle in reproductive women is orchestrated by steroids and lactobacilli abundance is strictly linked to estrogen levels [35].

The second "mechanistic hypothesis," the common function hypothesis, proposes that in nonhuman mammals, other bacteria may protect hosts via mechanisms other than lactic acid and low vaginal pH so that the presence of lactobacilli may not be a requirement for a healthy vaginal environment [34, 35].

In addition to these mechanistic hypothesis, two evolutionary explanation have been proposed, the first, referred to as the "disease risk hypothesis," proposes that humans have higher STDs risk than nonhuman mammals because species with promiscuous mating strategies are

predicted to have higher STD risk than those with only single, brief reproductive episode per breeding season [36, 37].

The last evolutionary explanation, the "obstetric protection hypothesis" suggests that selection for lactobacilli in the human vagina is due to the high risk of microbial complications associated with pregnancy and childbirth, thus lactobacilli and low vaginal pH may serve as a protective function during human birth, and these traits are unnecessary in mammals with less pregnancies and birth risks [38, 39].

An understanding of the diversity of the vaginal microbial environment during states of health and disease is essential for the identification of risk factors for disease and the development of appropriate treatment [26].

2.1. Vaginal microbiome in the healthy state

Nowadays, it is well established that the normal vaginal microbiome is dominated by lactobacilli species [16, 40, 41].

Years of research have clearly demonstrated that the vaginal microbiota represents the first barrier against obligatory or facultative pathogens in the female reproductive tract [43].

It is well known that women with low lactobacillus species in vaginal microbioma are at high risk for urogenital infective diseases and adverse pregnancy outcomes [42].

Lactobacilli help to prevent vaginal infection by producing lactic acid, hydrogen peroxide, bacteriocins, or through competitive exclusion of other bacteria [43–46].

Studies utilizing 16S rRNA PCR have demonstrated that the vaginal microbial environment is usually dominated by one or two lactobacilli species, most frequently *Lactobacillus iners*, *Lactobacillus crispatus*, *Lactobacillus gasseri*, or *Lactobacilli jensenii* [9, 47].

Of the 73% of women with lactobacilli-dominant environment, the most frequently detected organism was *L. iners*, which was the predominant organism in 34% of women sampled [9].

The second most common Lactobacilli-dominant environment was *L. crispatus* (26.2% of women) [9].

The identification of an *L. iners*-dominant microbial environment was in contrast to findings from early molecular-based and culture-dependent studies which suggested other dominant *Lactobacilli* spp, including *Lactobacillus acidophilus*, *L. crispatus*, and *L. jensenii* [48, 49].

It seems that the species of lactobacilli that dominate the vaginal environment may have implications for gynecologic health: different species may differentially predispose to dysbiosis [50, 51].

For example, it has been suggested that an *L. crispatus* vaginal microbiome is more stable and less likely to transition to bacterial vaginosis (BV) than *L. iners* or mixed lactobacilli environment [52, 53].

Culture-dependent and microscopy methods demonstrated that the composition of normal vaginal flora may also fluctuate within an individual woman: this "fluctuation" is related to the menstrual cycle or as result of sexual activities [54, 55].

During menses there is a decrease in lactobacilli and a relative increase in the proportion of bacteria associated with higher Nugent Scores [54, 55].

Recent sexual activity may also affects the microbial composition of the vagina by decreasing the proportion of the lactobacilli species present, which may predispose to dysbiosis with the loss of the protective effects of lactobacilli [53, 56].

Decreased of lactobacilli have also been observed in postmenopausal women, specifically those with vaginal dryness or atrophy [57–59].

The observed fluctuation throughout the menstrual cycle may be explained by evidence that high levels of E2 may favor a lactobacilli-dominant environment [53, 60, 61].

Evidence from culture-dependent and independent methods supported the dynamic nature of the vaginal microbiome [62–64].

A lot of studies have evaluated the vaginal microbiota in tandem by both culture-based and molecular techniques: the results demonstrate a moderate level of concordance providing similar but not identical vaginal microbiome profiles [62–64].

Also interesting is the fact that the quantity and proportion of specific microorganisms in the vagina may vary between women of different ethnic origins: African-American women may have an increased *L. iners* and decreased *L. crispatus* levels compared with Caucasian or Asian women [53].

This distinction is important because *L. iners* dominated flora may predispose to BV [53].

Molecular studies have also demonstrated that African-American and Hispanic women are more likely to harbor a vaginal microbiome dominated by bacteria other than lactobacilli species compared with Caucasian women [9, 66, 67].

These studies suggest that African-American women may have higher levels of *Gardnerella*, *Atopobium*, *Clostridiales*, and BV-associated bacterial species or be more likely to harbor a polymicrobial environment compared with Caucasian women [9, 65, 66].

Taken together, these data suggest that the differences in the microbiome between women of various races may alter woman's predisposition to infection and may at least in part explain the racial disparities in the incidence of BV and STDs [67, 68].

Concluding, vaginal microbiome in healthy women is a lactobacilli-dominated environment in which pH (under 4.5), lactic acid, hydrogen peroxide, bacteriocins, biosurfactants and co-aggregant activities counteract the growth of Gram-negative anaerobes bacteria such as *G. vaginalis*, Bacteroides, Mobiluncus spp, E. coli: when this equilibrium is broken (decreased of lactobacilli and increased of Gram-negative bacteria) vaginal pH increases and BV was detected.

2.2. Vaginal microbiome in pathologic state

2.2.1. Vaginal microbiome in bacterial vaginosis

From the beginning of 1900, the medical community accepted that a shift in the microbial environment of the vagina, specifically a decrease in "Doderlein's rods" (later identified as lactobacilli) can lead to symptomatic vaginitis with vaginal discharge [16, 40].

Subsequent studies by Gardner and Dukes demonstrated that nonspecific vaginitis (the old name of BV) was associated with a relative increase in rod-shaped bacteria on Gram stain, later identified as *G. vaginalis* [17, 69, 70].

These studies also described the "clue-cells" as characteristic of BV, resulting from vaginal epithelial cells with grainy cell borders [17, 69].

In order to implement standardized diagnostic criteria, researchers pointed out diagnostic clinical criteria (Amsel's criteria) and Gram stain criteria (Nugent scores): Amsel's criteria requires almost three of four "clinical conditions" to be present: (1) thin, white, vaginal discharge; (2) vaginal pH > 4.5; (3) "clue cells" on microscopy evaluation; (4) positive "whiff test" (10% KOH addition to sample produces fishy odor) [19, 71].

Are Amsel's criteria "Clinical Criteria"? Are we sure that pH values or clue cells or whiff test evaluation are "Clinical Criteria"?

The only "real" clinical criteria for women is "vaginal discharge" but, only taking into account TV and media campaigns for the use of panty-liners in women, it is easy to understand "the reason why" BV is underdiagnosed by gynecologists; on one side, the women feel their "vaginal discharge" like "physiological," and on the other side, vaginal symptoms are not "so urgent" for gynecological consultation.

This is the reason why BV is "a very dangerous pathology": the time from the beginning of the BV and the diagnosis is too much and, during this time, the local defense of the vagina disappeared leading, in the meanwhile, to more susceptibility to STDs and the bacteria facultative pathogen of vagina such as *Mobiluncus*, *Prevotella*, and *Escherichia coli* could determine an ascending pelvic inflammatory disease (PID) with subsequent infertility.

The Nugent score is evaluated by calculating the proportion of large, Gram-positive rods (*Lactobacilli*), small, Gram-variable rods (*Gardnerella*), and curved, Gram-variable rods (*Mobiluncus* species) on Gram stain [71].

The sensitivity and specificity of Amsel's criteria was estimated to be around 70% and 94%, respectively, and of the Nugent score were 89 and 83%, respectively [72].

Culture-dependent studies of BV demonstrate increased diversity of vaginal bacteria (including an increase in facultative anaerobes as *Gardnerella*, *Mycoplasma*, and *Prevotella*), with a simultaneous decrease of lactobacilli [73].

With an increasing use of molecular-based techniques to study the vaginal microbiome, bacteria that seemingly evaded detection using culture-based methods have now been associated with BV, including *Atopobium vaginae*, *Clostridiales*, and *Megasphaera* species [47, 66, 74, 75].

2.2.2. Vaginal microbiome in sexually transmitted infection

It seems universally accepted that increased bacterial diversity, as in BV, with the well-known vaginal ecosystem modifications, could be associated with gynecological infections, such as *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, human papilloma virus (HPV), and herpes simplex virus (HSV) 2 infections [21, 76–78].

It is unclear whether it is the altered levels of bacteria themselves that predispose to infection or whether the altered vaginal microbiome leads to BV, which predispose to these pathologies owing, for example, to altered pH (leading to less efficient neutralization of pathogen, decreased of immune response, loss of hydrogen peroxide activity).

Numerous studies have demonstrated the association between BV and an increased risk of HIV acquisition: hydrogen peroxide produced by lactobacilli is known to have viricidal activities and, consequently, the relative decrease in lactobacilli in BV women may increase susceptibility to HIV infection [23, 24, 39, 79].

A prospective cohort study evaluating the relationship between the vaginal microbial environment and infection risk, the absence of lactobacilli on culture, and the presence of abnormal vaginal flora on Gram stain were associated with an increased risk of HIV acquisition, even after controlling for risk factors [22].

Nevertheless, molecular-based techniques and culture methods confirmed that vaginal microbiome in STDs is usually modified and different from women in healthy status and that the absence of lactobacilli on culture and the presence of abnormal vaginal flora on Gram stain were associated with an increased risk of STDs.

2.2.3. Vaginal microbiome in upper genital tract infection

The vaginal microbiome modifications affect vaginal health, and pathology may also predispose to upper genital tract infection, such as pelvic inflammatory disease (PID) [79].

Subclinical PID (histological evidence of endometritis) was detected in 15% of women with BV diagnosed by clinical and Gram stain criteria [79].

Women with vaginal samples (Gram stain and culture) positive for "BV-associated bacteria (BVAB)" (*Gardnerella*, *Mycoplasma*, anaerobic Gram-negative rods, and Ureaplasma urealyticum) were at increased risk for PID [80].

Since isolation of BV-associated bacteria in the vagina has been demonstrated to increase the risk of sexually transmitted infection acquisition, the correlation of BV with PID may be related to altered vaginal flora that predisposes to STIs and subsequent ascending infection [76, 77].

Available data from upper genital tract structures confirm that BV-associated bacteria can be isolated from upper genital tract.

In a study on 89 women affected by acute salpingitis (45 with pathology and 44 as control group) 16SrDNA detected bacteria in the fallopian tubes of 24% of cases and none of controls [81].

There was a statistically significant difference in the proportion of upper genital tract microbiome based on race: African-American and Hispanic women were more likely to harbor an upper genital tract microbiome dominated by a nonlactobacilli species compared with Caucasian, and this is in contrast with those of vaginal microbiome.

The reasons of this "discrepancy" are not clear and, probably, future acquisitions on molecular-based techniques may facilitate to better understand these differences.

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3. Bacterial vaginosis

Bacterial vaginosis is a polymicrobial clinical syndrome resulting from replacement of the normal hydrogen peroxide producing Lactobacillus spp in the vagina with high concentration of anaerobic bacteria (e.g., *Prevotella* sp, *Mobiluncus* sp, *G. vaginalis*, *Ureaplasma*, and *Mycoplasma*) [10].

BV is a common vaginal condition among U.S. women and also worldwide, especially in childbearing age women [79].

A recent analysis of the National and Nutrition Examinations Surveys demonstrated that almost one-third of women were positive for BV [80].

BV is almost three times more common among black than white women [12], and it has been correlated with particular sexual behavior such as young age at coitarche, life time number of sex partners, a recent history of multiple sex partners, and a recent history of new sex partner [80–83].

The reason for the higher prevalence of BV among black women is unknown but the relationship between BV and race is remarked also from the number of the percentage of black infants born preterm in U.S. (17.5%) vs 10.2% of white infants [82].

BV could account for as much as 30% of the racial difference in premature birth and infant mortality [82].

Other risk factors for BV seem to be "vaginal douching" and use of intrauterine device (IUD) for birth control so that these practices must be limited in women of childbearing age [83].

BV seems not to be a self-limiting pathology since it has been consider a predisponent factor for PID, infertility, PPROM, preterm delivery, and neonatal small for gestational age [82, 83].

Since a lot of cross-sectional and prospective cohort studies have found that BV is associated with acquisition of both HIV and sexually transmitted infections, it seems to be almost interesting to fully understand this vaginal pathology to better clarify its role in determining STDs.

One of the most important "practical problems" of BV is that it is a "silent vaginitis" from a symptomatic point of view.

Comparing BV with specific vaginitis (e.g., vaginal infections clearly referred to vaginal obligatory pathogen such as *Candida vaginalis* or *T. vaginalis*), it is easy to understand why BV diagnosis is underestimated by gynecologists [84] (**Table 1**).

Approximately 50% of the women affected by BV are asymptomatic [82, 83].

In addition, also considering the standard clinical criteria for the diagnosis of BV (Amsel's criteria): almost three of four "clinical conditions" are to be present: (1) thin, white, vaginal discharge; (2) vaginal pH > 4.5; (3) "clue cells" on microscopy evaluation; and (4) positive "whiff test" (10% KOH addition to sample produces fishy odor) [19]. It seems to be obvious that the only real clinical condition is the vaginal discharge that can often be perceived by women like "a kind of physiologic condition."

	Bacterial vaginosis	Candidiasis	Trichomoniasis
Symptoms	Approx. 50% asymptomatic	10–20% asymptomatic	10–50% asymptomatic
	Offensive fishy smelling discharge	Vulval itching	Offensive vaginal discharge
		Vulval soreness	Vulval itching/irritation
		Vaginal discharge (nonoffensive)	Dysuria
		Superficial dyspareunia	Rarely low abdominal discomfort
Clinical signs	Thin white homogenous discharge, coating walls of vagina, and vestibule	Vulval erythema	Vulval erythema
	Absence of vaginitis	Vulval fissuring	Vaginitis
		Vaginal discharge may be curdy (nonoffensive)	Vaginal dsicharge in up to 70% frothy and yellow in 10–30%
		Satellite skin lesions	Approx. 2% "strawberry" cervix visible to naked eye
		Vulval oedema	5–15% no abnormal signs

Table 1. Symptoms and clinical signs of bacterial vaginosis, candidiasis, and trichomoniasis (adapted from) [85].

This means that the rupture of vaginal microbiome equilibrium determining BV could frequently happened without a real alarm in women and, consequently, this long-lasting vaginal imbalance in "apparently healthy women" could bring to invalidate ascending pathologies such as PID or STDs.

- Why the presence of BV could lead to such as improving urogenital pathologies?
- Why the "natural vaginal microbiome" of healthy people could protect against vaginal infections?
- Why the presence of lactobacilli seems to be pivotal in women health?

These Gram-positive bacteria possess a lot of activities that could be useful to counteract vaginal facultative and obligatory pathogen.

It is well known that the term lactobacilli means bacteria that are able to hydrolyze sugars (especially glycogen in the vagina) producing lactic acid and other acids (e.g., pyroglutamic acid): this happened because they try to conserve the optimal vaginal pH for their survival.

Vaginal pH < 4.5 is the optimal condition for their life but it is detrimental for other bacteria such as a lot of anaerobes Gram-facultative bacteria that are commonly present in vagina, also in the healthy women, but with low vaginal concentration: once that lactobacilli decrease, we assist to an increase in vaginal pH with a consequent increase in anaerobes facultative bacteria [82–84].

Low vaginal pH seems to possess also direct antiviral and antimicotic activities related to unfavorable vaginal condition for these infective agents [82–84].

Lactic acid is a potent broad-spectrum bactericide and virucide [83].

During the process of glycogen metabolism in the vagina, hydrogen peroxynitrite is produced: this molecule possesses antiviral, antibacterial, and antimicotic properties [82–84].

In addition, lactobacilli produced a lot of bacteriocins, substances that locally possess antibacterial activities [82–84].

Lactobacilli have demonstrated to possess some cosurfactant and antiaggregant activities that could be useful in controlling vaginal anaerobes growing-up [82–87].

Last but not the least, the ability of a lactobacilli predominants spp. to modulate local immunosystem [86, 87].

Genital epithelial cells and human microbiota seems to regulate the innate immune response: so that genital tract immune response plays a key role in the etiopathogenesis and pathophysiology of BV [87].

It has been demonstrated that the derangement of vaginal microbiota modifies pathogen's susceptibility encouraging HIV shedding/replication in women genital tract and consequently leading to an increase in the transmission of HIV from female to male [86–88].

It seems that activation of Toll-like receptors (TLRs) could lead to BV-associated inflammation [87].

Recurrence of BV in HIV-infected people seems to be associated with agenetic variation in TLR4, TLR9, and TLR2 in African-American adolescents [89].

BV has also been associated with a polymorphism in TLR2 suggesting that different BV-associated bacteria (BVAB) were able to control cytokines secretion and that activation of immunity in differentiate vaginal epithelial cells was related to different bacteria: an increase in proinflammatory cytokines and in epithelial cells has been associated with the presence of *A. vaginae* while *L. iners* seems to activate receptor signaling activity: on the contrary, *Prevotella bivia* and *L. crispatus* do not possess these effects [89].

BVAB infections could result in a proinflammatory immune response that disrupts barrier functions where other microbes could elicit different responses [90].

Recently, the pivotal role of Gamma Delta (GD) cells in the innate and adaptive immune system has been demonstrated: these cells are well represented in the female reproductive tract and seems to play a key role in the vaginal epithelial barrier against pathogens [91].

The decrease in cervical GD1 cells and increase in GD2 cells among women with abnormal vaginal flora predisposes women with BV to HIV acquisition [91].

Considering the high rate of correlation between these parameters, the authors proposed to use GDT cells as markers of female genital tract vulnerability to HIV [91].

GD1 cells and GD2 cells substantially differentiates in vaginal localization and functioning: GD1 cells are well represented in mucosal tissue and play a leading role in maintaining mucosal

structure, while GD2 cells are well represented in peripheral blood and are important in maintaining humoral immunity and in the development of the immune response to pathogens [91].

Increased cervical vaginal lavages seem to increase sialic acid residues leading to an increase in sialidase levels that are associated with BV [92].

BVBlue System method has been used to measure sialidase levels and to make diagnosis of BV [93].

Mucinases, sialidases, and biofilm production seem to be related with sialidase secretion by *G. vaginalis* and *Bacteroides* spp leading to STDs [94] by disrupting the integrity of the mucosa, facilitating the adhesion of pathogens to mucins, and underlying epithelial cells [95].

The relationship between sialidases and HIV infection has recently been evaluated [95–98], as gp 120 and CD4 seem to carry sialic acid residues: sialidases administration to HIV cells has demonstrated to enhance HIV infection [9, 97] suggesting that sialic acid disruption could help virus-binding and enhanced virus transmission.

In addition, the negatively charged sialic acid molecules at the terminal ends of the O-linked sugar chains determine changes in mucosal viscosity [98, 99].

Concluding, there are a lot of activities related to lactobacilli presence in the vagina that seems to be helpful in counteracting BV and preventing BV-associated pathologies.

Treatment of BV is recommended in order to relieve vaginal symptoms and signs of infection and to reduce the risk of acquiring *C. trachomatis*, *Neisseria gonorrhea*, *T. vaginalis*, HIV, and herpes simplex type 2 infections.

Obviously, the treatment is necessary also to avoid risk of PID, infertility, and pregnancy/newborn's complication.

Center for Disease Control's (CDC) recommended regimen for B.V. is [83]:

a. Metronidazole 500 mg orally twice a day for 7 days

OR

b. Metronidazole gel 0–75% one full application (5 g) intravaginally, once a day for 5 days

OR

c. Clindamycin cream 2% one full application (5 g) intravaginally at bedtime for 7 days

Women treated with nitroimidazoles must remember that alcohol consumption should be avoided during treatment [83].

To reduce the possibility of disulfiram-like reaction, abstinence from alcohol use should continue for almost 24 hours after completion of metronidazole [83].

We also have to remember that clindamycin cream is oil-based and consequently might weaken latex condoms and diaphragms for almost 5 days after use; women should be advised

to refrain from sexual activities or use condoms consistently and correctly during the treatment regimen [83].

Since no clinical data support the use of vaginal douching for symptoms relief or treatment of BV, and since vaginal douching increases recurrence of BV, this procedure must be avoided by gynecologists [83].

CDC's alternative regimens to treat BV are:

a. Clindamycin 300 mg orally bid for 7 days or

b. Clindamycin vaginal ovules 100 mg once a day (bedtime) for 3 days or

c. Tinidazole 1 g oral route once a day for 7 days or

d. Tinidazole 2 g oral route for 5 days

Also, in this case we have to remember the same disulfiram-like reaction for nitroimidazoles so that alcohol consumption should be avoided during treatment and almost 72 hours after the completion of the tinidazole regimen [83].

For Clindamycin ovules, since an oleaginous base that might weaken latex or rubber products is present (condom and diaphragma), we have to remember to avoid condoms and diaphragms use during treatment and within 72 hours following treatment [83].

Treatment of vaginal infections requires different drugs although the recurrence rate post-treatment remains high due to adverse effects on the beneficial microbiota [86].

Metronidazole and clindamycin treatment do not prevent recurrent BV infections as the lactobacilli population is rarely reconstituted [86].

Thus, there could be clear clinical advantages for the use of biotherapeutic agents (prebiotics and/or probiotics) for treating these infections [84].

Biotherapeutic agents have been defined by McFarland and Elmer in 1995 as living microorganisms that are used to prevent or treat human disease by interacting with natural microbial ecology of the host [84].

Probiotics could though be highly beneficial in modulating the mucosal flora, maintaining the integrity of the epithelial barrier and regulating the immune response [84].

Hydrogen peroxide–producing lactobacilli have been shown to be protective against a number of bacterial infections [84].

Reid et al. observed that a vaginal application of Lactobacillus casei sub-rhamnosus was able to survive in vagina after 7 weeks of exogenous application concluding that "it was surprising and it was the first and unique observation referring to exogenously-applied Lactobacilli vaginal application" [100].

Consistently with this information, a lot of clinical trials have recently been performed by using vaginal commercial probiotics containing selected Lactobacillus rhamnosus spp [100–109].

Since the recurrence rate of BV is higher also after treatment with CDC-recommended protocols, the "probiotic vaginal approach" with *Lactobacillus rhamnosus* BMX 54 has recently been tested in controlled, randomized clinical trial for the prevention of the recurrence rates of BV [100–109].

A review on its long-term use after CDC regimen administration seems to point out on a big sample size of women that its chronic use (almost 6 months) after CDC treatment administration in women affected by BV could significantly decrease the BV recurrence rates when confronted with the simple CDC regimen [104].

Lactobacillus rhamnosus BMX 54 has also recently demonstrated to be able to control HPV infection in women affected by BV [109].

While this "vaginal approach" with selected Lactobacilli spp seems to be encouraging, to lower the recurrence rates of BV, the oral approach with probiotics seems to be ineffective for BV treatment [110].

Considering the relationship between BV and STDs, it could be useful to consider this biotherapeutics approach to prevent and control STDs.

4. Sexually transmitted diseases

The term sexually transmitted diseases (STDs) refers to many diseases and the number keeps expanding with the discovery of new pathogens (e.g., HIV) or a new route of acquisition of a known pathogen (e.g., hepatitis C) [3].

Historically, all the diseases known to be transmitted only by sexual intercourse have been classified as "venereal diseases"; Other terms, such as "sexually transmitted infections" (STIs), "sexually transmitted diseases and infections" and "reproductive tract infections" have been used [3].

All these diseases will be included in this chapter under the term "sexually transmitted diseases."

STDs have complex political, social, and public health implications, in addition to their medical significance [3].

Syphilis continues to remain an important disease in spite of the introduction, more than 60 years ago, of effective treatment such as penicillin; its rate is on the rise in men who have sex with men (MSM) in some areas of U.S. [3].

STDs still remain the most common infectious diseases in developed and developing countries [3].

Considering the availability of effective therapies and that STDs could be prevented by changing one's behavior, it is surprising that these pathologies have been on the rise in

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developed and developing countries: only the complex nature of these diseases and the complex relationship between public health and social community could explain this continuous rise [3].

In this chapter, we divided the STDs in two main categories: diseases characterized by genital ulcers and diseases characterized by genital discharge; HIV infection will not be discussed in this chapter.

4.1. Sexually transmitted diseases with ulcers

Syphilis, herpes simplex virus (HSV), and chancroid are STDs with ulcer; each of these diseases has been associated with an increased risk of HIV infection [2, 3].

Genital ulcers diseases (GUD) facilitate enhanced HIV transmission among sexual partners. In the presence of genital ulcers, there is a fivefold increase in susceptibility to HIV, and HIV-infected individuals with genital ulcer disease may transmit HIV to their sexual partner more efficiently [2, 3].

A genital ulcer is defined as a breach in the skin or mucosa of the genitalia.

Genital ulcers may be single or multiple and may be associated with inguinal or femoral lymphadenopathy.

HSV is the most common cause of genital ulcers in U.S. among young, sexually active partners, *Treponema pallidum* (syphilis) is the next common cause of GUD, while chancroid, caused by H. ducreyl, has been infrequently associated with cases of GUD in U.S. [2, 3].

In developing countries, the most frequent genital ulcer disease is represented by "chancroid" [2, 3].

Travelers or native in the tropics could present *Lymphogranuloma venereum* (LFG) by *C. trachomatis* and Granuloma inguinale by *Calymmatobacterium granulomatis*: these GUDs are endemic in tropical countries [2, 3].

The relationship between GUD and pathogens are strictly related to patient population and geographic area [2].

There is a considerable overlap in the clinical presentation of herpes, primary syphilis, and chancroid [2].

Genital herpes typically presents with multiple, shallow ulcers and bilateral lymphadenopathy [2].

Primary syphilis can usually been differentiated from genital herpes by the presence of single deep, defined ulcer with induration [2, 3].

Also from chancroids and syphilis, a difference could be done according to the presence of a painful, undetermined ulcer with a purulent base that tender to lymphadenopathy [2, 3].

The cause of genital ulcers cannot be based on clinical findings alone because it possesses only 30–34% of sensitivity: this means that diagnostic testing should be performed [3].

4.2. Sexually transmitted diseases with vaginal discharge

Vaginal discharge is a frequent gynecologic complaint, accounting for more than 10 million office visits annually in U.S. [2, 3].

The three most common causes of vaginal discharge are BV (40–50% of cases), vulvovaginal candidiasis (20–25% of cases), and *T. vaginalis* (15–20% of cases); of these vaginitis, only Trichomoniasis is a STD, while BV is a "borderline" pathology that occurs in women with a high rate of STDs as well as in women who have never been sexually active [3].

Pelvic inflammatory diseases (PIDs), Gonococcal infections (*N. gonorrhea*), and *C. trachomatis* infections are three other STDs with vaginal discharge [2, 3].

Human papilloma virus (HPV) infections are the most common viral STDs worldwide; 1% of the sexually active persons in U.S. between the ages of 15 and 49 years are estimated to have genital warts from HPV [3].

Most genital HPV infections are subclinical and are transmitted primarily through sexual contact. HPV is a double-stranded DNA virus that causes a spectrum of clinical diseases ranging from asymptomatic infection to frank malignancy. External genital warts have various morphological manifestations such as condyloma acuminata, smooth dome-shaped papular warts, keratotic warts, and flat warts [3].

Because of the well-known relationship between HPV and cervical cancer, in June 2006, U.S. Food and Drug Administration approved a quadrivalent vaccine for HPV [2, 3].

Several states have already recommended HPV prevention making HPV vaccination mandatory for middle school girls [2, 3].

5. Bacterial vaginosis and sexually transmitted diseases: relationship

Several prospective studies have reported an association between abnormal vaginal microbiota, in particular BV and depletion of lactobacillus species and increased risk of STIs' acquisition [32, 111–121].

Human papilloma virus (HPV), human immunodeficiency virus (HIV), human herpes simplex virus (HSV), and PID infections/acquisition seem to be more frequent in women affected by BV [32, 111–121].

Also, *T. vaginalis*, *N. gonorrhea*, *C. trachomatis* genital infections, and PID seem to be more frequent in women with BV or depletion of vaginal lactobacilli [32].

The vaginal microbiome has been well characterized although cultivation-based and molecular methods and data from epidemiological studies indicate that the vaginal microbiota influences and enhanced STI susceptibility [20, 32, 113, 115, 116].

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Immunologic, enzymatic, and metabolic mechanisms could operate independently or in combination to enhance STI acquisition [20, 115].

An increasing number of evidences provide a strong foundation for a biologic relationship between BV and increased STIs susceptibility.

It is well known that vaginal Lactobacilli spp fermented local sugars (e.g., glycogen) producing an acidic vaginal pH that have been associated with decreased *in vitro* activity of *C. trachomatis* and *N. gonorrhea* [32, 118, 119].

This acidic environment seems to be unfavorable also for HPV, HIV, and HPS infections [32].

Hydrogen peroxynitrite produced in vagina by Lactobacilli spp through sugar fermentation seems to be a key point for reducing risk of STIs [32].

Hydrogen peroxynitrite possesses a well-known bactericidal and virucidal activity [32].

Cervical mucus has the ability to trap pathogens but, unfortunately, this mucus barrier may be compromised by mucin-degrading enzymes such as sialidase and mucinase, which are produced by BV-associated bacteria: loss of the protective mucus provides pathogens with unhindered access to target cells, increasing epithelial binding potential [32, 114].

Sialidase also cleaves terminal sialic acids from glycoproteins, exposing other sugar on their carbohydrate side chains, which can be used as energy for bacteria [32, 116].

Several BV-associated bacteria produced indole that is used by *C. trachomatis* to overcome the bactericidal effect of interferon gamma [32].

The relationship between genital epithelial cells in the vagina and vaginal microbiota seems to strongly influence the innate immune response suggesting a pivotal role of the reproductive tract immune response in determining BV and its compliances: vaginal microbiota derangement could decrease local immunity with a consequent increase of STDs risks in the women urogenital tract [87].

Lactobacilli have historically been considered keystone species of vaginal communities in reproductive-age women [32, 87].

Lactobacilli produce bacteriocins (low molecular weight proteins) that can inhibit the growth of a variety of bacteria reinforcing the concept of reducing susceptibility to STDs [32, 87].

BV may predispose to acquisition of STDs upon exposure because local cytokine production associated with BV may facilitate the acquisition of STDs [32].

Finally, lactobacilli exhibit cosurfactant and coaggregant activities that could envelop STDs virus or bacteria so that in BV the absence of these "mechanical inhibition" could facilitate the acquisition of STDs [32] (**Figure 2**).

Concluding, BV, a worldwide common vaginal infection, which is mostly asymptomatic, could be a predisposing factor, also if asymptomatic, to STDs acquisition and then to eradicate this very frequent pathology in developed and developing countries could represent a gold standard for STDs' primary prevention.

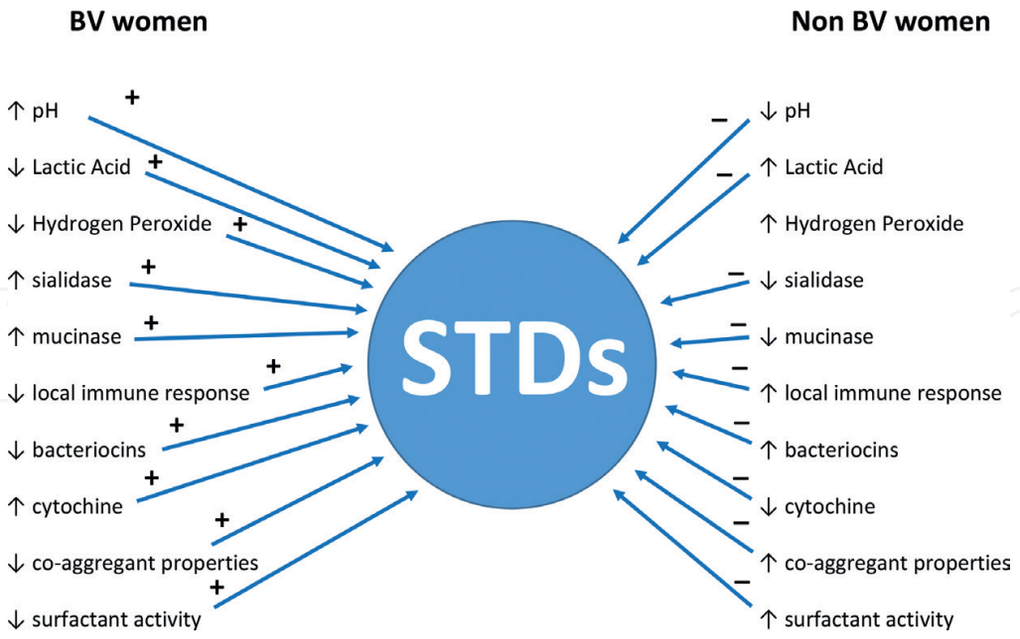

Figure 2. Differences in BV and non-BV in predisposing factors for STDs.

6. Bacterial vaginosis control for sexually transmitted diseases' primary prevention

Since BV is one of the key risk factors for STDs' acquisition, and since asymptomatic and symptomatic BV actually represents the most common vaginal infection worldwide (account for almost one-third of childbearing women), it seems obvious that BV treatment and definitive infection's eradication could be one of the most important plan for STDs' primary prevention.

Considering that BV increases susceptibility to STDs, two limiting factors are associated with BV treatment:

1. The fact that treatment for BV asymptomatic nonpregnant women is not currently recommended [118, 120–122];
2. The urgent need to develop more effective intervention for BV because the recurrence following current treatment is disappointingly high [42].

Women with an abnormal vaginal microbiota were at an increased risk of acquiring STDs compared to women with a normal vaginal microbiota; it seems that the risk of STD acquisition increased with higher Nugent score category [32].

Considering vaginal microbiome modifications as a predisposing factor for STDs acquisition, restoration of vaginal flora seems to be the crucial keystone for long-term BV treatment and, consequently, for STDs primary prevention.

Interventions that decrease the incidence and the recurrence rates of BV and promote a normal vaginal microbiota could potentially contribute to the reduction in STDs' incidence.

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Current available and recommended treatment for BV [83] as follows:

CDC's (Center for Disease Control) recommended regimens are:

a. Metronidazole tablet 500 mg oral route bid for 7 days or

b. Metronidazole vaginal gel 0.75% (5 g intravaginally every day for 5 days) or

c. Clindamycin vaginal cream 2% (5 g intravaginally every day at bedtime for 7 days)

CDC's alternative regimens to treat BV are:

a. Clindamycin 300 mg orally bid for 7 days or

b. Clindamycin vaginal ovules 100 mg once a day (bedtime) for 3 days or

c. Tinidazole 1 g oral route once a day for 7 days or

d. Tinidazole 2 g oral route for 5 days

Failure to produce sustained changes in the vaginal microbiota [113, 115, 120] clearly demonstrated that alternative regimens that improve cure rates and produce sustained changes in the vaginal microbiota are needed.

The CDC recommended therapies failed to control relapses of BV (almost 40% of recurrences rate at 3 months and 50% of relapses at 6 months), and this seems to be the most relevant problem in treating BV eradication in order to prevent STDs acquisition [104].

With >500 million new cases of STIs each year, the development of innovative strategies for STIs prevention is a global public health priority [32].

By using only Nugent score to classified and scoring BV, the relationship between BV and STDs seems to be clear: BV microbiota as gauged by Gram stain is associated with a significant elevated risk for acquisition of STDs [32].

Obviously, the Human Microbiome Project (http://nihroadmap.nih.gov/hmp/), providing also the genomic studies of the vagina, is expected to describe the structure of the complex microbial communities and how they contribute to disease susceptibility: when it will be available we will probably add more information to control BV recurrence.

Anyway, only by using Gram stain culture and Amsel's clinical criteria, which is available worldwide today, it is possible to make a BV diagnosis and to have a picture of the women "more susceptible" for STIs acquisition.

From the other side we have to manage the problem related to treatment recommendations that differentiate between women who report symptoms and those who do not; to our knowledge there are no published studies on differences in sequelae between asymptomatic and symptomatic BV [114, 115, 118].

Adverse outcomes linked to BV are probably caused by alterations in the vaginal flora that are seen in both [117, 118].

Screening and treatment for asymptomatic BV women would prevent STDs by restoring optimal vaginal flora, thus reducing susceptibility to STDs as supported by studies demonstrating a clear association between BV and an increase prevalence and incidence of STDs and HIV infection [114–118].

So the first recommendation is:

1. to treat also asymptomatic BV women in order to reduce STDs acquisition susceptibility;

However, recent largest study to evaluate the impact of treatment of BV on STD outcomes demonstrated that treatment of women with oral metronidazole does not affect the incidence of gonorrhea and chlamydia concluding that we are waiting for more effective therapies for BV [113, 119].

Standard of care for BV treatment is effective in the short term, and it is not able to restore vaginal microbiota. So by using this regimen, we obtain a clearance of BV more than a real eradication and, consequently, the long-term effect is detrimental with a high percentage (more than 50%) of recurrence after 6 months.

Nowadays, we could describe BV recurrences as a "drug-free pathology" for which every effort has to be done in order to restore vaginal pH and, obviously, to reduce the acquisition susceptibility of STDs.

If STDs acquisition is related to asymptomatic and symptomatic BV, and if standard of care seems to be unable to modify, almost in long term, STIs' epidemiology, the relationship between vaginal microflora modifications and STIs' susceptibility seem to be a key point to prevent STDs.

So that BV management in terms of restoring vaginal microflora such as in healthy women seems to be pivotal in STDs' primary prevention: taking into account that almost one-third of the women worldwide are affected by asymptomatic and symptomatic BV and that most of them are undiagnosed, untreated, or treated with the only available standard of care, BV management could represent a new/old cost-effective modality to primary prevent STDs.

Since sexual behaviors are changing year by year especially in young population, and since the percentage of sexually active girls/women that could have sexual intercourse with STDs people are increasing, we strongly believe that vaginal microbiota restoration could become the next milestone in STDs prevention.

So, the second recommendation is:

2. to restore vaginal microbiota in every sexually active women of childbearing age in order to reduce STDs acquisition susceptibility;

Since the standard of care (CDC recommended therapy) seems not to be able to restore vaginal microflora and possess a high rate of recurrences, alternative approaches are needed.

Biotherapeutic agents (living microorganism used to prevent or treat human disease by interacting with natural microbial ecology of the host) have been used to treat vaginal infections during the time [84].

Vaginal biotherapeutic agents can be divided in three classes:

1. Prebiotic (carbohydrates that topically stimulated the growth of the body's indigenous lactobacilli) [84];
2. Probiotic (living microorganisms—usually *Lactobacilli*) [84];
3. Synbiotic (a combination of the two concept) [84].

A lot of clinical trials have been done with vaginal probiotics; probiotics such as *Lactobacillus rhamnosus* GR-1, *Lactobacillus rhamnosus* Lcr 35, *Lactobacillus reuteri* RC-14, and *L. crispatus* CTV-05 taken orally or vaginally in various doses can improve vaginal flora without side effects [84].

Other strains such as *Lactobacillus rhamnosus* L60 and *Lactobacillus fermentum* L23 have been considered for probiotic development due to their specific characteristics including the production of bacteriocins, adherence properties, etc. [84].

Vaginal probiotics have been compared with vaginal metronidazole in a randomized clinical trial and the results show the superiority in terms of effectiveness for two intravaginal capsules of probiotic containing Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 taken once a day for 5 day vs 0.75% metronidazole vaginal gel applied daily for 5 days; another randomized clinical trial showed that there was no difference in BV treatment of patients administered Lactobacillus acidophilus and 0.03 mg estriol with vaginal metronidazole at 3–7 days [84].

Probiotics can be used as complementary to traditional therapies to improve the treatment of vaginal infections and to reduce recurrences of such episodes [84].

Probiotics can also be prophylactic in healthy subjects with a history of recurrent BV [84].

Unfortunately, a review published on EFSA Journal points out the ineffectiveness of probiotics for oral use in restoring vaginal microbiome [111], and another review published on Cochrane showed that probiotics clinical trials were inconsistent to demonstrate clinical efficacy of this approach in BV women [122].

The reported data on a vaginal tablet synbiotic containing *Lactobacillus rhamnosus* BMX 54 plus lactose seem to be interesting. Clinical data on a sample size of more than 800 women affected by BV and treated with standard of care (metronidazole) followed by a long-term course of this synbiotic clearly demonstrated a significant reduction in the recurrence rate of BV [101–109].

The results obtained in controlled trials [101–109] substantiated the effectiveness of the combination therapy (metronidazole 500 mg twice daily for 1 week followed by a once weekly application of vaginal tablets containing Lactobacillus rhamnosus and lactose for 6 months in preventing BV relapses, not only during the treatment time (6 months) but also during the 6-month follow-up without any treatment) (**Figure 3**).

Another controlled clinical trial performed by using the same synbiotic for 12 weeks once weekly in pregnant women vs no-treatment control group supported its effectiveness in preventing the

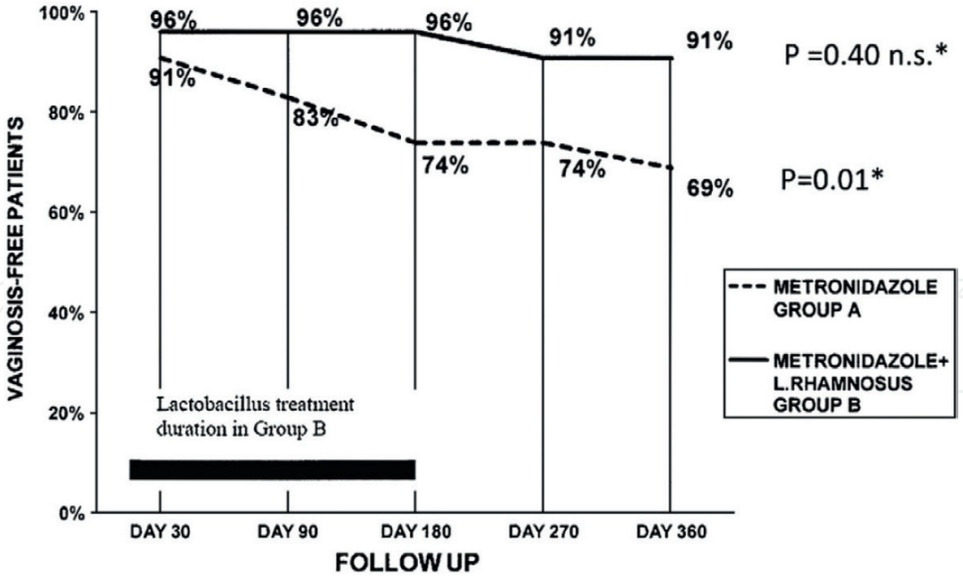

Figure 3. Trend of "vaginosis-free" patients in each group during follow-up. *A *p*-value for repeated measures in each group was considered to be significant if $p = 0.05$ [107].

development of abnormal vaginal microflora and in control of cervical parameters that could represent risk factors for preterm delivery [108].

A very long-lasting clinical trial (24 months) showed that the same combination between lactose and Lactobacillus rhamnosus BMX 54 via vaginal tablets was able to control vaginal pH in BV during the long-lasting treatment [103].

This "synbiotic vaginal approach" seems to be useful especially if administered for long time course (from 6 months to 3 years) to restore vaginal microflora and to prevent BV recurrences and mutually supported the standard of care for BV.

This probably means that the right Lactobacillus together with the right prebiotic could add complementary effectiveness vs the only therapy with vaginal probiotics resulting in an interesting option to prevent STDs' infections and acquisition.

A recent controlled clinical trial performed on 117 women affected by BV/vaginitis and associated HPV infection showed a significant decrease in HPV-related cytological anomalies (71.9 vs 36.6%: $p = 0.04$) and HPV clearance (33.3 vs 13.3%) in metronidazole or fluconazole plus *Lactobacillus rhamnosus* BMX 54/lactose long-term (8 months) treated group vs metronidazole or fluconazole plus a short-term course (2 months) of vaginal application of the same synbiotic [109].

Synbiotic vaginal tablets were administered after metronidazole or fluconazole treatment with a precise long-term schedule (once a day for 10 days, then every 3 days for a month, then once every 5 days till 2 months, and the last 6 months 1 vaginal tablet once a week) [109].

These results support the evidence from Mitra et al. [123] "there is emerging evidence which leads us to conclude that increased diversity of vaginal microbiota combined with reduced relative abundance of Lactobacillus species is involved in HPV acquisition and persistence and the development of cervical precancer and cancer."

Concluding, considering the lack of short-long term efficacy of standard of care in decreasing BV prevalence and recurrences, it seems that every effort must be done during the next years to control "microbioma modifications related to BV": selected biotherapeutic agents, using for long-term course, could be an interesting and cost-effective treatment to prevent STDs acquisition.

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