The Vaginal Microbiome in Health and Disease

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Thesis mentor: Prof. lvana Gobin, M.Sc.EPH, PhD

The graduation thesis was graded on _____ in _____, before the Committee composed of the following members:

1. Prof. Maja Abram, MD, PhD (President of the Committee)
2. Prof. Darinka Vuckovic, MD, PhD
3. Assoc. Prof. Tea Stimac, MD, PhD

The graduation thesis contains _____ pages, _____ figures, _____ tables, _____ references.

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List of abbreviations and acronyms

BV = bacterial vaginosis

CST = community state type

STD = sexually transmitted disease

VMB = vaginal microbiome

VVC = vulvovaginal candidiasis

1. Introduction

The vaginal microbiome is highly delicate and complex ecosystem. It undergoes continuous fluctuations throughout a woman's life and is influenced by Myriad factors, known and unknown alike. Keeping it in balance is of utmost importance for maintaining vaginal health, as imbalances, also known as dysbiosis, are associated with different diseases or adverse obstetric outcomes. Even if they are not always symptomatic, they still comprise the most common reasons for women to seek medical assistance.

Among these conditions, bacterial vaginosis (BV) stands out by far as the most prevalent. It is characterized by a decline in protective lactobacilli leading to an increasing pH, with a simultaneous increase in diversity of the resident bacteria. BV mainly affects women of childbearing age and can occur asymptomatically or with the typical clinical picture (vaginal malodor, discharge, and itching). Additionally, it increases the risk of contracting STDs and can lead to various complications in pregnancy. Although there are several treatment options with varying degrees of effectiveness, they often do not work in the long term and the recurrence rate is high.

The healthy vagina houses billions of microbes. This includes not only bacteria, but also other organisms such as fungi or protozoa. A higher diversity of the vaginal microbiota is linked to illnesses. Generally, the healthy microbiota is dominated by *Lactobacillus* species, producing various antimicrobials, occupying the available space, regulating the pH down and thus building an effective first line of defense against potential invaders. A decline in *Lactobacilli* and a simultaneous increase in anaerobes is known as a condition called "bacterial dysbiosis". It does not count as a disease by itself, still various illnesses can arise from it. However, as the vaginal microbiome even among women in reproductive age fluctuates and may differ greatly in between individuals, general statements are not always applicable.

The therapy, usually consisting of different antibiotics or antifungals may not always be the optimal solution to deal with BV and candidiasis respectively due to possible resistant strains, as well as biofilm formation. Furthermore, they potentially cause a disruption of the endogenous population of lactobacilli and thus could lead to a vicious cycle and the recurrence of the disease. The existing necessity for a better solution led, among other things, to the use of probiotics in maintaining the health or in restoring the normal microbiota after antibiotic therapy. However, as the results are not satisfactory yet, further research is required. [1,4,11]

2. Aims and objectives

The aim of this review is to provide an overview of the healthy vaginal microbiome, factors that influence its balance and bacterial vaginosis. Also, various strategies of diagnosis and treatment of bacterial vaginosis, with an emphasis on recent research progress will be covered.

3. The healthy vaginal microbiome

The vaginal microbiota previously regarded as relatively simple community is dynamic and complex. It changes throughout the woman's life from pre-puberty to post-menopause and is shaped even by small hormonal shifts. As it influences the women's health, reproduction, and disease, it is of great importance. During recent years our knowledge about the vaginal microenvironment greatly improved, mainly due to technologic advancements such as next-generation sequencing. The understanding of all members of the human vaginal microbiota (VMB) increased, including bacteria, viruses, archaea, fungi, and protozoa. Most available data treats bacteria and fungi. However, for full insight of its impact on vaginal health and disease classic cultivation still can prove useful as molecular techniques will not reveal its mechanism of action.

Histologically, the structure and function of the vagina appears to be similar between women. The mucosa is composed out of a stratified, squamous, nonkeratinized epithelium and covered by cervicovaginal secretions. Nutrients are acquired through diffusion from underlying tissue, as the blood supply is limited. An anaerobic environment is created, inhabited by different microorganisms that ideally coexist in a symbiotic relationship with the host.

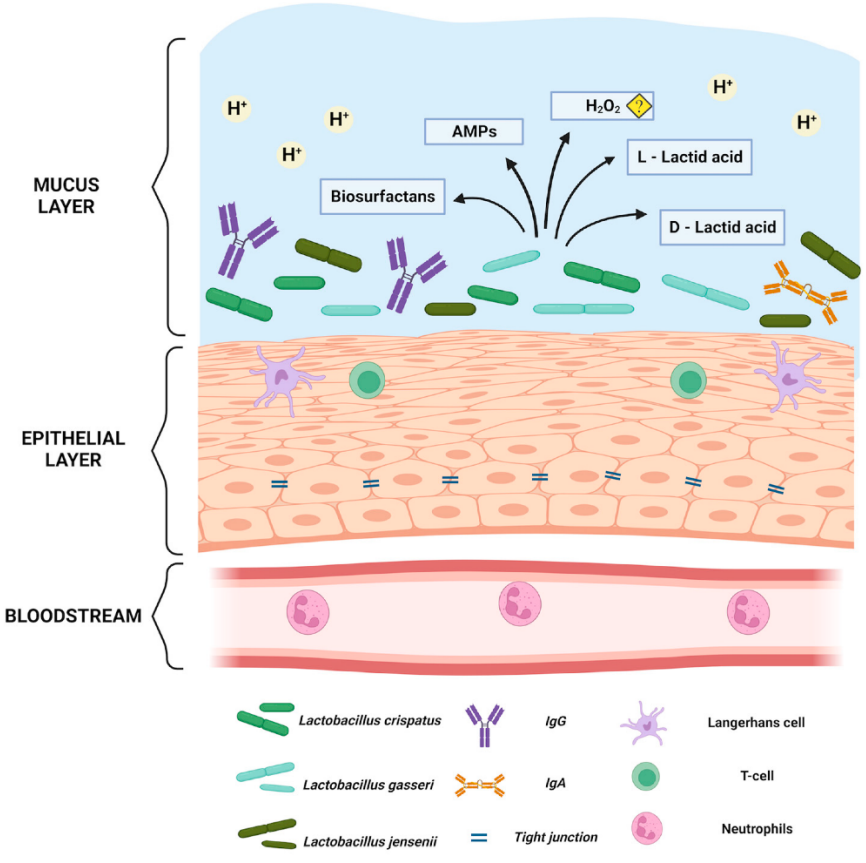

Figure 1. Microstructure of the vagina [Taken from source 12]

When we are talking about a healthy vagina, we are usually referring to one that is free from any symptoms indicating an infection. A healthy VMB is the first line of defense against pathogenic bacteria. Organisms coexisting in a mutualistic relationship with the host highly depend on the prevailing environment as well as host factors. There exist several hallmarks which are of importance. In contrast to the microbiota in the gastrointestinal system, here a low bacterial diversity should be found. Furthermore, a high occurrence of *Lactobacillus* spp. is desirable, as those bacteria play a fundamental role in the interaction with the hosts innate immune system and the general homeostasis of the VMB. To simplify a far more complex process: Lactobacilli produce lactic acid and create an acidic environment of low pH. Thus, they prevent the overgrowth of potentially harmful microbes. However, it is important to keep in mind that lactobacilli are not exclusively responsible for maintaining an acidic environment. Even women without a *Lactobacillus*-dominated microbiome can have healthy vaginal microbiota. These inhabit other lactic acid producing species such as *Atopobium, Megasphaera, Leptotrichia, Streptococcus*, and *Staphylococcus*. [13]

The host and the vaginal microbiota coexist in a symbiotic relationship. While the lactobacilli use the provided glycogen as energy source, they produce lactic acid thus lowering the pH and effectively preventing the overgrowth of potential pathogens. This can be seen through the fact that even slight changes in the pH may already predispose to diseases. During the female lifespan the VMB undergoes constant changes, initiated by hormonal changes, pregnancy or other factors.

Since the female reproductive tract is known to serve as one of the main entry points for many pathogens, host and microbiota-mediated protection is of utmost importance. The multilayered stratified squamous epithelium of the vagina is covered by dead flattened cornified cells. As they are only loosely attached, they may exfoliate and serve as decoy for pathogens. Together with the mucous produced by cervical glands they form the primary physical barrier. Additionally, the mucus may also act as chemical barrier. Vaginal epithelial cells and immune cells produce antimicrobial peptides (AMP). This includes, among others, secretory leucocyte protease inhibitors (SLPIs), elafin, calprotectin, lysozymes and defensins. For example, women suffering from an STD had lower levels of SLPIs in their vaginal fluid. The calprotectin and lysozymes produced by myeloid cells on the other hand, have direct antimicrobial properties by inhibiting bacterial growth or by degrading bacterial cell walls respectively. So do defensins, which also inhibit bacterial toxins. The AMPs are water soluble and can be delivered via the mucus. Immunoglobulins are another component of the vaginal mucus. Even though IgA

usually are the predominant isotype in mucous fluids, more IgG can be found in the vagina depending on the phase of the hormonal cycle. Their role is not yet completely clear, although there is evidence that they are involved in defense against viruses.

Lactobacillus spp. further contributes to the hosts immunity. As already described, they produce lactic acid, lower the vaginal pH and prevent the growth of pathogenic microorganism. Furthermore, lactic acid also protects against viruses and has direct antimicrobial effects.

Additionally, lactobacilli secrete hydrogen peroxide with the potential to inhibit the growth of pathogenic strains. However, as the *in vivo* measured levels are low its antimicrobial role is controversial.

It is important to note that not all species of lactobacilli are equally protective. Even though all are involved in producing other bacteriostatic and bactericidal components, *L. iners* is more readily correlated with BV. It can only produce L-lactic acid, which has less potency against uropathogens and it releases inerolysin, a cytolysin that potentially lyses eukaryotic cells including host epithelium. Furthermore, lactobacilli compete for the space and exclude competitors on this way. Other species commonly seen in non-pregnant woman are *Actinobacteria, Prevotella, Veillonellaceae, Streptococcus, Proteobacteria, Bifidobacteriaceae, Bacteroides*, and *Burkholderiales*. [14]

The composition of the VMB in humans is relatively unique, compared to other mammals. Lactobacilli for example typically dominate around 70% of the community in humans, while the percentage is much lower in other mammals, leading to a pH closer to neutral. Studies have shown that each woman has a unique vaginal microbiota, a core VMB common to all women seems not to exist. There is a high variety between individuals and over time, be it regarding to age or even regarding to the menstrual cycle. A high number of factors with direct influence on the VMB is known to us today. Among those already mentioned, there are additionally illnesses and antibiotics, lifestyle, ethnicity, diet, and genetics.

More than 20 different species of *Lactobacillus* were detected, a healthy vagina usually is dominated by 1-2 species. Ravel et al. were the first to talk about the so called “5 community state types”. The clustering of human vaginal bacterial communities was possible thanks to advanced molecular methods using 16S rRNA gene sequencing. CST I-V differ in composition, as well as in abundance. Overall CST I, II and V are most often associated with health. CST I is linked to *L. crispatus*, CST II to *L. gesseri*, CST III to *L. iners* and CST V to *L. jensenii* (Figure 2).

CST IV shows a higher diversity of species than the others and lacks significant numbers of lactobacilli. It can be further divided into subgroups, namely CST IV-A comprising mostly *L. iners* and strict anaerobes, CST IV-B with BV associated bacterial species and CST IV-C formed by facultative and strict anaerobes. CST IV-C can be subclassified again. To summarize, instead of lactobacilli, a higher number of strictly anaerobes can be seen, including bacteria like *Prevotella, Dialister, Atopobium, Gardnerella, Megasphaera, Peptoniphilus, Sneathia, Eggerthella, Aerococcus, Finegoldia*, and *Mobiluncus*. Still, CST IV is considered normal and healthy. It can be seen in around 25% of women. Bacteria capable of lactic acid production can be found. However, it should be noted, that it usually comes with a higher pH, a higher Nugent score and higher risks for STDs and preterm births. While the pH for CST I is around 4, the pH of CST IV is situated around 5.3. While CST IV is more common in black or Hispanic women, Asian and white women more commonly show CST I and II.

The role of fungi in the maintenance of a healthy vaginal microbiota is much less understood than that of bacteria. They seem to play a role in maintaining the homeostasis as well as contribute to the immune defense. There is a high diversity among the vaginal mycobiota, it is mostly formed by *Ascomycota* and *Basidiomycota*. Changes in the bacterial community leading to dysbiosis, may also lead to opportunistic fungal growth. Here the most predominant genus is *Candida*, where several different species have been detected including *C. albicans, C. krusei* and *C. tropicalis. C. albicans* is a well-known opportunistic pathogen reported in at least 20% of asymptomatic women. There are enormous variations to that number, depending on different sources and studies. Notably is that vaginal colonization seems to be more commonly in VMB dominated by *Lactobacillus* spp. [1,2,3,4]

The following illustration shows the community state types as well as their dominant species and the associated vaginal pH.

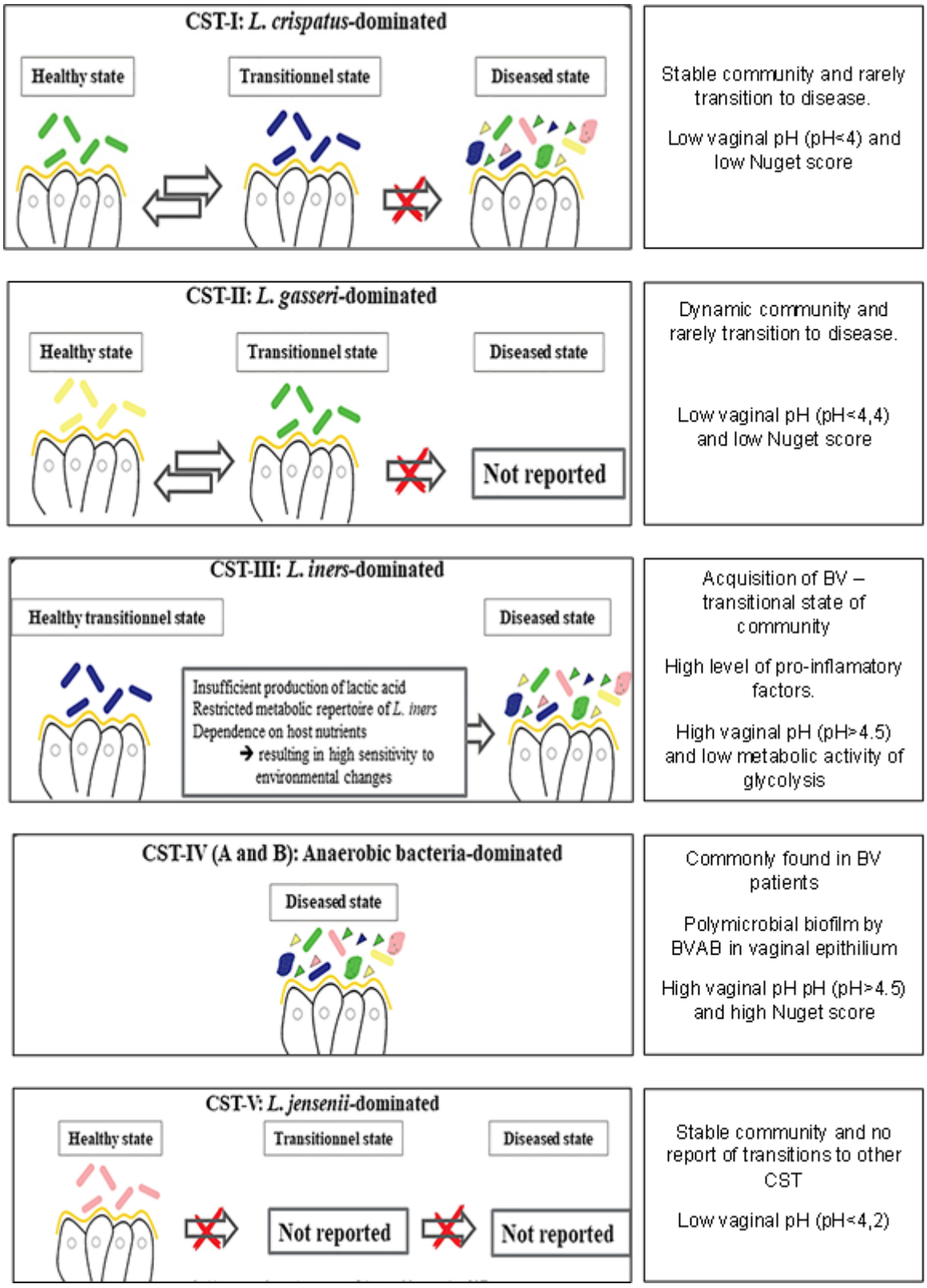

Figure 2. Community state types [Altered from source 18]

4. Influencing factors of the vaginal microbiome

As was already briefly mentioned above, there are many known factors influencing the VMB. In the following section some of the more important ones will be addressed shortly.

1.1 Ethnicity:

Genetic factors have been shown to play a role, evidenced by the fact that the CSTs vary among different ethnic groups. A dominance of *Lactobacillus* spp. was observed in asian and white women, while the VMB in black and hispanic women was significantly more diverse and contained several species associated with BV, such as *Gardnerella*. In general, it seems that the diagnoses of BV and PTB occur more frequently in black women. Interestingly, CST III was detected in almost equal proportions in all ethnic groups.

1.2 Menstrual cycle:

Reproductive hormones, in particular estrogen, shape the VMB throughout the woman's entire life. Estrogen leads to an increased glycogen storage in vaginal epithelial cells forming a great energy source for lactobacilli. The highest estrogen level can be measured during the follicular phase of the menstrual cycle and as expected, lactobacilli are dominant at this state, while one can also observe the lowest diversity. Interestingly the peaked estrogen seems to have a positive influence on the growth of *C. albicans*, this becomes apparent through increased symptoms. During menses however, the VMB is less stable, and the number of lactobacilli is lower. Often there also is an increase in *G. vaginalis* as well as in *L. iners*, potentially due to the iron content of blood.

Short term variations of the VMB can be observed, some seem to follow a specific pattern. CST IV-B often changes to CST III, CST I may convert also to CST III or to CST IV-A, while in case of CST II there are rarely any transitions present.

Despite the wide use of hormonal contraceptives, their effect on the VMB has not been sufficiently investigated. The existence of numerous different ways of contraception might be the root of this problem, study results are inconsistent. However, there seems to be a link between the use of oral contraceptives and a reduction in the risk for BV.

1.3 Lifestyle:

Personal hygiene practices like vaginal douching, using specific soaps and the choice of underwear can have a direct influence on the VMB composition and should not be

underestimated. Vaginal douching for example is strongly associated with an increased risk for BV, as are some sexual habits like having multiple partners. Something similar can be said about smoking, which influences estrogen production and alters the vaginal metabolite production profile. Among other things, it leads to an increase in nicotine and its derivatives, together with the biogenic amines. Also, alcohol is associated with an increased BV risk.

Today's modernization of our society has a strong influence on the VMB too. The most probable causes are the high amount of psychological stress, consumption of highly processed food and urbanization. Urbanization may increase the diversity of the vaginal microbiota (only limited data is available to underline this statement). Regarding the psychosocial stress, specifically the chronic type is of concern. It leads to a dysregulation of the immune system which in turn, together with increased levels of cortisol, decreases the vaginal glycogen. As previously discussed, the vaginal glycogen is an important energy source for lactobacilli, a decrease subsequently leads to a decrease in those, an increased pH and a proinflammatory state.

As with so many other things, diet also has an impact on human VMB. Research has shown that a healthy diet rich in nutrients and low in fat can also reduce the risk of BV. Of particular note here are folate, vitamin A and calcium. Furthermore, according to some studies, vegetarianism can lead to a more diverse vaginal microbiota. Also, obesity seems to play a role, as obese women mor often suffer from BV. A possible mechanism can be dysfunctions or disturbances in the host's metabolism, hormone system regulation and immune system regulation. Also, dysbiotic gut microbiota can have a direct effect on the VMB. Socioeconomically, a higher degree of education is more commonly linked to domination by *Lactobacillus* spp.

1.4 Immune system:

There is a very complex interaction between the VMB and the immune system. Epithelial and dendritic cells in the mucosa are responsible for homeostasis, while also scanning for potential pathogens. Antigens are detected via pattern recognition receptors, followed by the production of antimicrobial peptides and cytokines or chemokines. Dendritic cells further act as link between the innate and the adaptive immune system. Generally spoken, if an epithelial cell encounters endogenous lactobacilli, this event leads to a low level of antimicrobial production and homeostasis. Any detection of BV associated strains however leads in turn to higher levels, as well as a different profile of antimicrobials.

1.5 Vaginal infections and antimicrobial therapy:

BV is one of the most common disorders in reproductive aged women, affecting more than 30% every year. It comes with a sharp decline in lactobacilli and a concomitant rise in anaerobic bacteria. Even though patients can be asymptomatic, BV often comes with an increased risk for STDs, pelvic inflammatory disease, endometritis, chorioamnionitis and amniotic fluid infection. Regarding fungal infections *Candida* is the most common representative.

However, the major alterations of the VMB are usually caused by the therapy attempts with antibiotics or antifungals. The first line treatment for BV is composed of Metronidazole and Clindamycin, as Metronidazole seems to have no substantial effect on endogenous lactobacilli and the VMB recover within a few days. Due to resistance and biofilm formation, there is a very high recurrence.

1.6 Probiotics:

Probiotics are basically life microorganisms with a health benefit for the user if the dose taken is high enough. Regarding VMB, probiotics are usually composed of different strains of lactobacilli, even though there are some other strains under investigation. They lead to an increase in vaginal lactobacilli either in healthy subjects or in case of BV, where they may assist in recovery of the natural state after antimicrobial therapy. Their method of action is comparable to that of endogenous lactobacilli. They decrease the pH, produce antimicrobial compounds, and inhibit the vaginal space. Probiotics can be taken orally, or they can be introduced into the vagina directly. Both ways are efficacious. The oral intake also shows benefits for the gut-vagina axis. However, it is important to notice the heterogeneity among different studies on this subject.

5. Changes of the vaginal microbiota across the women's life span

During the female life span there are several changes of the VMB observable, from childhood over puberty and reproductive age up to menopause and post menopause. In the following section the main differences inbetween those age groups will be elaborated. Since the normal microbiota of women of reproductive age has already been discussed above, the focus now turns mostly to childhood, pregnancy and menopause.

Table 1 – Dominant species of VMB across female lifespan [Taken from source 4 and altered]

Dominant VMB in prepuberty	**Dominant VMB in premenopause**	**Dominant VMB in perimenopause**	**Dominant VMB in postmenopause**
Actinomyces	*L. crispatus*	*L. crispatus*	*Lactobacillus*
Bifidobacterium	*L. gasseri*	*L. gasseri*	*Gardnerella*
Peptococcus	*L. jensenii*	*L.jensenii*	*Prevotella*
Peptostreptococcus	*L. iners*		*Atopobium*
Propionibacterium	*Streptococcus anginosus*		
Bacteroides	*Prevotella bivia*		
Fusobacterium			
Veillonella			
Staphylococcus aureus			
Staphylococcus epidermidis			
Streptococcus viridans			
Enterococcus faecalis			
Note: In prepuberty shift towards Lactobacilli	Note: Changes within menstrual cycle and pregnancy	Note: Very similar to premenopause but with beginning shift towards postmenopausal VMB	

Depletion of maternal estrogen leads to thinner mucosa after birth and a decrease in glucose-fermenting microorganisms. Consequently, aerobic and facultative anaerobic bacteria are predominant. During childhood the VMB is formed by various representatives of Gram positive and negative anaerobes as well as aerobes. The pH changes and becomes neutral, the abundance of *Lactobacilli, G. vaginalis* or *P. bivia* is low. With puberty and the associated increased estrogen levels, the epithelium thickens again. The vaginal microbiota at this stage resembles that of an adult woman, dominated by various lactobacilli. A hereditary component of the VMB could not be determined, as there were no similarities between mothers and daughters

observable. Vulvovaginitis caused by either fecal organisms or Group A beta-hemolytic streptococci is the most common gynecological disease in young girls. From puberty onwards, these are replaced by *C. albicans* as usual cause.

When comparing pregnant and non-pregnant women, an increased dominance of lactobacilli and a higher stability of the VMB can be seen. Bacteria associated with BV are significantly reduced. After delivery the VMB shifts again becoming less stable and less diverse. This is most likely due to estrogen and progesterone, which stimulate glycogen accumulation during pregnancy and thus colonization by lactobacilli. Dysbiosis during pregnancy is linked to adverse outcome.

At menopause, as there are significant hormonal shifts observable, the VMB changes yet again. In healthy women at this age the most prevalent genera are *Lactobacillus, Gardnerella, Prevotella* and *Atopobium*. However, the portion of *Lactobacillus* spp seems to be much lower than before. Those changes lead to an increased pH and a higher risk for infections. The decline in estrogen may cause vulvovaginal atrophy in some women, coming with symptoms like itching, discharge, or bleeding. The condition also seems to be connected to the VMB, as women with CST IV-A are more prone to get it. An increased pH as well as the lower estrogen favor the growth of different harmful bacteria and increase the risk to develop BV. Hormone replacement therapy can affect the VMB, leading to a closer resemblance to the pre-menopausal state. However, since VVC is strongly related to estrogen, this should be considered as potential side effect.

As can be easily seen, the VMB is strongly related to the estrogen level. This is also shown again in the following figure. A low estrogen level is associated with fewer lactobacilli and a more neutral pH. Increased diversity and an increased occurrence of potential pathogens can be observed. As the estrogen level increases, however, the pH decreases, and the number of lactobacilli increases. This creates the first line of defense as described above. Furthermore, the VMB species that are dominant at different stages of life can be observed. [4]

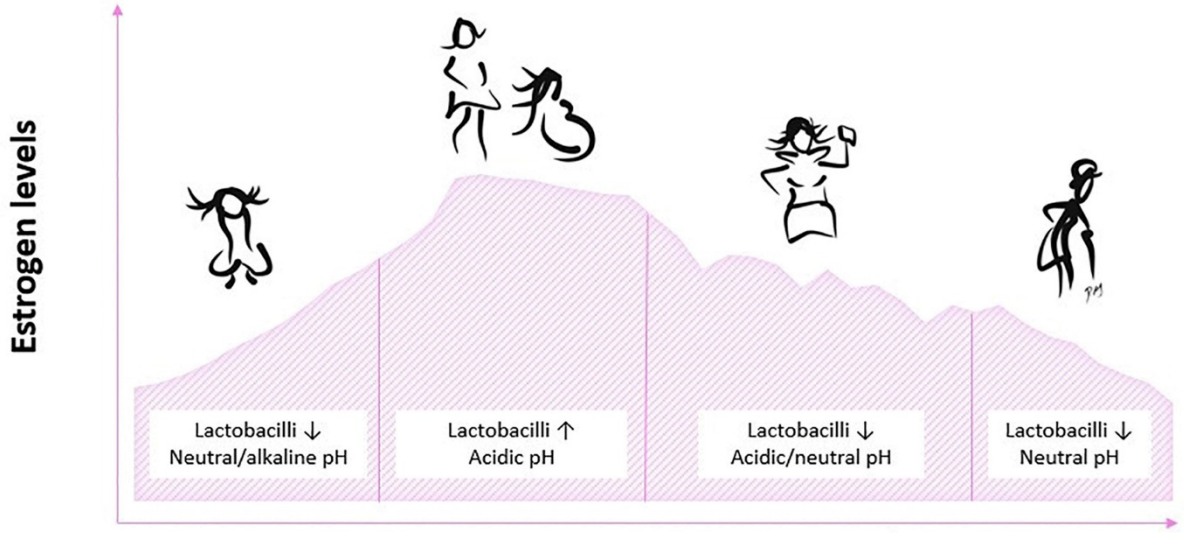

Figure 3 Influence of Estrogen on vaginal microbiota [Taken from source 4 and altered]

6. Vaginal Dysbiosis

The term "vaginal dysbiosis" refers to a disturbed VMB, featuring a loss of the domination by lactobacillus spp with a concomitant increase in the microbial diversity. Although the condition as such is not typically considered a disease by itself, it can be linked to a number of gynecological diseases. The vaginal microbiota typically does not only contain bacteria, but also a small number of fungi and parasites (Table 2).

Table 2 – Factsheet Vaginitis [Taken from source 15 and altered]

Diagnosis	Etiology	Symptoms	Signs	Risks	pH
Bacterial vaginosis	Anaerobic bacteria *(Prevotella, Mobiluncus, Gardnerella vaginalis, Ureaplasma, Mycoplasma)*	Fishy odor, discharge (homogenous, clear, white, or gray), pelvic discomfort	No inflammation	Increased risk of STDs	*>4.5*
Vulvovaginal candidiasis	*Candida albicans, Candida krusei,*	No odor, discharge (white, thick, cheesy),	Signs of inflammation, vulvar	vulvodynia	4.0

	Candida glabrata	vulvar itching, vulvar burning	erythema, and edema		
Trichomoniasis	*Trichomonas vaginalis*	Discharge (green or yellow, frothy), dyspareunia, vaginal soreness, dysuria	Signs of inflammation, "strawberry cervix", vestibular erythema	Increased risk for STDs and preterm labor	5.0 – 6.0

A well-balanced relationship among the microbial communities plays an important role in female health. Any kind of disruption may favor the development of adverse conditions such as BV, VVC or *Trichomonal vaginitis*, all of which will be further elaborated in the following chapters. But even STDs or viral infections like human papillomavirus or herpes simplex virus-2 may appear, together with their associated complications. Vaginal dysbiosis is additionally linked to non-infectious complications like abortions, intrauterine adhesions, miscarriage, preterm delivery, infertility, polycystic ovary syndrome or menstrual disorders, depending on the overgrowing microorganisms. [6]

7. Bacterial Vaginosis

Bacterial vaginosis was formally known as non-specific vaginitis. It presents changes in the vaginal microbiota composition, generally meaning a decrease in Lactobacilli due to a concomitant overgrowth in facultative anaerobes can be observed. Typical examples for bacteria associated with BV are *Gardnerella vaginalis, Atopobium vaginae*, *Ureaplasma urealyticum, Mycoplasma hominis, Prevotella, Peptoniphilus, Megasphera, Mobiluncus* or BVAB 1-3.

Also, the exact trigger still remains unknown, some research was able to link BV to an alkaline vagina, the increased pH being attributable to the loss of protection through endogenous *Lactobacillus* spp.

In 1955 it was stated that the causative agent is *Haemophilus vaginalis*, later renamed as *Gardnerella vaginalis*. This bacterial species forms a biofilm on the vaginal epithelium. It is able to kill the epithelial cells via secretion of cytotoxins and additionally, it produces proteolytic enzymes. All those features are significant in BV. Undegraded amine compounds cause the typical fishy odor. Due to the cytotoxicity, an increased vaginal discharge consisting of exfoliated epithelial cells can be observed. Furthermore, a formation characteristic of BV, of so called "clue cells" may appear. However, a few years later *G. vaginalis* was found to be present in 40% of healthy women, indicating that a colonization does not necessarily lead to BV. This concludes that *G. vaginalis* can be an important factor for the disease development, yet alone it is not sufficient. It points towards a possible polymicrobial origin of the condition. Another reason for this high percentage of colonized healthy women could be the existence of different species within *Gardnerella.* Until today 13 species have been found and only few of them are linked to BV. Healthy women may be colonized by non-pathogenic strands of the bacterium.

An updated conceptual model of disease development was proposed, based on a possible synergism between *G. vaginalis, P. bivia* and *A. vaginae*. Accordingly, sexual exposure leads to the displacement of vaginal lactobacilli and the formation of a biofilm on the epithelial cells. Proteolysis by the now growing population of *G. vaginalis* promotes the growth of *P. bivia*, which in turn produces ammonia, favoring *G. vaginalis*. Additionally, *P. bivia* degrade the mucin layer and increase the adhesion of BV associated bacteria as *A. vaginae*.

Diagnosing BV can be difficult, as the disease can be present without symptoms. Otherwise, an increased discharge and a fishy smell are typical. In addition, the polymicrobial features and the wide range of clinical features complicate the procedure. Usually, a sample is taken using a speculum for testing. There are two main strategies in the diagnosis of BV, namely the Amsel's criteria and the Nugent's score. While the Amsel's criteria can be taken at bedside and are a more clinical approach, the Nugent's score is laboratory based and the gold standard for studies. The main problem with Nugent's score is that there is no recognition of intermediate microbiota and that it is subjective and depends on the technician. However, in clinical practice gram stain microscopy according to Hay-Ison criteria is recommended, as it is easier, faster, and analogous to Nugent's score.

Molecular, enzymatic, and chromatographic techniques are being tested, an example is quantitative real-time PCR to measure the presence of bacteria and make a diagnosis based on the number. Another example is the measurement of sialidase, an enzyme that seems to have a

key role in BV. Even though both sensitivity and specificity are high, hurdles still exist such as a higher price or the fact that they are not point of care tests. For further information regarding emerging diagnostic methods, please refer to my sources [5].

BV can appear at any reproductive age. The occurrence varies geographically (generally spoken it is low in Europe, intermediate in the US and high in Africa), according to ethnicity and to socioeconomic status. As no single causative agent can be pinned down, it cannot be classified as STD, but its transmission is strongly associated with sex and the condition also shows some of the characteristics of STDs. It can be present in post pubertal virgins, but the prevalence is lower while it is higher in women who had sex with a higher number of different partners. Furthermore, bacteria associated with BV can be present in the male reproductive system. They do not cause the disease; men only act as reservoir. Therefor the use of condoms or even circumcision of the male penis can decrease the BV risk.

BV is more prevalent in women who have sex with women most likely due to the transmission of vaginal fluids. Genital hygiene may lead to VBM disbalance and predispose the disease, as well as all the factors discussed earlier.

Women with an active BV infection are more vulnerable and prone to several complications. Among those there are opportunistic infections as well as an increased incidence of STDs and preterm delivery with accompanying complications like an increased mortality. In pregnant women, a higher risk for preterm labor, miscarriage, intrauterine fetal death, PROM, amniotic infections, chorioamnionitis and postpartum infections can be observed, while in non-pregnant individual's cervicitis, endometritis, salpingitis and urinary tract infections pose the main complications. Bacteria may even ascend through the urogenital system and cause PID, cervical cancer or tubal infertility.

Concerning the treatment strategy, the main goal is to stop the proliferation of causative microorganisms and restore the normal healthy microbiota, typically by using broad spectrum antibiotics and probiotics. The first line recommendation is 500mg oral metronidazole twice a day for one week. Typical side effects of this therapy are gastrointestinal pain as well as nausea and vomiting. An oral alternative to metronidazole is tinidazole. Other possibilities are 300mg oral clindamycin twice a day for one week, 5 days of 100mg intravaginal clindamycin or 0,75% intravaginal metronidazole gel, also for 5 days. In the end either taken orally or applied locally, those antimicrobials have the same effect and achieve cure rates of 58% up to 92% after one month. Unfortunately, high recurrence or reinfection rates of above 50% within one year can be observed. The reason for this phenomenon is unclear. Possible causes can be biofilm

formation, reintroduction through the partner or failure of the antibiotic (especially in the presence of BVAB 1-3, Megasphera type 2 or *Peptoniphilus lacrimalis*. An application of acidic agents such as Vitamin C together with the antibiotic decreases the recurrence rate.

As the use of antimicrobials can have a negative impact on the vaginal microbiota, diverse *Lactobacilli* probiotics have been developed. As previously mentioned, probiotics are live organisms constituting benefits to the host if given in adequate amount. They are supposed to either help in the restoration of the status quo or participate in its maintenance. Oral and vaginal application are both effective. Furthermore, sucrose containing products might be considered too and assist in the lactobacilli recolonization.

All in all, it can be said that the therapy of BV urgently requires new alternatives. Several new methods are under research. [5]

8. Vulvovaginal candidiasis

The second most common cause of vaginal infection is vulvovaginal candidiasis (VVC), with *C. albicans* being the most common causative agent responsible for around 90% of cases. It is a symptomatic inflammation of the vagina and often involves the vulva, where it leads to swelling and erythema. The main symptom is itching, but abnormal vaginal discharge, burning, soreness, dysuria, and dyspareunia may also be present. To distinguish it from other forms of vaginitis yeast must be proven via microscopy of vaginal fluid. However, as *Candida* can be part of the normal microbiota its presence is not enough for a disease establishment. It has been detected in around 10% of asymptomatic women. [8]

Next to *C. albicans* other members of the *Candida*-family may also cause VVC. *C. glabrata* is responsible for most of the remaining cases. Other non-albicans *Candida* like *C. krusei, C. parapsilosis* and *C. tropicalis* are exceedingly rare. However, their symptoms seem to be milder compared to VVC caused by *C. albicans*. As the disease outbreak is probably triggered by overgrowth of *Candida* in case of vaginal dysbiosis, some risk factors can be seen. They include pregnancy as well as other conditions with an increase in estrogen, diabetes mellitus, immunosuppression, and the use of antibiotics. Although increased sexual activity is associated with an increased incidence of VVC, there is no evidence of sexual transmission. [8,9]

The exact pathogenesis of VCC is extremely complex because it is a multifactorial disease. Asymptomatic colonization of the vaginal epithelium with *C. albicans* (yeast form) is followed by conversion to hypha. The resulting reaction in the epithelium leads to morphogenesis and

immune activation. Polymorphonuclear leukocytes migrate into the vaginal lumen where they contribute to symptomatic infection.

To put it simply, one can say that an interaction between host, pathogen and environmental qualities is required to lead to symptomatic infection. This can be seen in the following graphic.

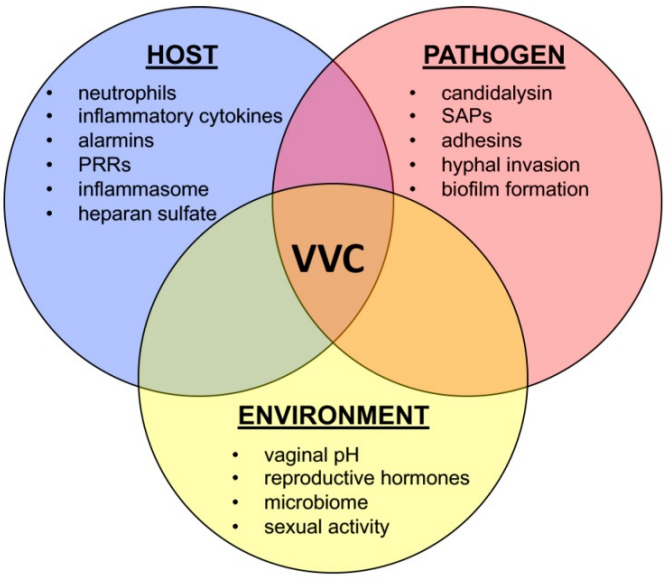

Figure 4. Interaction between host, pathogen and environmental in development of vulvovaginal candidiasis [Taken from source 9]

Candida is a fungal pathogen that can cause different superficial and systemic infections in humans. Thanks to its ability to form biofilms it may be difficult to eradicate. [7]

VVC is the most common candida infection in humans and estimated to afflict around 75% of women at least once in their life. In contrast to other forms of candida infection, those affected by VVC are immunocompetent and otherwise completely healthy women. Additionally, 8% worldwide are suffering from recurrent VVC. Recurrent VVC is defined as having more than 3 episodes of VVC per year and often indicates an antifungal maintenance therapy.

There are various options for therapy for VVC, depending primarily on the presentation and features of the disease. Oral medications, topical agents as well as vaginal suppositories are used. The most common class of antifungals used are Azoles however, due to their static effects, they rely on the help of the patient's immune system. Some over-the-counter examples are clotrimazole 1%-4% or miconazole 2%-4% topical cream, while fluconazole 150 mg or butoconazole 2% topical cream can be acquired with prescription. Patients with recurrent VVC often need longer therapy and in severe cases maintenance therapy, as previously mentioned.

The use of probiotics either as treatment or prevention of the disease has also been suggested. [9].

9. Trichomonal vaginitis

The causative agent of trichomoniasis is the parasite *Trichomonas vaginalis*. It is the most common STD of nonviral origin. As the infection remains asymptomatic in many individuals and male partners often serve as reservoir, the transmission rate is high. T. vaginalis can be associated with a variety of adverse health outcomes, including PROM and low birth weight, increased risk for the development of other STDs, pelvic inflammatory disease, infertility and cervical cancer. If symptomatic disease occurs, vaginitis is most common in women, while urethritis predominates in men. For women this means vaginal discharge, vaginal odor and irritation. Dysuria, dyspareunia and pruritus may also appear.

The WHO estimates the incidence of trichomoniasis at 156 million cases worldwide. Factors such as low socioeconomic status, lower level of education, older age and of course multiple sex partners promote infection.

Humans are the only known host for *T. vaginalis* and spread occurs through sexual intercourse. During its trophozoite stage active growth in preparation for replication can be observed. This is then done through longitudinal binary fission in the lower genital tract of women. The protozoa do not have a cyst form and it also does not survive in the environment for a longer period of time. The attachment of *T. vaginalis* in combination with the release of proteases triggers a host immune response.

Transmission of the disease is not only possible through penetrative intercourse alone. Sexual practices between women can also lead to trichomoniasis. Transmission without sex is also possible, although rare. These include, among other things, iatrogenic transmission, shared sex toys or suboptimal bathing conditions.

The diagnosis of trichomoniasis has traditionally been made by POC methods, among which wet mount microscopy of vaginal secretions was the most common. Moving Trichomonads can then be detected there. Although the specificity of this test is almost 100%, the sensitivity is much lower (44-68%). In recent years, other tests have often been used, such as the OSOM rapid test. Using antibodies to detect antigens of *T. vaginalis* in vaginal secretions, it does not require microscopy and has a significantly higher sensitivity.

Since 5-nitroimidazole is the only class of antimicrobials with trichomonacidal activity, representatives of this group are used for treatment. Among them we sometimes find metronidazole or tinidazole. Metronidazole is the first choice and is used in women at 500 mg twice daily for 7 days. To avoid reinfection, simultaneous therapy for all partners and the use of condoms are recommended. [10]

10. Probiotics

Recently, probiotics have come into focus because they seem to be able to both prevent and treat diseases. This is achieved through various properties of the probiotic bacterial strain. These include improving the mucus barrier, direct competition with potential pathogens, stimulating the immune system and more. By definition probiotics are living, non-pathogenic microorganisms that are beneficial for the host in adequate doses. In addition, they have specific properties which include resistance to acidic environments or adhesion and invasion capacity of epithelium. These ensure their survival. Despite being classified as safe dietary supplements; probiotics have not yet been approved for prevention or therapy. *Lactobacillus* and *Bifidobacterium* are generally the bacteria most commonly classified as probiotics, but various *Enterococcus* and *Streptococcus thermophilus* also have probiotic properties. [17]

Even though probiotics are readily used to treat diseases of the gastrointestinal tract, their effectiveness in the female reproductive system remains controversial. They seem to assist in maintaining the stability of the vaginal microenvironment, as well as improving the immune response and even influence the development of cancer.

An infection with HPV disrupts the balance of the microenvironment, decreases the number of Lactobacilli and promotes the development of cervical intraepithelial neoplasia. A study demonstrated that the use of *Lactobacillus curlicus* increases the HPV clearance. Additionally, lactobacilli participate in the inhibition of malignant tumor proliferation trough immune activation and secretion of several anti-tumor metabolites. Furthermore, probiotics seem to reduce side effects of radiotherapy, which is readily used to treat cervical cancer. It is not difficult to see how diverse probiotics can be used. Since this seminar is not primarily about cancer, the focus is on the benefits in vaginoses. However, it should not be forgotten that other areas can also benefit greatly.

Probiotics are one of the new therapeutic strategies in the treatment of BV. They ensure a larger number of good bacteria, reduce the harmful ones and contribute to the greater stability of the

vaginal microbiota. There is increasing awareness that probiotics contribute significantly to healing and reduce the recurrence rate. As can be seen from the table below, the majority of studies have been carried out on probiotics consisting of lactobacilli. There are also other species currently under observation with a view to possibly being used in the near future. Because even if the results are consistently positive, lactobacilli do not seem to be the miracle cure. [16]

Table 3. Clinical effect of different probiotics in BV therapy [Taken and altered from source 16]

Probiotics	Methods	Results
L. acidophilus GLA-14 and *L. rhamnosus* HN001	Patients received metronidazole for 7 days and were randomly assigned to receive probiotics plus lactoferrin or placebo	Probiotic plus lactoferrin improved symptoms, Nugent score and recurrence
L. rhamnosus GR-1 and *L. reuteri* RC-14	Patients received metronidazole for 7 days and probiotics and placebo for 30 days	Combined use of probiotics and antibiotics improves BV cure rates
L. crispatus LMG S-29995, *L brevis and L. acidophilus*	After completing metronidazole treatment patients received probiotics and placebo respectively	Probiotics reduced the rate of recurrence and prolonged the time to recurrence
L. rhamnosus BMX 54	After completing metronidazole treatment patients received probiotics and placebo respectively	Reduced BV recurrence and vaginal pH
L. crispatus LbV 88, *L. gasseri* LbV 150N, *L. jensenii* LbV 116 and *L. rhamnosus* LbV 96	After completing metronidazole treatment patients received probiotic yoghurt and placebo respectively	Yoghurt with probiotics improved BV recovery rate and vaginal microbes
L. brevis CD2, *L. salivarius* subsp. *salicinius, L. plantarum*	Patients were randomized to receive vaginal probiotics or pH tablets	Probiotics improved BV cure rates and reduced vaginal cytokines IL-1ß and IL-6

L. rhamnosus GR-1 and *L. reuteri* RC-14	Patients were treated with probiotics for 6 month and metronidazole for 10 days	Supplementation did not improve BV cure rates, but improved vaginal microbiota composition
L. brevis CD2, *L. salivarius* subsp. *salicinius* FV2 and *L. plantarum* FV9	Patients received probiotic containing vaginal tablets or placebo for 7 days	BV cure rates improved as well as vaginal microbiota

11. Discussion

While delving into this thesis, it became clear to me how complex the human vaginal microbiota actually is. Due to the multitude of influencing factors, it is exceedingly challenging to determine the “ideal” or “normal” vaginal microbiome. [19] There are differences that can be attributed to genetics as well as those that result from everyday environment, such as lifestyle or hygiene habits. [20] In addition, there are the usual hormonal fluctuations. Since dysbiosis can predispose to various diseases or unfavorable outcomes (e.g. in pregnancy), Establishing a standardized definition of “healthy” would be helpful and could undoubtedly aid in guiding therapeutic interventions. [1]

This leads me to another point where there is still potential for improvement in the future. Present therapeutic approaches rely primarily on various antimicrobial agents (metronidazole, clindamycin etc.), with or without the supplementation of probiotics. [21] Although these antibiotics are effective against BV-associated bacteria, the remission is usually temporary, and many patients relapse after treatment. [22, 23] The high recurrence rate (over 50%) may be because of lack of ability of antibiotics to affect the biofilm-associated bacteria of BV in vagina. [24, 25] Additionally, this very aggressive treatment option has the potential of inducing dysbiosis itself, as distinguishing between beneficial and harmful bacteria remains imprecise. Moreover, when looking at the high reinfection rate, it does not seem to be the ideal solution in the long term and suggest a need for more sustainable solutions. Whether attributed to the formation of biofilms, bacterial resistance, re-infection through sexual partners or something else, extensive further research on reinfection rate needs to be carried out. [26, 27] Microbial-based therapy have recently attracted an increasing amount of interest because of the beneficial effects to the host health. [28, 29] Probiotics, predominately Lactobacillus species seem to be promising candidates. [30] The idea of using bacteria that are beneficial to humans in order to restore the microbiota to an optimal state and to get potential pathogens under control seems sensible to me. [6, 16] However, the perfect strain has not yet been found and a lot of research is still needed, although studies of its use in conjunction with antimicrobials are quite promising.

Probiotics could bring a change not only in therapy. There is also potential in the prevention of dysbiosis itself. [17] The resulting improvements in women's quality of life would be immense, especially if keeping the psychological aspect in mind, be it discomfort or even reduced self-esteem. [31] However, the road to employing probiotics for preventive purposes remains paved with unanswered questions and requires substantial research efforts. [17]

Even though a number of factors (intrinsic and extrinsic in nature) that have a direct or indirect effect on the VMB have already been identified, there are many more that remain still unknown. Deepening the understanding of this could also contribute to the prevention of dysbiosis or offer us further options for therapy. [4]

12. Conclusion

In summary, the maintenance of a robust vaginal microbiota is of paramount importance for women and significantly impacts their overall health status. Serving as the primary line of defense against a myriad of potential pathogens, the vaginal microbiota constitutes a dynamic and intricately complex ecosystem comprising billions of microorganisms, encompassing bacteria, fungi, and protozoa. Various factors can disrupt this delicate equilibrium, predisposing individuals to a spectrum of ailments.

Predominantly, pathogens such as bacteria, viruses, fungi, and protozoa pose significant threats. The resultant diseases can inflict substantial physical and psychological distress upon women, imposing a considerable burden on healthcare systems, and in severe instances, culminating in infertility or mortality. Among these conditions, Bacterial Vaginosis stands as the most prevalent, typically characterized by a reduction in Lactobacilli accompanied by elevated pH levels and heightened microbial diversity.

Furthermore, the vaginal microbiome exhibits considerable dynamism throughout a woman's lifespan, with notable inter-individual variations, including among offspring. The conceptualization of an "ideal" microbial composition remains elusive, complicating therapeutic interventions. Current treatments, often aggressive in nature, suffer from high recurrence rates, underscoring the necessity for improved therapeutic modalities.

13. Summary

There are different compositions characterizing a healthy vaginal microbiota, typically characterized by a dominance of Lactobacilli and an acidic pH milieu, constituting the primary barrier against pathogenic intrusion. Even subtle perturbations can precipitate various clinical presentations or adverse obstetric outcomes. The vaginal microbial balance (VMB) is intricately modulated by multifaceted factors, encompassing the woman's age, hormonal milieu, lifestyle choices, prior infections and treatments, as well as the influence of probiotics, among others. Hence, it exhibits dynamic temporal fluctuations rather than static stability.

This discourse delves into three prevalent clinical entities incited by dysbiosis, namely Bacterial Vaginosis (BV), Vulvovaginal Candidiasis (VVC), and Trichomoniasis, each attributed to bacterial, fungal, and protozoal pathogens, respectively. Beyond hallmark symptoms such as malodor, pruritus, and increased vaginal discharge, these maladies pose obstetric complications and heighten susceptibility to sexually transmitted diseases (STDs) and urogenital afflictions during gestation. Treatment modalities entail aggressive measures, often entailing systemic antibiotics, inadvertently affecting both pathogenic and commensal flora, thus disrupting the delicate equilibrium and fostering recurrence.

In mitigating such recurrences, probiotics emerge as pivotal adjuncts, comprising non-pathogenic, symbiotic microorganisms beneficial to the host. While strides have been made in ameliorating reinfection rates and optimizing therapeutic outcomes, extensive research endeavors remain imperative to pinpoint the ideal bacterial strains, thereby furnishing patients with enhanced therapeutic regimens.

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15. CV

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