

The composition and stability of the vaginal microbiome of healthy women

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Abstract

Our knowledge of the composition of the vaginal environment in healthy women stands greatly improved. An imbalance in microbial communities is associated with a number of different diseases, disorders and other adverse health outcomes. Cultivation-independent studies have been published indicating that each woman has unique vaginal microbiota. The vaginal microbiome in pregnant women is more stable and associated with high level of *Lactobacillus*, particularly, *Lactobacillus crispatus* and low bacterial diversity. The current review was planned to provide a more complete picture of the abundance of various bacteria species in the vagina and how they impact women's reproductive health and pregnancy outcomes. This should provide a better understanding of what is considered a "healthy" or "unhealthy" vaginal microbiome and how the dysbiosis of the vagina affects the women. Additionally, it was planned to identify factors that influence the structure and / or composition of the microbial community.

Keywords: Vaginal microbiome, VMB, Bacterial vaginosis, Community state types, CST, *Lactobacillus*, Preterm birth, Pregnancy.

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Introduction

Understanding the Human Microbiome

The definition of the human microbiome has been complicated by confusion related to the terminology. 'Microbiome' refers to the genetic material of the microbiota that lives in close association with humans and all of their genes.¹ The term 'microbiota' refers to site-specific human bacteria that are associated with an environment and are revealed by 16s ribonucleic acid (RNA) sequencing.¹ This microbiome has many benefits for the human body which is responsible for 40% of the host's energy intake as well as is important for the development of the host's immune system.² However, any distribution in microbiome can result in disease.

Over the past decade, there has been an explosion of culture-independent technologies that have allowed for

the classification of millions of bacterial species through the analysis of the sequence of marker genes.

Many studies have investigated the human microbiome in different biological states via 16s ribosomal RNA (rRNA) sequencing. For example, Ley et al. discovered gut microbiome that revealed a significant diversity in the microbiota of obese³ individuals. Additionally, studies have described the microbiome in the oral cavity, vagina and skin using the 16s rRNA gene level.⁴⁻⁶

Vaginal microbiome of healthy women

A healthy vagina is typically defined as lacking symptoms of various infections, which results in good pregnancy outcomes. The vaginal microbiome (VMB) plays a critical role in the protection of the host from the growth of pathogenic bacteria.⁷ In addition, the VMB of mothers plays a critical role in the health of newborns.⁸ The VMB of healthy non-pregnant women is characterised by a variety of *lactobacillus* (*L.*) *species* (*sp.*) and many other organisms may be present in lower concentrations, including anaerobic and facultative anaerobic bacteria and *candida* *sp.* The *lactobacillus* *sp.* dominates this ecosystem with concentration of 10⁶-10⁷ colony forming unite (CFU) per gram of vagina fluid, and plays a key protective role by lowering the vaginal potential of hydrogen (pH) to 3.5-4.0 through lactic acid as a fermentation product, and creates an inhospitable environment for the growth of harmful bacteria. Moreover, *lactobacilli* also produce hydrogen peroxide (H₂O₂), bacteriostatic and bacteriocidal compounds that can lower pH.⁹ There is a clear correlation between the pH value and the floral species detected in the cervicovaginal liquid.¹⁰ The loss of *lactobacilli* dominance results in bacterial vaginosis (BV), which is caused by abnormal proliferation of facultative and strictly anaerobic microorganisms and results in relatively high vaginal pH (> 4.5).

Species-level classification of VMB

Over 20 species of *lactobacillus* have been detected in the vagina.¹¹ To date, healthy vagina is likely dominated by one or two *lactobacillus* species. Ravel et al. were the first to describe five community state types (CSTs) that are different in their microbial composition and abundance. The five main grades are *L. crispatus*, *L. gasseri*, *L. iners*, and *L. jensenii* (I, II, III and V) respectively, whereas other *lactobacillus* *species* are rare. CST IV lacks significant number

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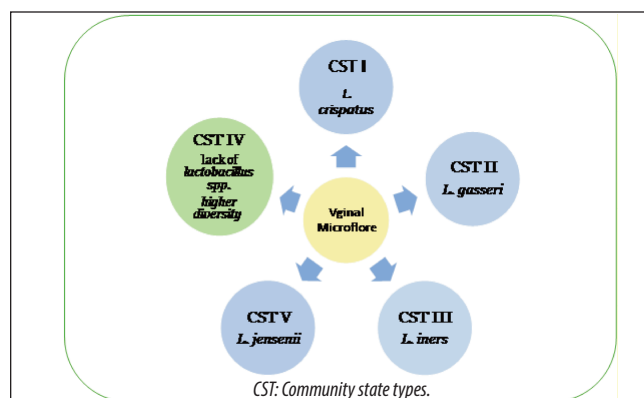


Figure-1: The composition of vaginal microbiome.

of lactobacillus-higher diversity (Figure 1).¹² Over 25% of women are a non-lactobacillus-dominated and are considered “normal” and healthy, though associated with high Nugent scores and higher pH. On the other hand, these women are prone to sexually-transmitted diseases, including human immunodeficiency virus (HIV), as well as preterm birth.

Prior cultivation-independent studies using molecular techniques have indicated that there no core vaginal microbiome common to all women; it largely differs by age and ethnicity. Several studies have shown significant difference in the vaginal microbiome in different ethnic groups. These studies demonstrated that Black and Hispanic women are more likely to be colonised with CST IV, whereas Asian and White women are more likely to be dominated by CST I and/ or CST II than any other lactobacillus species.^{13,14} However, many women lack appreciable vaginal lactobacilli without any health effects.

These findings were practically confirmed by Fettweis et al. who compared the differences in VMB in African-American women with women of European ancestry using the largest African-American cohort to date (1,268 subjects, 960 of whom were not pregnant). African-American women were more likely to be dominated by *L. iners* and more likely to have higher bacterial diversity, whereas women of European ancestry were likely to be dominated with *Lactobacillus species* CST I, II and V, and have lower bacterial diversity.¹⁵ This observation is important in understanding VMB composition and can help to protect a more fertile environment and develop appropriate treatments.

VMB in pathologic states

Bacterial vaginosis

BV may be defined by the disequilibrium in the vaginal microbiota and the microbial community composition shift from symbiotic to pathogenic (disease).¹⁶ Usually,

treatment is conducted with antibiotics, such as clindamycin or metronidazole, but the treatment is often ineffective, with high rates of recurrent bouts. In women of childbearing age, BV is a common reproductive tract infection and has been observed in 15-30% of pregnant women. Moreover, it has been associated with serious health issues, including infertility, pelvic inflammatory disease (PID), sexual-transmitted disease (STD) and increased risk of adverse pregnancy outcomes.¹⁷ BV is usually diagnosed using the Amsel criteria in research setting¹⁸ and by gram-stain Nugent scoring in research and laboratory settings.¹⁹

Culture-dependent studies of BV demonstrate alterations in the composition and structure of vaginal microbial communities, including an increase in *Gardnerella*, *Atopobium*, *Mobiluncus* and *Prevotella*, with decreased *Lactobacilli*.²⁰ On the other hand, VMB community composition of many women was previously thought to have asymptomatic BV resembles, but is now recognised as normal VMB using the culture-based methods. Using culture-independent techniques, Fredericks et al. confirmed the association of *Atopobium vaginae* with BV, and identified three potentially BV-associated bacteria with unique 16s RNA sequences that could not be identified by culture-dependent studies. These are now known as BV-associated bacteria (BVAB) 1, 2 and 3.²¹

Using high-performing techniques, Ceccarani et al. compared the alterations that occur in the VMB during common genital infections.²² The shift from healthy to BV was strongly associated with increased abundance of *L. iners* mainly characterised by *Gardnerella*, *Prevotella*, *Megasphaera*, *Roseburia* and *Atopobium*. *L. iners* is typically associated with dysbiosis of the VMB that leads to BV.²³ However, the mechanisms for this species in vaginal health is unclear, and much attention is required to explore these since it can be detected in normal conditions as well as in another fingerprint of the ongoing dysbiosis in infectious conditions. At present, *L. gasseri* and *L. crispatus* have been associated with health,²³ while *L. gasseri* and *L. iners* have negative connotations, suggesting that *L. iners* may be an opportunistic pathogen whose potential is enhanced by *L. gasseri*. More research is needed to investigate this relationship with the host.

In a small study, Lev-Sagie et al. reported the benefits of vaginal microbiome transplantation, which entails the transplanting of vaginal fluids from healthy women to women with BV. A non-randomised trial of 5 women with intractable BV was conducted. Before the transplantation, all patients received a course of intravaginal antibiotics. The results revealed that four of the recipients showed long-term full remission, and one recipient had a partial

remission. No adverse effects were observed in any of the patients.²⁴ Changes in the VMB composition were confirmed with metagenomic sequencing. The results are striking and are likely to spur a significant amount of research in this area.

The presence of certain bacteria can indicate whether the vaginal tract is healthy or not. Research has revealed that BV appears to increase risk of the HIV acquisition due to altered pH, while treatment of BV may reduce the risk of HIV, especially where rates of HIV are very high. Moreover, behavioural, genetic and environmental factors are also likely to impact the effect of the VMB on HIV risk. For example, using douches regularly that can change the vaginal pH have been reported to cause serious health problems, including increased risk of cervical cancer, PID and sexually-transmitted infections (STIs), including HIV.²⁵

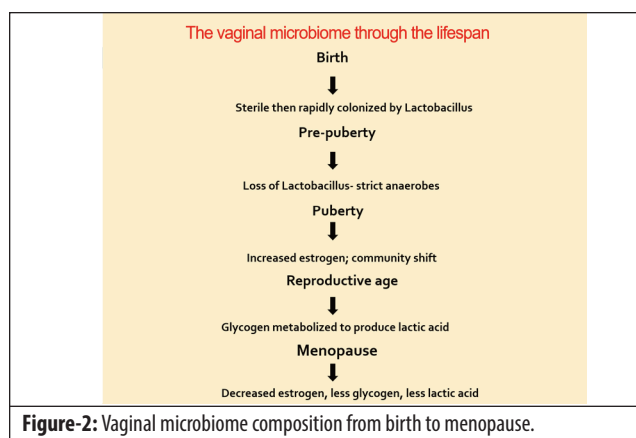
Physiological and environmental factors

Many studies have been performed to identify factors that may drive changes in the composition of the VMB. Age, pregnancy, sexual activity, number of sexual partners and smoking can affect the composition of the VMB.²⁶

During the menstrual cycle, physiological changes occur that can impact the VMB. Numerous studies have assessed the stability of the VMB of healthy women throughout the menstrual cycle. Some women have vaginal microbial communities that appear to be more stable when oestrogen levels are at their highest, whereas other experience fluctuations timed with the menses.²⁷ The high level of oestrogen causes a thickening of the vaginal epithelium and prompts the accumulation of glycogen and increased *Lactobacillus species*. Molecular-independent techniques have shown that vaginal microbial community varied among women, and each woman's VMB composition fluctuates throughout her reproductive lifespan (Figure 2).²⁸

Furthermore, very few studies have compared the composition of the VMB of postmenopausal women who have received hormone replacement therapy (HRT) and those who have not.²⁹⁻³¹ Notably, women not using HRT are less likely to be colonised with healthy *Lactobacillus species* than those using HRT, and HRT greatly improves vaginal atrophy scores and decreases vaginal pH.

Smoking has been identified as one of the factors that influence the composition of VMB and can lead to BV and other genital infections and contributes to a reduction in vaginal lactobacillus, particularly *L. crispatus*.³² In 2014, the evaluation of the vaginal microbiota of smokers and non-smokers was first done. It followed changes in the vaginal microbiota over time in a pilot study of smoking



cessation.³³ Smoking was found to be associated with low proportions of lactobacillus species, and, during smoking cessation, vaginal microbiota shifted from lactobacillus-deficient CST to lactobacillus-dominated CST. This provided important preliminary data for future studies. However, larger studies are needed to confirm these findings and establish if smoking is causally related to BV.

Very little research has been conducted on the microbiome of semen and how the seminal microbiome influences the vaginal microbiome after sexual intercourse. Some researchers have suggested that specific sexual partners can cause BV. The seminal microbiome of individual men might influence the health of their partners and the health of their offspring.^{34,35} Additionally, seminal microorganisms could influence pregnancy and miscarriage rates and be implicated in preterm births.³⁶ However, the mechanisms for this are unclear, and further studies are required to confirm these findings. The first next-generation sequencing (NGS) study measured the seminal and vaginal microbiome pre- and post-intercourse in 23 couples.³⁷ Seminal communities contained significantly fewer bacteria, but were significantly more diverse. Still, each shared many of the same bacteria, including lactobacillus, veillonella, streptococcus and atopobium. There was lower level of in the abundance of *L. crispatus* after intercourse. Interestingly, *gardnerella (G.) vaginalis* in women was related to inflammation in male genital tracts.

VMB during pregnancy

A growing body of research demonstrates that VMB in pregnancy, as characterised by new sequencing technologies, plays a critical role in both mother and neonatal health outcomes. In recent studies, the VMB during pregnancy has been examined in cross-sectional and longitudinal studies. These studies confirm that the VMB becomes more stable during pregnancy and is associated with high levels of lactobacillus, particularly *L. crispatus*, and low bacterial diversity.³⁸ The first longitudinal

study, by Romero et al., compared the VMB composition of 32 non-pregnant women with 22 pregnant women who delivered at term, by analysing the taxonomic 16s rRNA. The study found that normal pregnant women maintain vaginal CSTs dominated by *L. vaginalis*, *L. jensenii*, *L. crispatus* and *L. gasseri*, and alpha diversity is decreased. In contrast with the non-pregnant, there are fluctuations between CSTs. Interestingly, *L. iners* was not significantly different between the two groups.³⁸ However, large cohort is needed and further research is required to elucidate the correlation between the role of microorganism and adverse pregnancy outcomes. Ethical variations and geographical areas have shown significant differences in what constitutes the dominant vaginal organism.³⁹

MacIntyre et al. recently characterised the VMB of healthy pregnant women in the early weeks of gestation and compared it to those of non-pregnant women of similar ethnicities based on sequencing of the chaperonin (cpn)-60 universal target region.³⁹ Microbiome profiles of pregnant women were clustered into six CSTs; I, II, III, IVC, IVD and V. This observation has been recently confirmed by Freitas et al. Notably, they identified several differences in the composition between the two cohorts' higher lactobacillus abundance and less rich and diverse presence in healthy pregnant women.⁴⁰ Another recent study evaluated if the VMB was different between women during the first trimesters of their first pregnancies and women during subsequent pregnancies. Remarkably, there was a marked decrease in *L. crispatus* and a concomitant increase in *L. iners*, *L. gasseri* and *G. vaginalis* in women with prior pregnancies.⁴¹

As mentioned previously, the distribution of vaginal bacteria CST appears to vary by population during pregnancy. Dysbiosis of the VMB is associated with an increased risk of post-abortion infection, and early and late miscarriages as well as preterm premature rupture of membranes and preterm birth.⁴²⁻⁴⁴

VMB during preterm birth

Preterm birth (PTB), defined as a birth-occurring delivery before 37 weeks of gestation, is one the most common single direct causes of neonatal deaths.⁴⁵ PTB has multiple causes, including factors unrelated to the microbiota, such as age of the mother <20 or >35 years, STIs, obesity, short interpregnancy intervals, multiple gestations, Black ethnicity and tobacco and substance use.^{46,47} Over 90% of vaginal bacteria are uncultured and they play a critical role in PTB. The most prevalent species associated with PTB are ureaplasma (*U.*) urealyticum, mycoplasma (*M.*) hominis, bacteroides specie, *G. vaginalis* and *Fusobacterium* (*F.*) nucleatum. Ethnicity significantly influences the

composition of VMB, with a higher risk in Black women compared to non-Black women.¹² BV likely represents a dysbiosis, or abnormal restructuring of the VMB, and has been more strongly associated with PTB.¹²

At present, using culture-independent techniques to identify a cause of PTB within the VMB are similarly inconclusive. One of the first studies, by Nelson et al., reported that women with previous PTB, women with higher levels of leptotrichia/sneathia species, BVAB1 and megasphaera species in the first trimester of pregnancy have been considered risk factors to experience spontaneous PTB.⁴⁸

A later study by DiGiulio et al. characterised the composition of the vaginal community microbial in 49 pregnant women's duration of the pregnancy and tested the associations with complications of pregnancy, in particular PTB.²⁶ The major finding was that there was inversely relationship between PTB lower abundance of lactobacillus, while positive relationship between abundance of gardnerella and ureaplasma. The study indicated that *L. iners* often coexists with *G. vaginalis*. Studies have shown inverse correlation between *G. vaginalis* and *L. crispatus* via reciprocal interference in epithelial adhesion and biofilm formation.^{49,50} The relative abundance of *L. iners* is recommended as a mark of the dysbiosis of the vaginal microbiome and is identified as a risk of PTB, but *L. crispatus* is not.^{51,52} For these reasons, *L. crispatus* is a key indicator of a healthy microbiome and may also be protective against PTB.

Recently, a study was conducted comparing the VMBs of pregnant women at low risk for PTB and women who delivered preterm to examine differences in the microbial richness, diversity and differential abundances of specific taxa by 16s sequencing. The results provide further evidence that VMB composition is associated with PTB: women who delivered preterm had decreased lactobacilli species abundance and an increased abundance of BV-associated taxa.⁵³ Kindinger et al. revealed that CST III is correlated more specifically with a risk of early PTB rather than late PTB. Furthermore, non-lactobacillus species or altered vaginal microbial community in the 13-27 weeks of gestation does not appear to contribute to PTB.⁵⁴

As mentioned previously, the White race is more likely to harbour a lactobacillus-dominated microbiome, whereas the Black race is more likely to have a high diversity in the microbiome, but little to no lactobacillus and twice as likely to be diagnosed with BV and experience PTB. Thus, it is unclear whether dysbiosis in the vagina is an independent cause of PTB or merely an association.

Callahan et al. conducted a case-control study in two cohorts of pregnant women: Caucasians had a low risk for PTB, and African-Americans experienced high risk. In both cohorts, *L. crispatus* was associated with a low risk of PTB, while increased abundance of *Prevotella* was not.⁵⁵ These findings have been recently confirmed and extended.

The characterisation of the normal vaginal flora can help to protect a more fertile environment and development-appropriate treatment. To understand the pathological events related to vaginitis, it is necessary to understand the normal vaginal flora versus that related to PTB. The study of microbiome will detect the type of pathogenic microorganisms. Besides, it will allow the possibility to detect the recovery rate of pregnancy outcomes. Further work is needed to study ethnic and other genetic differences to elucidate the causes of PTB and how bacteria are capable of interacting with the vaginal flora and host.

Looking to the Future

It is evident that the mode of birth affects a newborn's microbiome composition. Large-scale epidemiological studies have reported that caesarean section (CS) can cause long-term health problems for infants, like asthma, allergies and other immune conditions, compared to babies born vaginally.^{56,57} Only one study reported that mode of birth could not affect a newborn's microflora.⁵⁸ However, different studies have confirmed the important role of vaginal delivery because it transfers positive bacteria strains to the infant. Crucially, a mother's VMB may play an important role in lifelong microbiome composition and health. Newborns delivered by CS lack strains of gut bacteria present in those delivered naturally, and their guts harbour harmful microbes that are common in hospitals. Recently, Li et al. found that different modes of birth delivery affect oral bacteria in healthy newborns.⁵⁹

Several studies have investigated how to reduce this risk by restoring the microbiome of CS babies. Recently, Domingues-Bello et al. transferred maternal vaginal bacteria to the infants delivered by CS with vaginal fluids, transferring maternal vaginal bacteria to the newborn, to restore any missing microbes within 2 minutes of birth via a swab over the newborn's body. The investigators found that, similar to vaginally-delivered newborns, skin, gut and oral microbiota during the first month was enriched within vaginal bacteria.⁵⁷ However, the safety and effectiveness of these procedures are unproven. Longitudinal studies need to explore whether these changes have any effect on infants' long-term health.

In 2019, a large-scale birth cohort study in the United Kingdom analysed gut microbiota using deoxyribonucleic acid (DNA) sequencing and genomic analysis from nearly

600 full-term babies born in British hospitals. The study found that the mode of delivery had an effect on the infant's gut microbes.⁶⁰ Eventually, the microbiomes of CS babies shifted to become more similar to those of vaginally-born babies after they had been weaned.

Conclusion

The vaginal microbiome composition of women fluctuates from birth to menopause, and it differs greatly between women. The dysbiosis of the vagina affects the women during pregnancy. Research is needed to elucidate the influence of microbiome in reproductive health outcome for each woman.

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