

Vaginal Microbiome and Reproductive Health

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ABSTRACT

The vaginal microbiome is a dynamic and diverse microbial community that plays a fundamental role in women's reproductive health. Dominated primarily by *Lactobacillus* species, it maintains vaginal homeostasis through acidification, antimicrobial compound production, and pathogen inhibition. Disruptions in this balance, influenced by hormonal fluctuations, sexual activity, hygiene practices, antibiotic use, and lifestyle factors, can lead to dysbiosis, bacterial vaginosis, and increased susceptibility to sexually transmitted infections. The composition of the vaginal microbiome varies across different stages of life, pregnancy, and ethnic groups, with distinct community state types (CSTs) conferring varying degrees of protection or risk. Growing evidence underscores its impact on fertility, pregnancy outcomes, neonatal health, and postpartum complications. Advances in sequencing technologies have deepened understanding of its composition and function, highlighting opportunities for microbiome-based diagnostics and therapeutic interventions to improve reproductive and maternal health.

Keywords: Vaginal microbiome, Reproductive health, *Lactobacillus*, Dysbiosis and Pregnancy outcomes.

INTRODUCTION

The vaginal microbiome is increasingly recognized as a key determinant of women's reproductive health. It influences fertility, adverse pregnancy outcomes, and postpartum complications. Despite accumulating evidence, substantial gaps and challenges remain in understanding this complex microbial ecosystem [1]. The vaginal microbiome comprises an array of bacteria, archaea, protozoa, fungi, and viruses inhabiting the female reproductive tract. Many of these microorganisms regulate food metabolism and maintain mucosal integrity. Their imbalance leads to diseases through altered absorption dynamics, impaired immune development, and compromised infection protection. Improvements in sequencing technologies have expanded comprehension of vaginal microbial composition and potential functional applications. Yet, resulting variations caused by genetic predisposition, diet, or environmental stimuli predispose to dysbiosis, inflammation, and adverse reproductive outcomes [2]. Under most circumstances, the vaginal flora is dominated by *Lactobacillus* species. These bacteria rapidly colonize epithelial cells and create a protective barrier against invading pathogens, actively inhibiting multiple microorganisms. The resulting acidic pH, hydrogen peroxide, and bacteriocin production further maintain eubiosis. The high d/l lactate ratio indicates bacteria rather than host cells as the main source of vaginal acidity [3]. Different species confer varying stability; for example, *L. crispatus* strongly promotes vaginal homeostasis, whereas *L. iners* and *L. gasseri* associate with fluctuating states that may lead to microbial imbalance and inflammation. Biological cycles contribute significant fluctuations. The vaginal microbial composition changes markedly upon puberty, during menses, throughout pregnancy, and at menopause. Metagenomic assessments of different populations identify five community state types, four dominated by specific *Lactobacillus* species (*L. crispatus*, *L. gasseri*, *L. iners*, *L. jensenii*) and a fifth characterized by mixed anaerobic communities. Frequent vaginal douching, commensurate with Asian or Hispanic ethnicity, appears to disrupt indigenous *Lactobacillus* populations and foster bacterial vaginosis. Temporal dynamics demonstrate a natural progression away from CST I (*L. crispatus*-dominance) during late pregnancy and lactation, gradually reverting to CST I predominance at 9 to 12 months postpartum. This interplay suggests mechanisms whereby the microbiome influences reproductive parameters throughout the lifespan.

Understanding the Vaginal Microbiome

The vaginal microbiome is a unique and dynamic ecosystem in the female host that plays a pivotal role in health and disease. It consists of different microorganisms, including bacteria, fungi, viruses and archaea, forming complex interactions with the host and amongst each other [2]. These interactions support vital functions such as immune regulation, protection against pathogens, and maintenance of the reproductive tract's physiological conditions. An imbalance in this microbiome can lead to various vaginal infections and sexually transmitted infections. The vaginal microbiota changes with different stages of the female reproductive and postreproductive life cycle due to variations in hormone levels and glycogen supply. Typically protective populations of *Lactobacillus* decrease markedly as women enter menopause, which made researchers inquire whether this decline contributes to postmenopausal health risks. In premenopausal women, *Lactobacillus* species are predominant in the vaginal microbial community [2]. However, disruption of this protective *Lactobacillus*-dominated microbiota can lead to vaginal dysbiosis, resulting in prevalence of anaerobic bacteria such as *Gardnerella*, *Prevotella*, and *Sneathia*. The vaginal microbiome plays a crucial role in reproductive and obstetrical health. It is involved in fertilization, maintenance of pregnancy, and parturition, offering protective effects against urogenital infections during pregnancy and/or puerperium. The presence of healthy vaginal flora is associated with better pregnancy and neonatal outcomes, while disturbance significantly increases the risk of complications during pregnancy. Understanding the vaginal microbiome is therefore crucial in the field of reproductive and obstetrical health [2].

Definition and Composition

The vaginal microbiome constitutes microorganisms that inhabit the vagina, including bacteria, fungi, viruses, and archaea [2]. Although the vaginal bacterial population is dominated by *Lactobacillus* species during reproductive-age women, healthy states may also be characterized by the absence of *Lactobacillus* dominance and an increased abundance of facultative anaerobes such as *Gardnerella*, *Atopobium*, or *Prevotella*, among others, which do not generally signify dysbiosis [4]. *Lactobacillus* species play a pivotal role in maintaining the vaginal microbial ecosystem primarily through the production of antimicrobial compounds. When dominance by *Lactobacillus* is absent, niche protection against vaginal pathogens is conferred by microbial diversity.

Role of *Lactobacillus*

The vaginal microbiota is dominated by *Lactobacillus* species in most women, but the composition of *Lactobacillus* species varies among individuals [5]. Loss of *Lactobacillus* dominance promotes colonization by anaerobic bacteria and increases microbial diversity. Competition among vaginal microbial species is regulated by a variety of mechanisms: vaginal *Lactobacillus* species strongly adhere to epithelial surfaces, prevent bacterial adhesion, promote autophagy of infected cells and modulate inflammatory processes through a variety of pathways. Sexual activity and pregnancy may influence the dominance of *Lactobacillus* species in the vaginal microbiota. Longitudinal studies show that certain *Lactobacillus* species, such as *L. crispatus*, promote stability, while others like *L. iners* and *L. gasseri* may be associated with an increased likelihood of abnormal flora [6]. Variations in vaginal microbial communities have been observed across different racial groups and between healthy women and those at risk for preterm birth. The vaginal microbiota influences reproductive health [7]. *Lactobacilli* produce lactic acid and short chain fatty acids with antimicrobial and immunomodulatory effects; through acidification of the vaginal milieu, *Lactobacilli* strongly inhibit a variety of aerobic and anaerobic bacterial pathogens.

Diversity of Microbial Species

The vaginal microbiome consists of a complex ecosystem of microorganisms that are integrated within a mucus layer deposited over the vaginal epithelial cells [3]. A healthy vaginal microbiome is commonly dominated by *Lactobacillus* species, which represent about 70% of healthy women worldwide. These bacteria contribute to host immunological and physiological homeostasis through the production of antimicrobial compounds such as bacteriocins and lactic acid, thereby preserving the low vaginal pH [5]. The low pH protects humans from colonization by pathogenic microbes and promotes the growth of bacterial species that produce the by-products responsible for maintaining this acidity. Relative to the mean number of species harbored, human vaginal communities are the least diverse at both the species and phylogenetic levels. However, a certain level of diversity, reaching the protective and steady-state colonization of different microbial members, is essential in microbiomes, including those of the vagina. The degree of proximity in microbial interaction networks predicts the stability of microbial communities. A diverse microbial network is better positioned to cope with personalized disturbances and to sustain post-disruption regrowth of eubiotic conditions. Different vaginal microbial groups, classified as community-state types (CSTs), dominate different women. High-throughput sequencing has identified at least five CSTs. CST-I is dominated by *Lactobacillus crispatus*; CST-II by *Lactobacillus gasseri*; CST-III by *Lactobacillus iners*; CST-V by *Lactobacillus jensenii*; and CST-IV consists of diverse anaerobic species such as *Gardnerella*, *Atopobium*, *Mobiluncus*, and others. CST-IV is more common among Black and Hispanic women, with a prevalence ranging from 10% to 60% depending on geographic location. Factors responsible for these racial and

geographic differences are not yet well defined but may involve sexual behaviour, hygienic practices, host genetics, or rectal colonization [5].

Factors Influencing the Vaginal Microbiome

The vaginal microbiome is a dynamic community of microorganisms whose composition can be modulated by factors including hormonal fluctuations, sexual activity, antibiotic use, and lifestyle characteristics. This section considers how such influences alter the microbiome and thereby shape women's reproductive health [1]. Sex hormone level is a key regulator of the vaginal microbial landscape, as illustrated by changes that occur during puberty, pregnancy, the late phase of the menstrual cycle, and menopause. Estradiol exerts a strong effect on the structure and function of the vaginal epithelium, driving the up-regulation of glycogen production. Intracellular glycogen is metabolized and excreted as maltose, maltotriose, maltopentaose, and alpha-amylase, which serve as energy sources and substrates for vaginal microorganisms. Microorganisms metabolize these substrates to lactic acid and other acids, thereby establishing a stable vaginal microbial community. Variations in glucose levels also influence microbial diversity, and dramatic changes in glucose supply can perturb the balance of commensal bacteria [8]. Microbial shifts also fluctuate during the menstrual cycle, responding to the progressive variations in vaginal glycogen, mucin, and bioavailable iron. Estrogen influences the vaginal environment through more complex mechanisms; recent studies have shown that many lactic acid-producing bacteria harbor the receptors ESR1 and ESR2 at different levels, making it possible to respond directly to circulating estrogens [2]. Other major exogenous factors also determine the vaginal microbial pattern. For instance, sexual activity is associated with fluctuating microbial profiles on the vulva and external inguinal region. Unprotected sexual intercourse leads to an increased number of genera typical of the male genital organ (*Escherichia*, *Shigella*, *Megasphaera*, and *Ureaplasma*), as well as to quantities of *L. crispatus*. Multiple sexual partners are associated with an increased prevalence of *G. vaginalis* and of *Ureaplasma*, while condom use is associated with a decrease of *G. vaginalis*. Increased frequencies of sexual intercourse correlate with higher quantity of *G. vaginalis* and the presence of *L. murinus*, whereas an increased time since the last sexual intercourse is associated with increased quantities of *L. jensenii* and overall lower concentrations of various genera and species [1]. Additional lifestyle factors can negatively influence the vaginal microbial community. Daily treatment with soap and bubble bath products is associated with increased abundance of *Pseudomonas* and *Myrsococcus*, respectively. Antiseptic solutions used for genital hygiene (chlorhexidine, hydrogen peroxide, or iodine) also profoundly modify the bacterial community and reduce the total bacterial load. Smoking modifies the vaginal ecology by increasing the presence of potential pathogenic agents such as species of *Candida*, *Staphylococcus*, and *Peptostreptococcus*. The use of vaginal lubricants clearly diminishes the abundance of *Lactobacillus* spp., similar to the effects of vaginal douching. Taken together, these effects can synergistically increase the risk of negative reproductive sequelae and prevent long-term vaginal homeostasis [12].

Hormonal Changes

The composition dynamics of the vaginal microbiome (VMB) are significantly influenced by female reproductive physiology and changing levels of female sex hormones [9]. Few studies have longitudinally characterized the VMB before and after menarche, but increases in *Lactobacillus* spp. are associated with rising estrogen levels rather than menarche itself. During menses, vaginal microbial diversity tends to increase while *Lactobacillus* abundance decreases, and hormonal contraceptives disrupt these cyclical changes. Some women show cyclical alterations in vaginal microbial community correlated with menstrual cycles, others maintain stable communities. Pregnancy is associated with a higher abundance of *Lactobacillus* spp. and more stable vaginal microbiota over time. Menopause involves changes in VMB composition, but specific details are not provided [5]. Reproductive hormones affect the vaginal microecology. Throughout life, physical development, health, emotional fluctuations, and disease alter endocrine. Abnormal endocrine leads to microecological imbalances closely related to the pathological state of different physiological and pathological stages such as puberty, menstruation, pregnancy, and menopause [2, 3, 8]. The estrogen and progesterone in female sex hormones strongly influence the vaginal microbiota during the reproductive period. The vaginal microbiota varies throughout reproductive life for estrogen and progesterone not only modulate epithelial cell proliferation and exfoliation but also increase the concentration of glycogen in vaginal epithelial cells [2]. The fertility factors associated with reproductive endocrine and the genital tract environment affect the vaginal microbiome. The bacterial species *Escherichia coli*, *Lactobacillus iners*, *Streptococcus agalactiae*, and *Prevotella intermedia* serve as biomarkers to reflect the pathological state of reproductive endocrine and genital tract [8].

Sexual Activity

Sexual activity has a significant effect on vaginal microbiota composition, with unprotected intercourse leading to a decrease in protective *Lactobacillus* species and greater microbial diversity. A 16S ribosomal RNA gene survey of South African women revealed that unprotected sexual intercourse correlates with lower proportions of *L. crispatus* and *L. jensenii*, allowing the proliferation of anaerobic communities dominated by *Gardnerella*,

Prevotella, and Atopobium [2]. Similar findings were reported in other studies, where sexual activity reduced Lactobacillus abundance and triggered the outgrowth of Prevotella, Streptococcus, Mycoplasma, and Sneathia species 9. The absence of contraception and consistent vaginal sex can increase the diversity of vaginal microbes, which in turn raises the risk of sexually transmitted infections. Condom use appears to protect against microbial shifts by reducing exposure to partner's semen, which is alkaline and can increase vaginal pH, thereby disturbing the microbial environment [3]. Loop-mediated isothermal amplification (LAMP) assays targeting Gardnerella vaginalis have been suggested as simple, rapid, and sensitive testing methods for routine clinical screening of vaginal infections, particularly in cases of abnormal vaginal discharge related to sexual activity.

Antibiotic Use

Antibiotic use has long been a cornerstone of disease management in the modern world, tackling severe disease while promoting somatic and reproductive health. However, antibiotic exposure can exacerbate vaginal dysbiosis during reproductive-aged women's symptom-free intervals, upregulating markers of genital inflammation and barrier damage that are associated with HIV risk. Despite this, there is no indication of vaginal inflammation in women who develop dysbiosis while on antibiotics [10]. Furthermore, reoccurrences of dysbiosis after treatment indicate a delay of approximately 30 days before the vaginal microbiome returns to its stable, Lactobacillus-replete state, illustrating the microbiome's substantial resilience [10]. The results underscore the necessity to consider the effects of antibiotics on clinical management practices, particularly in regions with high antibiotic consumption and increased risk for STIs and HIV transmission [10].

Diet and Lifestyle

Diet and lifestyle constitute modifiable factors with the potential to shape the vaginal microbiome. Lactic acid bacteria represent the first line of defense at the mucosal level, playing a crucial role in maintaining a balanced ecosystem, preventing invasion of pathogenic microbes, and modulating immune responses [11]. The existence of a close correlation between diet and the composition of the gut microbiota, together with the limited data relating to diet and the vaginal microbiota, has stimulated efforts towards a better understanding of the role of diet in maintaining a healthy vaginal microbiome. Existing data show that the ingestion of certain probiotics can influence the gut microbiota, promoting the maintenance of vaginal microbiota balance. Modulation of the vaginal microbiota through diet and oral probiotics has therefore the potential to promote women's overall health and wellbeing [11].

Vaginal Microbiome and Reproductive Health

The vaginal microbiome is recognized as a key biological system influencing women's reproductive health. During a healthy state, it is typically dominated by Lactobacillus species, which play a protective role by producing lactic acid that maintains a low vaginal pH environment [1]. Conversely, a vagina exhibiting a diverse microbial community depleted of Lactobacillus has been associated with adverse reproductive outcomes [3]. These observations are reviewed with a particular focus on the relationships between the vaginal microbiome and reproductive health. All living organisms are colonized by microorganisms, collectively referred to as microbiota; the combined genetic material of these microorganisms constitutes the microbiome. The human body, inclusive of external and internal body regions, harbors diverse microbial communities that contribute to the preservation of several fundamental functions. The microbial population is colonized in biological areas exposed to the exterior such as the skin, mouth, nose and vaginal mucosa [1]. The vaginal microbiome has been characterized using both cultivation-dependent and independent molecular techniques. Both approaches have revealed that the healthy vaginal microbiome depends on colonization by a handful of dominant Lactobacillus species, each of which promotes a low pH environment. The role of the vaginal microbiota in maintaining women's health is recognized as critical. Ruthless efforts have been made to understand the influence of the vaginal microbiota on improvement of female human well-being. Since it represents a complex environment, the microbial metabolism of the vaginal microbiota is still poorly unraveled. The host is recognized as a key factor shaping the vaginal microbiome and longitudinal surveys clearly demonstrated the dynamic nature of the vaginal microbiota during a woman's life span. Although the influence of sex hormones is well documented, other factors, including chemicals and personal hygiene products, which persist in the vaginal habitat, could be potentially harmful and require further in-depth investigations. A more comprehensive study of the vaginal microbiome using culture-based strategies will not only elucidate the diverse microbiota-host interactions and their kinetics in vaginal dysbiosis and disease but also help identify novel candidates that can be exploited in probiotic formulations for anti-pathogen-mediated diseases [3].

Impact on Fertility

Disruptions in the vaginal microbiome have been linked to several adverse fertility outcomes. In vitro fertilization (IVF) patients with a vaginal microbiota depleted of Lactobacillus spp. are less likely to become pregnant. Presence of certain organisms, such as Gardnerella vaginalis, may lessen the chances of implantation. Streptococcus viridans has been isolated from cases of tubal factor infertility following pelvic inflammatory

disease. Bacterial vaginosis has also been linked to an increased risk of infertility, miscarriage, preterm birth, low birth weight, and postpartum endometritis [1, 10]. Other disorders and infections can contribute to infertility. Yeast infections, although not directly linked with infertility, can increase the likelihood of acquiring human immunodeficiency virus (HIV) or human papilloma virus (HPV). These diseases have been associated with sterility and cause a host of other reproductive health issues including cervical cancer. Sexually transmitted infections such as chlamydia, gonorrhea, and mycoplasma can contribute to infertility, premature births, and even high-risk pregnancies [11].

Pregnancy Outcomes

The composition of the vaginal microbiota during pregnancy reflects a marked increase in *Lactobacillus* species coupled with enhanced stability throughout gestation. Elevated oestrogen concentrations may drive this persistence, while postpartum the microbial community shifts toward reduced *Lactobacillus* abundance and greater taxa diversity [11]. Positive pregnancy outcomes correlate with a predominance of *Lactobacillus* spp., whereas higher microbial diversity and diminished *Lactobacillus* presence associate with complications such as miscarriage and ectopic gestation. Abnormal vaginal microbiotas adversely affect clinical pregnancy rates in assisted reproductive technologies, with *Lactobacillus*-dominant profiles favoring in vitro fertilization success. Data on the microbiota's role in early pregnancy events remain limited; nonetheless, *Lactobacillus* depletion has been implicated as a risk factor for miscarriage and ectopic pregnancy [12].

Postpartum Health

The vaginal microbiome undergoes notable changes during and after pregnancy that can influence postpartum health [13]. Microbiota composition differs between pregnant and non-pregnant women, with pregnant individuals exhibiting greater community stability and lower diversity of certain microorganisms such as Mollicutes [6]. The immune system adopts a distinctive state of immune tolerance that facilitates the maintenance of pregnancy and protects the fetal semi-allograft during gestation. Structural properties of the cervical stroma are also relevant to postpartum recovery and health. During pregnancy the vaginal microbiota is dominated by *Lactobacillus* species, whereas postpartum samples contain fewer *Lactobacillus* and exhibit higher species diversity. Bacterial vaginosis associated bacteria such as *Gardnerella*, *Atopobium* and *Sneathia* are more abundant in postpartum vaginal communities. High abundance of *Bifidobacterium*, along with *Prevotella*, *Porphyromonas* and *Mobiluncus* also characterize the postpartum vaginal microbiota [3, 13].

Disruptions in the Vaginal Microbiome

A healthy vaginal microbiome, dominated by protective *Lactobacillus* species, supports reproductive health throughout the childbearing years [1]. Disruptions permit overgrowth of various pathogenic anaerobic bacteria and are associated with vaginal conditions such as bacterial vaginosis (BV), yeast infections, and sexually transmitted infections [4]. Bacterial communities associated with BV replace the protective lactobacilli, resulting in a heightened vaginal pH. Most women experiencing BV as a vaginal lactobacilli imbalance become symptomatic, and many experience recurrence after standard antibiotic treatment. Aerobic vaginitis (AV) and yeast infections have comparatively well-documented clinical etiology. Both have established ideas regarding pathogenesis and mechanisms of infection, but the microbial alterations accompanying these conditions remain poorly characterized [4]. BV has been reported to be the most common cause of vaginal discharge worldwide, complicated by unclear causation of the employed therapeutic antibiotic medication. If the vaginal microbiome is disrupted, the propensity for microbial transfer as a sexually transmitted disease to a sexual partner is greater, increasing the risk of developing pelvic inflammatory disease and adverse effects during pregnancy and labour. Disruptions to the healthy vaginal microbiome can therefore significantly adversely influence reproductive outcomes and overall well-being [4].

Bacterial Vaginosis

Bacterial vaginosis (BV) is the most common vaginal disorder worldwide, particularly affecting women during their reproductive years. It occurs when the vaginal flora shifts from a healthy, *Lactobacillus*-dominant state to an overgrowth of anaerobic bacteria, including *Gardnerella vaginalis*, *Prevotella bivia*, *Atopobium vaginae*, and *Mobiluncus mulieris* [14]. The resulting dysbiosis disrupts both the microbial and immunometabolic landscape of the cervicovaginal mucosa and mucosal immune defenses, increasing susceptibility to gynecologic and reproductive complications [15]. Immune-metabolic perturbations in the host become more pronounced as the number of BV-associated organisms proliferating in this polymicrobial mucosal environment increases, and the severity and complexity of clinical disease progress [16]. Motivated by the need to understand these pathogenic processes, an advanced three-dimensional cervical epithelial cell model was developed using rotating wall vessel bioreactor technology to characterize the immune responses and tolerant microenvironment induced by single, dual, and complex bacterial cervicovaginal infections in BV.

Yeast Infections

Microscopic yeasts from the *Candida* genus can cause infections affecting various tissues and organs of the body. For instance, although vaginal yeast infections (vulvovaginal candidiasis, VVC) can affect a large number of women, these infections lack a good causative agent. *Candida albicans* is the primary pathogen, but non-*albicans* *Candida* species such as *Candida glabrata* can be etiological agents, becoming more prevalent in recurrent or persistent episodes. Vulvovaginal candidiasis is one of the most common infections affecting women worldwide, with up to 75% experiencing it at least once in their lifetime. *Candida albicans* is a normal member of the vaginal microbiota in most women; without the protective role of lactobacilli, the yeast can outgrow, leading to VVC [14]. Severe VVC episodes cause cell exfoliation and adhesion, strong neutrophil infiltration, pronounced erythema, and intense burning in the vaginal region. However, a consensus on whether different *Candida* species affect the vaginal microbiota disparately remains elusive due to diverse study populations and methodologies. Nevertheless, research generally agrees that in yeast infections, the vaginal microbiome becomes less lactobacilli-dominated and more diverse. The specific bacteria co-occurring with either *Candida albicans* or non-*albicans* species in the vagina and the effect on infection recurrence are topics still under discussion [16].

Sexually Transmitted Infections

The vaginal microbiome influences the risk and transmission of sexually transmitted infections (STIs). Maintaining a *Lactobacillus* -dominated microbiome is associated with reduced risk of STIs: a beneficial effect that extends beyond bacterial vaginosis and conditions of reduced microbial diversity. This protective effect may be mediated by the ability of a *Lactobacillus* dominant community to acidify the mucosal environment, to produce bacteriocins that inhibit specific bacterial pathogens or to physically mediate pathogen adhesion. In sub-Saharan African populations, vaginal microbial communities dominated by species associated with bacterial vaginosis correlate with increased risk of HIV acquisition, in a pattern that may be generalizable across populations [14]. The protective effect conferred by *Lactobacillus* -dominated communities varies amongst these bacterial taxa. Bacterial communities dominated by *Lactobacillus crispatus* appear to be particularly resilient and associated with reduced risk of STI acquisition, while *Lactobacillus iners* – a transcriptomically distinct species that produces less lactic acid and lays dormant in the presence of a pathogen – does not confer a similar level of protection and often co-occurs with metronidazole-resistant bacterial vaginosis-associated anaerobes [14].

Diagnosis and Assessment

Microbiome testing can be performed on vaginal swabs to assess the abundance and composition of vaginal microbiota. The linear array method enables intermediate-resolution identification of microbial species, while next-generation sequencing platforms provide comprehensive, high-resolution data on the microbial community [17]. Advanced mass spectrometry techniques, such as desorption electrospray ionization mass spectrometry (DESI-MS), allow rapid, direct analysis of vaginal swabs, giving insights into the metabolomic profile associated with specific vaginal microbiota compositions [18]. In the clinical setting, reported symptoms include abnormal vaginal discharge, vaginal pruritus, offensive odor, burning sensation, and painful urination. Examination of vaginal discharge helps to identify its source: vulvar (e.g., lipids), vaginal (e.g., sexual lubricant), or cervical (e.g., cervical mucus). For a more comprehensive assessment of microbiota–host interactions at the vaginal mucosa interface, upper vaginal swabs can be collected for microbiomic, immunological, and metabolic profiling. Integration of data from these approaches may facilitate biomarker discovery, disease risk stratification, and mechanistic understanding of vaginal microbial dynamics during health and disease [17].

Microbiome Testing Methods

The vaginal microbiome comprises microorganisms residing in the female reproductive tract and plays a crucial role in reproductive health. *Lactobacillus* spp., dominant in 70% of healthy women, produces lactic acid that maintains acidic vaginal pH (~4.5), alongside bacteriocins and hydrogen peroxide that inhibit pathogenic growth [18]. Microbial diversity varies among ethnicities, with non-*Lactobacillus* dominance linked to increased risks such as preterm birth and pelvic inflammatory disease. Hormonal fluctuations, such as those during the menstrual cycle and pregnancy, influence the microbiome composition, often decreasing diversity and enhancing *Lactobacillus* prevalence. Sexual intercourse, antibiotic use, socioeconomic and hygiene factors further modulate the microbial balance [19]. The vaginal microbiome affects fertility, pregnancy outcomes, miscarriage rates, and postpartum health. Disruptions lead to conditions like bacterial vaginosis, yeast infections, aerobic vaginitis, STIs, and reproductive complications including miscarriage, preterm birth, low birthweight, and premature rupture of membranes. Diagnosing the microbiome status involves microbiome testing and assessment of clinical symptoms. Microbiological testing techniques include microscopy, culture, and specific assays for *Candida*, *Chlamydia*, *Trichomonas*, and signs of inflammation, whereas *G. vaginalis* identification requires molecular profiling [15]. Molecular approaches offer improved diagnostics for bacterial vaginosis, describing microbial composition and metabolic activities via taxonomic and functional unfold analysis. Quantitative PCR serves as a practical tool to evaluate microbiota associated with bacterial vaginosis. Sampling the vaginal mucosa followed by next-generation

sequencing, combined with immunological and metabolic profiling, enables detailed characterization of microbial composition and host response. Optimizing methodological and analytical workflows for these multi-omic platforms is essential to limit technical and experimental biases that compromise data interpretation and reproducibility. Ambient ionization mass spectrometry techniques such as DESI-MS allow rapid, direct analysis of vaginal swabs and detection of metabolite changes linked to vaginal microbiota composition [19].

Clinical Symptoms and Diagnosis

Diagnosis of vaginal microbiome problems is essential for identifying conditions such as bacterial vaginosis (BV) [17]. Vaginal microbiome testing can provide valuable information on the presence and abundance of specific microbes, aiding in the assessment of disruptions and guiding targeted interventions. Clinical symptoms play a significant role in the diagnosis of vaginal microbiome disorders. Women with BV often experience abnormal vaginal discharge, typically thin, greyish-white, and accompanied by a strong fishy odor, especially after sexual intercourse. Other symptoms may include vulvar itching, irritation, and dysuria. However, about 50% of women with BV may not exhibit any symptoms. BV is characterized by a thin, milky non-viscous discharge that adheres to the vaginal walls. During an active BV infection, a clinician may observe a “profuse and thin discharge coating the vaginal sidewall.” The vaginal pH usually rises above 4.5, reaching values upwards of 6.5. Several diagnostic methods are available to detect disturbances of the vaginal microbiome. One approach is direct urine dipstick examination, which, combined with vaginal discharge evaluation, can assess pH and amines. In many low-income settings, leucocytes and nitrites are often used alongside pH and amines to diagnose bacterial, fungal, or parasitic infections. Microscopy remains the official gold standard for vaginal microbiome diagnosis, with the Nugent scoring system frequently employed due to its time and cost efficiency. This method classifies vaginal smears by counting bacteria at $\times 100$ magnification; a Nugent score greater than 7 indicates BV, while scores between 4 and 6 are considered transitional. Microscopy-based diagnosis enables further evaluation of other infections, including candidiasis and *Trichomonas vaginalis* [17].

Management and Treatment

Management and treatment strategies for bacterial vaginosis emphasize diagnosis aligned with established guidelines. Probiotics such as *Lactobacillus crispatus* may restore healthy vaginal microbiota; however, their efficacy can decline with sexual activity [1]. Antibiotic therapies remain a common prescription. Insight into vaginal microbiota dynamics during pregnancy further informs management approaches. Restoring and sustaining a balanced vaginal microbiome is integral to women's health and disease prevention.

Probiotics and Prebiotics

The use of probiotics and prebiotics as strategies to prevent and treat vaginal dysbiosis has garnered increasing attention. Probiotics are live microorganisms providing health benefits when administered in adequate amounts. Prebiotics consist of non-digestible substrates that selectively promote the growth of beneficial bacteria [1]. Probiotic strains considered suitable for vaginal administration often originate from *Lactobacillus* species isolated from the vaginal environment, although other microorganisms such as *Bifidobacterium* may also be included. Probiotics serve various functions related to the maintenance of vaginal homeostasis and the prevention of imbalances. Initial clinical trials evaluating *Lactobacillus* probiotics for bacterial vaginosis highlight challenges; preliminary evidence does not conclusively support their effectiveness as adjunctive or replacement therapy. Uncertainties persist regarding optimal species, strains, combinations, timing of administration, and mechanisms of action whether colonization is necessary or if transient activity suffices to restore a *Lactobacillus*-dominated low-pH environment. Further research aims to identify formulations capable of successfully reestablishing a protective vaginal microbiota [1]. Growing evidence also underscores the presence of microorganisms throughout the reproductive tract, where each tissue harbours a unique microbiome. These reproductive microorganisms play a key role in fertility and fitness by maintaining ecological balance; their alteration can induce dysfunction and adverse pregnancy outcomes. Probiotics offer promising therapeutic potential by modulating the composition of gut and reproductive microbiota, regulating host metabolism, enhancing epithelial barrier function, and modulating immune responses. Dietary supplementation with probiotics may thus improve reproductive function and offspring health, influence bacterial transmission from gut to milk and female reproductive tract, and impact maternal and infant well-being [20].

Antibiotic Therapy

The vaginal microbiome is crucial for health and can be disrupted by antibiotics [21]. Bacterial communities dominated by *Lactobacillus* species maintain homeostasis, whereas bacterial vaginosis (BV) is characterized by *Gardnerella vaginalis*, *Prevotella*, *Atopobium*, *Sneathia*, and *Megasphaera* dominance. Tinidazole combined with *Lactobacillus reuteri* RC-14 and *L. rhamnosus* GR-1 restores indigenous *L. iners* or *L. crispatus*, supporting antibiotic-probiotic synergy for BV treatment. Despite a stable understanding of optimal and perturbed vaginal microbial communities, therapies have remained largely unchanged for over 40 years, resulting in frequent recurrences and challenges including drug resistance, inadequate biofilm penetration, and failure to reestablish a

balanced bacterial community. High BV recurrence rates after metronidazole treatment underscore the need for improved prevention strategies, especially given BV's associations with HIV acquisition and adverse pregnancy outcomes [22]. A Rwandan pilot study evaluated the safety and efficacy of intermittent metronidazole administration and vaginal probiotics containing *L. rhamnosus*, *L. acidophilus*, *L. brevis*, and *L. plantarum* following initial metronidazole therapy. All interventions were safe; although *Lactobacillus*-based probiotics temporarily increased beneficial lactobacilli and decreased BV-associated anaerobes, substantial inter-individual variability and rapid loss of effect after treatment cessation were observed. Probiotic approaches appear promising since they are less likely to disturb gut microbiota or promote antimicrobial resistance compared with repeated antibiotic courses. The prevalence of BV and other dysbioses, as well as the common practice of empirical antibiotic or antifungal treatment without diagnostic testing, further highlights the need for effective and safe vaginal microbiome management options [21, 22].

Lifestyle Modifications

Lifestyle factors play an important role in shaping the vaginal microbiome, offering a means to enhance vaginal health and potentially contribute to reproductive success [3]. Diet and oral probiotics have been extensively examined for their influence on vaginal microbial communities. Probiotic therapy and nutritional interventions are associated with lower rates of bacterial vaginosis, pre-eclampsia, preterm birth, and improved pregnancy outcomes. During pregnancy, the intake of probiotic milk is effective in reducing the likelihood of preterm delivery. Vaginal microbiota changes over the course of a pregnancy, with particular probiotic strains implicated in maintaining microbial balance. Interventions involving vaginal administration of *Lactobacillus* strains during the perinatal period decrease the risk of preterm birth through mechanisms that may include the immunomodulation of inflammatory responses and pathogen antagonism [3]. Additional lifestyle modifications can be initiated to reinforce probiotic supplementation or monotherapies, operating under the premise that they reduce biotic and abiotic perturbations to microbial communities. Abstaining from smoking, balancing adult-onset diabetes, achieving a healthy body mass index, and eliminating sugar-sweetened beverages are associated with a lower concentration of vaginal microbes linked to inflammatory conditions. Moreover, limiting vaginal douching and using vaginal lubricants are recommended to protect epithelial barriers [3]. A critical reduction of the vaginal microbiota and enhancement of diverse anaerobic populations have been demonstrated with the use of panty liners, diapers, and menstrual cups, indicating their limited compatibility with maintaining

Research and Future Directions
Several critical areas of ongoing and future research remain before vaginal microbiome data can be effectively translated into clinical treatments [1]. Investigations into the influence of pregnancy or other physiological states on vaginal microbial transition, the role of vaginal microbiota in increasing susceptibility to HIV, the protective nature of *Lactobacillus*-dominated communities against bacterial vaginosis or sexually transmitted infections, and the utilization of vaginal microbiota in screening for HIV-1 or cervical cancer are actively pursued [23]. A nuanced understanding of factors that maintain or disrupt the vaginal ecosystem has the potential to prevent or reduce the rates of bacterial vaginosis and other vaginal infections. Correspondingly, several candidate live biotherapeutics are undergoing clinical development. As bioinformatics tools evolve, it will be feasible to integrate full-length 16S rRNA gene amplicon sequences with metagenomic and metatranscriptomic profiles to gain enhanced insights into the vaginal microbiome. A personalized approach matching treatment to the vaginal microbiome and host genome also merits investigation; potential interventions include modifying the mother's vaginal microbiota during or prior to pregnancy or exposing a newborn to beneficial microbiota immediately after birth. Further research is required to identify beneficial vaginal microbiota and elucidate how the mother's vaginal microbiota influences her daughter's microbiota and health outcomes. The complex interaction between microbiota at other body sites and the vaginal microbiota, such as a potential gut–vagina axis, represents an additional consideration. Efforts to develop strategies that restore a healthy vaginal environment comprise the overarching objective of these endeavors [1, 23].

Current Studies

Research on the composition of the vaginal microbiota has identified important factors associated with its maintenance and health. The key role of *Lactobacillus crispatus* and *L. vaginalis* in supporting vaginal health was confirmed, and associations with cervical microbiota composition were noted for ethnicity and host genetics. Risk factors most strongly linked to bacterial vaginosis include sexual activity, the absence of hydrogen peroxide-producing lactobacilli, black race, and herpes simplex virus type 2 serology [3]. External influences on vaginal health encompass cigarette smoking, which alters the vaginal metabolomic profile, as well as psychosocial stress through neuroendocrine and inflammatory pathways related to preterm birth. In sub-Saharan Africa, the prevalence of bacterial vaginosis varies among sub-populations and sexual risk factors have been identified. Extravaginal reservoirs of vaginal bacteria can increase the risk of bacterial vaginosis. Intravaginal practices such as vaginal douching are associated with bacterial vaginosis and HIV infection, and various hygiene products affect

the vaginal mucosal biome. These insights contribute to a comprehensive understanding of the vaginal microbiome and its implications for gynecological, obstetric, and sexual health [3].

Potential Therapies

Maintenance of a healthy vaginal microbiota is critical to reproductive health and failure to sustain *Lactobacillus* dominance is associated with greater risk of pelvic inflammatory disease, miscarriage, preterm birth, and HIV acquisition [1]. Probiotic administration is widely regarded as a safe treatment for recurrent vaginal dysbiosis and antibiotic-resistant bacterial vaginosis, but orally delivered strains appear unable to durably colonize the vagina and most clinical trials to date have found only modest benefit from their use [11]. Oral probiotics failed to reduce either prematurity or vaginal dysbiosis during pregnancy and there was minimal colonization following vaginal application. Conversely, vaginal application of a live biotherapeutic comprised of *Lactobacillus crispatus* was more effective, preventing recurrence of bacterial vaginosis after treatment with metronidazole, a result congruent with the protective effect of *L. crispatus* in pregnancy and elsewhere. Vaginal probiotics thus remain a promising avenue for intervention but they require additional clinical investigation and development. Research may also profitably explore vaginal microbiome transplantation. Transfer of fecal microbiota can durably correct microbial dysregulation underlying chronic *C. difficile* infection and the demand for vaginal therapies is similarly pronounced. Vaginal microbiome transplantation has been shown in an exploratory study to produce clinical remission in patients with symptomatic, intractable bacterial vaginosis, an encouraging observation that motivates further study and clinical development [1, 11].

Personalized Medicine Approaches

A woman's vaginal fluency, encompassing the openness of the vagina, genital secretions, and vaginal discharge, provides pertinent indications of the state of the vaginal microbiota. These clinical manifestations are qualitatively assessed from basic examinations such as swab examinations and medical histories [1]. The initial clinical examination employing microscopy (a simple, rapid, and cost-effective technique) includes measuring the vaginal pH, observing discharge and inflammation, performing a whiff test, and viewing Gram-stained vaginal smears when indicated. The vaginal ecosystem is a complex biological system. The usual standard methods for microbial diagnosis of disturbance and infection include vaginal pH measurement, vaginal fluid amines testing (the whiff test), quantification of white blood cells, bacterial morphotyping (with Gram stain), and culturing [1]. Candidate therapeutics soon to be evaluated for treatment of vaginal disruption include prebiotics and biogenics. If prebiotics are administered intravaginally, their ineffective components can be absorbed rapidly and reliably by the vaginal mucosa, and they are expected to act only on the vaginal microbiota. Although several prebiotic agents have shown promise in vitro or in animal models, only Polydextrose has thus far been tested in women [1].

Public Health Implications

The vaginal microbiota plays a vital role in dictating the health of the female reproductive tract and continues to be one of the key players in vaginal health [9]. It is therefore essential to understand how the vaginal microbiota can affect various reproductive-health outcomes, such as fertility, abortion, and pregnancy outcomes. An imbalance in the vaginal microbiota can often become a source of reproductive-health problems including infertility, irregular menstruation, abortion, and preterm birth [24]. Moreover, vaginal microbiome perturbations early in pregnancy can predict a higher risk of miscarriage, while distinct vaginal microbiome profiles at mid-gestation are associated with a higher risk of spontaneous preterm birth [1]. Despite the importance of the vaginal microbiota and the need to address these issues, awareness, proper education, and healthcare access for vaginal health remain poorly suited to handle instances of microbial imbalance. It is therefore necessary to increase efforts in these areas through public health interventions, workshops, and primary healthcare protocols to minimize the impact of vaginal microbiome dysbiosis.

Education and Awareness

Education and awareness are vital for empowering women to make informed choices about vaginal health. Increasing knowledge about the vaginal microbiome and reproductive well-being enables the adoption of healthy habits that support lactobacilli maintenance, thereby reducing the risk of infection [1]. Educational efforts are especially important for young women, notably those under 25 years, and for individuals with chronic health conditions. Monitoring awareness helps identify knowledge gaps and informs targeted interventions. Higher education levels correlate with enhanced understanding, underscoring the role of tailored communication in promoting microbiome and probiotic literacy [25]. A well-balanced vaginal microbiota, dominated by *Lactobacillus* species alongside constrained populations of anaerobic bacteria and *Candida* yeast, maintains vaginal homeostasis and prevents infections. Dysbiosis predisposes to conditions such as bacterial vaginosis and vulvovaginal candidiasis. *Lactobacillus* dominance is therefore crucial in preventing recurrent vulvovaginal infections. The vaginal microbiome also influences reproductive health, pregnancy outcomes, postpartum recovery, and newborn health. Given its impact on dietary choices and antibiotic use, awareness of microbiota and probiotics constitutes a valuable tool for enhancing women's health across the lifespan [25].

Access to Healthcare

Access to healthcare is a critical determinant for maintaining good health and preventing disease. Equitable access necessitates tackling obstacles related to socioeconomic status, geographic location, and healthcare infrastructure. Improved access often leads to enhanced health outcomes, especially among vulnerable groups [1]. Challenges in guaranteeing widespread healthcare availability include disparities in affordability, cultural acceptance, and the presence of necessary facilities. Policy initiatives often aim to dismantle these barriers and broaden coverage so more individuals receive essential medical interventions.

Policy Recommendations

Public health programs and clinical practice guidelines incorporating up-to-date diagnostic methods and effective microbiome-targeted therapy can contribute to the improvement of women's reproductive health. Increased awareness about the importance of vaginal health and vaginal microbiome can improve the uptake of preventive screening and timely treatment of vaginal infections and help reduce maternal morbidity and mortality, and racial/ethnic disparity in pregnancy complications. Public-funded microbiome testing and targeted therapy can alleviate the health inequity resulting from the cost of microbiome-associated prevention and treatment. Additional public health efforts are needed to increase education, improve access, and update national and state recommendations on vaginal care for the general public and healthcare providers [24, 25].

CONCLUSION

The vaginal microbiome is a critical determinant of reproductive and obstetrical health, influencing fertility, pregnancy maintenance, and protection against urogenital infections. While *Lactobacillus*-dominated microbiota are often associated with stability and favorable outcomes, shifts toward diverse anaerobic populations predispose women to adverse reproductive events and disease. Variations across hormonal stages, lifestyle behaviors, and ethnic populations emphasize the complexity of host-microbiome interactions. Future research should focus on identifying predictive microbial biomarkers, clarifying mechanisms underlying dysbiosis, and developing targeted interventions such as probiotics, prebiotics, and personalized therapies. A deeper understanding of the vaginal microbiome will not only advance reproductive medicine but also contribute to improved maternal and neonatal outcomes globally.

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