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Review Article

THE VAGINAL MICROBIOME IN GYNECOLOGICAL DISEASES: CLINICAL IMPLICATIONS, PRECISION DIAGNOSTICS AND FUTURE THERAPEUTIC STRATEGIES

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ABSTRACT

The vaginal microbiome is a dynamic microbial ecosystem that plays an essential role in maintaining female reproductive tract homeostasis, mucosal immunity, epithelial integrity and resistance to pathogenic colonization. In many healthy reproductive-age women, the vaginal environment is characterized by low microbial diversity and dominance of selected *Lactobacillus* species, whereas reduced lactobacilli, increased anaerobic diversity and altered microbial metabolism are commonly associated with vaginal dysbiosis. Growing evidence suggests that vaginal microbial alterations are related not only to bacterial vaginosis and recurrent vaginal infections but also to persistent human papillomavirus infection, cervical intraepithelial neoplasia, cervical cancer, endometriosis, polycystic ovary syndrome, infertility, implantation failure and gynecological malignancies. This review summarizes current knowledge regarding normal vaginal microbial ecology, host–microbe interactions, disease-associated dysbiosis, microbiome-derived biomarkers and emerging therapeutic strategies. Particular attention is given to advances in 16S ribosomal RNA sequencing, shotgun metagenomics, metabolomics, multi-omics and artificial intelligence-based microbial analysis. Current evidence indicates that vaginal microbial signatures have potential applications in disease-risk stratification, diagnosis, prognosis and treatment monitoring; however, considerable methodological heterogeneity and uncertainty regarding causality continue to limit clinical translation. Probiotics,

Lactobacillus-based live biotherapeutic products, biofilm-targeted therapies and vaginal microbiota transplantation represent promising approaches for ecological restoration. Future research should prioritize standardized sampling, longitudinal multicentre studies, functional microbiome analysis and adequately powered clinical trials to support the development of precision microbiome-based gynecology.

Keywords: Vaginal microbiome; vaginal microbiota; dysbiosis; *Lactobacillus*; gynecological diseases; human papillomavirus; infertility; precision medicine

INTRODUCTION

The human microbiome is increasingly recognized as an important biological determinant of health and disease. Microbial communities interact with epithelial surfaces, host metabolism, immune pathways and local tissue environments. Within the female reproductive system, the vaginal microbiome represents a highly specialized microbial ecosystem with direct relevance to gynecological and reproductive health.[1–3] Unlike the gastrointestinal microbiome, in which high microbial diversity is commonly associated with ecological resilience, the vaginal environment of many healthy reproductive-age women is characterized by relatively low bacterial diversity and dominance of selected *Lactobacillus* species.[1,3] The most frequently identified dominant organisms include *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus iners* and *Lactobacillus jensenii*.[1,2] These organisms contribute to vaginal homeostasis through lactic acid production, maintenance of an acidic vaginal pH, competitive exclusion of potentially pathogenic microorganisms and regulation of epithelial and mucosal immune responses.[3,4]

Vaginal Community State Types:

Molecular profiling of the vaginal microbiome has led to the classification of vaginal bacterial communities into distinct community state types. The landmark study by Ravel et al. identified major microbial patterns in reproductive-age women.[1] Community state type I is predominantly associated with *L. crispatus*, community state type II with *L. gasseri*, community state type III with *L. iners* and community state type V with *L. jensenii*. Community state type IV is characterized by reduced *Lactobacillus* abundance and increased representation of diverse anaerobic organisms.[1,5]

The community state type framework substantially changed the understanding of vaginal microbial ecology by demonstrating that apparently healthy women may possess different microbial community structures. Subsequent longitudinal investigations showed that these microbial states are not necessarily permanent and may change over relatively short periods.[6,7] Menstruation, hormonal status, pregnancy, menopause, sexual activity, antibiotic exposure, smoking, metabolic conditions and vaginal hygiene practices may influence the composition and stability of the vaginal microbiome.[6–8]

The dynamic nature of the vaginal microbiome has important methodological implications. A single sample may provide only a temporary representation of vaginal microbial composition. Therefore, longitudinal sampling may be more informative than single-time-point assessment when investigating disease development, treatment response or microbial recurrence.

Protective Functions Of Lactobacilli:

The protective effects of vaginal lactobacilli are mediated through several complementary biological mechanisms. Lactic acid production contributes to maintenance of a vaginal pH commonly below 4.5 in healthy reproductive-age women. This acidic environment restricts the growth of several potentially pathogenic microorganisms and influences microbial competition within the vaginal niche.[3,4]

Certain lactobacilli may also produce bacteriocins and other antimicrobial compounds, compete for epithelial adhesion sites and interact with vaginal epithelial cells to regulate local immune activity.[4,9] These mechanisms support colonization resistance and may reduce susceptibility to vaginal infections and inflammatory disorders.

However, individual *Lactobacillus* species are not functionally identical. *L. crispatus* is generally associated with greater ecological stability, lower vaginal pH and reduced abundance of dysbiosis-associated anaerobes.[3,4] In contrast, *L. iners* may be detected in both apparently healthy and dysbiotic communities and is frequently present during microbial transitions.[6,7] Consequently, total *Lactobacillus* abundance may not adequately define vaginal microbial health.

The functional activity of bacterial communities may be more clinically important than taxonomic composition alone. Two women with apparently similar bacterial profiles may demonstrate different clinical outcomes because of strain-level differences, microbial gene expression, metabolite production, hormonal conditions or host immune responses. This consideration has encouraged the transition from descriptive bacterial profiling toward functional metagenomic, transcriptomic and metabolomic investigation.

Vaginal Dysbiosis:

Vaginal dysbiosis broadly refers to disruption of the physiological microbial ecosystem. It is commonly characterized by reduced protective lactobacilli, increased microbial diversity, altered microbial metabolism and enrichment of anaerobic organisms such as *Gardnerella*, *Prevotella*, *Fannyhessea*, *Sneathia* and related taxa.[3,10–12]

Bacterial vaginosis represents the most extensively investigated clinical manifestation of vaginal dysbiosis. Contemporary evidence indicates that bacterial vaginosis is not caused by a single microorganism but instead represents a polymicrobial ecological disorder.[10–13] Interactions among *Gardnerella* and other anaerobic bacteria may facilitate biofilm formation, microbial persistence and reduced susceptibility to antimicrobial therapy.[11–13]

Polymicrobial biofilms attached to the vaginal epithelium may contribute to recurrent bacterial vaginosis after apparently successful treatment.[12,13] Conventional antimicrobial therapy may suppress dysbiosis-associated organisms and improve symptoms without restoring a stable, protective *Lactobacillus*-dominant ecosystem. This limitation has stimulated growing interest in microbiome-restoration strategies.

Vaginal dysbiosis may affect gynecological health through several interconnected mechanisms. Reduced lactic acid production may increase vaginal pH, while microbial enzymes and metabolites may alter epithelial integrity, vaginal mucus and local inflammatory signaling. Dysbiotic communities may also

influence cytokine production, immune-cell recruitment and antiviral immune responses.[9–11]

Nevertheless, microbial dysbiosis should not automatically be interpreted as a primary cause of gynecological disease. Hormonal abnormalities, inflammation, tissue damage, malignancy, antibiotic exposure and therapeutic interventions may themselves alter vaginal microbial composition. The relationship between the microbiome and gynecological disease is therefore likely to be bidirectional.

Vaginal Microbiome And Human Papillomavirus-Related Cervical Disease:

Persistent infection with high-risk human papillomavirus is the principal biological driver of cervical carcinogenesis. However, most human papillomavirus infections are transient and are eliminated by the host immune system. Only a proportion of affected women develop persistent infection, cervical intraepithelial neoplasia or invasive cervical cancer. Viral genotype, host immunity, smoking, sexual and reproductive factors and local tissue characteristics influence this progression.

The vaginal microbiome has emerged as a potential modifier of human papillomavirus persistence and cervical disease.[14–18] Systematic reviews and clinical studies have reported associations between non-*Lactobacillus*-dominant communities, increased microbial diversity, persistent high-risk human papillomavirus infection and cervical dysplasia.[14–16]

Specific anaerobic organisms, including *Gardnerella*, *Prevotella* and *Sneathia*, have been investigated in relation to persistent viral infection and cervical abnormalities.[14–18] Proposed mechanisms include disruption of epithelial barrier function, alteration of vaginal metabolites, increased mucosal inflammation and modification of local antiviral immune responses.

Longitudinal studies have demonstrated interactions between temporal changes in the vaginal microbiome and human papillomavirus detection.[15] Increased vaginal microbial diversity has also been associated with progression of cervical intraepithelial neoplasia in selected populations.[16]

However, most available evidence remains observational. Human papillomavirus infection and cervical epithelial changes may themselves alter the local immune environment and subsequently modify microbial composition. Therefore, causality cannot be established from cross-sectional microbial comparisons alone.

At present, vaginal microbiome profiling cannot replace established cervical screening methods such as human papillomavirus testing, cytology, colposcopy and histopathological examination. Its future role may involve complementary risk stratification within integrated prediction models combining viral genotype, cytology, microbial characteristics, immune biomarkers and clinical factors.

Vaginal Microbiome and Endometriosis:

Endometriosis is a chronic estrogen-dependent inflammatory disorder characterized by endometrial-like tissue outside the uterine cavity. It is commonly associated with pelvic pain, dysmenorrhea, infertility and impaired quality of life. Despite extensive research, its pathogenesis remains incompletely understood.

Recent studies have investigated possible relationships between the vaginal, cervical, endometrial

and intestinal microbiomes and endometriosis.[19–21] Differences in microbial composition have been reported between women with endometriosis and healthy controls, although the identified bacterial taxa have varied considerably.

Potential mechanisms include microbial modulation of inflammatory pathways, epithelial-barrier dysfunction, altered immune regulation, microbial metabolite production and interactions with estrogen metabolism.[19,20] However, current findings remain inconsistent and do not establish a disease-specific vaginal microbial signature.

Anatomical sampling represents an important methodological issue. The vaginal microbiome cannot automatically be considered representative of the cervical, endometrial, peritoneal or intestinal microbiome. Each anatomical compartment possesses distinct biological and environmental characteristics. Studies should therefore report the sampling site clearly and avoid combining results from different reproductive-tract locations without appropriate stratification.

At present, vaginal microbiome testing cannot independently diagnose endometriosis. Nevertheless, microbial or metabolomic signatures may eventually contribute to multimodal diagnostic models when combined with symptoms, imaging findings and circulating inflammatory or molecular biomarkers.

Vaginal Microbiome and Polycystic Ovary Syndrome:

Polycystic ovary syndrome is a heterogeneous endocrine and metabolic disorder characterized by variable combinations of hyperandrogenism, ovulatory dysfunction and polycystic ovarian morphology. Insulin resistance, obesity and chronic low-grade inflammation frequently coexist with the syndrome.

Recent studies have reported vaginal microbial alterations and pro-inflammatory characteristics in women with polycystic ovary syndrome.[22–24] However, a consistent disease-specific microbial signature has not been established.

Interpretation of these findings is complicated by several confounding variables. Hyperandrogenism, insulin resistance, obesity, irregular menstruation, metformin use and hormonal treatment may independently influence microbial composition. Microbial differences observed between women with polycystic ovary syndrome and control participants may therefore reflect metabolic or hormonal characteristics rather than the syndrome itself.

Future studies should integrate vaginal microbial profiling with androgen concentrations, insulin resistance indices, body mass index, inflammatory biomarkers, menstrual patterns and established polycystic ovary syndrome phenotypes. Longitudinal evaluation before and after metabolic or hormonal treatment may clarify whether microbial alterations are reversible and whether these changes correlate with clinical improvement.

Vaginal and Endometrial Microbiomes In Infertility:

The potential influence of the female reproductive-tract microbiome on infertility and assisted reproductive treatment has received increasing attention.[25–30] Several studies suggest that *Lactobacillus*-dominant vaginal or endometrial microbial profiles may be associated with more favorable implantation and

pregnancy outcomes.[25–27]

Conversely, increased microbial diversity and non-*Lactobacillus*-dominant communities have been associated with implantation failure, recurrent pregnancy loss or less favorable assisted reproductive outcomes in selected populations.[25–30]

The endometrial microbiome remains particularly controversial because the upper reproductive tract is a low-microbial-biomass environment. Endometrial samples are vulnerable to contamination from the vagina, cervix, sampling instruments, laboratory reagents and environmental microbial DNA. Detection of bacterial DNA does not necessarily confirm the presence of a stable or metabolically active microbial community.

Clinical heterogeneity also complicates interpretation. Maternal age, infertility cause, ovarian reserve, embryo quality, assisted reproductive protocols and underlying gynecological disorders may independently influence pregnancy outcomes.

Routine vaginal or endometrial microbiome testing before assisted reproductive treatment cannot currently be universally recommended. Prospective multicentre studies evaluating implantation, clinical pregnancy and live birth are required before microbiome-based reproductive testing can be incorporated into clinical practice.

Vaginal Microbiome and Gynecological Malignancies:

The concept of the gynecological oncobiome describes the potential contribution of microbial communities to cancer development, chronic inflammation, immune regulation and treatment response.[18,31–38]

In cervical cancer, microbiome research is closely connected with persistent high-risk human papillomavirus infection and cervicovaginal immune regulation.[14–18] In endometrial cancer, microbial differences have been reported in the uterus and lower reproductive tract.[31] Vaginal, cervical, endometrial and tumor-associated microbial communities have also been investigated across other gynecological malignancies.[32–38]

Potential mechanisms include chronic inflammatory signaling, altered immune surveillance, epithelial barrier dysfunction, microbial metabolite production and modification of the tumor microenvironment.[18,32–35]

However, cancer-associated bleeding, tissue necrosis, antibiotic exposure, hospitalization, surgery, chemotherapy and radiotherapy may independently alter microbial communities. Consequently, microbial differences identified in patients with cancer may represent secondary ecological changes rather than causal oncogenic mechanisms.

At present, vaginal microbiome-derived biomarkers cannot independently screen for or diagnose gynecological cancer. Their greatest future value may involve integration with tumor genomics, immune profiling, imaging, histopathology and conventional clinical variables.

Advances in Microbiome Analysis:

Traditional vaginal microbiology has relied on microscopy, culture, biochemical testing and clinical diagnostic criteria. These approaches remain important in routine practice but provide limited characterization of complex microbial ecosystems.

The introduction of 16S ribosomal RNA gene sequencing substantially transformed vaginal microbiome research by enabling culture-independent characterization of bacterial communities.[1,39] However, this method has limited strain-level resolution and provides incomplete information about microbial function.

Shotgun metagenomic sequencing can provide species-, strain- and gene-level information and may identify microbial functional pathways that cannot be determined through 16S ribosomal RNA analysis alone.[40] Metatranscriptomics evaluates active microbial gene expression, whereas metabolomics characterizes microbial and host-derived metabolites.

Integrated multi-omics approaches may combine microbial taxonomy, microbial genes, gene expression, metabolites, host immune biomarkers and clinical data. These methods are shifting vaginal microbiome research from the question of which organisms are present toward an understanding of what these organisms are doing and how their functions interact with the host.[41–43]

Machine-learning and artificial intelligence methods may support analysis of these high-dimensional datasets. Potential applications include prediction of human papillomavirus persistence, recurrent bacterial vaginosis, infertility outcomes and gynecological cancer risk.

However, small sample sizes, population heterogeneity, inconsistent sampling and overfitting can produce models that perform poorly in external populations. Computational models therefore require transparent methodology, biological validation and independent external assessment.

Microbiome-Directed Therapeutic Strategies:

Conventional antimicrobial therapy remains central to the management of appropriately diagnosed bacterial vaginosis and other vaginal infections. However, recurrent dysbiosis and incomplete ecological recovery remain important limitations.

The recognition of vaginal dysbiosis as an ecological disorder has stimulated development of therapeutic strategies aimed at restoring protective microbial communities. Probiotics containing selected *Lactobacillus* strains have been investigated as adjunctive or preventive treatments, although their effects vary according to strain, formulation, administration route and treatment duration.[44]

Defined live biotherapeutic products represent a more standardized approach. A randomized clinical trial evaluating vaginal *L. crispatus* CTV-05 demonstrated the potential of microbiome-directed therapy for reducing recurrence of bacterial vaginosis.[45]

Vaginal microbiota transplantation has also been investigated in women with severe recurrent bacterial vaginosis.[46] This approach aims to transfer a complete microbial community from a screened healthy donor to a recipient with persistent dysbiosis. Nevertheless, donor selection, pathogen transmission,

regulatory requirements, microbial stability and long-term safety remain unresolved.

Other emerging strategies include biofilm disruption, selective antimicrobial treatment, bacteriophage-based interventions, microbial metabolite therapy and engineered beneficial microorganisms.[47,48]

The future therapeutic model may therefore shift from broad microbial eradication toward individualized ecological restoration. However, microbial modification alone should not be considered evidence of therapeutic success. Clinical trials should evaluate meaningful outcomes such as symptom resolution, recurrence reduction, human papillomavirus clearance, reproductive outcomes and long-term safety.

Knowledge Gap And Objective Of The Review:

Despite rapid scientific progress, several important knowledge gaps remain. There is no universally accepted definition of an optimal vaginal microbiome, and healthy women may demonstrate different microbial community structures.[1–3] Vaginal microbial composition is dynamic, and a single sample may not accurately represent long-term ecological status.[6,7]

Similar patterns of reduced lactobacilli and increased anaerobic diversity have also been reported across multiple gynecological diseases.[38] This overlap limits the diagnostic specificity of individual bacterial organisms.

Most available disease-associated studies remain observational, and causal relationships are difficult to establish. Methodological variation in sample collection, storage, DNA extraction, sequencing and bioinformatic analysis further limits reproducibility.[39–43]

Accordingly, this review aims to critically summarize current evidence concerning the vaginal microbiome in major gynecological diseases. It examines physiological microbial ecology, vaginal dysbiosis, host–microbe interactions, disease-associated microbial alterations, emerging diagnostic technologies and microbiome-directed therapeutic strategies. The review also identifies the principal methodological barriers and future research priorities required for the development of precision microbiome-based gynecology.

DISCUSSION

The vaginal microbiome has emerged as a biologically important component of female reproductive health and disease. Current evidence supports the concept that vaginal microbial communities influence local pH, epithelial integrity, mucosal immunity, pathogen resistance and microbial metabolite production. A *Lactobacillus*-dominant ecosystem, particularly one enriched in *Lactobacillus crispatus*, is generally associated with greater ecological stability and a lower risk of dysbiosis, whereas reduced lactobacilli and increased anaerobic diversity are recurrent features of several gynecological disorders.[1–4,10–13]

However, the relationship between vaginal microbial composition and gynecological disease is complex. Microbial dysbiosis may contribute to disease initiation or progression, but it may also develop

secondary to hormonal abnormalities, inflammation, tissue injury, infection, malignancy or therapeutic exposure. Therefore, microbial associations should not be interpreted as evidence of causation unless they are supported by longitudinal, mechanistic and interventional studies.

Principal Findings:

The strongest and most reproducible evidence concerns bacterial vaginosis. Bacterial vaginosis is increasingly understood as a polymicrobial ecological disorder rather than an infection caused by a single organism.[10–13] The condition is commonly characterized by depletion of protective lactobacilli, increased vaginal pH, enrichment of anaerobic bacteria and formation of adherent polymicrobial biofilms.

Biofilm formation is especially important because it may facilitate bacterial persistence, reduce antimicrobial susceptibility and contribute to recurrent disease.[11–13] Conventional antimicrobial treatment can suppress dysbiosis-associated bacteria and improve symptoms, but it may not consistently restore a stable *Lactobacillus*-dominant ecosystem. This distinction between clinical response and ecological recovery is central to understanding recurrent bacterial vaginosis.

Substantial evidence also supports an association between vaginal dysbiosis and persistent high-risk human papillomavirus infection.[14–18] Women with non-*Lactobacillus*-dominant communities or increased vaginal microbial diversity may demonstrate a greater likelihood of persistent human papillomavirus detection, cervical intraepithelial abnormalities or disease progression.

Several biological mechanisms may explain this relationship. Vaginal dysbiosis may disrupt epithelial integrity, alter microbial metabolites, increase local inflammatory signaling and impair antiviral immune responses. These changes may create a cervicovaginal environment that is less favorable for viral clearance.

Nevertheless, the relationship is likely bidirectional. Human papillomavirus infection and cervical epithelial disease may themselves alter local immunity and microbial composition. Therefore, current evidence supports the vaginal microbiome as a potential disease modifier or complementary biomarker rather than an established independent cause of cervical cancer.

Evidence involving endometriosis, polycystic ovary syndrome, infertility and gynecological malignancies remains less consistent.[19–38] Microbial alterations have been described in each of these conditions, but the bacterial taxa and diversity patterns differ substantially among studies.

This inconsistency may reflect differences in patient populations, disease stage, hormonal status, treatment history, sampling site and sequencing methodology. It may also indicate that general ecological disturbance is more readily detectable than disease-specific microbial signatures.

Comparison with Previous Literature:

The findings of this review are consistent with foundational studies demonstrating that vaginal bacterial communities can be classified into distinct community state types.[1,5] These studies established a conceptual framework in which vaginal health could no longer be defined solely by the presence or absence of individual bacterial species.

Longitudinal investigations subsequently demonstrated that vaginal microbial communities are

dynamic and may transition between ecological states over relatively short periods.[6,7] This observation has important implications for clinical interpretation. A single sample may not accurately represent long-term microbial stability, particularly in women undergoing menstruation, hormonal treatment, antibiotic exposure or changes in reproductive status.

Previous reviews have often focused on a single clinical condition, such as bacterial vaginosis, human papillomavirus infection, endometriosis or infertility. In contrast, the present review evaluates vaginal microbial alterations across a broader range of gynecological diseases.

This cross-disease perspective demonstrates that several microbial features recur across different disorders. These include reduced *Lactobacillus* abundance, increased anaerobic diversity, inflammatory activation and altered epithelial function.[10–24,38]

The repetition of these findings across different diseases suggests that vaginal dysbiosis may represent a common ecological response to pathological conditions. However, this overlap also limits the specificity of individual microbial taxa as diagnostic biomarkers.

The literature concerning bacterial vaginosis supports an ecological and biofilm-based model of disease.[10–13] This model is clinically relevant because it explains why treatment based exclusively on broad bacterial suppression may fail to prevent recurrence.

Similarly, studies concerning human papillomavirus and cervical disease support associations between microbial diversity, local immune changes and viral persistence.[14–18] However, the available evidence does not yet establish whether modifying the vaginal microbiome can improve viral clearance or prevent cervical disease progression.

The reproductive microbiome literature has generated considerable interest, particularly in relation to implantation and assisted reproductive outcomes.[25–30] Some studies suggest that *Lactobacillus*-dominant reproductive tract profiles are associated with improved implantation or pregnancy outcomes. However, differences in sampling and contamination control remain major concerns.

The controversy is particularly important in endometrial microbiome research because the upper reproductive tract is a low-biomass environment. Even small amounts of contamination may substantially alter sequencing results. Therefore, future studies must include rigorous negative controls and standardized transcervical sampling protocols.

Clinical Implications:

The growing understanding of vaginal microbial ecology has several potential implications for clinical gynecology.

First, vaginal disorders should increasingly be understood within an ecological framework. Traditional diagnostic approaches often classify microorganisms as normal flora or pathogens. However, microbial function, community structure and host response may be more clinically important than the presence of a single organism.

This concept is especially relevant to recurrent bacterial vaginosis. Repeated courses of antimicrobial

therapy may reduce symptoms while failing to restore ecological stability. Future management strategies may need to evaluate whether protective lactobacilli and functional microbial homeostasis have been re-established.

Second, vaginal microbiome profiling may eventually contribute to risk stratification in women with high-risk human papillomavirus infection. Women with similar viral genotypes may have different probabilities of viral clearance or progression.

An integrated model combining human papillomavirus genotype, cervical cytology, vaginal microbial composition, inflammatory biomarkers, smoking status and age may potentially improve individualized risk prediction. However, microbiome testing should presently be considered investigational and should not replace established cervical screening methods.

Third, vaginal microbial biomarkers may contribute to multimodal diagnosis of endometriosis. The current diagnosis of endometriosis may involve clinical assessment, imaging and, in selected cases, surgery. A validated non-invasive microbial signature could potentially complement symptoms, imaging and circulating biomarkers.

However, the lack of consistent disease-specific findings currently prevents clinical use. Future studies should clearly distinguish vaginal, cervical, endometrial and intestinal microbial profiles and should control for hormonal treatment, menstrual phase and disease stage.

Fourth, vaginal and endometrial microbiome analysis may eventually contribute to reproductive medicine. Microbial patterns could potentially assist in predicting implantation or treatment outcomes. Nevertheless, routine testing should not be adopted until prospective studies demonstrate improved pregnancy and live-birth outcomes.

Fifth, microbiome research may contribute to gynecological oncology. Microbial profiles may eventually supplement established diagnostic or prognostic tools. However, no current vaginal microbial signature is sufficiently validated for independent cancer screening.

The clinical value of the gynecological oncobiome will probably depend on integration with tumor genomics, pathology, immune profiling and clinical outcomes rather than microbial analysis alone.

Diagnostic and Technological Advances:

The introduction of molecular sequencing has substantially expanded understanding of vaginal microbial communities.[39–43] Although 16S ribosomal RNA sequencing remains widely used, it provides incomplete strain-level and functional information.

Shotgun metagenomics offers improved taxonomic resolution and allows assessment of microbial genes and potential functional pathways. Metatranscriptomics can identify actively expressed microbial genes, while metabolomics provides information about biologically active microbial and host-derived metabolites.

The integration of these technologies may identify functional signatures that are more disease-specific than bacterial abundance patterns. For example, two women may have similar bacterial communities

but different metabolic or inflammatory profiles. Functional multi-omics may therefore improve biological interpretation.

Artificial intelligence and machine-learning approaches may further support analysis of complex microbial datasets. These methods can integrate microbial taxa, microbial genes, metabolites, immune markers, hormonal data and clinical characteristics.

However, the value of computational models depends on the quality of the underlying data. Small sample sizes, inconsistent sampling, population bias and inadequate validation may produce unreliable models. Transparent model development and external validation are essential before artificial intelligence-based microbiome tools can be used clinically.

Current Controversies:

One major controversy concerns the definition of a healthy vaginal microbiome. Although *Lactobacillus*-dominant communities are generally associated with favorable vaginal characteristics, some asymptomatic women demonstrate diverse microbial communities without clinically apparent disease.[1–3] Therefore, reduced *Lactobacillus* abundance should not automatically be classified as pathological in the absence of symptoms, inflammation or adverse clinical outcomes.

A second controversy concerns the role of *Lactobacillus iners*. Unlike *L. crispatus*, *L. iners* is commonly observed in both healthy and transitional microbial states.[6,7] Its presence may represent ecological adaptation, instability or recovery following treatment. Its precise clinical significance remains uncertain.

A third controversy concerns causality. Most gynecological microbiome studies are cross-sectional. Such studies can identify differences between groups but cannot determine whether microbial dysbiosis precedes disease development.

Longitudinal studies are required to establish whether microbial changes occur before human papillomavirus persistence, endometriosis progression, infertility or cancer development.

A fourth controversy concerns the upper reproductive tract microbiome. Detection of microbial DNA in the endometrium does not necessarily confirm the presence of viable organisms or stable microbial communities. Contamination and sequencing noise remain major concerns.

A fifth controversy concerns probiotics. Commercial products contain different bacterial strains, doses and formulations. Results obtained with one strain cannot be generalized to all probiotics.[44]

Similarly, vaginal microbiota transplantation raises important concerns regarding donor screening, pathogen transmission, long-term ecological effects and regulatory oversight.[46]

Microbiome-Directed Therapy:

Microbiome-directed treatment represents one of the most promising translational areas in vaginal microbiome research. Conventional antimicrobial therapy primarily aims to suppress disease-associated microorganisms. Emerging strategies instead aim to restore microbial function and ecological stability.

The randomized trial of vaginal *L. crispatus* CTV-05 provided evidence that a defined live biotherapeutic product may reduce recurrence of bacterial vaginosis following standard treatment.[45] This

trial supports the concept that restoration of protective microbial communities may improve long-term outcomes.

However, important questions remain regarding patient selection, timing, duration and durability of treatment. It is also unclear whether similar approaches will be effective across different forms of vaginal dysbiosis.

Vaginal microbiota transplantation represents a more comprehensive ecological strategy.[46] It may be considered conceptually similar to microbiota transplantation in other organ systems, although the vaginal environment has distinct biological and safety considerations.

Potential benefits include transfer of a complete protective microbial community rather than a single bacterial strain. However, donor screening, infectious safety, reproducibility and long-term follow-up require further investigation.

Biofilm-targeted therapy may be particularly useful in recurrent bacterial vaginosis. Disruption of adherent polymicrobial communities could improve antimicrobial susceptibility and reduce persistence.

Selective antimicrobial therapy, bacteriophage-based interventions, microbial metabolite replacement and engineered beneficial organisms are additional emerging approaches.[47,48] These strategies may allow targeted treatment while preserving protective microbial communities.

Nevertheless, microbiome modification alone should not be considered therapeutic success. Clinical trials should assess symptom resolution, recurrence, quality of life, human papillomavirus clearance, pregnancy outcomes and long-term safety.

Future Recommendations:

Future vaginal microbiome research should prioritize methodological standardization. Studies should report the exact anatomical sampling site, sampling device, storage conditions, menstrual phase, hormonal status, antibiotic exposure and recent use of vaginal products.

Standardized laboratory and bioinformatic protocols are also required. Variation in DNA extraction, sequencing platforms, amplified regions, taxonomic databases and analytical pipelines can influence microbial findings.

Large multicentre longitudinal studies should replace small single-centre cross-sectional investigations whenever feasible. Serial sampling can clarify temporal relationships between microbial changes and disease development.

Population diversity should also be considered. Vaginal microbial composition varies across geographical and ethnic populations. Diagnostic models developed in one population may not perform adequately in another.

Future studies should increasingly evaluate microbial function rather than taxonomic abundance alone. Metagenomics, metatranscriptomics, metabolomics and host immune profiling may identify clinically relevant biological pathways.

Microbiome biomarker models should undergo independent validation before clinical use. Studies

should report sensitivity, specificity, predictive values and calibration in external populations.

Randomized clinical trials are required to evaluate probiotics, live biotherapeutic products, vaginal microbiota transplantation and biofilm-targeted interventions. These trials should use clinically meaningful endpoints and standardized safety monitoring.

Multidisciplinary collaboration will be essential. Gynecologists, reproductive specialists, oncologists, microbiologists, immunologists, bioinformaticians and biostatisticians should contribute to study design and interpretation.

Ultimately, precision microbiome-based gynecology may involve individualized assessment of microbial composition, microbial function, host immunity, hormonal status and clinical phenotype. Such integration could support personalized prevention, diagnosis, surveillance and treatment.

However, precision microbiome medicine should be introduced only after robust validation and demonstration of clinical benefit.

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