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Dysbiosis of Vaginal Microbiome, Impact on Reproductive Health and Emerging Therapeutic Strategies

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ABSTRACT

Women's reproductive health is of paramount importance, given their central roles in family and societal well-being. During the reproductive years, women are particularly vulnerable to physiological and psychosocial challenges arising from dynamic hormonal fluctuations, emotional stress, sexual practices, and complex interpersonal relationships. This vulnerability is further exacerbated by limited awareness of reproductive health and low socioeconomic status. Lifestyle factors, including dietary habits, sleep patterns, personal hygiene, and inadvertent exposure to pathogens, also significantly influence reproductive outcomes. The human vagina harbours a highly specialised and dynamic microbial ecosystem, predominantly composed of beneficial Lactobacillus species, including Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus iners, and Lactobacillus jensenii. These species play a crucial role in maintaining vaginal health by preserving an acidic pH, sustaining epithelial integrity, and regulating mucosal immune homeostasis. Disruption of this microbial balance leads to dysbiosis, which is associated with conditions such as bacterial vaginosis (BV), vulvovaginal candidiasis, and sexually transmitted infections (STIs). BV-associated microorganisms, including Gardnerella vaginalis, Prevotella bivia, bacterial vaginosis-associated bacteria (BVABs), and Sneathia species, along with STI-causing pathogens such as Neisseria gonorrhoeae, Chlamydia trachomatis, Treponema pallidum, Mycoplasma genitalium, human papillomavirus (HPV), herpes simplex virus (HSV), and human immunodeficiency virus (HIV), contribute significantly to reproductive morbidity. Additionally, opportunistic pathogens such as Escherichia coli, Enterococcus faecalis, and Group B Streptococcus further complicate vaginal health. This review comprehensively examines the vaginal microbiota in both healthy and diseased states, with particular emphasis on the mechanistic pathways that underlie its associations with epithelial barrier disruption, immune modulation, biofilm formation, microbial ascension, and chronic inflammation. These interconnected processes are implicated in adverse reproductive outcomes, including infertility, preterm labour, recurrent pregnancy loss, and cervical carcinogenesis. Furthermore, emerging multi-omics approaches such as metagenomics, metatranscriptomics, metabolomics, and organ-on-chip technologies hold significant promise for advancing diagnostic precision and therapeutic interventions. Finally, the limitations of conventional antimicrobial therapies are critically evaluated, and novel microbiota-targeted strategies, including strain-specific probiotics, vaginal microbiota transplantation, bacteriophage therapy, and partner-inclusive interventions, are highlighted. It is anticipated that this review will raise awareness and promote strategies to restore the functional balance and resilience of the vaginal microbiome, thereby improving women's reproductive health and overall quality of life.

Key Words: *Vaginal health, Microbial dysbiosis. Sexually transmitted infection, vaginal fauna, vaginosis, and candidiasis.*

I. INTRODUCTION

The human vaginal microbiome is a highly specialized and dynamic ecosystem that is essential for maintaining reproductive health. Traditionally, this microbial community has been ccharacterised by the predominance of Lactobacillus species, which contribute to maintaining an acidic vaginal environment and inhibiting the growth of pathogenic organisms. While this compositional perspective has provided foundational insights, it offers a limited understanding of the complex biological processes that govern vaginal health and related diseases (Huang et al. 2014). Recent advances in high-throughput sequencing and multi-omics technologies have revealed that microbial communities cannot be fully understood through taxonomic profiling alone. Instead, increasing evidence highlights the importance of functional attributes, including microbial metabolite production, host–microbe signalling, and immune modulation. These

functional interactions regulate key processes such as epithelial barrier integrity, mucosal immunity, and inflammatory responses, which are critical for maintaining vaginal homeostasis. Within this context, vaginal dysbiosis is no longer viewed simply as a shift in microbial composition but rather as a disruption of functional homeostasis (Ravel et al. 2011). This includes alterations in metabolic activity, dysregulated immune signalling, and compromised epithelial barriers, collectively contributing to disease susceptibility. Such functional dysregulation has been implicated in a wide range of clinical conditions, including bacterial vaginosis, sexually transmitted infections, infertility, and adverse pregnancy outcomes (Ojo et al. 2025). Despite substantial progress in ccharacterizing the vaginal microbiome, current therapeutic strategies remain limited by high recurrence rates and inconsistent clinical outcomes. These limitations reflect an incomplete understanding of the underlying mechanisms driving dysbiosis and highlight the need for a more integrative approach (Amabebe et al. 2018). In response to these challenges, there is a growing shift toward a systems-level perspective, which integrates microbial composition with functional activity and host responses. This approach provides a more comprehensive framework for understanding the dynamic interactions that define vaginal health and diseases. Therefore, the present review aims to move beyond a descriptive, composition-based framework, instead proposes a functional model of vaginal dysbiosis,emphasizing the interplay between microbial metabolism, host immune responses, and epithelial integrity. By encompassing current evidence across molecular, clinical, and translational domains, this review aims to provide new insights into the mechanisms of dysbiosis and highlight emerging opportunities for precision microbiome-based therapeutics

II. CONCEPTUAL FRAMEWORK FROM FUNCTIONAL EUBIOSIS TO DYSBIOSIS

Historically, vaginal health has been linked to the predominance of lactobacilli, while dysbiosis was characterized by the absence of lactobacilli and a greater number of microorganisms. Yet, recent findings indicate that microbial content alone cannot be used to predict an individual's risk for illnesses, recurrence of diseases, and susceptibility to treatment. Rather, vaginal health should be viewed as a functional process modulated by metabolic and ecological factors (Borrego et al. 2025). Thus, vaginal dysbiosis is not merely a binary transition from a "normal" to an "abnormal" microbiome. It is, in fact, a gradient of functional instability where metabolic, immunologic, epithelial, and microbial balance is disturbed. Patients with identical vaginal microbiomes can present with diverse symptoms based on the functional status of their microbiomes and individual reactions (Graeber et al. 2025). To understand this phenomenon, we have devised a system-based "Functional Dysbiosis Axis," which includes the following four dimensions given hereunder.

- *Metabolic Axis:* The microbial metabolites like lactic acid keep the pH value low in the vagina to prevent any pathogens from growing. In dysbiosis, metabolite changes like SCFAs and bioactive amines cause inflammation and epithelial dysfunction.
- *Immune Axis:* Microbiota control cytokine production and immune response within the mucosa. Dysbiosis activates inflammation via the Toll-like receptor and nuclear factor-kappa B pathways, causing chronic inflammation and poor infection resolution.
- *Epithelial Barrier Axis:* The normal vaginal microbiota maintains epithelial cell structure and tight junctions. Dysbiosis causes damage to the mucosal barrier via sialidase activity, proteases, and oxidative stress, allowing pathogen entry and ascending infections.
- *Ecological Axis:* Vaginal Microbiome is a Dynamic Ecosystem. Dysbiosis causes ecosystem instability, biofilm production, and decreased microbial resilience, causing ongoing disease.

Together, all of the above constitute the new axes for redefining vaginal dysbiosis as a functional condition, rather than just an imbalance. This could help explain recurrent disease, treatment outcomes variability, and the shortcomings of composition-based approaches to treating dysbiosis (Graeber et al. 2025).

III. VAGINAL DYSBIOSIS AND SYSTEMIC DISEASE ASSOCIATIONS

- *HPV Persistence and Cervical Carcinogenesis:* A sustained infection with high-risk HPV alone does not result in cervical carcinogenesis since most infections clear up on their own. More and more studies have shown that vaginal dysbiosis, especially those that are not dominated by Lactobacilli, can lead to HPV persistence and progression to cervical intraepithelial neoplasia and cervical cancer. The presence of CST IV microbes, which have lower Lactobacillus density and higher microbial diversity, is significantly correlated with HPV persistence and inflammation induction (Bautista et al. 2025).

- *Vaginal Dysbiosis and Implantation Failure in ART:* The dysbiotic vaginal microbial composition is linked with decreased implantation and pregnancy outcomes in assisted reproductive technologies (ARTs). There is evidence that women with vaginal microbiota non-dominant for Lactobacillus have significantly fewer implantations than Lactobacillus-dominant vaginal microbiotas. The functional interplay between vaginal and endometrial microbes results in poor endometrial receptivity, chronic inflammation, and altered immunological tolerance. Microbiome models have shown excellent predictability for implantation success and recurrent implantation failure (Su et al. 2024).
- *Metabolic and Immune Mechanisms of Implantation Failure:* There is accumulating evidence indicating that metabolism alteration resulting from dysbiosis plays a role in preventing implantation. The presence of an over-representation of L-lysine metabolism pathways, deficiency in metabolites for Lactobacilli, and microbial migration to the endometrium cause a lack of endometrial receptivity (Su et al. 2024).
- *BV and Recurrent Pregnancy Loss:* The lack of dominance by *L. crispatus* is linked to pro-inflammatory polarization of Th1 and Th17 cells, decreased regulatory T cell function, and fetal immune intolerance. The inflammatory pathways activated by bacterial dysbiosis products, mediated by TLRs, increase the chances of recurrent pregnancy loss and ascending infection (Garmendia et al. 2024).
- *Preterm Birth and Premature Rupture of Membranes:* Bacterial vaginosis is an important risk factor for preterm labor and PROM/PPROM. Dysbiosis-linked bacteria synthesize sialidases, inflammatory factors, and matrix metalloproteinases, contributing to fetal membrane degradation and ascending polymicrobial infection in the uterine cavity. The extent of dysbiosis is highly correlated with poor pregnancy outcomes (Deng et al. 2025) (**Table I**).

TABLE I. COMPARATIVE EFFECTS ON EUBIOTIC AND DYSBIOTIC GUTS

S. NO.	Domains	Eubiotic Gut Microbiota	Dysbiotic Gut Microbiota
1	Microbial composition	Dominated by anti-inflammatory bacteria including *Faecalibacterium prausnitzii*, *Roseburia spp.*, *Bifidobacterium spp.*, and *Lactobacillus spp.*	Enriched with pro-inflammatory and pathogenic bacteria such as *Escherichia coli*, *Bacteroides fragilis*, and pathogenic *Clostridium spp.*
2	Key microbial metabolites	Short-chain fatty acids (butyrate, acetate), lactic acid, H_2O_2	Lipopolysaccharide (LPS), enterotoxins
3	Immune regulation	Enhances regulatory T cells (Tregs), suppresses Th17-driven inflammation, and maintains immune tolerance	Increased Th17 cells with reduced Tregs, elevated pro-inflammatory cytokines (IL-6, TNF-α, IL-1β), and chronic immune activation
4	Inflammatory signalling pathways	SCFAs suppress NF-κB signalling, maintain epithelial barrier integrity, and limit inflammasome activation	NF-κB pathway activation, TLR4-LPS signalling amplification, and NLRP3 inflammasome activation leading to IL-1β production
5	Macrophage and dendritic cell activity	Balanced macrophage polarization and controlled antigen presentation	M1-polarized macrophage infiltration and dendritic cell activation with enhanced antigen presentation and tissue reactivity
6	Hormonal regulation (estrobolome activity)	Physiological modulation of estrogen metabolism and preservation of progesterone sensitivity	Estrogen dominance due to excess β-glucuronidase activity, altered estrogen receptor β (ERβ) signalling, and progesterone resistance

7	Stress and neuroendocrine modulation	Modulates the HPA axis, supporting reproductive hormone homeostasis	Hormonal imbalance with disruption of neuroendocrine regulation
8	Endometrial receptivity markers	Promotes expression of LIF, HOXA10, integrins, and balanced mucins essential for implantation	Reduced LIF and HOXA10 expression, impaired integrin αvβ3 function, and altered MUC1 and glycodelin affecting implantation
9	Decidualization and implantation	Supports decidualization and embryo–endometrial crosstalk	Impaired decidualization and defective embryo attachment
10	Gut–uterus microbial cross-talk	Maintains stable molecular communication between gut and endometrial microbiota	Altered gut–endometrium signalling with microbial metabolite translocation
11	Endometrial microbial environment	Stable endometrial microbial community with pathogen control	Possible biofilm formation and dysbiotic shift in endometrial microbiota
12	Nutrient synthesis and reproductive support	Produces folate and vitamin B12 critical for early embryogenesis	Reduced nutrient availability compromising early embryonic development

IV. THE QUANTITATIVE STI RISK GRADIENT ASSOCIATED WITH DYSBIOSIS

Bacterial vaginosis (BV) is a well-established and potent predictor of sexually transmitted infection (STI). The reason behind that is due to a woman's permissive behavior, which leads to dysbiosis of the vagina. The evidence from the epidemiological studies confirms the dose-dependent relationship wherein women with high vaginal dysbiosis (Nugent score of 9-10) had an increased risk of 2.7 times in acquiring STIs compared to women with a normal vaginal microbiota (**Table II**).

TABLE II. RELATIONSHIP BETWEEN SEXUALLY TRANSMITTED INFECTION (STI), RISK ENHANCEMENT AND MICROBIAL DYSBIOSIS

S.N.	STI Pathogen	Risk Elevation	Mechanistic Basis	References
1.	*Neisseria gonorrhoeae*	1.8-fold increased risk	Epithelial damage; pathogen access to upper tract	Wiesenfeld et al. 2003
2.	*Chlamydia trachomatis*	1.9-fold increased risk	Loss of protective lactobacilli; epithelial permeability	Wiesenfeld et al. 2003
3.	*Trichomonas vaginalis*	Elevated susceptibility	Epithelial damage; inflammatory milieu facilitates parasitic invasion	Shipitsyna et al. 2020
4.	Herpes Simplex Virus-2	BV is independent risk factor	Epithelial ulceration; inflammatory cell recruitment	CDC STI Guidelines (2021)
6.	*Mycoplasma genitalium*	Enhanced susceptibility	Epithelial barrier loss; immune dysregulation	Shipitsyna et al. 2020
7.	HIV	6-fold increased shedding	Maximum risk elevation; multiple mechanisms	CDC STI Guidelines (2021)

V. MECHANISTIC PATHWAYS OF FUNCTIONAL EUBIOSIS

Several interconnected mechanisms contribute to microbiome dysfunction and increased susceptibility to sexually transmitted infections (STIs).

- *Epithelial Barrier Disruption: Dysbiotic bacteria, especially G. vaginalis, produce sialidase and proteases to break down cervical mucus and interfere with tight junctions of the epithelium, hence enhancing mucosal permeability. The high levels of reactive oxygen species (ROS) also contribute to epithelial barrier dysfunction (Deng et al. 2025).*
- *Impaired Local Innate Immunity: In the presence of a dominant lactobacilli microbial community, cervicovaginal immunity is enhanced by the production of lactic acid, hydrogen peroxide, and bacteriocins. Dysbiosis disrupts all these activities, reducing colonization resistance and impairment of leucocytes.*
- *Facilitated Ascension to the Upper Reproductive Tract: The polymicrobial biofilm development associated with bacterial vaginosis helps in generating anaerobic microenvironments that favour the persistence and ascending spread of microbes. G. vaginalis is the main biofilm founder, while anaerobes like A. vaginae contribute to biofilm stability and survival.*
- *Pro-inflammatory Recruitment of Pathogen Target Cells: Dysbiosis initiates an inflammatory cascade involving TLRs, resulting in the production of pro-inflammatory cytokines and the recruitment of $CCR5^+$ $CD4^+$ T cells. This predisposes the individual to infections such as HIV. The chronic inflammatory response damages the mucosal immune system (Zozaya et al. 2016).*
- *Sexual Transmission and Partner-Centred Dynamics: Accumulating evidence supports bacterial vaginosis as a sexually transmissible dysbiotic condition. Bacterial vaginosis can be viewed as a dysbiosis condition that involves the transmission of microbes through sexual intercourse. In this regard, the male genitourinary microbiome, which includes anaerobes found on the foreskin, urethra, and semen, plays a vital role in the transmission of bacteria. It has been established that partner therapy significantly lowers the recurrence rate of the infection (Zozaya et al*. 2016) (**Figure I**).

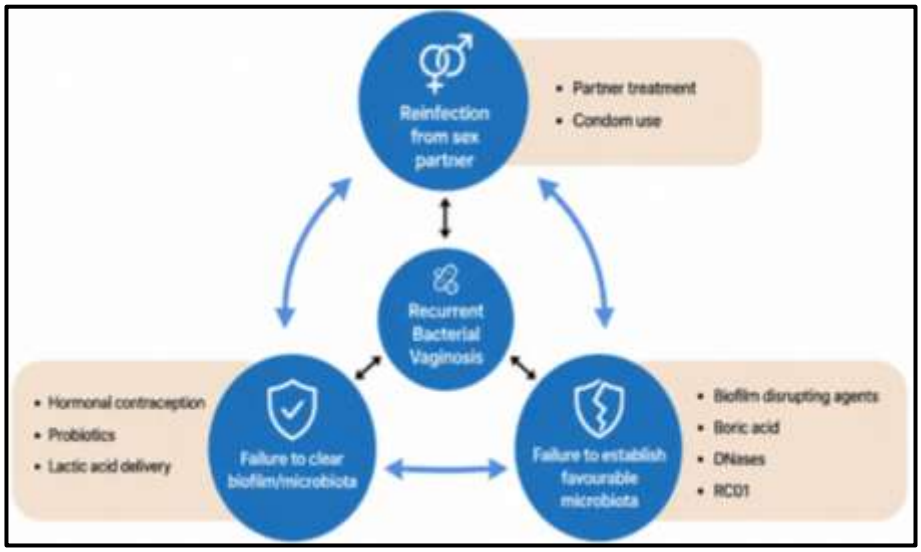

FIGURE I. DIAGRAMMATIC ILLUSTRATION SHOWING DETAILS OF RECURRENT BACTERIAL VAGINOSIS

VI. NOVEL AND CONVENTIONAL THERAPIES FOR VAGINAL DYSBIOSIS

- *Conventional Therapies for Vaginal Dysbiosis:* Antimicrobial resistance contributes only partially to treatment failure in bacterial vaginosis (BV), with metronidazole resistance remaining low (<0.3%), although clindamycin resistance is comparatively higher (~17%). Emerging evidence suggests clade-specific resistance among *Gardnerella* species, indicating strain-level variability in therapeutic response. Antibiotic therapy also disrupts microbial succession, with post-treatment recolonization often dominated by *Lactobacillus iners* rather than more protective species such as *Lactobacillus crispatus*, predisposing to recurrent dysbiosis. In

vulvovaginal candidiasis, increasing azole resistance, particularly in *Candida glabrata*, is mediated through target enzyme mutations, efflux pumps, and stress-response pathways, contributing to multidrug resistance (Bradshaw et al. 2015).

- *Live Biotherapeutic Strategies: Probiotic efficacy is highly strain-specific, limiting the effectiveness of non-targeted therapies. Lactobacillus crispatus (LACTIN-V) has demonstrated reduced BV recurrence in clinical trials; however, sustained remission remains challenging due to persistent biofilms and ecological instability. Vaginal microbiota transplantation (VMT) represents a promising ecosystem-based approach capable of restoring Lactobacillus-dominant communities and reducing inflammation. Similarly, synthetic bacterial consortia are emerging as scalable alternatives for microbiome restoration (Van et al. 2020).*
- *Novel and Emerging Approaches: Prebiotics and synbiotics require cautious application in vaginal health due to the "SCFA paradox," where short-chain fatty acids exhibit anti-inflammatory effects in the gut but pro-inflammatory effects in the vagina. This highlights the limitations of directly translating gut-derived microbiome therapies to vaginal ecosystems (Amabebe et al. 2020) (Figure II).*
- *Lifestyle and Hygiene Interventions: Lifestyle and hygiene practices significantly influence vaginal microbial stability. Douching and use of fragranced vaginal products disrupt protective microbiota and increase BV risk, whereas water-based hygiene practices are considered safer. Behavioural strategies including condom use, partner treatment, and limiting new sexual partners remain effective non-pharmacological interventions for reducing recurrence and maintaining vaginal health (Bradshaw et al. 2016).*

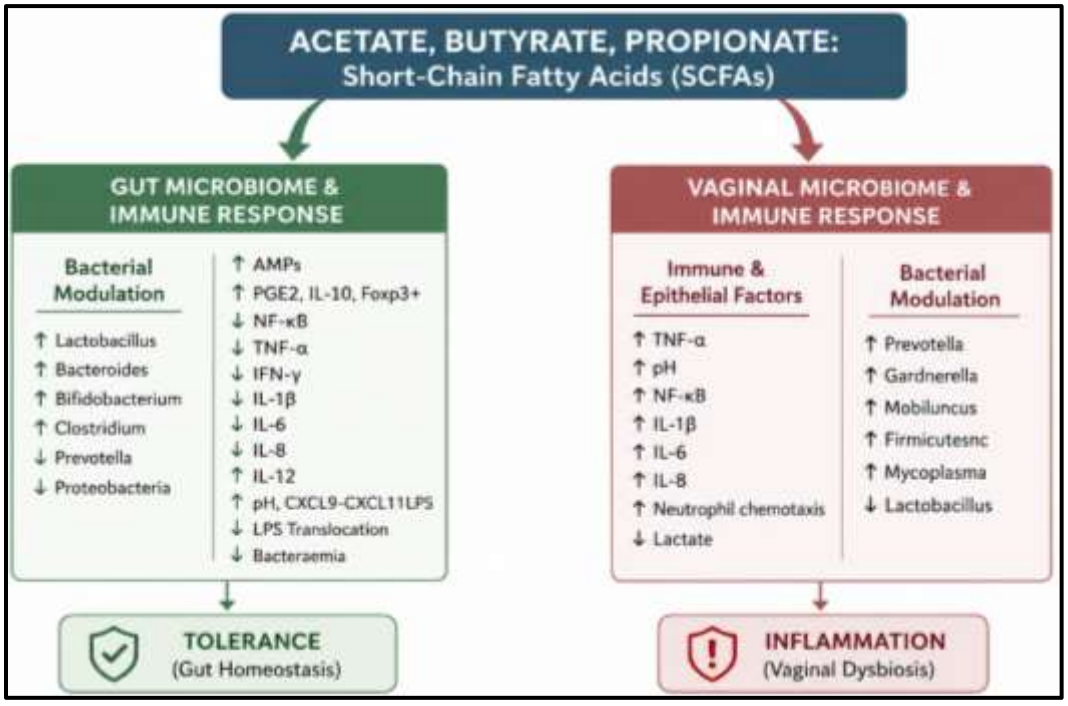

FIGURE II. DIFFERENTIAL IMPACT OF SCFA ON GUT AND VAGINAL MUCOSAL IMMUNITY MICROBIOTA

VII. MECHANISTIC ROLE OF ANIMAL MODELS IN MICROBIOME RESEARCH

Animal and murine models provide mechanistic evidence linking microbiome alterations with host immune, metabolic, and epithelial outcomes. These studies also support the development of microbiome-targeted therapeutic strategies (Table III).

TABLE III. MOUSE AND ANIMAL MODELS LINKING MICROBIOMES TO HOST OUTCOMES: A MECHANISTIC EVIDENCE AND THERAPEUTIC INSIGHTS

S.N.	Model / Section	Experimental System	Key Dysbiotic Features	Primary Mechanisms Identified	Host Outcomes	Translational Insight	Reference
1	POI – Bacterial Dysbiosis	Cyclophosphamide-induced POI mice	↓ *Helicobacter, Odoribacter, Alistipes*; ↑ *Clostridium XIVa, Barnesiella, Bacteroides, Mucispirillum*	Microbiota–endocrine coupling; hormone–taxa correlations	Ovarian failure, follicular atresia, infertility	Dysbiosis is mechanistically linked to ovarian endocrine dysfunction	Cao et al. 2020
2	POI – Probiotic Intervention	Oral 12-strain probiotic gavage (28 days)	Restoration of commensal taxa	Immune reprogramming; cytokine normalization; lipid metabolism correction	Partial ovarian recovery; ↑ follicles; ↓ atresia	Proof-of-concept for microbiome-targeted POI therapy	Cao et al. 2025
3	Gut–Vaginal Axis	Oral probiotics with vaginal microbiome profiling	Vaginal Lactobacillus restoration	Immune cell trafficking; systemic metabolite signalling	Reduced vaginal inflammation	Systemic microbiota modulation affects distal reproductive sites	Xu et al. 2024
4	Gut Virome Dysbiosis in POI	Human POI virome profiling	↓ viral α-diversity; ↑ phage stress genes (AcrIF1); altered AMGs	Virome-driven bacterial metabolic reprogramming	Hormonal imbalance, ovarian dysfunc-tion	Virome is an independent regulator of reproductive health	Jin et al. 2026
6	*Gardnerella vaginalis* Inflammation	Macrophage & vaginal mouse models	Keystone BV pathogen dominance	ROS production; NLRP3 inflammasome → caspase-1 → pyroptosis	Severe vaginitis; IL-1β, IL-18 release	Dysbiosis drives immune-mediated tissue damage	Xiang et al. 2021
8	Bacterial Extracellular Vesicles (gEVs)	Vaginal exposure to *G. vaginalis* EVs	EV-mediated inflammatory cargo	NF-κB activation without live bacteria	Vaginitis, systemic and neuroinflammation	Explains CNS effects of reproductive tract dysbiosis	Shin et al. 2025
9	*Lactobacillus johnsonii* Protection	Immune cell & mouse models	Protective Lactobacillus dominance	TLR1/2 → STAT3 → M2 macrophage polarization	IL-10 production; immune tolerance	Molecular basis of probiotic-mediated protection	Jia et al. 2022

10	*Lactobacillus plantarum* Immune Activation	Early immune response models	Context-dependent probiotic signalling	JAK-STAT, NF-κB → transient M1 polarization	Enhanced early pathogen defence	Strain-specific probiotic immune effects	Duan et al. 2022
11	Estrobolome Dysfunction	Dysbiotic & germ-free mice	↓ β-glucuronidase activity	Impaired estrogen deconjug-ation & reabsorption	Reduced circulating estradiol; infertility	Microbial control of estrogen homeostasis	Escorcia et al. 2025
12	Endometriosis Dysbiosis Model	Endometriosis-induced mice	↓ SCFA producers	SCFA depletion; immune metabolic hyperactivation	Chronic inflammation; lesion persistence	Metabolic–immune coupling in estrogen-driven disease	Chadchan et al. 2021
13	Acute LPS Exposure	IV LPS-injected mice	Opportunistic pathogen blooms	TLR4-dependent loss of colonization resistance	Pathogen expansion without colitis	Explains infection risk during systemic inflammation	Kroon et al. 2025
16	*Chlamydia trachomatis* Ascension	Intravaginal infection in mice	Immune competence-dependent outcomes	APC recruitment; tissue-level immune resolution	Clearance or chronic PID	Dysbiosis + immune defects permit ascension	Stagg et al. 1988
17	*E. coli* Ascending Infection	Pregnant mouse model	Vaginal pathogen ascension	Intrauterine infection & inflammation	Preterm birth; fetal neuroinflammation	Direct model of dysbiosis-driven obstetric risk	Boyle et al. 2025
18	Vaginal Microbiota Transplantation	Dysbiotic rats + healthy VMT	Lactobacillus restoration	Barrier repair; cytokine normalization	Durable dysbiosis reversal	Feasibility of VMT as therapy	Luo et al. 2024

VIII. THERAPEUTIC FAILURE: WHY CURRENT TREATMENTS DO NOT RESTORE FUNCTIONAL HOMEOSTASIS

Although antibiotics, antifungals, and probiotics have been used for years, recurrences are frequent, indicating that current management strategies are unable to provide an alternative for normal vaginal homeostasis. Standard approaches focus on microbial ecology and symptom alleviation rather than restoration of proper functioning of the vagina (Machado et al. 2016). However, an important drawback involves the survival of polymicrobial biofilm communities, especially *G. vaginalis* biofilm. Such biofilm formation protects dysbiotic bacteria from antibiotics and immune destruction. Further, recolonization following treatment is often characterized by the presence of *Lactobacillus iners*, which is not stable and is related to recurrent dysbiosis, rather than sustained eubiosis (Petrova et al. 2017). It is noteworthy that current approaches do not account for immune impairment in the host, loss of integrity of the epithelial barrier, and impaired metabolic signalling. While inflammation may persist and microbial metabolite dysregulation may be present, regardless of whether there is a temporary correction of microflora. All these studies, together, imply that vaginosis is not just a matter of composition but one of functionality at a systemic level. Thus, any approach to treatment of this condition must be directed towards reinstatement of metabolic, immune, epithelial, and ecological homeostasis (Muzny et al. 2015).

XI. MICROBIAL RESILIENCE AND ECOLOGICAL INSTABILITY

The critical characteristic of vaginal homeostasis is microbial resilience, which is described as the ability of vaginal microbiome to withstand any external or internal disruption and return back to normalcy once it ends. Under eubiotic conditions, the dominance of Lactobacilli leads to increased ecological stability and their effective resistance to pathogens by preventing them from colonization by maintaining low pH and producing antimicrobial metabolites (Ma et al. 2012). Under dysbiotic conditions, low microbial resilience is shown by increased microbial diversity and biofilm formation. Such vulnerability to ecological imbalances could also account for the frequent cases of recurrences after treatment with antibiotics, which involves the suppression of microbes, which is immediately followed by the reappearance of dysbiosis. It is likely that microbial resilience should be considered an important target in future treatments with the use of the microbiome (France et al. 2016).

X. VAGINAL DYSBIOSIS, CHALLENGES IN CLINICAL MANAGEMENT AND FUTURE RESEARCH DIRECTIONS

- *Sequencing Technologies and Diagnostic Challenges in Vaginal Dysbiosis:* Current diagnostic approaches for vaginal dysbiosis are constrained by a trade-off between feasibility and biological resolution. 16S rRNA gene sequencing remains widely utilized due to its cost-effectiveness and rapid turnaround; however, it is limited by reduced taxonomic resolution and a lack of functional insight. In contrast, shotgun metagenomic sequencing enables strain-level characterization and comprehensive functional profiling, including detection of antimicrobial resistance genes and viromes, albeit at the expense of higher cost, greater sequencing depth, and substantial computational demands. Emerging evidence positions metatranscriptomics as a potential gold standard, given its ability to selectively capture metabolically active microbial populations. This approach enhances community state type (CST) classification, improves biomass estimation, and circumvents the confounding influence of DNA derived from inactive or dead organisms inherent to DNA-based methodologies. Commonly employed gynaecological swabs and DNA extraction methods produce largely comparable microbiome profiles, while host DNA depletion techniques enhance the accuracy of shotgun sequencing without altering microbial community composition. Diagnostic complexity is further exemplified by cytolytic vaginosis, a condition characterized by excessive *Lactobacillus* (often *Lactobacillus crispatus*) mediated epithelial cytolysis that clinically mimics candidiasis. As current sequencing modalities do not capture epithelial damage, diagnosis remains reliant on indirect measures such as vaginal pH, microscopy, and exclusion of pathogenic organisms. These limitations underscore the urgent need for integrated molecular biomarkers capable of linking microbiome composition with host epithelial pathology (Dos et al. 2024).
- *Personalized Microbiome Therapeutics and Pharmacomicrobiomics:* Pharmacomicrobiomics translates the role of host-microbiota interactions into the field of precision medicine. It acknowledges microbiota as an important factor that influences the pharmacokinetics, pharmacodynamics, and toxicity of drugs. Microbial enzymes directly affect the metabolism of drugs, whereas microbial metabolites affect the signalling pathways of the host. Unlike the host's genetic makeup, the microbiota is dynamic and can be altered, which makes it a promising target for personalized medicine. However, the clinical application of the microbiota is hindered by the presence of high level of individual and strain variability. Further, the absence of strain-level efficacy information makes it difficult to use probiotics as a treatment option. To overcome these issues, patient-specific drug testing platforms, such as the use of an organ-on-chip device that co-cultures the patient's microbiota with human vaginal epithelial cells, provide a promising preclinical tool for assessing individualized responses to drugs and probiotics (Zimmermann et al. 2019).
- *Multi-Omics Integration and Systems-Level Understanding:* The taxonomic composition of microbes alone is insufficient for understanding dysbiosis. Multi-omics analyses have shown that host-derived metabolites and immune mediators are better predictors of inflammation, vaginal pH, and microbial community structure than microbial composition itself. While multi-omics analyses provide better functional resolution, their benefit over single-omics analysis is often small, which can be attributed to redundancy in the different layers of data (Bokulich et al. 2022).
- *Global Health Disparities and Research Equity:* The African vaginal microbiota has been shown to have unique features, which are often represented by a high abundance of *Lactobacillus iners* and a higher baseline microbial diversity, with varying prevalence of bacterial vaginosis. Importantly, ancestry-related microbiota profiles are retained even after geographical migration, indicating a

strong host-microbiota association (Kullin et al. 2025). Sub-Saharan Africa is disproportionately affected by microbiota-related outcomes, such as preterm birth and enhanced susceptibility to STIs and HIV. However, most current diagnostic criteria and treatment modalities are based on non-African populations, representing a critical "missing microbiome" gap. In an attempt to fill this gap, the Vaginal Microbiota Research Consortium for Africa (VMRCA) encourages African-led longitudinal research to define resilient microbiota profiles, develop probiotics tailored to the population, and enhance African research capacity (Bayigga et al. 2019).

XI. CRITICAL KNOWLEDGE GAPS AND RESEARCH PRIORITIES

The essential knowledge gaps are categorized into the following diagnostic, therapeutic, mechanistic, and population-level gaps. The diagnostic knowledge gaps include the requirements for population-specific thresholds, cost-effective point-of-care technologies, and non-invasive cervicovaginal health biomarkers. The therapeutic knowledge gaps include the validation of strain-specific probiotic efficacy, vaginal microbiota transplantation, and partner-inclusive treatment approaches. The mechanistic knowledge gaps include the uncertainties associated with polymicrobial interactions, estrobolome and disease correlations, the paradoxical role of vaginal short-chain fatty acids, and immune system reprogramming. The population-level knowledge gaps include the characterization of African vaginal microbiota dynamics, representation of transgender and non-binary populations, and dysbiosis in post-menopausal conditions.

XII. FUTURE RESEARCH DIRECTIONS AND IMPLEMENTATION STRATEGIES

A standardized diagnostic approach should focus on RNA-based tests as reference standards, use population-stratified thresholds, leverage cost-effective point-of-care technologies, and develop reliable molecular biomarkers of cervicovaginal health. For therapeutic advances, the application of organ-on-chip technology for strain-level testing, the incorporation of pharmacomicrobiomics into personalized therapies, the development of targeted bacteriophage therapies, and the adoption of couple-based care models may be explored. From a research and public health community point of view, continued investment in longitudinal multi-omics studies, the standardization of organ-on-chip technology, the expansion of African-led research efforts, and the integration of health equity frameworks would be critical. Notably, the integration of dysbiosis screening into gynaecologic practice and the adoption of partner-centered treatment approaches may be useful in controlling the global burden of cervicovaginal disease.

XIII. CONCLUSION

A perusal of literature suggests that dysbiosis is the key responsible for compromised vaginal health. However, African data suggests that *Lactobacillus* species may show ethnic specific vaginal flora. If this point is proved beyond doubt employing systematic global studies, some very valuable information may be generated. The fact that migration does not alter association of vaginal microbiota with host means their lateral inheritance. Assuring that this is true, one may then expect that all well-defined ethnic groups would show ethnicity specific vaginal flora. Notwithstanding these speculations, it would be worthwhile to conduct global study on the overall health of vaginal biome, BV and overall heterogeneity of *Lactobacillus* species across the spectrum of the society. Similarly, paradox on SCFA between vaginal and gut biome may also need to be addressed to be able to enhance our undemanding on the women health in general and a specific population in particular.

XIV. ACKNOWLEDGMENT

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XV. AUTHOR CONTRIBUTIONS

Shilpa Sharma: Conceptualization and design. Asma Imran Ansari, Wajiha Zehra: Writing- Original draft preparation, Zoya Hussain: Data curation, Sher Ali: Supervision, reviewing.

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