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Review

Bacterial Vaginosis and Dysbiosis: Insights into Pathogenic Infections and Trichomoniasis

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Abstract

Background/Objectives: Bacterial vaginosis (BV) is the leading cause of vaginal dysbiosis and remains a public health concern due to its high prevalence, recurrence, and association with adverse reproductive and infectious outcomes. Advances in next-generation sequencing (NGS) and microbiome characterization have redefined BV by revealing microbial composition profiles known as Community State Types (CSTs). Once regarded primarily as a polymicrobial infection, BV is now recognized as a multifactorial ecological disorder marked by microbiome instability, *Lactobacillus* depletion, anaerobic overgrowth, host–microbe interactions, and clinical heterogeneity. Methods: This narrative review critically analyzes recent evidence on vaginal microbial ecology, the transition from eubiosis to dysbiosis, and the mechanisms underlying BV development, persistence, and recurrence. This review focuses on interactions between BV-associated microorganisms and *Trichomonas vaginalis*, including microbial persistence, immune modulation, and susceptibility to Sexually Transmitted Infections (STIs). Results: BV involves ecological, metabolic, and immunological changes, including polymicrobial biofilm formation, alterations in the mucosal metabolome, and impaired resistance to colonization by other microorganisms. These changes may facilitate microbial persistence, recurrence, and *T. vaginalis* persistence. This is reflected in the association between BV and an increased risk of STIs, although the cause has not yet been fully established. Microbial resistance and recurrence underscore the need for new interventions, while probiotics, live biotherapeutic products, vaginal microbiota transplantation (VMT), targeted therapies, and vaccines have shown promising results. Conclusions: Integrating microbiological, immunological, and clinical evidence, this review provides a framework for BV pathophysiology and its association with *T. vaginalis*, supporting personalized therapeutic and prophylactic strategies.

Keywords: bacterial vaginosis; sexually transmitted infections; dysbiosis; *Trichomonas vaginalis*; antimicrobial resistance

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1. Introduction

Bacterial vaginosis (BV) is recognized as the most prevalent form of vaginal dysbiosis among women of reproductive age. Global estimates range from 23–29%, corresponding to approximately one-quarter of women in this population, although prevalence varies substantially across populations and geographic regions [1–3]. In Brazil, official health information systems such as DataSUS do not provide consolidated national prevalence estimates, as BV is not a condition subject to mandatory notification. Available clinical and population-based studies report a mean prevalence of 18.1%, with estimates ranging from 15% to 20% across the populations investigated. These Brazilian estimates are lower than the reported global range; however, differences in study populations, geographic coverage, and diagnostic methods may limit direct comparisons [4,5]. BV recurrence is clinically significant because it affects quality of life and increases the risk of gynecological and obstetric complications [1,6]. However, the literature still lacks standardized epidemiological studies that allow consistent comparisons of recurrence rates across populations and geographic settings. This limitation highlights the need for multicenter studies to better characterize the condition's epidemiology [1,7].

Unlike a classic infectious disease, BV is not caused by an external pathogen but by an imbalance in the bacteria naturally present in the host. The vaginal microbiota is a highly specialized and dynamic ecosystem that plays a fundamental role in maintaining women's reproductive health [8]. Under physiological conditions, this environment is colonized, albeit in smaller numbers, by various bacterial genera in addition to the traditionally predominant *Lactobacillus*, including *Prevotella* spp., *Sneathia* spp., *Leptotrichia* spp., *Megasphaera* spp., and *Mobiluncus* spp. Other species include *Gardnerella vaginalis*, *Fannyhessea vaginae* (formerly *Atopobium vaginae*), and *Hoylesella timonensis* [9].

The state of vaginal eubiosis has traditionally been characterized by the presence of the genus *Lactobacillus*, which is considered a key protective component of the microbiota. In this microenvironment, the main species identified are *Lactobacillus crispatus*, *Lactobacillus jensenii*, *Lactobacillus gasseri*, and *Lactobacillus iners*. These bacteria exert protective effects by producing lactic acid (LA), hydrogen peroxide, and bacteriocins, which help maintain an acidic vaginal pH and limit colonization by opportunistic pathogens, thereby preserving microbial community balance [10]. Recent pan-genomic studies have shown that these mechanisms are associated with specific genes, such as ldh (lactate dehydrogenase), pox (pyruvate oxidase), and nox (NADH oxidase), which are involved in synthesizing these metabolites. In addition, genes related to epithelial adhesion, biofilm formation, and exopolysaccharide synthesis confer a competitive advantage against invading microorganisms and help modulate the vaginal mucosal immune response, promoting a balance between immune tolerance and protection against infection [11].

This classical understanding of vaginal eubiosis, centered on *Lactobacillus* predominance, has been challenged and expanded by recent studies, prompting a reassessment of the strict distinction between eubiosis and dysbiosis within the Community State Types (CSTs), which had long been regarded as an established consensus, without considering that the definition of CSTs reflects the dynamic nature of the vaginal ecosystem. Recent evidence has shown that the five CSTs (Figure 1) represent dynamic ecological states rather than fixed categories corresponding to health and disease [12]. Taxonomic composition alone is insufficient to explain the behavioral complexity of the vaginal microbiota, as factors such as genetic variability among species, the functional potential of microorganisms, interactions with viral components, and the community's temporal dynamics can significantly influence vaginal health [13].

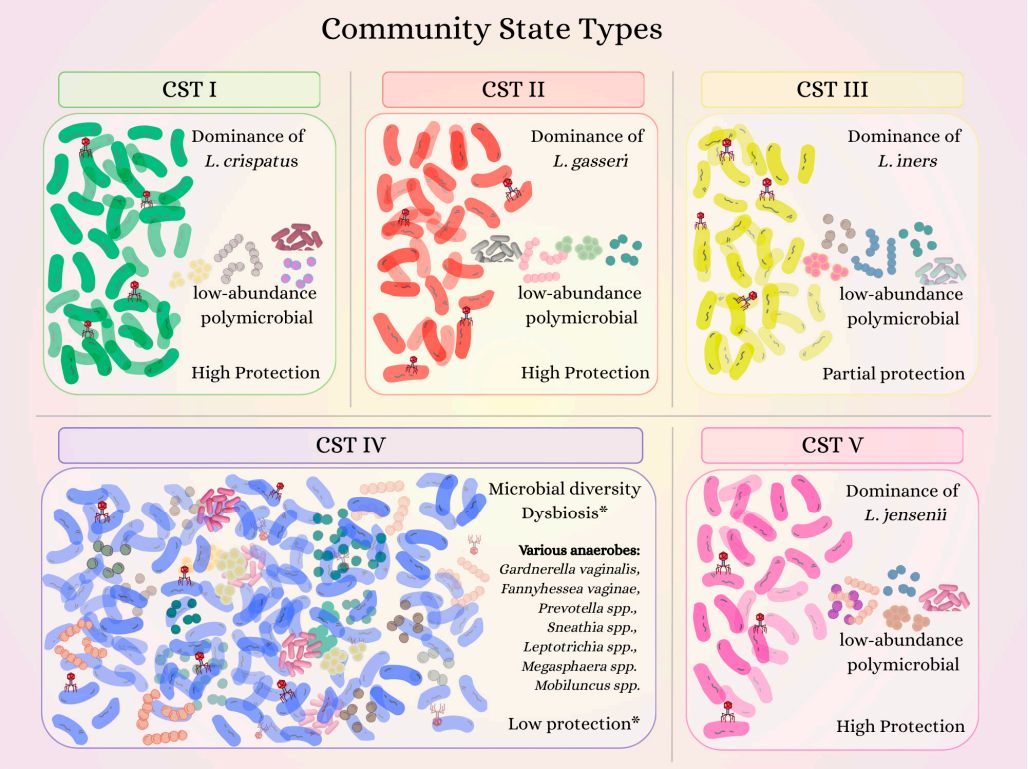


Figure 1. Community State Types (CSTs) of the vaginal microbiome. Each CST represents a distinct microbial profile: CSTs I, II, and V are dominated by *Lactobacillus* species (*L. crispatus*, *L. gasseri*, and *L. jensenii*, respectively) and are associated with greater protection; CST III is characterized by *L. iners*, which provides partial protection and may be linked to the transition between eubiosis and dysbiosis; CST IV shows high microbial diversity, dominated by anaerobes such as *Gardnerella vaginalis*, *Prevotella* spp., and *Mobiluncus* spp., and is generally associated with dysbiosis and reduced protection. * However, there is ongoing debate regarding the direct classification of CST IV as a representative of dysbiosis.

Dysbiosis and BV have traditionally been associated with CST IV because of its high microbial diversity and reduced abundance of protective *Lactobacillus*. Recent studies indicate that this CST exhibits greater biological heterogeneity than previously recognized. However, according to the literature, it remains frequently associated with BV and other adverse gynecological outcomes [14]. There is evidence that some asymptomatic women may exhibit profiles consistent with CST IV without overt clinical manifestations, given the dynamic nature of the vaginal microbiome and its susceptibility to physiological and environmental fluctuations. These findings suggest that different physiological and ecological states can coexist within this same category, which encompasses heterogeneous microbial communities with distinct functional and immunological profiles, challenging the traditional interpretation that highly diverse communities necessarily represent a pathological state [15–17].

A microbiome study provided a high-resolution characterization, revealing that CST IV comprises distinct subgroups with specific bacterial taxa, as well as potential intermediate profiles between *Lactobacillus*-dominated communities and more diverse states. This also suggests that profiles classified as CST IV may represent different ecological states within the same category, reinforcing the dynamic nature of the vaginal microbiome [18]. Using 16S rRNA amplicon sequencing validated by RT-qPCR confirmed the heterogeneity of vaginal profiles classified by CST and demonstrated the ability to distinguish *Lactobacillus*-dominated communities from those associated with bacterial vaginosis. This reinforces that

CST-based classification remains a useful tool for characterizing microbial patterns linked to various ecological and clinical conditions within the vaginal environment, even when accounting for the dynamic and transient nature of states within this ecosystem [19]. In addition, recent longitudinal analyses have demonstrated substantial temporal variability, with daily transitions between CSTs within this microbiome, influenced by factors such as menstruation, bacterial genetics, and interactions with bacteriophages, underscoring the importance of this temporal transience [20].

Similarly, the interpretation of CST III has also been refined. Although characterized by a predominance of *L. iners*, CST III is considered a dynamic microbial community that may remain relatively stable in some women or function as a transitional state, evolving toward a more diverse bacterial profile associated with dysbiosis [21,22]. Unlike species such as *L. crispatus*, which are more closely associated with greater ecological stability and sustained LA production, *L. iners* exhibits a high capacity to adapt to different environmental conditions, conferring ecological plasticity that is strongly associated with lower lactic acid production and reduced capacity for competitive exclusion of microorganisms associated with dysbiosis, which contributes to its ability to coexist in diverse microbial communities. These characteristics may have contributed to this transitional interpretation of CST III, which is often viewed as an intermediate state preceding dysbiosis [23,24]. Longitudinal analysis further supports the importance of considering the temporal dynamics of vaginal microbial communities, as different community states exhibit distinct probabilities of persistence and transition. In a cohort of South African women, *L. iners*-dominated communities were identified as an intermediate state between *L. crispatus*-dominated communities and more diverse bacterial communities associated with adverse reproductive outcomes [22]. Although this does not diminish the importance of more diverse CSTs in dysbiosis, these communities have also been associated with other health consequences. Studies have linked vaginal microbiome profiles characterized by *Lactobacillus* depletion to HPV infection and cervical cancer, although the mechanisms underlying these associations remain incompletely understood [24].

In summary, although BV remains strongly associated with reduced protective *Lactobacillus* species and increased bacterial diversity, recent evidence suggests that the presence of a specific CST alone may not be sufficient to characterize a pathological state. Thus, the interpretation of BV is shifting from a model based exclusively on microbial composition to an integrated approach that considers community stability, functional activity of microbiota, the host inflammatory response, and clinical manifestations. In this context, this review synthesizes current evidence on the microbiological and molecular mechanisms underlying the transition from a physiological to a dysbiotic state, with an emphasis on BV pathogenesis, including biofilm formation, antimicrobial resistance, and factors associated with disease recurrence. Furthermore, it addresses the association with the STI caused by *Trichomonas vaginalis* (Tv), a condition frequently linked to BV that presents with partially overlapping clinical manifestations, particularly in cases of coinfection. To this end, the review covers the composition of the vaginal microbiota, dysbiosis, available therapeutic strategies for both conditions, and clinical challenges such as treatment failure and recurrence, highlighting the clinical similarities between conditions that, in fact, have distinct pathogenic mechanisms.

2. Literature Search and Selection Criteria

The literature search was conducted primarily in PubMed and Google Scholar, complemented by searches in Scopus and Web of Science. The initial identification of articles was performed through these databases, and the organization and management of the references were supported by the Sciscape platform, which was used for citation tracking, thematic grouping, and avoiding duplicate records. In this initial search phase, a bibliographic search

was conducted, primarily on PubMed, using the following keywords within the title/Abstract fields: ("vaginosis" [Title/Abstract]) AND ("bacterial vaginosis" [Title/Abstract]) AND (dysbiosis[Title/Abstract] OR pathogenic[Title/Abstract]). We evaluated studies for eligibility using the Sciscape tool, based on the predefined inclusion and exclusion criteria established earlier. The review included studies in human subjects that investigated bacterial vaginosis and provided information regarding dysbiosis, pathogenicity, epidemiology, microbiota, or prevalence. Duplicate studies unrelated to the review question and ineligible publication types were excluded. This process yielded 20 initial records, which served as the theoretical foundation for developing the outline of this narrative review. In the second search phase, additional searches addressed the specific topics covered in each section of the review. Search terms were combined according to the subject under investigation, including "vaginal" AND "healthy microbiota" AND "virome"; "bacterial vaginosis" AND "bacteriophages" AND "virome"; and "bacterial vaginosis" AND "coinfection" AND "*Trichomonas vaginalis*", among others. Searches focused on publications from 2019 to 2026 in English and Portuguese. The final search was conducted in October 2026. This period was intended to capture recent advances in understanding bacterial vaginosis, Tv infection, and vaginal microbial ecology. The search timeframe was determined by the availability and relevance of publications in women's health, particularly those related to bacterial vaginosis (BV) and *Trichomonas vaginalis* (Tv) infection, the association between these two conditions, and their interactions with the vaginal microbiome, resulting in the inclusion of 77 studies in the manuscript. Although research on the vaginal microbiome has progressed significantly since the characterization of community state types, important gaps remain regarding an integrated understanding of microbiological dynamics, host responses, and their clinical implications. As this was a narrative review, the literature search aimed to identify and critically discuss relevant publications rather than conduct an exhaustive systematic search. The selected publications were considered for their relevance to the review objectives and the consistency and limitations of the available evidence. No formal systematic-review selection process or quantitative risk-of-bias assessment was performed.

3. Healthy Vaginal Microbiota and States of Dysbiosis

3.1. Physiological State of the Vaginal Microbiota Associated with Other Microorganisms

BV is a complex condition resulting from profound alterations in the vaginal ecosystem. Although bacteria are the predominant components of the vaginal microbiome, current metagenomic studies are increasingly highlighting the importance of the virome and mycobiome in vaginal physiology, despite their lower relative abundance compared with bacteria [7,25]. The mechanisms by which virome and mycobiome components contribute to the maintenance of vaginal eubiosis remain poorly understood. Current hypotheses are primarily supported by observational evidence and ecological concepts drawn from studies of the gut microbiome, which is often used as a reference for understanding other complex microbial ecosystems [26]. In these ecosystems, ecological balance is shaped by a dynamic network of interactions, including selective predation, niche competition, and cross-kingdom interactions among bacteria, fungi, viruses, and the host [25,27].

Bacteriophages constitute a significant fraction of the vaginal virome and actively contribute to the dynamics of the microbial communities that comprise the vaginal microbiota [28]. These viruses may contribute to the ecological stability of these communities by regulating bacterial populations through selective predation on bacteria susceptible to bacteriophage infection [27]. They may also do so through superinfection exclusion, a mechanism in which prophages integrated into some bacterial genomes reduce susceptibility to subsequent viral infections [28].

However, it is important to emphasize that these bacteriophages should not be considered inherently beneficial or harmful; as such, a distinction is not applicable in this context. These microbial communities continuously interact within the ecosystem, and depending on the conditions and factors present, certain changes may occur in the microenvironment that may shift the community toward eubiosis or dysbiosis [29]. The ecological effects of prophages are thought to depend on the bacterium harboring the phage and the microbial context in which they occur (e.g., phages with a lysogenic cycle in protective bacteria or a lytic cycle in pathogenic bacteria). Thus, mechanisms such as superinfection exclusion may influence both eubiotic populations and bacteria associated with dysbiosis [28,29].

In addition, phages attached to vaginal mucus may provide a form of non-host-derived immunity, targeting pro-dysbiotic bacteria before they reach and damage the vaginal epithelium [30]. Another proposed mechanism involves pattern recognition receptors (PRRs) on vaginal mucosal cells, such as Toll-like receptor 9 (TLR9), recognizing bacteriophage DNA in the virome. Activation of these receptors triggers signaling pathways involved in the innate immune response, promoting the production of type I interferons (IFN-I) and inflammatory mediators that modulate the host antiviral response (Figure 2) [28,31]. These findings suggest that these mechanisms may play an important role in virome–microbiota–host interactions, contributing to vaginal mucosal immune homeostasis. However, these hypotheses still require experimental validation in the vaginal environment [28,32].

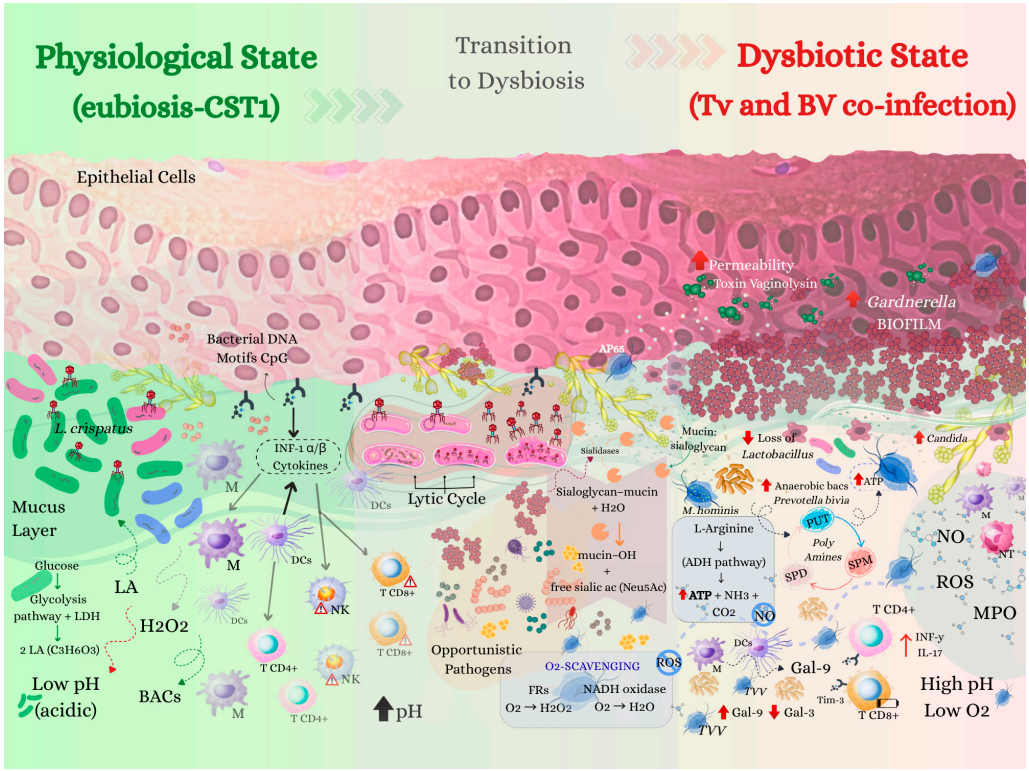


Figure 2. Schematic representation of the biological processes underlying the transition of the vaginal microbiota from a physiological to a dysbiotic state during bacterial vaginosis (BV) and *Trichomonas vaginalis* coinfection. On the left, the physiological state (eubiosis–CST I) shows dominance of *L. crispatus*, which produces lactic acid (LA) and hydrogen peroxide (H_2O_2) via the lactate dehydrogenase (LDH)-dependent glycolytic pathway, maintaining acidic pH and mucosal integrity. Resident immune cells—macrophages, dendritic cells (DCs), NK cells, and $CD4^+$/$CD8^+$ T lymphocytes—recognize CpG motifs in bacterial DNA and induce type I interferons (IFN-1α/β). The virome and mycobiome regulate the ecosystem through selective bacteriophage predation and immune surveillance by commensal fungi such as *Candida* spp. At the center, the transition to dysbiosis involves increased pH,

loss of *Lactobacillus*, activation of the lytic cycle, and proliferation of opportunists. On the right, the dysbiotic state (BV + Tv) shows epithelial damage by vaginolysin, increased epithelial permeability, and biofilm formation involving *Gardnerella* (a key contributor to BV). In cases of concomitant vulvovaginal candidiasis, *Candida* may also be present and, under specific conditions, contribute to inter-kingdom biofilm. Furthermore, anaerobic metabolism by *P. bivia* produces ATP and polyamines. L-arginine consumption through the arginine dehydrogenase (ADH) pathway limits nitric oxide (NO) synthesis, weakening antioxidant defense. The immune response includes reactive oxygen species (ROS), myeloperoxidase (MPO) activity, and expression of Gal-9 and Tim-3, leading to $CD8^+$ T-cell exhaustion, dysregulated IFN-γ and IL-17, chronic inflammation, tissue damage, and persistence of coinfection. The dashed arrows help clarify which microorganisms participate in the indicated processes and how these processes may be interconnected, relationships that might not otherwise be readily apparent. The separate core blocks describe specific processes carried out by individual microorganisms, such as Tv (involved in scavenging), the Tv symbiont M. hominis (associated with L-arginine metabolism), and G. vaginalis (involved in mucin manipulation). The color transition in the background of the image represents the shift from a healthy vaginal microbiome state to a state of microbial imbalance (dysbiosis). Furthermore, in each case, the microorganism responsible for or involved in the described process is shown.

Similarly, the mechanisms of cross-kingdom interactions, which have been developed and explored most extensively within the gut microbiome, are gradually being applied to the female reproductive tract [25,26]. The mycobiome and the bacterial community establish a regulated coexistence that contributes to vaginal homeostasis. Under physiological conditions, *Lactobacillus* species regulate the fungal population, primarily by producing LA and, possibly, hydrogen peroxide [33], which help maintain an acidic vaginal pH and restrict the proliferation of opportunistic fungi such as *Candida* spp. They may also inhibit the fungus's transition into its invasive filamentous form; suppress the expression of fungal virulence genes; produce biosurfactants and bacteriocins; compete for nutrients; and occupy adhesion sites on the vaginal epithelium, thereby limiting excessive fungal colonization (Figure 2) [34].

Among the components of the vaginal mycobiome, yeasts of the genus *Candida* are the predominant members, with Candida albicans as the dominant species. Other species, such as *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, *Candida krusei*, *Candida dubliniensis*, and *Candida guilliermondii*, may also be present at lower abundance [35]. In addition to *Candida* spp., sequencing and metagenomic studies of the vaginal microbiome have identified other fungal genera, also present in lower abundances, such as *Saccharomyces* and *Cladosporium*, among others. However, the ecological contribution of these taxa in vaginal homeostasis remains poorly understood [36].

Although eubiosis is predominantly associated with *Lactobacillus* species, these yeasts also form part of the vaginal ecological network and may persist as commensal organisms under conditions of microbial balance [37]. Evidence suggests that *Candida* colonization involves continuous interaction with the mucosa and remains under ongoing surveillance by the local immune system. Although this mechanism is not yet well established in the vaginal context because of a lack of experimental validation in this environment, this state of recognition and control can be hypothesized as a form of basal immune surveillance that may limit fungal expansion and prevent excessive inflammation [38]. Taken together, these interactions among fungi, bacteria, and the host also contribute to the dynamic balance of microbial communities within the vaginal ecosystem [1,39].

3.2. Bacterial Vaginosis Associated with Various Microorganisms

During the transition from a healthy vaginal microbiota to BV, bacteria play a central role, particularly species of the genus *Gardnerella*, which are recognized as key drivers of this condition [40]. Unlike other anaerobic bacteria associated with dysbiosis, *G. vaginalis*

has the unique ability to adhere to the vaginal epithelium and initiate the formation of a structured biofilm that provides a physical and metabolic scaffold for the recruitment of secondary colonizers, such as *F. vaginae* and *Prevotella bivia* [1].

Concomitantly, *G. vaginalis*, the predominant species identified within the genus, secretes sialidases that degrade sialoglycans and mucins that protect the vaginal mucosa, exposing new adhesion sites and favoring the persistence and stability of the dysbiotic biofilm [41–43]. At the same time, *G. vaginalis* produces vaginolysin, which causes cytotoxic damage to the epithelium, while cooperative metabolic interactions among biofilm members increase nutrient availability and promote the stability of the dysbiotic community [42–44].

Thus, *Gardnerella* is not merely another component of the altered microbiota but acts as the keystone pathogen that initiates and shapes the pathogenic process of BV. In addition to biofilm formation, the genus expresses a broad repertoire of virulence factors involved in epithelial adhesion, mucin degradation, biogenic amine production, and immune modulation, thereby promoting a microenvironment conducive to persistent dysbiosis [45]. These microorganisms occur in different strains, whose characteristics may influence whether the vaginal microenvironment tends toward eubiosis or dysbiosis. While certain *Lactobacillus* taxa are associated with maintaining vaginal homeostasis, other microorganisms may contribute to the establishment and perpetuation of dysbiosis. In this context, unlike *Gardnerella*, which may act as a secondary keystone pathogen, remodeling the vaginal microenvironment in a way that favors the persistence of dysbiosis without triggering BV on its own [46,47]. Regarding the mechanisms of viral pathogenesis, bacteriophages may contribute to vaginal dysbiosis depending on the bacterium harboring the phage, the phage's characteristics, and the ecological context in which they occur. This process can be initiated through two similar pathways that differ only in the bacteriophage's origin. In the first, lysogenic phages integrated into *Lactobacillus* genomes may be induced to enter the lytic cycle by environmental and physiological stressors, such as chemical agents and mucosal disturbances associated with menstruation, sexual intercourse, and intimate hygiene practices. Second, exogenous bacteriophages, potentially introduced during sexual activity, can infect susceptible *Lactobacillus* strains [27,30,48]. Although their origins differ (endogenous or exogenous), both mechanisms promote lysis of protective *Lactobacillus* populations, reducing the production of LA and other metabolites that maintain acidic pH and mediate the competitive exclusion of pathogens. As a result, the microbiota's chemical and ecological barriers weaken, creating conditions favorable for the establishment and persistence of dysbiotic communities [1,49]. Similarly, the protozoan *T. vaginalis* can disrupt the vaginal microenvironment by inducing simultaneous changes in the mucosa's physical, immunological, and biochemical components. Infection with this protozoan creates a microenvironment conducive to the proliferation of anaerobic microorganisms [50]. In some cases, the presence of *Trichomonas vaginalis virus* (TVV) leads to galectin-mediated modulation and exacerbated activation of inflammatory pathways (Figure 2). These processes may reduce *Lactobacillus* abundance and weaken the microbial containment mechanisms characteristic of the physiological state. This review includes a section dedicated to the protozoan *Trichomonas vaginalis* [32].

Fungi, although rarely considered initiators of BV, may also disrupt homeostasis when environmental changes enable them to shift from a commensal state to pathogenic behavior. The loss of regulatory roles exerted by *Lactobacillus* and an increase in vaginal pH promote hyphal formation, virulence factor expression, and the establishment of inter-kingdom biofilms [7]. These processes intensify local inflammation, increase microbial resistance, and reinforce feedback loops that hinder the return to eubiosis [49]. Thus, viruses, protozoa, and fungi may weaken or disrupt the ecological, immunological, and biochemical barriers

that maintain vaginal microbiota stability, creating conditions conducive to the onset and progression of dysbiosis [51].

4. Relationship Between Bacterial Vaginosis and *Trichomonas vaginalis*

According to the WHO, Tv is the causative agent of the world's most common nonviral STI: trichomoniasis. More than an invader, this parasite modulates the vaginal ecosystem by increasing vaginal pH and reducing *Lactobacillus* dominance and its protective effects, creating a favorable niche for the overgrowth of anaerobic bacteria associated with BV [52]. In this context, trichomoniasis should not be viewed as an isolated infection, but rather as part of a complex network of interactions in which the protozoan and the dysbiotic microbiota reciprocally influence each other's survival and persistence.

This endosymbiotic interaction is supported by complementary metabolic and structural mechanisms. Among these, the endosymbiotic relationship between the parasite and *Mycoplasma hominis* stands out, with the proportion of clinical isolates of Tv harboring the symbiont exceeding 80% [53]. In this context, the sharing of metabolic pathways, including arginine metabolism, may contribute to increased ATP production. While Tv benefits from enhanced proliferation, M. *hominis* receives protection against the host immune system's effector mechanisms (Figure 2). Tv may act as a potential "Trojan horse" for *M. hominis*, functioning not only as a host but also as a vehicle for bacterial dissemination to niches more favorable for its colonization. For example, by remaining inside the protozoan, *M. hominis* can be transported while maintaining viability, facilitating dissemination to other compartments of the female genital tract [54]. However, beyond this potential for transport and dissemination, it is important to consider the biological consequences of endosymbiosis for both microorganisms.

This relationship is bidirectional. The intracellular presence of *M. hominis* significantly influences the biology and pathogenicity of Tv, promoting metabolic changes previously associated with pathway sharing, increased ATP production, and greater proliferative capacity in the protozoan [53]. In contrast, the bacterium occupies a stable intracellular niche within the parasite, gaining protection from the host's immune effector mechanisms and, to some extent, from antimicrobial agents through a physical barrier [55].

Beyond effects on microbial survival and proliferation, this relationship is also linked to changes in host interactions. The presence of *M. hominis* has been associated with an amplified inflammatory response in experimental models, compared with strains lacking the bacterium, and with increased parasite cytotoxicity toward host cells [55,56]. Together, both microorganisms may interfere with the host immune response, promoting its modulation and evasion [57]. This interaction is also associated with increased adhesion to epithelial cells, greater cell-lysing capacity [56], and exacerbated stimulation of pro-inflammatory cytokine production, which may contribute to an environment of tissue damage and persistent inflammation [55].

These effects also relate to metabolic processes shared by the two microorganisms. Shared arginine metabolism increases the local consumption of this amino acid, potentially reducing its availability for nitric oxide (NO) synthesis, an important microbicidal mechanism of macrophages [53,58]. In parallel, infection with symbiont-harboring Tv activates interferon-ε (IFNε)- mediated responses, which have been associated with protection of the genital epithelium against parasite adhesion and cytolysis. However, Tv releases extracellular vesicles that can reduce this protective response, potentially favoring conditions associated with infection persistence and host colonization [57].

Tv interactions with a dysbiotic vaginal microbiota are not limited to endosymbiosis with *M. hominis*. *Prevotella bivia* also establishes a commensal relationship with Tv, providing metabolites such as polyamines that promote parasite growth and induce expression of the

AP65 adhesion protein, thereby enhancing its ability to colonize the vaginal epithelium [52]. After adhesion, Tv secretes cysteine proteases and other virulence factors that degrade extracellular matrix components, compromising mucosal integrity [52,59]. Damage to host epithelial cells releases nutrients that become available to both the protozoa and the anaerobic bacteria associated with BV, reinforcing the dysbiotic community and hindering restoration of the protective vaginal microbiota [1,60]. Immune mechanisms represent another essential component of this cooperation. During infection, Tv releases extracellular vesicles (exosomes) containing proteins, small regulatory RNAs, and, in some strains, the TVV, which can alter communication among the parasite, the microbiota, and the host [59]. Recognition of the double-stranded RNA of TVV by the Toll-like receptor 3 (TLR3) triggers the production of pro-inflammatory cytokines and interferons that amplify the local inflammatory response. Although this is an innate antiviral immune response, its persistent activation may promote tissue damage and sustained inflammation, which also contributes to the persistence of the dysbiotic environment, benefiting both the protozoan and bacteria associated with BV [50].

This cooperation also affects the therapeutic response. The recommended treatment for trichomoniasis remains metronidazole (MTZ), a prodrug activated under highly reducing conditions, such as those present during the parasite's anaerobic metabolism [61,62]. For BV, the treatment of choice also involves MTZ, although clindamycin is a widely used therapeutic alternative [7]. However, the efficacy of these antimicrobials may be compromised by the very cooperation that has been established. In the parasite, downregulation of enzymes such as flavin reductase 1 (FR1) reduces intracellular MTZ activation, representing one mechanism underlying clinical resistance [63]. In BV, polymicrobial biofilms produced by *Gardnerella* spp. and other anaerobes hinder the diffusion of MTZ and clindamycin, partially protecting biofilm-associated bacteria and the protozoan from antibiotic activity. Together, these mechanisms promote infection persistence, increase the likelihood of treatment failure, and contribute to higher recurrence rates of these STIs. In BV, recurrence rates can reach approximately 50% within the first 12 weeks after treatment [1,64,65]. In Tv infection, approximately 20% of women experience reinfection within three months after treatment, according to the Centers for Disease Control and Prevention (CDC) [66].

Although Tv has traditionally been described as anaerobic, it is microaerophilic. This metabolic versatility is crucial during the early stages of infection, when the pathogen is exposed to fluctuating oxygen (O_2) concentrations in the urogenital tract, highlighting its adaptive mechanisms for coping with oxidative stress [67]. Nevertheless, Tv contains O_2-sensitive organelles such as hydrogenosomes, which are the primary sites of ATP production, and therefore requires an integrated oxygen-scavenging system (Figure 2) to maintain energy metabolism and protect these sensitive structures [68].

This system consists of an enzymatic arsenal for removing molecular O_2, thereby preserving the reducing environment that is essential for hydrogenosome function. Key components include flavin reductases (FRs), which comprise eight isoforms that catalyze the reduction of O_2 to hydrogen peroxide (H_2O_2), and NADH oxidase, which directly reduces O_2 to water (H_2O) (Figure 2) [53,69]. Recent studies suggest that putative proteins of the Modulator of Drug Activity B (*Mda*B) family may be components of this redox network, as they possess conserved oxidoreductase domains and show increased expression under oxidative stress and after MTZ exposure, although their specific function still requires experimental validation [68]. Oxygen reduction generates reactive oxygen species (ROS), forming a second line of defense through an integrated antioxidant system [63,69]. This system includes thioredoxin reductase (*Trx*R), other thioredoxins, peroxiredoxins, osmotically inducible protein C (*Osm*C), and superoxide dismutase (TvFeSOD), which neutralize

peroxides and superoxide radicals, thereby maintaining redox homeostasis and protozoan cellular viability [63,68].

From this perspective, adaptation to the microaerophilic environment is another survival mechanism of Tv. Its ability to maintain redox homeostasis despite fluctuations in oxygen availability enhances parasite viability across different stages of infection, prolonging persistence and facilitating colonization, transmission, and establishment of infection. Furthermore, by limiting damage from host-derived ROS (Figure 2), these mechanisms help the parasite evade the innate immune response, which also favors persistent infection [59,63]. However, this high metabolic plasticity suggests the parasite may function as an organism adapted to fluctuating atmospheric conditions that indirectly favor a microenvironment conducive to vaginal dysbiosis [60,67].

5. Clinical Implications, Recurrence, and Antimicrobial Resistance

The interaction between Tv and BV-associated dysbiosis may have important clinical implications. Persistent vaginal dysbiosis, which may develop into BV and involve pathogenic microorganisms, has been associated with a chronic inflammatory environment and increased susceptibility to the acquisition of other STIs, particularly HIV and HPV. Furthermore, BV and Tv infections have been associated with adverse reproductive outcomes, including pelvic inflammatory disease (PID), infertility, and preterm birth. However, these associations should not be interpreted as evidence that BV–*T. vaginalis* coinfection independently causes these outcomes [1,7]. In recent years, evidence has further highlighted the potential role of sexual partner reinoculation in the recurrence of BV and the reinfection of *T. vaginalis*. This issue is particularly relevant to trichomoniasis, for which treatment regimens differ between men and women. Consequently, the traditional understanding of these diseases is evolving, prompting ongoing discussions about partner treatment and updated therapeutic recommendations [70].

Although conventional treatments for BV have initial cure rates of about 80%, recurrence remains a major clinical challenge. Over a longer follow-up period, approximately 70% of women experience new episodes of abnormal vaginal flora (in some cases, BV) within one year [1]. The problem of recurrent BV is not simply the failure to eliminate bacteria associated with BV, but also the reintroduction of these bacteria from sexual partners and the inability to restore a stable vaginal microbial community [71]. However, recurrence should not be attributed solely to reinfection. Recent studies suggest that BV may also recur due to intrinsic biological and microbiological factors, even without new exposure to the initiating agents [7,65].

Antimicrobial resistance (AMR) is a major clinical challenge in urogenital infections, resulting from distinct biological mechanisms across different microorganisms. In Tv, reduced susceptibility to 5-nitroimidazoles, particularly MTZ, results from a coordinated multifactorial process involving remodeling of redox metabolism, altered expression of enzymes responsible for prodrug activation, hydrogenosome adaptation, and mutations and transcriptional changes associated with oxidative stress. This resistance cannot be explained solely by reduced FR1 expression [61,68]. In BV, treatment failures stem primarily from high heterogeneity among *Gardnerella* species [72], the organization of polymicrobial communities into biofilms, the presence of tolerant bacteria, and the limited penetration of MTZ and clindamycin into biofilm-associated communities [73,74].

The main factor associated with clinical treatment failure of BV is the persistence of an adherent polymicrobial biofilm, dominated by *Gardnerella* spp., which impedes antibiotic penetration, protects bacteria from the host immune system, and promotes the persistence of subpopulations in a metabolically tolerant state (persister cells), allowing them to survive after drug exposure. Beyond biofilm protection, recent studies have identified 11

genospecies within the genus Gardnerella, with the core genome comprising approximately 18% of the total pangenome across the 129 samples analyzed [72]. This high genomic diversity is reflected in significant differences among genospecies and strains (isolates), such as ATCC 14018, ATCC 14019, ATCC 49145, JCP7276, HMP9231, and UGent 25.49, which exhibit distinct profiles of biofilm formation, virulence factor production, and antimicrobial susceptibility [45]. In a separate taxonomic analysis based on 312 genomes, the authors proposed a revised classification of the genus *Gardnerella*, comprising 11 species and 15 subspecies [75]. Although these results highlight the importance of genomic diversity when investigating the biological and clinical characteristics of *Gardnerella*, they should be interpreted separately because they come from different genomic datasets and analytical approaches. At the same time, certain *Gardnerella* species have been detected in asymptomatic women whose vaginal microbiota tested negative for bacterial vaginosis (BV), suggesting that the mere presence of this genus is not sufficient to explain disease development [76]. These findings reinforce the idea that virulence potential varies among different species and strains [45]. Consequently, some genospecies show greater potential for clinical persistence, whereas others remain susceptible to clindamycin, underscoring the potential of genomics for personalized therapy and identifying predictive biomarkers of resistance [45,72].

In parallel, researchers have identified AMR genes in bacteria associated with BV, including *nim* (nitroimidazole) and *ermG* or *ermTR* (lincosamide) genes. However, current evidence indicates that these genetic determinants alone do not explain the high rates of treatment failure and clinical recurrence [77]. Another important factor is the possibility of reinfection from sexual partners, who may serve as reservoirs for bacteria associated with BV, reintroducing these microorganisms after the patient completes treatment [71]. However, treatment failure results from the interplay among antimicrobial resistance, biofilm persistence, the genetic heterogeneity of genospecies and their strains, the presence of persister cells, and the reintroduction of dysbiotic microbiota from sexual partners, thereby promoting the rapid reestablishment of the bacterial community after treatment.

6. Therapeutic and Immunoprophylactic Perspectives

Standard BV treatment, established decades ago, focuses primarily on symptom relief and reducing the anaerobic bacterial load through antimicrobial therapy [78]. Currently, MTZ and clindamycin remain the most used antibiotics for BV, available in both oral and intravaginal formulations [7]. More recently, secnidazole was introduced as a single-dose alternative, aimed at improving patient adherence to treatment [78].

The recommended treatment for trichomoniasis is MTZ, with tinidazole (TNZ) as an alternative. Secnidazole (SEC) has also been approved by the U.S. Food and Drug Administration (FDA) for the treatment of trichomoniasis in patients aged 12 years and older. However, MTZ and TNZ remain established treatment options [79]. Although these treatment options are available for both conditions, the current approach is often criticized for focusing solely on pathogen eradication, without ensuring the stable restoration of a healthy microbiome [71].

MTZ and its alternative, TNZ, belong to the nitroimidazole class and interfere with bacterial DNA synthesis and integrity; they are highly effective against free-living anaerobic bacteria when these organisms are not attached to the vaginal mucosa [1]. SEC has a similar mechanism of action but a longer half-life, allowing effective single-dose therapy. Clindamycin inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit and has a broader spectrum of activity that includes both anaerobic bacteria and some aerobic species [7]. Adjuvant therapies such as LA also promote re-acidification of the vaginal environment, which the literature suggests inhibits pathogen growth and reduces the perception of unpleasant odors by protonating biogenic amines [80].

These limitations highlight the need for new therapeutic approaches and restoration of the native microbiota. In this context, emerging therapeutic strategies, still under investigation, are progressively moving away from an exclusively antimicrobial approach and toward restoring vaginal homeostasis, including live biotherapeutic products (LBPs), such as *Lactobacillus crispatus*-based formulations (LACTIN-V) in phase 2b studies, as well as vaginal microbiota transplantation (VMT), which is still in the experimental stage [71]. In parallel, more precise therapies, including targeted approaches such as metastable iron sulfides (mFeS) for resistant *Gardnerella* and bacteriophage endolysins (such as PM-477 and BNT331-EL) that penetrate biofilm through their cell wall-degrading activity, are being developed to selectively eliminate bacteria associated with BV while sparing beneficial microorganisms, which show promising results in preclinical studies [71,81].

This perspective becomes particularly relevant given the heterogeneity of the vaginal microbiota, which comprises bacteria, fungi, and other microorganisms that interact and coexist. *C. albicans* can adopt a pathogenic phenotype in response to microecological changes, while non-*albicans* species, such as *C. glabrata* and *C. tropicalis*, are gaining clinical relevance because of their lower susceptibility to antifungal agents and greater propensity for recurrence. In the context of emerging therapies for *Candida* associated with BV, researchers have also investigated adjunctive probiotic strategies to modulate the microbiota, with a focus on reducing recurrence of vulvovaginal candidiasis [37,82]. Furthermore, newer antifungal agents, such as ibrexafungerp and oteseconazole, expand the treatment options for recurrent infections and infections associated with azole resistance [83]. Therefore, the emerging trend is to develop precision interventions while promoting the restoration of a functional and resilient vaginal ecosystem.

In this context, women with BV are approximately twice as likely to acquire Tv infection, underscoring the clinical significance of coinfection [52,84]. From a therapeutic standpoint, the main challenge driving the development of new strategies for treating this STI is the continued reliance on the 5-nitroimidazole class, which is increasingly affected by resistance and limited alternatives [85]. Among the emerging approaches, the most promising ones involve mechanisms of action distinct from those of the drug class currently in use. One leading strategy is inhibition of the 20S proteasome of Tv, particularly the β5 subunit, which can be selectively targeted because it contains a threonine at the binding site that is absent from the human proteasome. This structural difference between the proteasomes may be exploited to achieve selective toxicity against the parasite. In a library of 373 compounds, proteasome inhibitors active against Tv were nearly 200-fold more active against the parasite than against mammalian cells, demonstrating high in vitro potency at concentrations ranging from 10–20 nM [86,87].

Synthetic antimicrobial peptides are another promising strategy because they remain active against metronidazole-resistant strains. Candidates for new therapies, such as D-TN1, D-TN3, and D-TN6, have shown in vitro activity against both susceptible and resistant isolates [88], while Epinecidin-1 has also shown activity in a murine model of trichomoniasis, achieving 100% inhibition at approximately 62.5 μg/mL [89]. These approaches aim to move beyond strategies focused solely on optimizing nitroimidazoles, instead seeking to identify new pharmacological vulnerabilities in the parasite and expand therapeutic options for treating resistant infections (Table 1) [68].

Although the current landscape of diseases associated with the vaginal ecosystem is driving a shift away from therapies aimed exclusively at eliminating the pathogen, this shift has not yet been fully realized for Tv. Therefore, future research should prioritize developing strategies that simultaneously address parasite resistance, interactions with BV-associated bacteria, and restoration of a vaginal microenvironment less conducive to persistent infection [90]. Indeed, this pathogen has received comparatively little attention;

despite the growing number of experimental candidates, translating these findings into advanced therapeutic candidates remains limited [68].

Taken together, immunoprophylactic approaches are receiving increasing attention in global public health and are being extensively explored in STI-related research. Currently, no vaccines are approved for human use against BV and Tv infection, but advances in immunoinformatics and reverse vaccinology have expanded vaccine development research [91]. Recent studies have used computational tools to analyze the genomes of these microorganisms and identify highly conserved and immunogenic surface proteins as potential targets. One such study on *G. vaginalis* identified vaginolysin (VLY), a key cytotoxin involved in epithelial cell destruction, and adhesion proteins such as OMALP as candidate antigens for a multi-epitope vaccine designed to enhance antigen presentation to immune cells [92].

Another study on Tv highlighted and used various surface and secreted proteins as candidates for the development of a multi-epitope vaccine. These include adhesins (AP33-1, AP33-3, AP51-2, AP65-1, and AP65-3), which bind to the vaginal epithelium. We also included a cytotoxic cysteine protease involved in extracellular matrix degradation and immune evasion. In addition, P270, LP07818P1, and proteins with a calponin-homology (CH) domain exhibited high predicted antigenicity and lacked predicted allergenicity. However, the multi-epitope vaccine was specifically composed of epitopes derived from four proteins: AP33-1, AP33-3, LP07818P1, and the CH-domain-containing protein [93]. The goal of these multi-epitope vaccines is to induce a robust cellular and humoral immune response in the vaginal mucosa that can neutralize pathogen virulence factors and prevent initial colonization, potentially limiting biofilm establishment, in conditions such as BV associated with trichomoniasis [94]. This leads us to an emerging approach in vaccine development that has gained prominence in recent years. This strategy integrates multiple organisms into a single vaccine construct, enhancing immunological reach and the efficacy of protective responses. In the context of diseases caused by multiple infectious agents, this approach has shifted the traditional perspective on vaccine development, paving the way for more comprehensive and promising prophylactic solutions. Consequently, it emerges as a potential alternative for controlling polymicrobial infections and reducing the recurrence of complex diseases [95].

Looking ahead, medical research aims to break the cycle of reinfection and treatment failure. Recent studies suggest that simultaneous treatment of the sexual partner may be important to prevent recurrent infection, although clinical trials are still underway to determine the optimal treatment protocols [64]. From a technological standpoint, nanomedicine holds considerable promise; for example, metronidazole-coated nanoliposomes may enhance drug efficacy and induce parasite apoptosis more rapidly and directly [96]. Therefore, understanding Tv not merely as an isolated infection, but as a key driver of profound dysbiosis, is the path to therapies aimed at restoring the body's natural balance [52,97].

Therefore, the need for new treatments and vaccines stems from BV's serious public health burden. The persistence of dysbiosis is directly linked to an increased risk of acquiring HIV and other STIs, in addition to being associated with adverse obstetric outcomes such as preterm birth, spontaneous abortion, and infertility. Treatment with broad-spectrum antibiotics often results in unstable dominance by *L. iners*, a species considered less protective and more permissive to subsequent dysbiotic states [71]. Therefore, prophylactic and therapeutic vaccines represent a promising strategy for generating mucosal immune memory, preserving epithelial barrier integrity over the long term, and potentially protecting female fertility [92].

Table 1. Therapeutic approaches for bacterial vaginosis and *Trichomonas vaginalis* infection: mechanisms, levels of evidence, and clinical applicability.

Therapeutic Approach	Proposed Mechanism	Evidence Level Reported in the Review	Clinical Applicability and Limitations
Metronidazole 500 mg for 7 days, and clindamycin for BV; secnidazole single dose as an alternative	Metronidazole and secnidazole interfere with microbial DNA integrity. Clindamycin inhibits bacterial protein synthesis through binding to the 50S ribosomal subunit.	Established clinical treatment	Currently used for BV. Recurrence and persistence of dysbiosis remain important limitations [7].
Metronidazole 500 mg for 7 days and tinidazole for trichomoniasis; single dose secnidazole as an alternative	Nitroimidazoles interfere with parasite metabolic processes and DNA integrity.	Established clinical treatment	Metronidazole is the principal treatment, with tinidazole as an alternative. Secnidazole has limited approval according to the age groups described in the review. Antimicrobial resistance remains a challenge [79].
Lactic acid (LA) as an adjunctive approach	It promotes vaginal reacidification, helping inhibit pathogen growth and potentially reducing unpleasant odors by protonating biogenic amines.	Adjunctive approach discussed in the literature	May support restoration of the vaginal environment but is not presented as a replacement for antimicrobial treatment [80].
Live biotherapeutic products (LBPs), including LACTIN-V (*L. crispatus* CTV-05)	Reinforcement of protective vaginal microbial communities and restoration of vaginal homeostasis.	Clinical evaluation, including phase 2b studies	Promising microbiome-based strategy, but clinical implementation and long-term benefits require further evaluation [71,98].
Vaginal microbiota transplantation (VMT)	Transfer of vaginal microbial communities to promote restoration of eubiosis.	Experimental investigation	Limited evidence; safety, donor selection, standardized protocols, and long-term efficacy remain to be established [99].
Targeted approaches against BV-associated bacteria, including metastable iron sulfides (mFeS) and bacteriophage endolysins (PM-477 and BNT331-EL)	Selective targeting of resistant Gardnerella and degradation of bacterial cell walls within biofilms.	Preclinical studies	Potential to target BV-associated bacteria while preserving beneficial microorganisms; clinical efficacy and ecological safety remain unestablished [71,100,101].
Antifungal agents for concomitant vulvovaginal candidiasis, including ibrexafungerp and oteseconazole	Antifungal activity against Candida species, expanding treatment options for recurrent infections and infections associated with azole resistance.	Clinical trials	These agents have limited applicability due to safety concerns, particularly during pregnancy, as well as high costs and restricted availability. Furthermore, both are still being evaluated in clinical trials to investigate broader therapeutic applications [82].

Table 1. *Cont.*

Therapeutic Approach	Proposed Mechanism	Evidence Level Reported in the Review	Clinical Applicability and Limitations
Proteasome inhibitors targeting *T. vaginalis*	Selective inhibition of the parasite's 20S proteasome, particularly its β5 subunit, exploiting structural differences from the human proteasome.	In vitro studies	Promising experimental activity, but clinical efficacy, safety, pharmacokinetics, and therapeutic applicability remain unestablished [80,81].
Synthetic antimicrobial peptides (D-TN1, D-TN3, and D-TN6)	Antimicrobial peptides destabilize and disrupt the parasite's cell membrane and may also interfere with nucleic acid and protein synthesis. D-peptides are more resistant to protease degradation and therefore more biologically stable.	In vitro studies	Activity has been reported against susceptible and metronidazole-resistant isolates; clinical validation is still required [88].
Epinecidin-1	It destabilizes and disrupts the cell membrane of *T. vaginalis*, leading to loss of cellular integrity and parasite death, including in strains resistant to metronidazole.	In vitro and murine-model evidence	Experimental candidate; efficacy has not been established [89].
Multi-epitope vaccines and reverse vaccinology approaches.	Computational identification of antigenic proteins and epitopes intended to induce protective cellular and humoral immune responses.	In silico studies	No approved vaccines are available for BV or trichomoniasis. Immunogenicity, safety, and protective efficacy require experimental and clinical validation [92–95].
Partner treatment and metronidazole-coated nanoliposomes	The formulation showed greater antiparasitic activity, with a faster reduction in cell rupture, potentially involving programmed cell death mechanisms.	In vitro studies	Optimization of the antiparasitic activity of MTZ, accelerating the elimination of *T. vaginalis*. Although increased metacaspase expression was observed, its role in cell death remains hypothetical and does not support the proposed apoptotic mechanism [90].

7. Limitations and Future Directions

The available literature still contains significant gaps in our understanding of the mechanisms governing the interactions between bacterial vaginosis, Tv infection, and the vaginal microbiota. Research on the vaginal microbiota has benefited from broader advances in microbiome research, including experimental approaches initially developed in other microbial systems. However, the distinctive features of the vaginal environment require adapting and validating these approaches for vaginal communities [102]. In addition, researchers have often investigated the gut and vaginal microbiomes separately, highlighting the need for integrated approaches to understand their potential physiological connections [103].

Another major limitation is our incomplete understanding of the complex interactions between vaginal microorganisms, the host immune response, and various environmental and behavioral factors. Although advances in molecular techniques have substantially expanded our knowledge of the vaginal microbiome, significant questions remain about the mechanisms underlying transitions within microbial communities, susceptibility to coinfections, and recurrence after treatment. Differences in study populations, diagnostic criteria, sampling strategies, and microbiome characterization methods may also limit direct comparisons across studies [104].

This study provides a critical synthesis of evidence from complementary research areas, rather than a systematic assessment of all available studies. The findings should therefore be interpreted in the context of the scope of the literature examined and the methodological differences among individual studies. Nevertheless, this approach supports an integrated discussion of BV, Tv infection, and vaginal microbiome dynamics, helping identify converging evidence, unresolved questions, and directions for future research.

Future research should prioritize longitudinal, multicenter studies that employ standardized methodologies for diagnosis and microbiome characterization. Such studies could clarify temporal changes in microbial communities and distinguish associations from the mechanisms that contribute to the onset, persistence, or recurrence of each disease. Further investigation into interactions among the host, the microbiome, and pathogens, as well as microbial metabolic pathways and biomarkers associated with disease progression, could contribute to a more comprehensive understanding of vaginal health. Furthermore, developing and evaluating microbiome-based therapies, including live biotherapeutic products, vaginal microbiota transplantation, and other precision medicine approaches, represents a promising avenue for improving prevention and treatment strategies. However, vaginal microbiota transplantation remains experimental, and its safety, efficacy, and clinical applicability require further investigation [105].

8. Conclusions

The interpretation of the vaginal microbiota should extend beyond static classifications. Recent evidence calls into question the direct association of CST III and, particularly, CST IV with dysbiosis, showing that these profiles are heterogeneous and transient, and that microbial composition alone does not necessarily define a disease state. The complexity of this topic increases further when considering the less-studied components of the vaginal microbiome. The mycobiome and virome appear to participate in ecological networks in which bacteria, fungi, viruses, and the host engage in interactions that can alter community stability and the mucosal immune response. The effects of bacteriophages on bacterial populations and the potential interactions between fungi and bacteria suggest that vaginal physiology cannot be explained solely by *Lactobacillus* abundance. These gaps therefore represent not only a limitation of the current literature but also an important frontier for understanding vaginal physiology and disease. In the context of BV and Tv coinfection, the transition from eubiosis to dysbiosis involves changes in the vaginal environment and interactions among microorganisms that promote adhesion, metabolic adaptation, immune evasion, and persistence. This involves a gradual reorganization of the microenvironment, particularly within commensal or opportunistic communities that establish mutually beneficial relationships when ecological balance is disrupted.

The relationship between BV and Tv warrants particular attention, especially given the parasite's ability to exploit an environment already structured by the bacterial biofilm, contributing to coinfection persistence and limiting the effectiveness of available treatments. However, this association extends beyond the mere coexistence of distinct microorganisms. These agents share similar mechanisms that drive the transition toward dysbiosis, creating a

synergistic interaction. The metabolism of other microorganisms, such as *P. bivia*, provides nutrients that support Tv energy metabolism, while *G. vaginalis* contributes to mucus degradation. This functional convergence enhances the persistence of coinfection and poses a significant challenge to women's reproductive health. Given this, significant gaps remain in our understanding of vaginal microbiota and the parameters used to define what constitutes a "normal" state. Given the importance of female reproductive health, there is an urgent need to expand research on vaginal physiology and reevaluate long-established concepts. Our current, overly simplistic understanding of CSTs, vaginal pH, and vaginal microbiota composition reflects, in part, a history of limited scientific investment in studying the female body. This situation is rooted in social and historical contexts that have hindered the development of research focused on women's health, thereby restricting the generation of knowledge about conditions fundamental to reproductive health.

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Abbreviations

The following abbreviations are used in this manuscript:

STIs	Sexually Transmitted Infections
WHO	World Health Organization
CST	Community State Types
Tv	*Trichomonas vaginalis*
BV	Bacterial Vaginosis
PRRs	Pattern Recognition Receptors
VMT	Vaginal Microbiota Transplantation
MTZ	Metronidazole
TNZ	Tinidazole
SEC	Secnidazole
CDC	Centers for Disease Control and Prevention
DNA	Deoxyribonucleic Acid
TLR9	Toll-Like Receptor 9
IFN-I	Type I Interferons
TVV	*Trichomonas vaginalis Virus*
TLR3	Toll-Like Receptor 3
FR1	Flavin Reductase 1
FRs	Flavin Reductases

H_2O_2	Hydrogen Peroxide
H_2O	Water
O_2	Molecular Oxygen
LA	Lactic Acid
ROS	Reactive Oxygen Species
OsmC	Osmotically Inducible Protein C
TrxR	Thioredoxin Reductase
TvFeSOD	Superoxide Dismutase
LDH	Lactate Dehydrogenase
DCs	Dendritic Cells
MPO	Myeloperoxidase
ADH	Arginine Dehydrogenase
NO	Nitric Oxide
HIV	*Human Immunodeficiency Virus*
HPV	*Human Papillomavirus*
Gal-9	Galectin-9
Tim-3	T-Cell Immunoglobulin and Mucin Domain-3
IL-17	Interleukin-17
PID	Pelvic Inflammatory Disease
ATP	Adenosine Triphosphate
MdaB	Modulator of Drug Activity B
AMR	Antimicrobial Resistance
FDA	U.S. Food and Drug Administration
mFeS	Metastable Iron Sulfides
VLY	Vaginolysin
NGS	Next-Generation Sequencing
LBPs	Live Biotherapeutic Products

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