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Harnessing Probiotics and Prebiotics for the Prophylaxis and Treatment of Bacterial Vaginosis

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Bacterial vaginosis (BV) is the prevalent condition of reproductive-age women in the general population. BV increases the risk of miscarriage, low birth weight, preterm birth, pelvic inflammation, sexually transmitted diseases, etc. Generally, antibiotics like metronidazole and clindamycin were used for the treatment of this recurrent infection. The use of antibiotics only gives temporal relief and may cause serious conditions of antibiotic resistance, and they kill a wide range of beneficial vaginal microbes. Hence alternative treatment methods like the use of probiotics and prebiotics for the prevention of bacterial vaginosis and other bacterial infections will be more effective. They help to optimize, maintain, and restore the vaginal acidic environment and eubiosis of the vaginal ecosystem. The current review focuses on the role of probiotics and prebiotics and the combined effect of both for the treatment and prophylaxis of bacterial vaginosis and other bacterial infections. Also, the review emphasizes the understanding of the role of vaginal microbiome in modulating the health of women and as a promising solution for recurrent vaginal infections.

Keywords: Bacterial vaginosis; Probiotics; Prebiotics; Vaginal microbial milieu; Vaginal Probiotics.

The human vaginal microbiome forms a close association with the host and serves as a critical determinant of vaginal health. This dynamic vaginal microflora has critical implications for preventing urogenital infections. The vaginal micro milieu is composed of Lactic acid bacteria (LAB) and the lactic acid produced by them maintains the low pH of the vagina, and the antimicrobial, anti-inflammatory products inhibit the growth of harmful bacteria. The glycogen-rich vaginal milieu of high estrogen states stimulates the proliferation of Lactobacilli in the vaginal microenvironment[1]. However, factors like antibiotic usage, hormonal changes, menses, pregnancy, immune status of the patient, chronic stress, sexual practices and vaginal douching can alter the vaginal microbial community[2]. Dysbiosis of this microenvironment lacking significant numbers of Lactobacillus spp. and elevated pH may lead to the proliferation of various anaerobic and facultative anaerobic pathogenic bacteria such as Gardnerella, Prevotella, Atopobium, Mobiluncus, Mycoplasma, Ureaplasma, Streptococcus, Dialister, Bacteroides etc. and cause bacterial vaginosis (BV) and other urinary tract infections[2].

The use of antibiotics against these bacterial infections only gives temporal relief and may cause serious conditions of antibiotic resistance and kill a wide range of beneficial vaginal microbes[3]. Hence the use of probiotics and

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prebiotics could be a potent alternative remedy for bacterial vaginosis and other bacterial infections and helps to optimize, maintain, and restore the vaginal acidic environment and vaginal ecosystem.

Many single and multi-probiotic strains, such as *Lacticaseibacillus rhamnosus*, *Lact. acidophilus*, *Limosilactobacillus reuteri*, *Lact. gasseri*, *Lactiplantibacillus plantarum*, *Limosilactobacillus fermentum*, *Levilactobacillus brevis*, *Lact. delbrueckii*, *Ligilactobacillus salivarius*, *B. longum*, *Bifdobacterium bifdum*, etc are well-studied and characterized and commercially available for the treatment of bacterial vaginosis and vaginitis[3]. Prebiotics such as polysaccharides, oligosaccharides, and carbohydrates can also optimize, maintain, and restore the vaginal acidic environment and vaginal ecosystem[4]. However, a combination of probiotics and prebiotics (biotherapeutics) to populate healthy vaginal microbiota to prevent or treat infection will be perhaps more realistic.

Worldwide studies on probiotics, prebiotics, and commercial synbiotic products for vaginal health are in a nascent state. There must be a need for a good understanding and validation of vaginal microbes for the prophylaxis and treatment of urinary tract infections. Hence, interventions in prebiotics and probiotics for urinary tract infections and bacterial vaginosis are inevitable, as many women are suffering from this recurrent and chronic problem.

The current review focuses on the state of the art in preventing and treating BV using probiotics and prebiotics by systematically reviewing studies from 2010 to 2024 showing impact results.

Human Vaginal Microflora

The women's vaginal microflora is diverse, and it changes continuously throughout a woman's lifetime. More than 200 bacterial species make up the niche, and they inhabit the vaginal surfaces and cavities and maintain eubiosis in the vaginal ecosystem. Various factors like genetic, immunological as well as race and social habitats influence the composition of this vaginal microbiome. Hence a thorough understanding of the vaginal microbiome, age-to-age transition of the microbiome and host-pathogen interaction is necessary for accurate diagnosis and better prophylaxis of bacterial vaginosis and to boost women's health.

The human vaginal microbiome is a determinant of reproductive health and is fully developed in puberty[1]. Estrogen/progesterone exposure at puberty, the vaginal microflora significantly changes to *Lactobacillus spp*., *Atopobium*, and *Streptococcus spp.* High estrogen state in reproductive age stimulates the vaginal epithelial cell's maturation and proliferation and the accumulation of glycogen. The human á-amylase catabolizes the glycogen to maltose, dextrins and maltotriose which were further metabolized to lactic acid by Lactobacillus species. As a result, vaginal pH reduces to 3.5-4.5, which is ideal for Lactobacillus and other bacterial species to adhere and colonize in the vagina[5].

In healthy reproductive age women, the vagina was colonized mainly by various lactic acid bacteria (LAB) such as *L. gasseri*, *L. crispatus*, *L. iners*, and *L. jensenii*[1]. As less glycogen and estrogen levels are seen in prepubertal, menopause[6] and menstrual women[7], anaerobes and pathogenic bacteria were the dominant ones. During menstruation, there is a rapid decrease in *Lactobacillus crispatus*, accompanied by an increase in *Lactobacillus iners*, *Gardnerella vaginalis, Atopobium vaginae*, and *Prevotella bivia*. However, in normal pregnancy, the vaginal microbiota remains more stable and dominated by Lactobacilli due to elevated glycogen and estrogen levels[8].

Sexual intercourse, douching, contraceptive devices, smoking, stress, the use of antibiotics[9] and probiotics[10] also significantly influence the composition of the vaginal microbiota. BV is more prevalent among immunocompromised women, who generally exhibit reduced levels of immune mediators in their vaginal fluid[11].

Host characteristics might be more important in determining the vaginal microbiome among racial groups than behavioural and cultural differences. The host genetic variables, including the immune system, the amount makeup of vaginal discharge and ligands on the epithelial cells may be the cause of the variances in vaginal microbiomes among women of different races[1,11]. A study in 2022 discussed about the relationship between the vaginal microbiome, race and spontaneous

preterm birth (SPTB)[11]. The results showed that race was significantly associated with vaginal microbiome composition, as black and Hispanic women having a higher prevalence of bacterial vaginosis-associated bacteria. Furthermore, vaginal microbiome composition was associated considerably with SPTB, with certain bacteria being more prevalent in women who experienced SPTB[11].

There are five distinct community state types (CSTs) associated with the vaginal microbiome. *L. gasseri*, *L. crispatus*, *L. iners*, and *L. jensenii* are the predominant species in CSTs I, II, III, and V, respectively. In contrast, CST IV is characterized by a high diversity of obligate anaerobic bacteria. CSTs I, II, III, and V are observed in 89.7% of white women and 80.2% of Asian women within these categories. However, the prevalence of these CSTs is lower in black and Hispanic women, at 61.9% and 59.6%, respectively. When CST IV was dominant, it was clear that ethnic groups had changed[12].

Vaginal microbes could also serve as potential biomarkers for cervical cancer[13]. The composition of vaginal microbes varies across different cervical lesions, suggesting that these microbial profiles have diagnostic potential for distinguishing between healthy women, those with high-risk HPV infections, and those with cervical lesions [13]. Hence vaginal microbiome study offers new avenues for treating HPV infections and related cervical abnormalities.

Mechanism of action of Vaginal Lactic acid bacteria

The Lactic acid bacteria maintain vaginal eubiosis by several mechanisms of action. Lactobacilli contain various surface proteins like fibronectin, mucin and collagen-binding proteins, which aid in the attachment of lactobacilli to pathogens. They also produce various active defence metabolites such as lactic acid, H_2O_2, and biosurfactants[14]. The lactic acid produced by them maintains an acidic pH of the vagina and inhibits the growth of pathogenic microorganisms[15]. H2O2 inhibits the growth of most catalase-negative bacteria, including anaerobes, lowering the risk of BV, preterm delivery, and human immunodeficiency virus (HIV) infection[16]. The other potent metabolite, bacteriocin, were effective against a wide range of bacteria and some fungi[17]. The biosurfactant produced by the lactobacilli prevents biofilm formation and pathogen adherence to the host cell[18].

Lactobacilli strongly adheres to vaginal epithelium due to the presence of adhesins and result in the exclusion of harmful pathogens by competitive adhesion[19]. Lactobacilli can elicits innate immune response through cytokine production and by the activation of Toll-like receptors (TLR). They enhance the generation of IL-10, which helps to prevent both acute and systemic inflammation[14].

Vaginal lactobacilli also play a crucial role in maintaining the integrity of epithelial cell tight junctions, particularly in mucosal surfaces in the genital tract, by re-epithelizing vaginal epithelial cells by enhancing the production of vascular endothelial growth factor[14].

Many recent studies show that lactobacilli have potent antimicrobial activity against BV pathogens [16,19]. The defence metabolites produced by them alter the morphology of BV-causing pathogens (*A. vaginae, G. vaginalis* and *P. bivia*), making the cells shrink or burst[19]. The lactic acid and acetic acid synthesized by the lactobacilli can disrupt the Na+/K+-ATPase activity, leading to abnormal ATP metabolism and inhibiting pathogen growth and reproduction[15]. Cell-free supernatant from lactobacilli could also reduce the biofilm formation of the pathogen, and the most significant inhibitory effect was observed when introduced early in the biofilm formation process of *G. vaginalis*[19]. Overall, Lactobacilli can inhibit the genes associated with biofilm formation, adhesion, virulence factors and antimicrobial resistance of the pathogen.

Bacterial vaginosis

The Indigenous vaginal microbiome is essential for preventing the colonization of anaerobic and microaerophilic pathogens in the vagina. Dysbiosis of the vaginal microecosystem, which occurs as a result of depletion of Lactobacilli or unbalanced vaginal microbiome, results in the growth of pathogenic microorganisms and leads to bacterial vaginosis (BV)[20]. BV is a commonly occurring condition affecting the lower genital tract, and it increases the risk of miscarriage, low birth weight, preterm birth, sexually transmitted diseases, pelvic inflammation, etc. In BV, it is noted that there is an exponential rise in obligate

and facultative anaerobic microbes, such as *Gardnerella*, *Mobiluncus*, *Atopobium*, *Prevotella, Sneathia*, *Bifidobacterium*, *Clostridials* etc[19]. In addition to these, in 2020, scientists isolated the novel *Megasphaera vaginalis* sp. and *Anaerococcus vaginimassiliensis* sp from the vagina of a French woman with bacterial vaginosis[21]. The overgrowth of pathogenic organisms leads to an increased vaginal pH, vaginal discharge, a fishy odour, and the vaginal flora covered by epithelial cells[22].

Gardnerella vaginalis is the most commonly isolated microorganism from vaginal lesions in women with BV and is regarded as the primary etiological agent of the condition. It has been implicated as a cause of non-specific vaginosis and a sexually transmissible organism. It is a slow-growing, non-motile, gram-variable bacillus first isolated by Leopold in 1953. In 1955 Gardner and Dukes found it to be associated with bacterial vaginosis and initially named it *Haemophilus vaginalis*. It was later reclassified into the genus *Gardnerella* and renamed *Gardnerella vaginalis*[23]. *G.vaginalis* may also reported from healthy or asymptomatic women, indicating that its presence in the vagina does not necessarily lead to BV. However, *Gardnerella vaginalis* can considered as the primary causative agent, as they adhere to vaginal epithelial cells by biofilm formation, and initiate the pathogenesis of BV. Further to the polymicrobial biofilm, BV-associated anaerobes such as *Prevotella bivia*, *Mobiluncus mulieris*, *Fusobacterium nucleatum, Atopobium vaginae*, and *Peptoniphilus sp*. adhered [20]. The biofilm provides a physical barrier against antimicrobial agents and the human immune response, enhancing tolerance to adverse conditions and increasing the severity of the infection.

Diagnosis of BV can be done using several tests, such as clinical indicators, point-of-care tests and molecular assays[24]. Molecular techniques for the diagnosis of Bacterial vaginosis (BV) utilize molecular markers for BV diagnosis, offering distinct advantages over traditional methods such as OSOM BV Blue, Fem Exam and Nugent score. These molecular approaches are characterized by their objectivity, applicability to self-collected vaginal swabs, and their ability to quantify and detect fastidious bacteria[25].

For the treatment or management of BV, antibiotics like clindamycin and metronidazole are generally used for patients present with symptoms. In addition to these, Tinidazole, Secnidazole and Rifaximin are also effective in the treatment of BV[26]. Both oral and vaginal roots are recommended for the treatment of bacterial vaginosis. Recurrence may occur in 80% of the cases, and if patients present with recurrent infection, a second dose of antibiotics are prescribed for the treatment. The biofilm formation of pathogens can affect the effectiveness of the treatment by preventing the penetration of antimicrobial compounds[27]. Also, the use of antibiotics can alter the normal lactobacillus dominant vaginal flora and change the vaginal acidic pH. The emergence of antibiotic resistance may also be a major concern for the treatment[26].

Here emerges the significance of formulating an alternative therapeutic approach that can eliminate pathogens and effectively penetrate the polymicrobial biofilm without disrupting the vaginal commensals. Antimicrobial peptides (such as retrocyclin and subtilisin), antiseptics (including dequalinium chloride, povidone iodide, hydrogen peroxide, chlorhexidine, and octenidine), surfactants, natural compounds, acidifying agents, probiotics, and prebiotics (Table 1) are currently under extensive research and utilization for the management of BV[28].

Probiotics for bacterial vaginosis

Probiotics are active microorganisms that colonize the human intestinal and reproductive tract and provide health benefits to the host. It has been widely accepted in the treatment and prophylaxis of various diseases related to the digestive system and reproductive problems in women, including vaginosis, polycystic ovary syndrome, human papillomavirus (HPV) infection, Genitourinary Syndrome of Menopause (GSM) and preterm delivery. There is growing evidence that probiotics are more effective than other treatment aids for the management and prevention of bacterial vaginosis[29]. *L. crispatus*, *L. gasseri*, *L. jensenii*, *L. helveticus*, *L. delbrueckii*, *L. johnsonii*, *L. acidophilus*, *L. plantarum*, *L. fermentum*, *L. paracasei*, *L. brevis, L. reuteri*, *L. salivarius* and *L. rhamnosus* have been studied and used to treat vaginal infections[30]. The most often used species are *L. crispatus* and *L. rhamnosus*. *L. crispatus* is one of the predominant Lactobacilli found in the healthy vaginal flora and is associated with maintaining vaginal health and promoting a

Table 1. Some of the recently studied and commercially available probiotic and prebiotic treatment aid for bacterial vaginosis

Probiotic and prebiotic treatment aid for bacterial vaginosis	Composed off	Probiotic/prebiotic components used	Root of administration
Soy beverages containing encapsulated vaginal probiotics[40]	Soy beverage	*Lactobacillus acidophilus*	Oral
Gelatin oil probiotic suppository[41]	Gelatin oil	Spores of *Bacillus coagulans*	Vaginal
Probiotic nanozyme hydrogel[42]	rGO@FeS2, Pt3Fe, Fe3O4, pyrite FeS2 nanozymes and hyaluronic acid (HA) hydrogel	*Lactobacillus capsules* (S20030005)	Vaginal
Bioprints with *Lactobacillus crispatus*[43]	Bioink composed of alginate, and gelatin	*Lactobacillus crispatus*	Vaginal
Electrospun fibers loaded with *Lactobacillus crispatus*[44]	poly (lactic-co-glycolic acid) (PLGA) and Polyethylene oxide (PEO)	*Lactobacillus crispatus*	Vaginal
Core-shell nano gel (CSNG)[57]	Prebiotic inulin and antimicrobial peptide Cath 30	Inulin	Vaginal
Bovine lactoferrin, *L. rhamnosus HN001* and *L. acidophilus* La-14, (Respecta(®) complex)[61]	Probiotics and prebiotics	Bovine lactoferrin, *L. rhamnosus HN001,* and *L. acidophilus* La-14	Oral
SYNBIO(®)[45]	Probiotic blend	*L. rhamnosus IMC 501®* and *L. paracasei* IMC 502®	Oral
MED-01 Probiotics[47]	Probiotic blend	*Limosilactobacillus fermentum MG901, Ligilactobacillus salivarius* MG242, *Lacticaseibacillus paracasei* MG4272, *Lactiplantibacillus plantarum* MG989, and *Lacticaseibacillus rhamnosus* MG4288	Oral
Immunovag®[48]	Hyaluronic acid and polycarbophil	*Propionibacterium acnes*	Vaginal
Lactogyn vaginal capsule[46]	Probiotic blend	*Lactobacillus reuteri RC-14* and *Lactobacillus rhamnosus* GR-1	Oral
SymbioVag®[62]	Synbiotic	*Lactobacillus acidophilus*, *Lactobacillus gasseri* and inulin	Vaginal
VagiBIOM Lactobacillus suppository[63]	Synbiotic vaginal suppository	*L. crispatus Bi16, B. coagulans Bi34*, *L. acidophilus* Bi14, *L. gasseri* Bi19, prebiotic complex, hyaluronic acid, lactic acid, coconut oil fatty acids, and silica gel.	Vaginal

balanced microbial environment[31]. *L.rhamnosus*, on the other hand, is less common in the natural vaginal microbiome compared to *L.crispatus*. However, *L.rhamnosus* is extensively studied and widely used in probiotic formulations due to its potential health benefits. It has been investigated for its role in preventing and treating various vaginal conditions, including bacterial vaginosis and vaginal yeast infection[30,2].

L.iners is a common vaginal inhabitant; however, there are currently no commercial probiotic products specifically containing *L. iners* available in the market[32]. In a recent study, a novel lanthipeptide, inecin L (bacteriocin) with posttranslational modifications from *L.iners*, was studied and found to have activity against *G. vaginalis* and *S. agalactiae* at nanomolar concentrations [33]. The use of *L. iners* as a probiotic for vaginal health is a new area of research, and more studies are needed to fully understand its effectiveness, safety and optimal dosing and delivery methods. In addition to these, *L. acidophilus*[34], *L.fermentum* and *L. plantaram are* often found in probiotic formulations targeting women's health[35].

The application of lactobacilli in treating vaginal infections has been subject of several *in vitro* and *in vivo* investigations. *In vitro* studies demonstrate the antimicrobial and immunoregulatory role of Lactobacilli in genitourinary pathogens[36]. *In vivo,* studies show promising results for confirming the probiotic potential of Lactobacilli in the treatment of bacterial vaginosis. Randomized controlled clinical trials found that women receiving probiotics had a significant increase in Lactobacillus abundance and reduction in recurrent bacterial vaginosis when compared to the placebo group[37].

Both oral and vaginal route administration of probiotics has proved its effectiveness. It is reported that the use of vaginal *Lactobacillus paracasei* LPC-S01 (DSM 26760) is suitable for oral intake and can be reisolated from the vagina[38]. A randomized placebo-controlled pilot study showed that orally administered multispecies probiotic formulations containing *L. reuteri* (F_1), *L. plantarum*, *L. acidophilus*, *L. rhamnosus* and *Bifidobacterium animalis* have antimicrobial properties and prevent urogenital infections[39].

Researchers also explore the potential of using fermented soy beverages as a carrier for

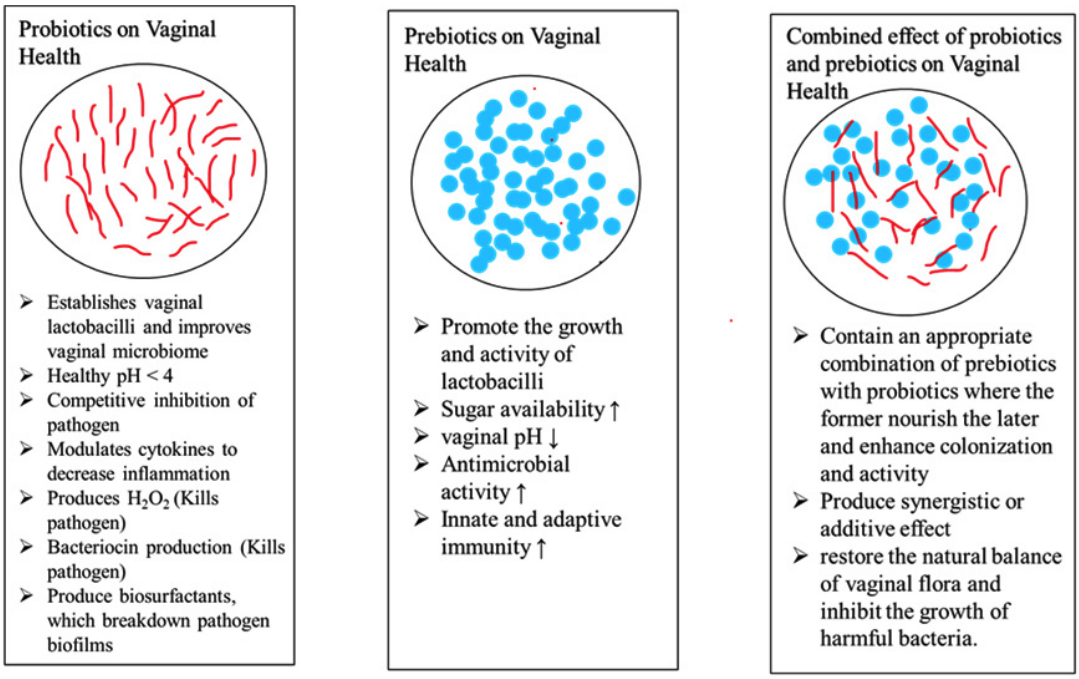

Fig. 1. Effect of probiotics, prebiotics and the combined effect on vaginal health

delivering probiotics to the vaginal tract[40]. The researchers formulated two different fermented soy beverages, one containing encapsulated *L. acidophilus* and another containing non-encapsulated *L. crispatus*. The study concluded that fermented soy beverages could serve as an alternative vehicle for delivering probiotics to the vaginal tract, particularly when the probiotics are encapsulated to improve their survival and stability. This finding has significant implications for the development of functional foods for women's health, especially for those with vaginal dysbiosis or infections[40].

Intravaginal application of drugs for the treatment of BV was as effective as the oral administration. Recent research developed a gelatin oil probiotic suppository containing probiotic spores of *B.coagulans* for combating vaginal fungal infections[41]. The suppositories exhibited efficacy in inhibiting pathogenic bacteria, maintained the stability of the probiotics, and showed good safety profiles[41]. Probiotic nanozyme hydrogel is also found to be effective as a therapeutic intervention to regulate the vaginal microenvironment and counteract the overgrowth of *C. albicans*[42]. The hydrogel is designed to contain probiotic bacteria, which can produce beneficial metabolites and modulate the local immune response. Additionally, the hydrogel incorporated enzymes, which are nanomaterials with intrinsic enzymatic activity that can promote the degradation of biofilms formed by *C. albicans*[42].

Recent development in bioprinting technology is employed in the vaginal delivery of probiotics. Bioprinting technology was used to fabricate three-dimensional structures containing *L. crispatus*[43]. In 2023 a study involved creating a bio-ink composed of *L.crispatus*, alginate, and gelatin, which was then used to print 3D structures using a bioprinter[43]. The printed structures were then characterized to evaluate their viability, morphology, and mechanical properties. The researchers found that the bioprinted structures had high cell viability, maintained their structural

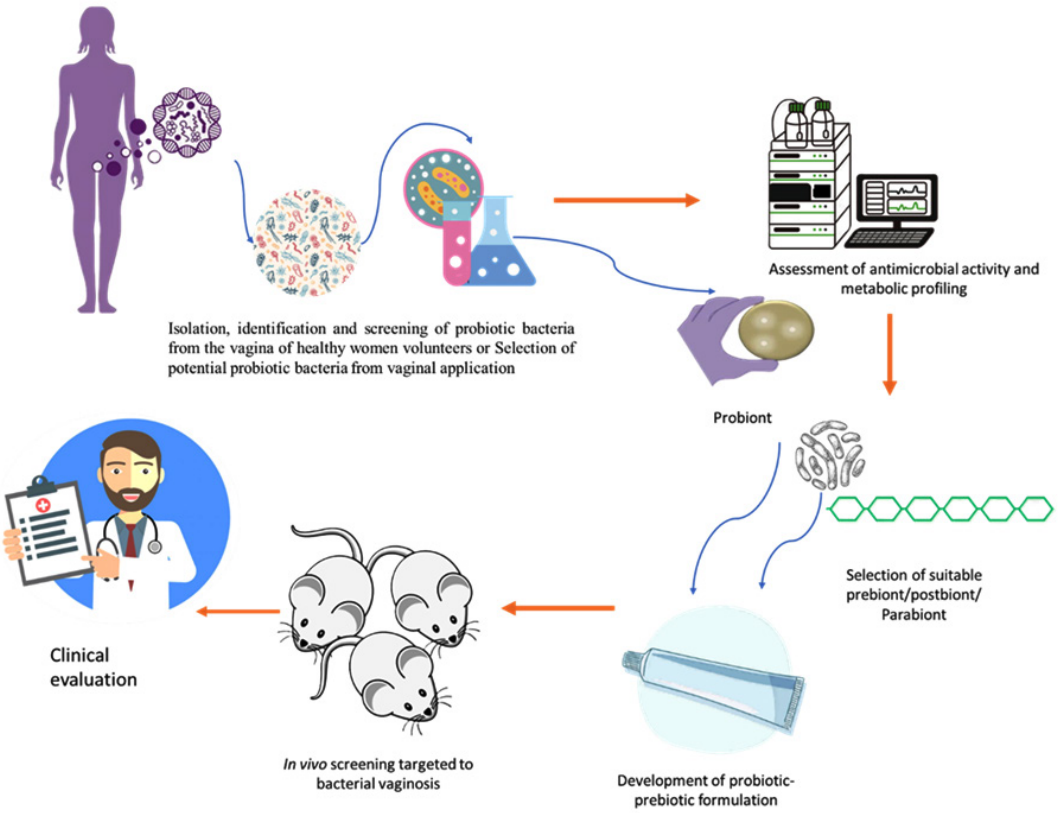

Fig. 2. Steps involved in the development of symbiotic formulation for bacterial vaginosis

integrity, and could be manipulated without damage. They also observed that the structures released *L. crispatus* over a period of several days, indicating the potential for sustained release of probiotics. Hence it is feasible to use bioprinting technology to create 3D structures containing live bacterial cells for potential use in vaginal probiotic applications. This approach may have potential applications for the treatment of conditions such as bacterial vaginosis and urinary tract infections[43].

Electrospun fibres loaded with *L. crispatus* for the treatment of bacterial vaginosis were developed by researchers[44] using a blend of poly lactic-co-glycolic acid (PLGA) and polyethylene oxide (PEO). The results showed that the *L. crispatus* loaded fibres were able to maintain high levels of bacterial viability and metabolic activity for up to 14 days. Moreover, the fibres were able to significantly reduce the number of *G. vaginalis* cells *in vitro*, indicating their potential as a treatment for bacterial vaginosis. Overall, the study suggests that electrospun fibres loaded with *L. crispatus* have the potential to be an effective treatment for bacterial vaginosis[44].

There are many commercial probiotic product blends like SYNBIO(®) gin[45], Lactogyn[46], Med-01[47] and Immunovag®[48] available for the treatment and prophylaxis of bacterial vaginosis. These formulations of different bacterial strains provide a broader range of potential benefits for BV treatment. Post-biotics are also found to be effective in preventing vaginal infections[49].

Even though probiotics are incorporated into solid dosage forms or vaginal rings, the effectiveness of probiotics is contingent on the number of viable cells. Ensuring an efficient and sustained release of probiotics from the matrix while maintaining cell viability throughout the storage period can be challenging. The potency and safety of probiotic organisms for reproductive health are still controversial. Studies revealed the non-effectiveness of oral probiotics *L. rhamnosus* GR-1 and *L. reuteri* RC-14 as an adjunctive treatment for bacterial vaginosis[50]. Also, a recent study demonstrated that the vaginal probiotic *L. crispatus* significantly affect sperm activity and could reduce pregnancies through its adhesive property, which might contribute to some unexplained infertility[51].

Hence, further research is required to validate the efficacy and safety of this therapeutic strategy using an appropriate sample size and experimental design.

Prebiotics for bacterial vaginosis

Prebiotics are substances that foster the selective growth of beneficial microorganisms. prebiotic such as polysaccharides, oligosaccharides, and carbohydrates optimize, maintain, and restore the vaginal acidic environment and vaginal ecosystem [6]. Prebiotics such as lactitol, lactulose, raffinose, and oligofructose are found to specifically stimulate vaginal lactobacilli and prevent the growth of BV-associated organisms[52]. By promoting the growth and activity of lactobacilli, prebiotics helps to restore the natural protective barrier against pathogens, lower vaginal pH, and produce antimicrobial substances that inhibit the growth of harmful pathogenic microorganisms.

Vaginal administration of á-galacto oligosaccharide prebiotics found to be, act as a nutritional support for lactobacilli, along with xylitol and hyaluronic acid, which effectively controls the symptoms and pathogenic microbial load, unlike the antibiotic and/or antifungal treatment[53]. This will restore the healthy vaginal flora of lactobacilli and thus prevents the opportunistic development of pathogens[53]. Galacto-oligosaccharides (GOS) and Fructo-oligosaccharides (FOS) have also been shown to enhance the growth and activity of lactobacilli in the vagina and to prevent vaginal infections[54].

In addition to these, administration of prebiotic lactoferrin, an iron-binding glycoprotein with bactericidal and bacteriostatic properties has been found to improve the vaginal microbiome composition in women with BV[55]. Vaginal suppositories and oral prebiotic lactoferrin were found to be effective in patients who were refractory to conventional therapeutic for vaginosis and had a history of late miscarriages and very early preterm delivery due to refractory vaginosis and chorioamnionitis [55]. A study showed that *L. helveticus*, not previously detected in the vaginal milieu, was the most dominant species found during and after the vaginal lactoferrin treatment, and proposed lactoferrin as an alternative treatment aid for BV[56].

As a new strategy for the treatment of vaginitis, researchers designed a core-shell nano gel (CSNG) which encapsulates antimicrobial

peptide Cath 30 and prebiotic inulin [57]. The nano gel allows for the sequential release of drugs by the enzyme gelatinase produced by aerobic vaginal pathogens and selectively kills the pathogenic bacteria, and helps in the proliferation of beneficial bacteria in the vagina. Adjuvant treatment of prebiotic vaginal gel with oral metronidazole is also found to be effective in the prevention of BV[58].

The combined effect of probiotics and prebiotics for bacterial vaginosis

Probiotics in combination with prebiotics (Synbiotics) are found to be effective in the prevention of recurrent BV with higher clinical and microbiological cure rates. When used together, probiotics and prebiotic formulations are expected to have a synergistic effect, where probiotics can restore the natural balance of vaginal flora and inhibit the growth of harmful bacteria, prebiotics provide nourishment for these bacteria, enhancing their colonization and activity (Figure 1). Thus, combination can help restore the natural balance of vaginal flora and inhibit the growth of harmful bacteria.

Recent development in formulation strategies contributes to enhancing the efficacy of the release of active substances and improving drug retention and adhesion to the vaginal epithelium. A case study showed that oral probiotics containing Lactobacilli species and prebiotic Lactoferrin 100mg could prevent recurrent preterm delivery by the prevention of intrauterine infection and inhibiting the production of inflammatory cytokines[59]. Studies also showed the synergistic effect of Mannan oligosaccharides (MOS) extracts with Lactobacillus spp. pool. for the vaginal prevention of *C. albicans*. MOS extracts along with *L. crispatus*, *L. gasseri*, and *L. jensenii* have been found to have potential role in the prevention of vaginal candidiasis than Lactobacilli alone in preventing *C.albicans* infections[60].

Synbiotics are also studied as an adjuvant to drug therapy. The oral synbiotic mixture, including *L. rhamnosus* HN001 and *L.acidophilus* GLA-14 (Respecta® complex) with bovine lactoferrin, has been shown to be an effective adjunct to metronidazole in preventing bacterial vaginosis. Research indicates that administering multiple courses of oral probiotics in combination with lactoferrin is effective in preventing the recurrence of BV, leading to higher clinical and microbiological cure rates[61].The synbiotic vaginal suppository SymbioVag®, which contains *L. acidophilus* and *L. gasseri* along with the prebiotic inulin, has been shown to reduce vaginal pH in 80% of test patients and decrease BV-associated pathogens by 60%[62].

VagiBIOM a lactobacillus suppository containing Lactobacillus spp. comprising 1–2% *L. acidophilus* Bi14, *L. crispatus* Bi16, *B. coagulans* Bi34, *L. gasseri* Bi19, prebiotic complex (0.05–2%), coconut oil fatty acids, hyaluronic acid (0.1–0.3%), lactic acid (0.01–0.025) and silica gel (0.1–0.3%), has been reported to improve vaginal health of peri and premenopausal women with bacterial vaginosis. A clinical study showed that VagiBIOM can significantly relieve vaginosis by lowering vaginal pH and Nugent scores and can improve the overall vaginal health in BV patients[63]. The microbiome-based suppository can serve as a natural preventive aid for mild vaginal infections and help to reseed *Lactobacillus* following antibiotic treatment for vaginosis. Its use could be tailored across different age groups by assessing its effectiveness in restoring the diversity of the beneficial commensal vaginal microbiome[63].

Studies also proved that antibiotics like clindamycin along with synbiotics were more effective for bacterial vaginosis than antibiotics without synbiotics[64]. It's important to note that while probiotics and prebiotics show promise in the treatment of BV, further research is still needed to establish their effectiveness and to identify the most suitable strains and formulations for the treatment and prevention. Steps involved in the development of a synbiotic formulation for bacterial vaginosis are depicted in Figure 2. There were considerable variations among prebiotic and probiotic interventions, including differences in dosage, administration routes, duration of therapy, strains or species of probiotics, and types of prebiotics, which complicate comparisons across different studies. Furthermore, commercially available products may also exhibit variability in their ingredients. And still, the combination of probiotics and prebiotics (biotherapeutics) to populate healthy vaginal microbiota to prevent or treat infection will be perhaps more realistic, and more research is inevitable in this field.

CONCLUSION

BV has a significant impact on women's self-esteem because it is associated with extreme discomfort and implies alterations in daily activities. Conventional treatment methods for BV are based on the use of antibiotics, especially Metronidazole and Clindamycin and are associated with therapeutic failure and recurrence. Recent studies on probiotics and prebiotics showed that they are effective in treating recurrent bacterial vaginosis and other urogenital infections. Development in the formulation strategies also contributes to improving the efficacy of the release of active substances and improving drug retention and adhesion to the vaginal epithelium. Therefore, it is emergent to study and develop probiotic-based biotherapeutic formulations for the prophylaxis and treatment of bacterial vaginosis and the betterment of women's lives.

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Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Authors Contribution

Nayana A R: Conceptualization, Methodology, Data collection, Writing-Original draft; Keerthi T R: Visualization, Supervision, Review and Editing, Funding Acquisition

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