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Exploratory Pilot Study of a Spray-Type Probiotic Delivery System for Vaginal Health in Women at Risk for Bacterial Vaginosis

Sunwha Park
Ewha Womans University Medical Center
Juan Kim
Yonsei University
Young-Ah You
Ewha Womans University Medical Center
Young Min Hur
Ewha Womans University Medical Center
Yongwun Jung
PH4 co.ltd.
Gain Lee
Ewha Womans University Medical Center
Dahye Son
Ewha Womans University Medical Center
Hae Ni Lee
Ewha Womans University Medical Center
Min Jin Ha
Yonsei University
Young Ju Kim
kkyj@ewha.ac.kr

Ewha Womans University Medical Center

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Abstract

Background

Bacterial vaginosis (BV) is a common gynecological condition affecting women of reproductive age, characterized by disruption of vaginal microbiota. Current probiotic interventions, including oral supplements and vaginal suppositories, face limitations in vaginal colonization and user compliance. This study evaluated the efficacy and safety of a novel spray-type probiotic powder (BalanceTalk Probiotic Feminine Hygiene Powder) containing multiple Lactobacillus strains for BV prevention and treatment.

Methods

This single-arm exploratory clinical study was conducted at Ewha Womans University Hospital from December 2023 to June 2025. Premenopausal women aged 19–55 years were enrolled and instructed to apply the spray-type probiotic powder (containing *L. plantarum, L. rhamnosus, L. paracasei, and L. crispatus*) to underwear or panty liners in contact with the vulvovaginal area once daily for 4 weeks. Primary outcomes included vaginal symptom improvement and Nugent score changes. Secondary outcomes assessed vaginal microbiome composition using 16S rRNA gene sequencing. Statistical analyses included Wilcoxon signed-rank test, McNemar's test, and mixed-effects logistic regression.

Results

Of 60 enrolled participants, 45 completed the study (75% completion rate). Overall improvement in vaginal symptoms approached statistical significance ($p = 0.051$), particularly in high-risk group (Nugent score ≥ 4; $p = 0.020$). In the high-risk group (n = 8), mean Nugent scores decreased significantly from 6.13 to 4.63 ($p < 0.001$), with 50% achieving score improvement. Lactobacillus abundance demonstrated a significant negative correlation with Nugent score (OR = 0.077, 95% CI: 0.009–0.634). Alpha diversity indices correlated significantly with Nugent score ($p < 0.05$). Beta diversity and overall Lactobacillus abundance showed no significant changes. Three mild adverse events were reported with no serious safety concerns observed.

Conclusions

The spray-type probiotic was associated with improvement in vaginal symptoms, and reduced Nugent scores, particularly in high-risk women, with an excellent safety profile. The significant correlation between Lactobacillus abundance and vaginal health suggests a potential association between Lactobacillus abundance and vaginal health of this intervention. Larger randomized controlled trials are warranted to confirm these findings and establish clinical utility.

1. BACKGROUND

Bacterial vaginosis (BV) represents the most prevalent vaginal infection among women of reproductive age, affecting approximately 29% of women globally (1). BV is characterized by a shift in vaginal microbiota from Lactobacillus-dominant flora to polymicrobial communities dominated by anaerobic bacteria (2). This dysbiosis results in vaginal discharge, odor, discomfort, and increased susceptibility to sexually transmitted infections, pelvic inflammatory disease, and adverse pregnancy outcomes (3). The Nugent scoring system, based on Gram stain evaluation of vaginal smears, remains the gold standard for BV diagnosis, with scores of 7–10 indicating BV, 4–6 indicating intermediate flora, and 0–3 indicating normal flora (4).

Current treatment strategies for BV primarily rely on antibiotic therapy, particularly metronidazole and clindamycin. However, antibiotic treatment is associated with high recurrence rates, up to 50–70% within 12 months (5, 6). Antibiotic use can disrupt beneficial vaginal microbiota and contribute to increasing antimicrobial resistance, and these limitations have prompted investigation of alternative therapeutic approaches, particularly probiotic interventions aimed at restoring healthy Lactobacillus-dominant vaginal microbiota (7).

Lactobacillus species, particularly *L. crispatus, L. jensenii, L. gasseri, and L. iners*, play crucial roles in maintaining vaginal health through multiple mechanisms: production of lactic acid, which maintains an acidic vaginal pH (3.8–4.5), generation of hydrogen peroxide and bacteriocins with antimicrobial properties (8, 9). Based on these mechanisms, Lactobacillus-based probiotics have been widely investigated for the prevention and treatment of bacterial vaginosis, emerging as a promising therapeutic strategy.

Existing probiotic delivery methods include oral capsules and vaginal suppositories. Oral probiotics face significant challenges in vaginal colonization, with studies demonstrating low survival rates during gastrointestinal transit and limited migration to the vaginal environment (10, 11). Vaginal suppositories, while achieving direct delivery, present practical limitations including inconvenience, interference with daily activities, and reduced user compliance (12). These limitations emphasize the need for alternative delivery methods that improve convenience and user compliance, which remain underexplored.

The spray-type probiotic delivery system represents a novel approach designed to overcome these limitations. By applying probiotic powder directly to underwear or panty liners, this method enables continuous, non-invasive delivery of viable Lactobacillus strains to the vulvovaginal area throughout daily activities. The powder formulation protects bacterial viability while allowing gradual release and potential colonization. This approach may enhance user compliance through improved convenience and discretion compared to traditional delivery methods.

Despite the theoretical advantages of spray-type probiotic delivery, clinical evidence supporting its efficacy remains limited. No previous studies have systematically evaluated the impact of spray-type probiotic application on vaginal symptoms, Nugent scores, and vaginal microbiome composition in

women at risk for BV. Understanding the clinical and microbiological effects of this novel delivery system is essential for determining its potential role in BV prevention and treatment strategies.

Study Objectives

This exploratory clinical study aimed to:

1. To evaluate the exploratory clinical and microbiome-associated outcomes of a spray-type probiotic powder (BalanceTalk Probiotic Feminine Hygiene Powder) in improving vaginal symptoms and reducing Nugent scores in pre-menopausal women
2. To Assess changes in vaginal microbiome composition, including Lactobacillus abundance and microbial diversity, following 4-week intervention
3. To examine the correlation between Lactobacillus abundance and Nugent score
4. To evaluate the safety profile and tolerability of the spray-type probiotic delivery system

We hypothesized that daily application of spray-type probiotic powder would improve vaginal symptoms, reduce Nugent scores in women with intermediate or abnormal vaginal flora, increase Lactobacillus abundance, and demonstrate acceptable safety and tolerability.

2. METHODS

2.1 Study Design and Setting

This was a single-arm, open-label, exploratory clinical study conducted at the Department of Obstetrics and Gynecology, Ewha Womans University Hospital, Seoul, Republic of Korea. The study was conducted from December 1, 2023, to June 30, 2025. The study followed a pre-post intervention design without a control group, with participants serving as their own controls. All study procedures were conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The study protocol, informed consent form, and all study materials were reviewed and approved by the Institutional Review Board (IRB) of Ewha Womans University Hospital prior to study initiation (IRB No. 2023-12-037). All participants provided written informed consent after receiving comprehensive verbal and written information about study objectives, procedures, potential risks and benefits, confidentiality measures, and the right to withdraw at any time without penalty.

2.2 Participants

Eligible participants were pre-menopausal women aged 19–55 years who provided written informed consent to participate in the study. Participants were required to be willing and able to comply with study procedures, including daily product application and scheduled follow-up visits. Women were excluded if they met any of the following criteria: age < 19 or > 55 years; post-menopausal status; current pregnancy or potential pregnancy; immunocompromised status; cardiac disease (including valvular abnormalities or history of endocarditis); substance abuse; antibiotic use within 2 weeks prior to enrollment; or any other condition deemed by investigators to preclude safe study participation.

2.3 Intervention

The investigational product was BalanceTalk Probiotic Feminine Hygiene Powder, a spray-type powder formulation containing four Lactobacillus strains: *L. plantarum, L. rhamnosus, L. paracasei, and L. crispatus*. Participants received detailed verbal and written instructions on product use. The standardized application protocol required participants to: (1) shake the spray container 3–4 times before each use; (2) pump the dispenser 5–7 times to apply powder to the inner surface of underwear or panty liner in the area that contacts the vulva; (3) apply the product at least once daily, preferably in the morning; (4) wear the treated underwear or panty liner throughout the day without washing off the powder; and (5) allow direct contact between the powder and the vulvovaginal area during normal daily activities. The intervention period was 4 weeks, with participants instructed to apply the product daily throughout this period. Compliance was assessed through participant diaries and product return at the final visit.

2.4 Outcome Measures

Primary Outcomes

1. **Vaginal Symptom Improvement**: Assessed using a standardized symptom questionnaire evaluating vaginal discharge, odor, itching, and discomfort. Symptoms were rated on a Likert scale, with defined as a reduction in symptom severity from baseline to week 4. Statistical significance was evaluated using McNemar's test for categorical outcomes.
2. **Nugent Score Changes**: Vaginal swabs were collected at baseline (Visit 1) and week 4 (Visit 2) for Gram stain and Nugent scoring. Nugent scores were calculated based on standardized morphological criteria: 0–3 (normal), 4–6 (intermediate), 7–10 (BV). Changes in Nugent scores were analyzed overall and stratified by baseline risk category (normal: 0–3; high-risk: ≥4).

Secondary Outcomes

1. **Lactobacillus Abundance**: Quantified through 16S rRNA gene sequencing of vaginal microbiome samples collected at baseline and week 4. Lactobacillus dominance was defined as relative abundance > 50% of total bacterial composition.
2. **Vaginal Microbiome Composition**: Comprehensive characterization of vaginal bacterial communities, including alpha diversity and beta diversity metrics.

Safety Outcomes

Participant safety was monitored throughout the study. All adverse events (AEs) were documented, assessed for severity and causality, and reported to the IRB according to institutional policies. AEs were classified by severity (mild, moderate, severe), relationship to study product (related, possibly related, unrelated), and outcome. Serious adverse events (SAEs) were defined according to ICH-GCP guidelines and required immediate reporting to the IRB.

2.5 Microbiome Analysis

Sample Collection

Vaginal swab samples were collected by trained clinical staff at baseline (Visit 1, Day 0) and endpoint (Visit 2, Week 4). Samples were collected from the mid-vaginal wall or posterior fornix using sterile swabs, immediately placed in sterile collection tubes, and stored at -80°C until batch processing.

DNA Extraction and Sequencing

Genomic DNA was extracted from vaginal swabs using standardized protocols. The V3-V4 hypervariable region of the 16S rRNA gene was amplified using universal bacterial primers and sequenced on the Illumina platform. Raw sequencing reads underwent quality filtering, denoising, and chimera removal using QIIME2 pipeline.

Taxonomic Classification

Amplicon sequence variants (ASVs) were classified taxonomically using the SILVA reference database (version 138). Lactobacillus species were identified to species level where possible, including *L. crispatus, L. iners, L. jensenii, L. gasseri, L. acidophilus*, and *L. johnsonii.*

Diversity Analysis

Alpha diversity was calculated using four metrics: Observed ASVs (richness), Shannon index (diversity accounting for richness and evenness), Simpson index (probability that two randomly selected reads belong to different taxa), and Pielou's evenness index. Beta diversity was assessed using Aitchison distance (compositional data analysis accounting for compositionality) and Bray-Curtis dissimilarity. Principal Coordinates Analysis (PCoA) was performed to visualize between-sample differences.

2.6 Statistical Analysis

All statistical analyses were performed using R software (version 4.0 or higher) and SPSS (version 25.0). Statistical significance was set at two-sided $\alpha = 0.05$. Continuous variables were summarized as mean ± standard deviation (SD) or median (interquartile range) depending on distribution normality assessed by Shapiro-Wilk test. Categorical variables were presented as frequencies and percentages.

Primary and Secondary Outcome Analysis

Nugent score changes were analyzed using Wilcoxon signed-rank test for paired samples, given non-normal distribution of scores. Subgroup analyses were conducted stratifying participants by baseline Nugent score category (normal: 0–3; high-risk: ≥4). Categorical symptom improvement was evaluated using McNemar's test for paired proportions. Changes in Lactobacillus abundance were assessed using paired t-test (for normally distributed data) and Wilcoxon signed-rank test (for non-normally distributed data). Alpha diversity changes were evaluated using paired Wilcoxon signed-rank test. Beta diversity

differences were tested using PERMANOVA (Permutational Multivariate Analysis of Variance) with 999 permutations.

Correlation Analysis

The relationship between Lactobacillus abundance and Nugent score was examined using mixed-effects logistic regression, adjusting for potential confounders including age and vaginal pH. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. Similar analyses were conducted for alpha diversity indices and Nugent score.

Multi-task learning analysis

To identify microbial taxa commonly associated with vaginal health and Nugent score across different time points, a tree-based multi-task learning (MTL) approach was employed. This method enables the simultaneous modeling of multiple related outcomes, allowing the identification of shared predictive features across tasks (13).

3. RESULTS

3.1 Participant Flow and Baseline Characteristics

Enrollment and Completion

Between December 2023 and June 2025, 60 pre-menopausal women were enrolled in the study. Of these, 45 participants (75.0%) completed all study procedures. Fifteen participants (25.0%) discontinued the study prematurely. Reasons for discontinuation included withdrawal of consent (n = 8, 53.3% of dropouts), antibiotic use during the study period (n = 4, 26.7%), and loss to follow-up (n = 3, 20.0%). The completion rate of 75% exceeded the anticipated retention rate used in sample size calculations (Table 1).

Baseline Characteristics

Among the 45 completers, the mean age was 36.4 years (SD 6.8). All participants were pre-menopausal women of reproductive age. At baseline (Visit 1), the mean Nugent score was 2.56 (SD 2.9), indicating overall normal vaginal flora in the study population. However, substantial heterogeneity was observed, with scores ranging from 0 to 10 (Table 1).

Baseline Nugent Score Distribution

Participants were stratified into two groups based on baseline Nugent scores:

- **Low-Risk Group** (Nugent score 0–3): n = 29 participants (82.2%), mean baseline Nugent score 0.59 (SD 0.78)

- **High-Risk Group** (Nugent score ≥ 4): n = 16 participants (17.8%), mean baseline Nugent score 6.13 (SD 1.63)

The high-risk group included women with intermediate flora (Nugent score 4–6) and bacterial vaginosis (Nugent score 7–10).

Table 1
Baseline Participant Characteristics (N = 45 Completers)

Characteristic	All Participants (N = 45)	Low-Risk Group (Nugent < 4, n = 29)	High-Risk Group (Nugent ≥ 4, n = 16)	*P*-value
Enrollment & Completion				
Enrolled, n	60	—	—	—
Completed, n (%)	45	29 (64.4%)	16 (35.6%)	—
Dropout, n	15	—	—	—
Withdrawal of consent	8	—	—	—
Antibiotic use	4	—	—	—
Lost to follow-up	3	—	—	—
Clinical Characteristics				
Age, years ± SD	36.4 ± 6.8	36.0 ± 7.1	37.2 ± 6.5	0.583
History of Vaginitis, n (%)	11 (24.4)	4 (13.8)	7 (43.8)	0.025
Vaginal symptoms, n (%)	8 (17.8%)	3 (10.3%)	5 (31.3%)	0.195
Baseline Vaginal Characteristics				
Vaginal fluid pH, mean ± SD	4.6 ± 1.0	4.4 ± 1.0	4.9 ± 1.0	0.095
Nugent score, mean ± SD	2.6 ± 2.9	0.6 ± 0.8	6.1 ± 1.6	< 0.001*

Values are expressed as mean ± SD unless otherwise stated. *Statistically significant ($p < 0.05$). Wilcoxon signed-rank test used for continuous variables; McNemar's test used for categorical variables. BV = Bacterial Vaginosis. SD = Standard Deviation.

Baseline Microbiome Characteristics

At baseline, 29 participants (64.4%) demonstrated Lactobacillus dominance (relative abundance > 90%) (Fig. 1). Alpha diversity was generally low, consistent with Lactobacillus-dominant vaginal microbiota.

3.2 Primary Outcomes: Symptom Improvement and Nugent Score Changes

Vaginal Symptom Improvement

Overall vaginal symptom improvement was observed following 4-week intervention with the spray-type probiotic. The proportion of participants reporting symptom improvement approached statistical significance in the total population ($p = 0.051$). When stratified by baseline risk status, significant symptom improvement was observed in the high-risk group ($p = 0.020$), while the normal group showed modest improvement that did not reach statistical significance (Table 2).

Nugent Score Changes

In the total study population (n = 45), mean Nugent scores increased slightly from 2.56 (SD 2.91) at baseline to 2.82 (SD 3.16) at week 4. This change was not statistically significant and reflected divergent responses in the two baseline risk groups (Table 2).

In participants in the low-risk group, mean scores increased from 0.59 (SD 0.78) at baseline to 1.83 (SD 2.78) at week 4 ($p = 0.003$). Despite this statistically significant increase, the mean score remained within the normal range (0–3) at week 4. Individual-level analysis revealed that approximately 70% of participants in the normal group maintained or improved their Nugent scores, while 30% experienced score increases (Table 2).

In the high-risk group, mean Nugent scores decreased significantly from 6.13 (SD 1.63) at baseline to 4.63 (SD 3.10) at week 4 ($p < 0.001$). This represents a mean reduction of 1.50 points, indicating improvement in vaginal flora composition. Individual-level analysis showed that 50% of high-risk participants (4 of 8) achieved Nugent score improvement, defined as reduction of ≥ 1 point from baseline. Although the mean score at week 4 remained in the intermediate range (4–6) rather than reaching the normal range (0–3) (Table 2).

Table 2
Vaginal pH, Symptoms, Nugent Score Changes Before and After Spray-Type Probiotic Intervention

Variable	Visit 1 (Baseline)	Visit 2 (Week 4)	Change	p-value
Vaginal fluid pH, mean ± SD				
Overall Vaginal fluid pH	4.56 (1.04)	4.73 (1.00)	+ 0.17	0.352
Low-risk group (score 0–3, n = 29)	4.38 (1.01)	4.55 (0.87)	+ 0.17	0.408
High-risk group (score ≥ 4, n = 16)	4.93 (1.03)	5.06 (1.18)	+ 0.13	0.638
Vaginal symptoms, n (%)				
Overall Vaginal symptoms	8 (17.8)	3 (6.7)	-5	0.051
Low-risk group	3 (10.3)	2 (6.9)	-1	0.489
High-risk group	5 (31.3)	1 (6.3)*	-4	**0.020***
Nugent Score Changes				
Overall Nugent Score	2.56 ± 2.91	2.82 ± 3.16	+ 0.26	0.412
Low-risk group	0.59 ± 0.78	1.83 ± 2.78	+ 1.24	**0.003***
High-risk group	6.13 ± 1.63	4.63 ± 3.10	−1.50	**< 0.001***

Values are expressed as mean ± SD unless otherwise stated. *Statistically significant ($p < 0.05$). Wilcoxon signed-rank test used for continuous variables; McNemar's test used for categorical variables. BV = Bacterial Vaginosis. SD = Standard Deviation.

Correlation Between Lactobacillus Abundance and Nugent Score

Mixed-effects logistic regression analysis, adjusting for age and vaginal pH, revealed a significant negative correlation between Lactobacillus abundance and Nugent score (OR = 0.077, 95% CI: 0.009–0.634). This finding indicates that higher Lactobacillus abundance is associated with lower Nugent scores and reduced risk of bacterial vaginosis.

3.3 Microbiome Analysis

Lactobacillus Abundance Changes

Overall Lactobacillus abundance did not change significantly from baseline to week 4 in the total study population (p = 0.535, Fig. 1). At baseline, 29 participants (64.4%) demonstrated Lactobacillus

dominance (> 90% relative abundance), compared to 25 participants (55.6%) at week 4. This difference was not statistically significant ($p > 0.05$, Fig. 2).

Despite the lack of significant change in overall abundance, most participants maintained Lactobacillus-dominant microbiota throughout the study period. Subgroup analyses by baseline Nugent score category did not reveal significant abundance changes in either the low or high-risk groups.

Species-Level Lactobacillus Analysis

Analysis of individual Lactobacillus species revealed no significant changes in the relative abundance of L. iners, L. jensenii, L. gasseri, L. acidophilus, or L. johnsonii from baseline to week 4 (all $p > 0.05$). However, correlation analyses demonstrated that increased abundance of L. iners, L. johnsonii, L. acidophilus, and L. jensenii was associated with decreased odds of elevated Nugent scores, and Aerococcus, Prevotella, Gardnerella, Dialister were associated with increased odds of elevated Nugent scores (Fig. 3).

Diversity Analysis

Alpha diversity metrics and Principal Coordinates Analysis (PCoA) of beta diversity revealed no significant differences in overall microbial community composition between baseline and week 4 samples ($p > 0.05$, Supplementary Fig. 1, 2)

The majority of participants exhibited low alpha diversity at both timepoints, consistent with Lactobacillus-dominant vaginal microbiota. Although alpha diversity did not change significantly with intervention, mixed-effects logistic regression revealed significant correlations between diversity indices and Nugent score ($p < 0.05$). These findings indicate that higher alpha diversity is associated with increased odds of elevated Nugent scores.

The absence of a significant difference in beta diversity indicates that the spray-type probiotic intervention did not induce substantial shifts in overall vaginal microbiome community structure at the population level.

Multi-task learning Analysis

The multi-task learning analysis identified several microbial genera that were consistently associated with vaginal health outcomes across both baseline and follow-up time points. The consistent selection of Lactobacillus across tasks suggests that this genus represents a shared key microbial determinant of vaginal health, irrespective of temporal variation (Fig. 4).

3.4 Safety and Adverse Events

Overall Safety Profile

The spray-type probiotic demonstrated excellent safety and tolerability throughout the 4-week intervention period. No serious adverse events (SAEs) were reported during the study. No participants discontinued the study due to adverse events.

Adverse Events

Three mild adverse events were reported among the 45 study completers (6.7% incidence): Two participants reported subjective increase in vaginal discharge volume during the intervention period. The discharge was described as non-malodorous and not associated with other symptoms. Clinical examination revealed no signs of infection or inflammation. One participant reported increased vaginal discharge with cheese-like appearance, potentially suggestive of vulvovaginal candidiasis. However, clinical evaluation and laboratory testing did not confirm fungal infection.

Product Tolerability and Compliance

Participant feedback indicated high acceptability of the spray-type delivery system. The application method was reported as convenient, discreet, and easily integrated into daily routines. No participants reported difficulty with product application or discomfort related to the powder formulation. Compliance with daily application was high based on participant diaries and product return assessment.

4. DISCUSSION

This exploratory pilot study provides preliminary evidence regarding the feasibility and potential clinical and microbiome-associated effects of a spray-type probiotic delivery system for vaginal health management. Our findings demonstrate that 4-week application of BalanceTalk Probiotic Feminine Hygiene Powder, containing multiple Lactobacillus strains, resulted in significant improvement in vaginal symptoms and Nugent scores among women at high risk for BV, with excellent safety and tolerability. These results support the potential utility of spray-type probiotic delivery as an innovative approach to vaginal health management.

The most clinically significant finding was the substantial improvement observed in women with elevated baseline Nugent scores (≥ 4). In this high-risk group, mean Nugent scores decreased significantly from 6.13 to 4.63 ($p < 0.001$), with 50% of participants achieving individual score improvement. This 1.50-point mean reduction represents meaningful improvement in vaginal flora composition, moving participants from the BV/intermediate range toward normal flora. The significant symptom improvement in this group ($p = 0.020$) further supports clinical benefit. These findings are particularly relevant given the limitations of current BV treatment approaches. Antibiotic therapy, while effective for acute BV treatment, is associated with recurrence rates of 50–70% within 12 months (12). The high recurrence rate reflects failure to restore healthy Lactobacillus-dominant microbiota following antibiotic-induced disruption. Probiotic interventions offer a complementary or alternative approach focused on microbiota restoration rather than pathogen elimination.

Previous studies of vaginal probiotics have shown variable efficacy (14). However, several meta-analyses have found that probiotic administration reduced BV recurrence compared to placebo (15–18). Our findings align with this evidence base, demonstrating benefit specifically in women with abnormal vaginal flora. The present meta-analysis demonstrated that the efficacy of probiotics in preventing BV recurrence was not significantly influenced by the route of administration, suggesting that microbiome modulation rather than delivery route may be the key determinant of therapeutic effect (14, 17, 19, 20). Although the optimal route of administration remains to be established and warrants further investigation, our findings suggest that alternative delivery approaches, including spray-type probiotics, may also be effective beyond conventional oral and vaginal routes.

Previous studies have demonstrated a significant correlation between Lactobacillus abundance and Nugent score, suggesting that Lactobacillus plays a key role in the development and progression of bacterial vaginosis (1, 2, 21). Our study showed a significant negative correlation between Lactobacillus abundance and Nugent score (OR = 0.077, 95% CI: 0.009–0.634), which confirms the central role of Lactobacillus in maintaining vaginal health. This strong inverse relationship validates the biological rationale for Lactobacillus-based interventions and suggests that strategies enhancing Lactobacillus colonization may effectively prevent or treat BV. Similarly, the positive correlations between alpha diversity indices and Nugent score support established understanding that healthy vaginal microbiota are characterized by low diversity and Lactobacillus dominance. The finding that higher diversity is associated with elevated Nugent scores aligns with the concept that BV represents a shift from low-diversity (Lactobacillus-dominant communities) to high-diversity (polymicrobial communities). These correlations provide mechanistic insight into the relationship between microbiome composition and clinical outcomes. However, caution is warranted when interpreting Nugent scores in non-reproductive-age women, as their validity may be limited in such populations (22).

Despite clinical improvements in the high-risk group, we did not observe significant changes in overall Lactobacillus abundance, alpha diversity, or beta diversity at the population level. Several factors may explain this apparent discrepancy between clinical and microbiological outcomes. First, the study population was predominantly composed of women with normal baseline Nugent scores (64.4%), who already exhibit Lactobacillus-dominant microbiota. In this group, the intervention may have maintained existing healthy microbiota rather than inducing substantial compositional changes. The lack of change in women with already-optimal microbiota is not unexpected and may actually represent a positive finding, suggesting the intervention does not disrupt healthy vaginal ecosystems. Second, the small sample size of the high-risk group (n = 16) limited statistical power to detect microbiome changes in subgroup analyses. While Nugent score improvements were significant in this group, microbiome sequencing analyses may have been underpowered to detect corresponding compositional shifts. Future studies with larger high-risk populations are needed to definitively assess microbiome changes in women with abnormal vaginal flora. Third, the 4-week intervention period may have been insufficient to induce substantial, detectable changes in established vaginal microbiota. Vaginal microbiome composition is relatively stable over time, and longer intervention periods may be required to achieve measurable shifts in community structure. Previous studies have reported considerable variability in

probiotic treatment duration, ranging from short-term interventions of less than two weeks to longer regimens extending up to several months (15). Although the optimal duration remains to be established, longer treatment durations may be associated with improved clinical outcomes. Fourth, the spray-type delivery system provides external application to the vulvovaginal area rather than direct intravaginal delivery. While this approach offers practical advantages, it may result in lower bacterial loads reaching the vaginal environment compared to intravaginal suppositories. The clinical improvements observed suggest that even modest bacterial delivery may be sufficient to influence vaginal health, but higher doses or longer duration may be needed for detectable microbiome changes.

The statistically significant increase in mean Nugent scores in the normal group (0.59 to 1.83, $p = 0.003$) requires careful interpretation. While statistically significant, this change remained within the normal range (0–3) and may reflect several factors including natural variation in the vaginal microbiota may occur across menstrual cycles and in response to behavioral and hormonal factors. The observed increase may represent normal physiological variation rather than intervention-related effects. Importantly, no participants in the normal group developed BV (Nugent score ≥ 7) during the study, and symptom reports did not indicate adverse effects. This suggests that the intervention did not induce clinically significant dysbiosis in women with healthy baseline microbiota. Nevertheless, these findings warrant attention in future studies, and longer-term monitoring would be valuable to assess whether score increases persist or resolve over time.

The excellent safety profile observed in this study is a key strength of the spray-type delivery system. Only three mild adverse events were reported (6.7% incidence), all self-limited and not requiring intervention. This compares favorably to intravaginal suppositories, which can cause messiness, leakage, and local irritation. The convenience and discretion of spray application may enhance long-term compliance, a critical factor for probiotic efficacy given the need for sustained use.

Several limitations should be considered when interpreting these findings. First, the single-arm design without a control group limits causal inference. Observed improvements may reflect natural history, regression to the mean, or placebo effects rather than true intervention effects. Randomized controlled trials with placebo controls are essential to definitively establish efficacy. Second, the small sample size, particularly in the high-risk group, limited statistical power and generalizability. While significant effects were detected in this subgroup, larger studies are needed to confirm findings and enable more detailed subgroup analyses. Third, the microbiome analysis focused on 16S rRNA gene sequencing, which provides taxonomic composition but limited functional information. Metabolomic or metatranscriptomic approaches could provide deeper insight into microbiome functional changes.

Despite these limitations, our findings have important clinical implications. The spray-type probiotic delivery system represents a novel, convenient, and safe approach to vaginal health management. For women with intermediate vaginal flora or recurrent BV, this intervention may offer a valuable adjunct or alternative to antibiotic therapy. The preliminary improvements in symptoms and Nugent scores in high-risk women suggest potential clinical utility, particularly for BV prevention in susceptible populations.

Several research priorities emerge from this study. First, randomized controlled trials with adequate sample sizes are essential to definitively establish efficacy. Studies should include placebo controls, stratification by baseline Nugent score, and sufficient power to detect clinically meaningful differences in BV incidence and recurrence. Second, longer intervention periods should be evaluated to assess sustained effects and optimal treatment duration. Studies should include extended follow-up to evaluate durability of effects and prevention of BV recurrence. Third, dose-response studies are needed to optimize bacterial load and application frequency. The current protocol used once-daily application; more frequent application or higher bacterial concentrations may enhance efficacy. Fourth, comparative effectiveness studies should evaluate spray-type delivery against intravaginal suppositories and oral probiotics to determine relative efficacy, safety, and user preference. Fifth, mechanistic studies using metagenomic, metabolomic, and immunological approaches could elucidate mechanisms of action and identify biomarkers predictive of treatment response. Sixth, studies in specific populations at high risk for BV (e.g., women with recurrent BV, pregnant women, women with HIV) would assess efficacy in clinically relevant subgroups.

5. CONCLUSIONS

This exploratory clinical study demonstrates that a novel spray-type probiotic delivery system containing multiple Lactobacillus strains was associated with improvement in vaginal symptoms and Nugent scores in women at higher risk for BV, with excellent safety and tolerability. The significant negative correlation between Lactobacillus abundance and Nugent score confirms the biological importance of Lactobacillus in maintaining vaginal health and supports the rationale for Lactobacillus-based interventions.

However, the single-arm design, small sample size, and short intervention period limit definitive conclusions about efficacy. Randomized controlled trials with larger sample sizes, longer intervention periods, and extended follow-up are warranted to confirm these preliminary findings and establish the clinical utility of spray-type probiotics for BV prevention and treatment.

Abbreviations

AE
Adverse event
ASV
Amplicon sequence variant
BV
Bacterial vaginosis
CI
Confidence interval
DNA
Deoxyribonucleic acid

ICH-GCP
International Council for Harmonisation–Good Clinical Practice
IRB
Institutional Review Board
MTL
Multi-task learning
OR
Odds ratio
PCoA
Principal Coordinates Analysis
PERMANOVA
Permutational Multivariate Analysis of Variance
QIIME2
Quantitative Insights Into Microbial Ecology 2
rRNA
Ribosomal ribonucleic acid
SAE
Serious adverse event
SD
Standard deviation
SRA
Sequence Read Archive

Declarations

Ethics Approval and Consent to Participate

This study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The study protocol was reviewed and approved by the Institutional Review Board (IRB) of Ewha Womans University Hospital (IRB approval number: 2023-12-037). All participants provided written informed consent prior to enrollment and any study procedures.

Consent for Publication

This manuscript does not contain any individual person’s data in any form.

Availability of Data and Materials

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request. Microbiome sequencing data have been deposited in SRA under

accession number PRJNA1467324. Due to privacy and ethical restrictions, individual participant data cannot be made publicly available.

Competing Interests

YJ is the CEO of PH4 Co., Ltd., which provided the investigational product used in this study and financial support. However, the company had no role in study design, data analysis, interpretation, or manuscript preparation.

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Authors' Contributions

YJK conceived and designed the study, supervised all study procedures, interpreted results, and critically revised the manuscript. SP coordinated participant recruitment, conducted clinical assessments, and drafted the manuscript. JK, MJH performed microbiome analyses and statistical analyses. Y-AY contributed to study design and data interpretation. YMH assisted with participant recruitment and data collection. YJ provided the investigational product but was not involved in data analysis or interpretation. GL contributed to microbiome sample processing and analysis. DS, HNL assisted with data collection. All authors read and approved the final manuscript.

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Figures

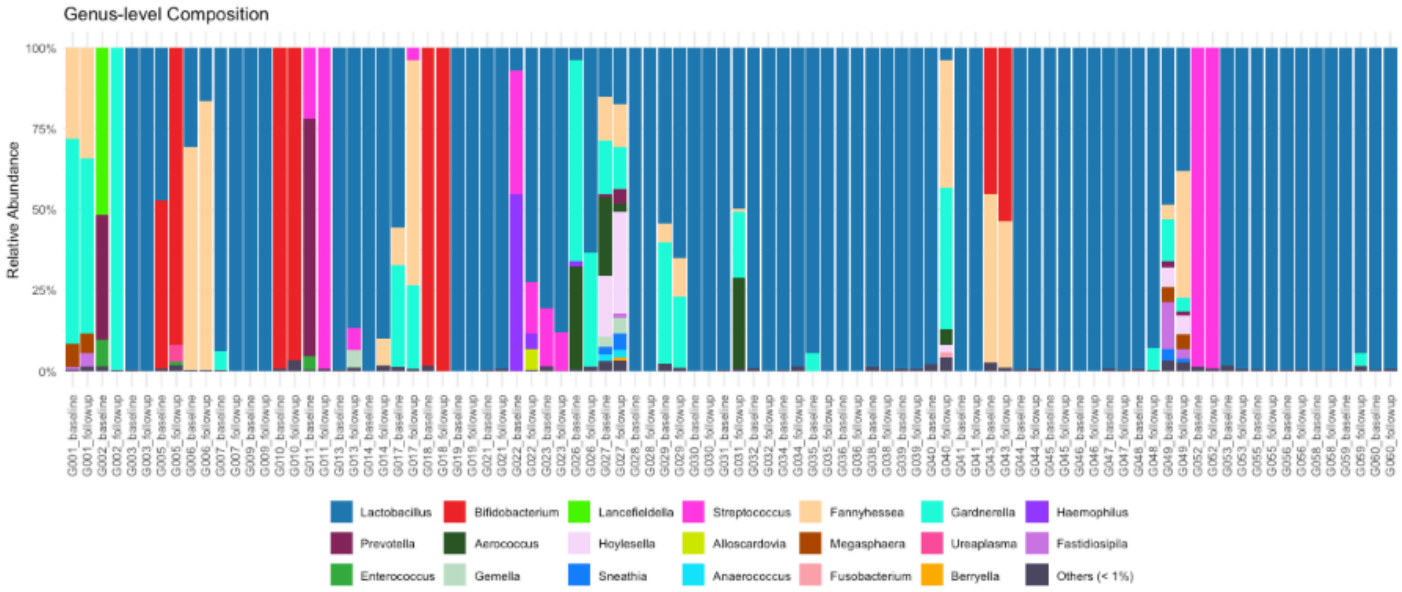


Figure 1

Changes in the vaginal microbiome with Genus-level after probiotic use

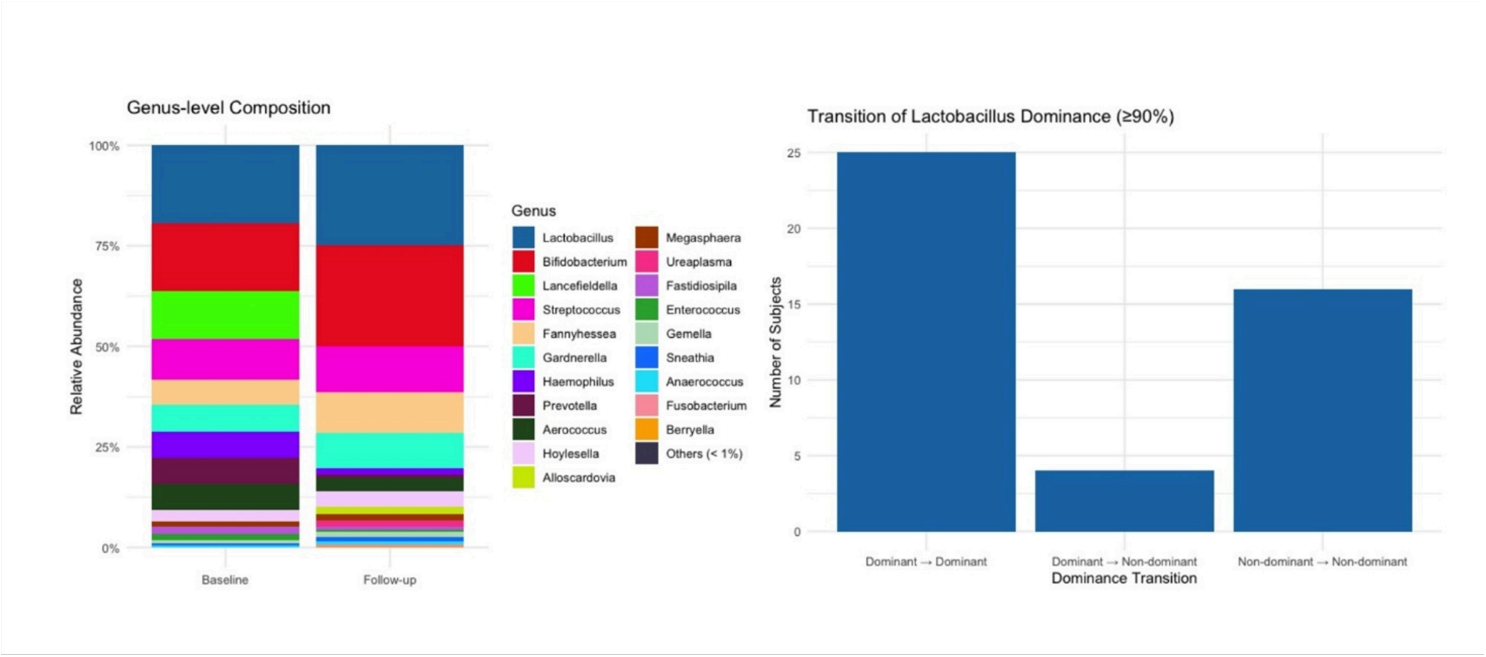


Figure 2

Changes in the Genus-level Composition (a) and Transition of Lactobacillus Dominance (b) after probiotic use

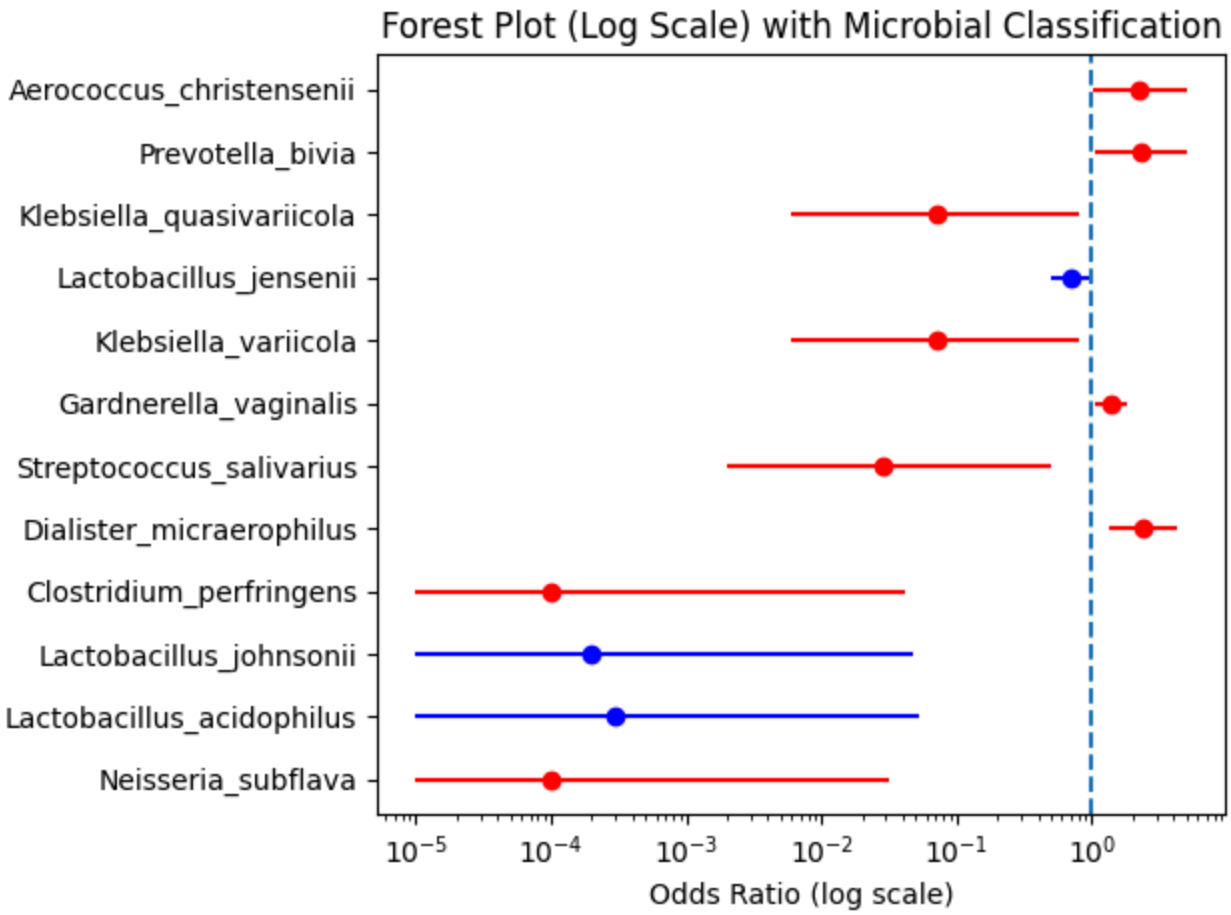


Figure 3

Forest plot showing odds ratios (ORs) and 95% confidence intervals for microbial species. The x-axis is presented on a logarithmic scale. Lactobacillus species are shown in blue, while non-Lactobacillus species, including anaerobic bacteria, are shown in red. The vertical dashed line indicates OR = 1.

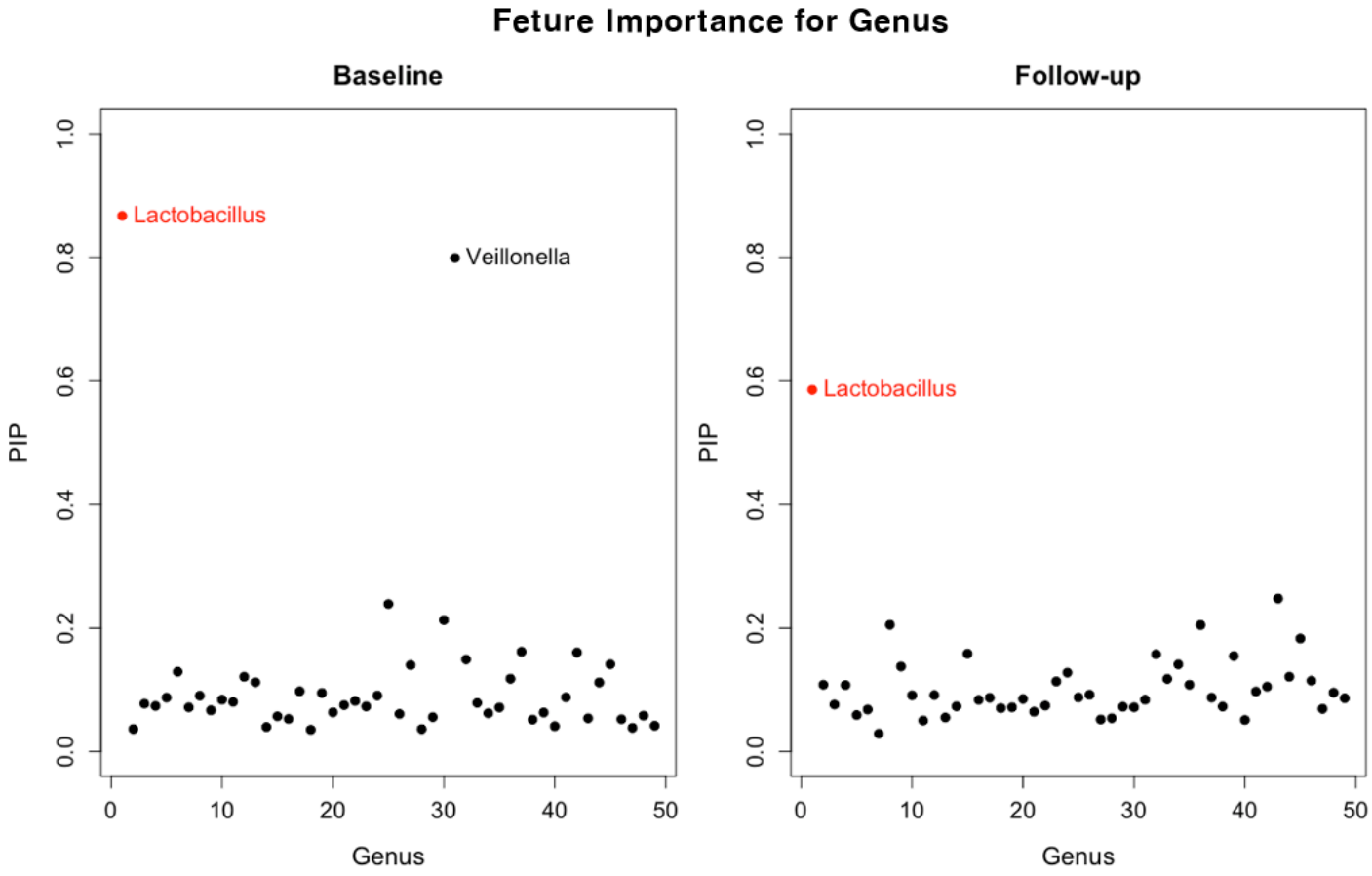


Figure 4

Feature Importance for Genus. Posterior Inclusion Probability (PIP) plot using Multi-task learning Analysis

Supplementary Files

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