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The Impact of Oral Lactoferrin on Managing Bacterial Vaginosis

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Abstract

Background : Bacterial vaginosis (BV) is a common cause of vaginal discomfort. It results from an imbalance of microbiota—reduced lactobacilli and increased anaerobes such as Gardnerella vaginalis. Although antibiotics work initially, recurrent infections often pose an ongoing problem.

Objectives: This study examined vaginal bacteria in women with BV and compared the effects of combined oral lactoferrin and metronidazole versus placebo with metronidazole alone.

Methods: A double-blind randomized trial at Ain Shams University Maternity Hospitals (Dec 2021- Dec 2023) included 60 women with BV, divided into two groups: Group A received 100 mg oral lactoferrin plus Metronidazole, while Group B received a placebo plus Metronidazole. After excluding seven participants, 27 women in the experimental group and 26 in the placebo group completed the study.

Results: Both treatments effectively managed BV, but the lactoferrin group showed significantly better results after 10 days. Only 7.4% had vaginal discharge compared to 30.8% in the placebo group ($p<0.05$). The cure rate was 85.2% with lactoferrin versus 69.2% with placebo, and recurrence rates were 14.8% versus 30.8%, respectively. No significant differences were observed between groups regarding age, BMI, contraceptive use, recurrent BV history, sexual activity, or baseline symptoms. The findings suggest that lactoferrin improves clinical outcomes and reduces recurrence in BV treatment.

Conclusions: This study assessed oral lactoferrin supplementation with antibiotics for bacterial vaginosis treatment versus standard care. While BV symptoms improved, differences between groups were statistically insignificant. Due to the small sample size, no firm conclusions can be drawn regarding the efficacy of the intervention compared to standard care.

Key words: Bacterial Vaginosis, Oral Lactoferrin, Metronidazole.

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INTRODUCTION

Bacterial vaginosis (BV) affects 40-50% of women, causing discharge, odor, and discomfort. It arises from an imbalance in vaginal microbiota, particularly a reduction in Lactobacillus species, which help maintain vaginal health. The absence of these protective bacteria can lead to BV development, negatively impacting women's quality of life. (3).

Despite the common use of antibiotics like Metronidazole for treating BV, recurrence rates are notably high(4). About 25% of patients face a second episode within four weeks, with long-term recurrence exceeding 70% (5). Furthermore, antibiotics can lead to antibiotic resistance and disrupt natural vaginal flora, emphasizing the urgent need for effective alternative treatments with fewer side effects. (6).

Lactoferrin, an iron-binding glycoprotein belonging to the transferrin family, has emerged as a promising candidate in this context (7). Produced and stored in secondary neutrophil granules, lactoferrin is released during neutrophil activation and degranulation. It exhibits bacteriostatic and bactericidal properties by binding to iron, thereby limiting its availability to bacteria and inhibiting their growth (7). Moreover, lactoferrin has immunomodulatory effects and can disrupt bacterial cytoplasmic membranes, enhancing its antimicrobial efficacy. The synergistic effects of lactoferrin when combined with immunoglobulin A, lysozyme, antibiotics, and other drugs further support its potential utility in treating infections (5, 8).

Given these properties, lactoferrin's role in managing BV warrants thorough investigation. This study aims to evaluate the effectiveness of oral lactoferrin combined with Metronidazole in treating BV compared to the standard antibiotic treatment alone. By characterizing the bacterial biota in women affected by BV and assessing clinical outcomes, this research seeks to determine whether lactoferrin can enhance treatment efficacy and reduce recurrence rates.

PATIENTS AND METHODS

- A double-blind randomized trial approved by ethics and involving 60 women with bacterial vaginosis (BV) was conducted at Ain Shams University Maternity Hospital from December 2021 to December 2023. The women were randomly divided into two groups: Group A received 100 mg of lactoferrin (Pravotin®) twice daily for 5 days followed by once daily for 10 days, alongside metronidazole (500 mg twice daily for 7 days). Group B received a placebo with the same antibiotic regimen.

The study included women aged 18-45 with a BMI of 18-25, regular cycles, and symptomatic acute BV diagnosed per Amsel's and Nugent's criteria.

Exclusion criteria included women with medical conditions as diseases like diabetes mellitus led to decrease the immunity and increase the risk of bacterial infection, women with active infections from other pathogens due to change in the vaginal PH and mixed infection can't test the effect of lactoferrin on two modifiable risk factors, pregnant or breastfeeding due to hormonal fluctuations, women who had recent antibiotic or hormone treatments as they lead to decreased immunity and change in the vaginal normal bacterial flora, women with Nugent score <7, women with gynecological conditions causing bleeding as they interfere with the result of vaginal smear and change the vaginal PH and women with allergies to study medications, or refused participation.

Diagnostic criteria of BV:

Amsel's criteria diagnose bacterial vaginosis (BV) with any three of four signs: abnormal discharge, fishy odor, pH over 4.5, and clue cells (vaginal cells with G. vaginalis) (9). BV diagnosis is confirmed through Nugent's scoring, which analyzes bacterial types in Gram-stained vaginal samples. The Nugent score ranges from 0 to 10, with scores of 7-10 indicating BV, 4-6 considered intermediate

(partial BV or clearing), and 0-3 representing normal flora (10). The scoring assesses the prevalence of Lactobacillus, G. vaginalis, Bacteroides, and Mobiluncus, based on cell counts. While intermediate scores may suggest developing BV or resolution, their significance remains debated (11).

Sample size Justification:

Based on Pino et al. (2017) [5], a total of 52 participants (26 per group) were initially calculated to achieve 80% power to detect an effect size of 0.80 at a 0.05 significance level using a two-sided t-test. To compensate for potential dropouts, a 10% increase was incorporated, resulting in a total sample size of 60 participants, evenly divided between the two groups. This approach ensures sufficient statistical power to detect meaningful differences while accounting for attrition, aligning with recommended sample size estimation practices.

Randomization was performed using a computer-generated randomization sheet to ensure unbiased allocation. Allocation concealment was maintained through sealed, opaque envelopes, which were handed to a neutral third party (a nurse). Each participant selected an envelope, revealing a unique number that determined her assignment to either group 1 or group 2, based on the predetermined random list. This method ensured that both participants and investigators remained blinded to group assignments, minimizing selection bias and maintaining the study's integrity. The process promoted fairness and unpredictability in group allocation, enhancing the reliability and validity of the trial outcomes.

Detection Bias Prevention:

Detection bias was minimized by blinding both patients and assessors to group assignments. Sachets in both groups were identical in shape and packaging, labeled anonymously with codes managed by a colleague unaware of the codes. Another blinded colleague with no group information conducted the final assessment.

Ethical Considerations:

Patient data remained anonymous, identified only by diagnosis and confidential codes. Informed consent was obtained from all participants in Arabic. Confidentiality was maintained by assigning numbers to initials, known only to the investigator. The OB/GYN department approved the study protocol at Ain Shams University.

Study Procedures:

Clinical assessment:

After obtaining consent, women provided detailed personal, obstetric, medical, and family histories. They underwent comprehensive clinical examinations, including general, abdominal, and pelvic checks, and assessments for vaginosis. Vaginal discharge samples were taken at the beginning and after 10 days of treatment.

Investigations:

Laboratory tests included blood count , LFTs , creatinines, coagulation profile, and transvaginal ultrasound.

Sample collection:

Vaginal discharge samples were obtained from the lateral vaginal wall and the posterior vaginal fornix using sterile cotton-tipped swabs. For each participant, two vaginal smears were collected at baseline and after 10 days of treatment.

Vaginal smears were analyzed for bacterial vaginosis (BV) during each visit using microscopic examination, the whiff amine test, and pH measurement with test strips. The whiff test utilized 10% KOH to reveal a fishy odor, indicative of volatile amines. Amsel's criteria and Nugent scoring were applied at the start and after 10 days. Nugent scoring assigns values to Lactobacilli and other bacteria, with scores of 0-3 showing normal flora, 4-6 as intermediate, and 7-10 as confirming BV (9-10).

Outcome measures:

- **Primary outcome:** Complete cure rate.

- **Secondary outcomes:** Non-recurrence of infection within 4 weeks

Statistical analysis: Data analysis utilized SPSS for Windows v20.0. Numerical parametric data were summarized using range, mean, and standard deviation, while non-parametric data were represented by range, median, and interquartile range. Categorical variables were analyzed by frequency and percentage. Independent t-tests assessed differences in parametric data, and chi-squared tests evaluated categorical data. Associations were examined through binary logistic regression, and ROC curves determined predictive validity, with significance set at $p<0.05$.

RESULTS

In this study, 85 patients were evaluated for eligibility, with 60 patients participating (30 in each group). Sixteen eligible patients were excluded, and nine refused to join. During follow-up, three patients from the lactoferrin group and four from the placebo group dropped out. Ultimately, data analysis included 27 women from the lactoferrin group and 26 from the placebo group.

Table (1): Comparison of clinical signs of vaginosis in the study and control groups at baseline and after 10 days.

Baseline	Study group (n=27)	Control group (n=26)	Test value	P-value
Vaginal discharge	27 (100.0%)	26 (100.0%)	0.000	1.000
Signs and symptoms of vaginitis after 10 days of treatment				
No signs and symptoms	23 (85.2%)	18 (69.2%)	1.122	0.289
Vaginal discharge	4 (14.8%)	8 (30.8%)		

(t) Student t-test; (p) probability value.

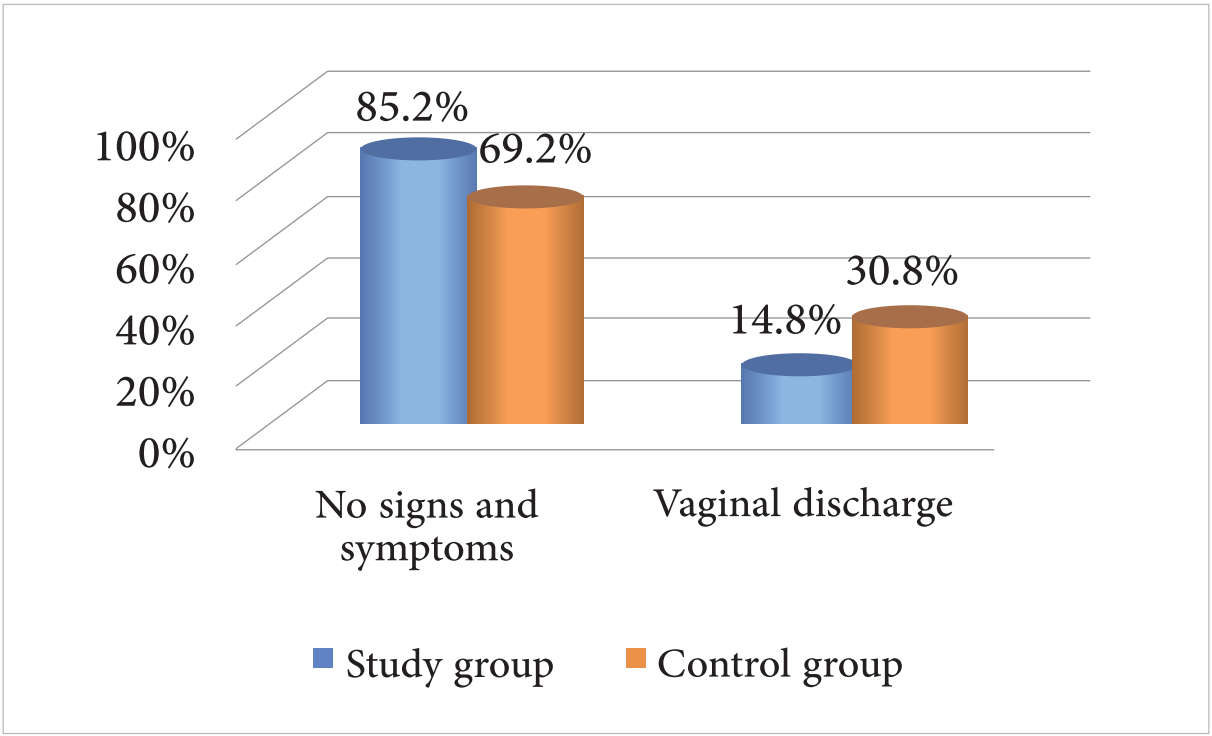

Figure (1): Comparison between the two studied groups according to clinical criteria after 10 days of treatment.

Table (1) and Figure (1) show that all studied patients presented with vaginal discharge. However, signs and symptoms after 10 days of treatment were improved compared to baseline in both groups.

Table (2): Comparison between baseline and after 10 days of treatment according to clinical criteria of vaginosis in the study group.

Clinical criteria	Study group (n= 27)		t	p
	Baseline	After 10 days of treatment		
• No signs and symptoms	0 (0.0%)	23 (85.2%)	13.587	**<0.001***
• Vaginal discharge	27 (100.0%)	4 (14.8%)		

(t) Student t-test; (p) probability value; (*) statistically significant result.

Table (2) Table (2) shows a significant improvement in clinical symptoms after 10 days of treatment in the study group, with $p < 0.001$ indicating a highly statistically significant reduction in signs and symptoms of vaginosis.

Table (3): Comparison between baseline and after 10 days of treatment according to clinical criteria in the control group.

clinical criteria of Vaginosis	Control group (n= 26)		t	p
	Baseline	After 10 days of treatment		
No signs and symptoms	0 (0.0%)	18 (69.2%)	32.8	**<0.001***
Vaginal discharge	26 (100%)	8 (30.8%)		

(t) Student t-test; (p) probability value; (*) statistically significant result.

Table (3) The control group showed significant improvement after 10 days, with $p < 0.001$, as the proportion without symptoms increased and vaginal discharge decreased, indicating effective symptom reduction over the treatment period (Table 3).

Table (4): Comparison between baseline and after 10d of treatment according to vaginal discharge sampling in the study group.

Vaginal discharge sampling	Study group (n=27)		Test value	p-value
	Baseline	After 10d of treatment		
pH Baseline				
pH 4	0 (0.0%)	2 (7.4%)	15.373	0.002*
pH 5	5 (18.5%)	17 (63.0%)		
pH 6	21 (77.8%)	8 (29.6%)		
pH 7	1 (3.7%)	0 (0.0%)		
pH 8	0 (0.0%)	0 (0.0%)		
Clue cells Baseline				
No clue cells	14 (51.9%)	24 (88.9%)	7.194	0.007*
Positive clue cells	13 (48.1%)	3 (11.1%)		
Fishy Odour Baseline				

Fishy Odour	19 (70.4%)	1 (3.7%)	22.95	<0.001**
No Fishy Odour	8 (29.6%)	26 (96.3%)		

Using: McNemar's test *p-value <0.05 is significant; **p-value <0.001 is highly significant.

Table (4) shows that there was statistically significant reduction mean of pH the after treatment than baseline. Also, there was high frequency of improved fishy odor after treatment compared to baseline with significant reduction in the positive clue cells after treatment comparing to baseline.

Table (5): Comparison between baseline and after 10d of treatment according to vaginal discharge sampling in the control group.

Vaginal discharge sampling	Control group (n=26)		Test value	p-value
	Baseline	After 10d of treatment		
pH Baseline				
pH 4	0 (0.0%)	1 (3.8%)	15.021	0.005*
pH 5	3 (11.5%)	14 (53.8%)		
pH 6	21 (80.8%)	10 (38.5%)		
pH 7	2 (7.7%)	0 (0.0%)		
pH 8	0 (0.0%)	1 (3.8%)		
Clue cells Baseline				
No clue cells	13 (50.0%)	23 (88.5%)	7.312	0.007*
Positive clue cells	13 (50.0%)	3 (11.5%)		
Fishy Odour Baseline				
Fishy Odour	19 (73.1%)	5 (19.2%)	13.077	0.002*
No Fishy Odour	7 (26.9%)	21 (80.8%)		

Using: McNemar's test *p-value <0.05 is significant; **p-value <0.001 is highly significant.

Table (5) shows that Significant changes were observed after 10 days, with p-values < 0.01. Notably, pH shifted towards normal, clue cells decreased, and fishy odor reduced, indicating treatment effectiveness.

Table (6): Comparison between two groups according to outcome.

Outcome	Study group (n=27)	Control group (n=26)	Test value	P-value	Sig
Cured	23 (85.2%)	18 (69.2%)	1.925	0.165	NS
Resistant	**4 (14.8%)**	**8 (30.8%)**			

Using: x^2: Chi-square test for Number (%) or Fisher's exact test, when appropriate.

Table (6) shows that the higher frequency of cured cases was 23 patients (85.2%) in the study group, compared to 18 patients (69.2%) in control group, with p-value (p>0.05).

Table (7): Comparison of Infection Recurrence Within 4 Weeks Between Study and Control Groups.

Recurrence of infection within 4 weeks	Study group (n=23)	Control group (n=18)	Test value	P-value	Sig
No Recurrence of infection	19 (82.6%)	14 (77.8%)	2.073	0.355	NS
Recurrence after 4 weeks	4 (17.4%)	4 (22.2%)			

Using: x^2: Chi-square test for Number (%) or Fisher's exact test, when appropriate.

Table (7) shows that the recurrence of infection after 4 weeks was 4 patients (17.4%) in the study group, compared to 4 patients (22.2%) in the control group, but insignificant difference, with p-value (p>0.05).

DISCUSSION

The current standard treatment for bacterial vaginosis (BV) involves antibiotics, particularly metronidazole and clindamycin, which are effective in about 80% of cases (12). Recurrence occurs in more than half of the patients within one year, and repeated antibiotic use can lead to resistance, particularly to these medications in G. vaginalis strains. This highlights the need for alternative therapies, such as oral lactoferrin, a glycoprotein known for its antimicrobial and immunomodulatory effects, which are currently under investigation(13-15).

Bacterial vaginosis (BV) poses significant obstetric and gynecologic risks, including preterm labor, premature membrane rupture, spontaneous abortion, chorioamnionitis, endometritis, infections after Cesarean and surgeries, and subclinical pelvic inflammatory disease (16).

Our results and comparison to similar studies

This study aimed to analyze the bacterial flora in women with bacterial vaginosis (BV) and evaluate the effects of combined oral lactoferrin and Metronidazole therapy versus placebo on vaginal bacteria.

The current study revealed no significant demographic differences between the groups, including age, BMI, contraceptive use, infectious disease history and sexual activity among the study groups. This ensured comparability for assessing the intervention's impact on BV.

Both groups initially had vaginal discharge. After 10 days, symptoms significantly improved, with 14.8% in the study group and 30.8% in the control group (p < 0.001). No significant difference was observed between groups post-treatment (p = 0.289).

At baseline, no significant differences existed between groups in pH, clue cells, or fishy odor (p > 0.05). After 10 days, both groups showed significant improvements in these parameters, with no significant difference between them (p > 0.05).

The study showed 85.2% cure in the study group versus 69.2% in controls. Recurrence was 17.4% and 22.2%, respectively, with no significant difference between groups (p > 0.05).

Vaginal Lactobacillus species form a protective barrier against pathogens by secreting metabolites that inhibit bacterial and viral infections (17). Their production of lactic acid contributes to a low vaginal pH (< 4.5), which inhibits pathogenic microorganisms that cause vaginal dysbiosis. This low pH is associated with a higher incidence of bacterial vaginosis (BV) in women of reproductive age (5,18).

The bacterial vaginosis (BV) treatment is not fully effective until its causes are better understood. Clinicians typically prescribe antibiotics like metronidazole or clindamycin, but 11–29% of cases recur within a month due to resistant biofilms from G. vaginalis created post-therapy, which are more complex to combat than free-floating bacteria (19).

Pino et al. (5) conducted a randomized trial to evaluate the effects of lactoferrin on vaginal microbiota in women with BV, using 100 mg and 200 mg pessaries. Results showed that lactoferrin significantly decreased pathogenic bacteria such as Gardnerella, Prevotella, and Lachnospira, while promoting Lactobacillus species. The microbial balance was maintained for up to two weeks in women receiving the 200 mg dose. However, the study had limitations, including the absence of a control group and the use of vaginal administration, which may differ in effectiveness compared to oral lactoferrin, as investigated in our study.

Lactoferrin primarily binds iron with high affinity (Kd 10–20), creating an iron-depleted, bacteriostatic environment (17). Pino et al. (20) examined 71 G. vaginalis isolates' susceptibility to metronidazole and clindamycin, assessing the antimicrobial activity of bovine lactoferrin (MTbLF). Results indicated that G. vaginalis strains utilize iron sources like salts, hemin, hemoglobin, and human lactoferrin, but not bovine lactoferrin. The specificity of G. vaginalis proteins for human lactoferrin suggests bovine lactoferrin may be more effective for BV treatment. Additionally, combining MTbLF with clindamycin showed a synergistic effect, enhancing antimicrobial efficacy and potentially addressing resistance issues. These findings support bovine lactoferrin as a promising adjunct therapy for BV.

Russo et al. (11) conducted a double-blind trial evaluating a probiotic mix with Lactobacillus acidophilus GLA-14, L. rhamnosus HN001, and bovine lactoferrin alongside metronidazole in women with recurrent BV. The combination significantly improved symptoms, Nugent scores, and reduced recurrence, indicating it as a safe, effective alternative for restoring healthy vaginal microbiota. Additionally, Bertuccini et al. (21) found bovine lactoferrin enhances biofilm formation of these Lactobacillus strains on surfaces and HeLa cells, suggesting that combining lactoferrin with probiotics may promote vaginal health and prevent BV by supporting beneficial microbiota growth.

A clinical study by De Alberti et al. (22) found that administering Respecta orally significantly boosted vaginal colonization by L. acidophilus GLA-14 and L. rhamnosus HN001 in healthy women after two weeks. In contrast, the placebo did not increase lactobacilli levels. Remarkably, levels continued to rise one week after treatment stopped, indicating prolonged lactobacilli presence in the vagina.

Research indicates that G. vaginalis plays a pathogenic role, as vaginal Gardnerella spp. biofilms contribute to antibiotic treatment failures and high recurrence rates. These biofilms may host other bacteria acting as opportunistic invaders. Bacteria within biofilms respond differently to antibiotics than planktonic forms; antibiotic resistance may lead to persistent BV. Saunders et al. (23) found that certain Lactobacillus strains could disrupt and eliminate BV biofilms, potentially decreasing the need for antibiotics.

Lactobacilli supplements significantly reduced symptoms like discharge and odor, and improved Nugent scores during treatment (24). Anukam et al. (25) found that women receiving probiotics with L. rhamnosus GR-1 and L. reuteri RC-14 after metronidazole had an 88% initial cure rate versus 40% in the placebo group ($P < 0.001$), with increased vaginal lactobacilli.

However, due to limited data, probiotics are not yet standard for BV. Factors like diet, douching, contraception, and menstrual cy-

cle influence BV risk. Managing BV requires both short- and long-term strategies, but challenges such as unclear causes and high recurrence persist (19, 26). Women often use self-help remedies, while researchers continue exploring new therapies.

Our study differs from others by using a probiotic mix of Lactobacillus acidophilus GLA-14, L. rhamnosus HN001, combined with bovine lactoferrin (Respecta), with a more extended treatment duration and different methods. Russo et al. (11) gave Respecta or placebo daily for 10 days over six months, starting at menstrual cycle onset to reduce pH and recurrence risk. Despite treatments, vulvovaginal infections often recur, leading to repeated ineffective courses. However, alternative strategies like probiotics may improve outcomes for chronic or recurrent cases. This approach could offer a promising therapeutic option for women with persistent infections.

Clinical implications of the study: This study suggests that combining lactoferrin with standard antibiotic therapy may enhance clinical and microbiological improvement in BV, potentially reducing symptoms and recurrence. Incorporating lactoferrin could offer a safe, adjunctive treatment to improve patient outcomes, though further studies are warranted.

The strengths and limitations of the study:

This study boasts several strengths, including its double-blind randomized controlled trial design, its conduct at a single tertiary care center, and the inclusion of a comparison control group. Importantly, there is a notable absence of research assessing lactoferrin for treating bacterial vaginosis with a control group, underscoring the significance of our findings. However, the study has limitations; the relatively small sample size compared to prior research and the single-center nature raise concerns about publication bias. Additionally, the exclusive use of an oral form and a limited dosage of lactoferrin (100 mg) could potentially underplay its antimicrobial properties, given that these properties are dose-dependent (Pino et al., 2022). Finally, the duration of treatment and follow-up was also brief.

Recommendations for further studies

Future studies should involve larger, more diverse populations and long-term follow-up to assess sustained efficacy. Comparing oral and vaginal administration routes, exploring underlying mechanisms, and evaluating combination therapies are essential. Additionally, investigating the influence of lifestyle factors and establishing standardized protocols can enhance management of recurrent BV.

Conclusions

This study evaluated oral lactoferrin with antibiotics for bacterial vaginosis versus standard care. Improvements in symptoms occurred, but results were statistically insignificant due to small sample size. Findings suggest the need for further research with larger samples, higher lactoferrin doses, probiotics, and extended treatment duration.

Availability of data and material

The datasets utilized and analyzed in this study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare no conflicts of interest.

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