



Research Article

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Efficacy Of Multi-Strain Lactobacillus Treatment in Reducing Pathogen Load And Symptoms Associated with Vaginal Infections

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Abstract

This study aimed to assess the therapeutic potential of multi-strain Lactobacillus treatment (Lactobacillus fermentum, Lactobacillus acidophilus, Lactobacillus jensenii, and Lactobacillus rhamnosus) in the management of vaginal infections, including bacterial vaginosis, vaginal candidiasis, and urinary tract infections. The study included 30 women, aged 25-45 years, who were diagnosed with infections caused by pathogens such as Escherichia coli, Streptococcus agalactiae, Staphylococcus aureus, Candida albicans, and Gardnerella vaginalis. Pathogens were identified through standard microbiological culturing methods and PCR analysis, while clinical symptoms were assessed using a standardized questionnaire both before and after a four-week treatment with vaginal capsules containing 10^8 CFU/ml of Lactobacillus strains. The results indicated significant changes in microbial populations. Escherichia coli, a major causative agent of urinary tract infections, was detected in 10 of the participants before treatment, with a baseline concentration of 10^5 CFU/ml, which significantly decreased to 10^2 CFU/ml after the treatment. Similarly, Candida albicans, known for causing vaginal yeast infections, showed a reduction from a high initial concentration of 10^6 CFU/ml to 10^2 CFU/ml post-treatment. Gardnerella vaginalis, a pathogen frequently associated with bacterial vaginosis, also demonstrated a marked decrease in concentration from 10^5 CFU/ml to 10^2 CFU/ml. Streptococcus agalactiae and Staphylococcus aureus, responsible for various bacterial infections, were present in lower counts, with reductions from 10^4 CFU/ml to 10^2 CFU/ml for Streptococcus agalactiae, and from 10^5 CFU/ml to 10^3 CFU/ml for Staphylococcus aureus. These reductions indicate the significant antimicrobial activity of Lactobacillus strains in suppressing pathogen growth. Symptomatically, the treatment led to a considerable reduction in common symptoms associated with vaginal infections.

The most prevalent symptoms before treatment were itching and irritation, reported by 10 participants each, followed by discomfort (9), unpleasant odor (9), pelvic pressure (8), and pain during sexual intercourse (7). After the treatment, significant improvements were observed across all symptoms. Irritation and unpleasant odor showed the most substantial reduction, with average scores dropping from 4.8 ± 0.7 and 4.7 ± 0.6 to 1.5 ± 0.4 and 1.3 ± 0.3 , respectively. Other symptoms such as itching, discomfort, pelvic pressure, and dryness also decreased significantly. Statistical analysis confirmed that all observed changes in both pathogen concentrations and symptoms were statistically significant ($p < 0.05$). These findings align with previous research, supporting the role of Lactobacillus as an effective therapeutic approach in managing vaginal infections caused by common pathogens like Escherichia coli, Candida albicans, and Gardnerella vaginalis. The significant reductions in both microbial load and clinical symptoms suggest that Lactobacillus supplementation may help restore vaginal health by promoting the growth of beneficial bacteria, thereby reducing pathogen colonization and associated discomfort. This study further demonstrates the potential of Lactobacillus strains, particularly in combination, as a viable and effective treatment for vaginal infections, improving both microbiological and symptomatic outcomes for affected women.

Keyword: Participants; Bacteria; Vaginal Infection; Microbiological Isolation; Microbiota Field; Pharmacoeconomics; Pharmacy

Introduction

The vaginal microbiota is a complex ecosystem that plays a crucial role in maintaining the health of the female reproductive tract. A balanced microbiota, predominantly composed of *Lactobacillus* species, is essential for protecting against vaginal infections. Among the many *Lactobacillus* species, *Lactobacillus fermentum*, *Lactobacillus acidophilus*, *Lactobacillus jensenii*, and *Lactobacillus rhamnosus* have emerged as key probiotics in the prevention and treatment of vaginal infections, particularly those caused by pathogenic microorganisms such as *Escherichia coli*, *Streptococcus agalactiae*, and *Staphylococcus aureus* (1). These *Lactobacillus* strains contribute to the maintenance of vaginal health through various mechanisms, including the production of lactic acid, hydrogen peroxide, bacteriocins, and the competitive exclusion of pathogens. *Lactobacillus acidophilus* is one of the most studied and widely used strains in probiotic therapies due to its ability to lower vaginal pH and inhibit the growth of pathogenic bacteria.

By producing lactic acid, it helps maintain an acidic environment in the vagina, which is unfavorable for the growth of most pathogenic microorganisms. Additionally, *L. acidophilus* has been shown to strengthen the epithelial barrier, reducing the likelihood of pathogen adhesion to vaginal cells. *Lactobacillus rhamnosus* has demonstrated strong antimicrobial properties, particularly against *Escherichia coli* and *Staphylococcus aureus*. This strain is known for its ability to survive in the harsh vaginal environment and for its positive impact on immune modulation, further enhancing its therapeutic potential. *L. rhamnosus* also promotes the production of immune factors that help protect against infections and support overall vaginal health. *Lactobacillus jensenii* is another significant species in the vaginal microbiota, known for its ability to adhere to vaginal epithelial cells and produce hydrogen peroxide, which has antimicrobial effects (2). It plays an essential role in maintaining the integrity of the vaginal ecosystem and preventing the overgrowth of harmful pathogens. Its presence in the vaginal environment is closely associated with the prevention of bacterial vaginosis and other vaginal infections (3). *Lactobacillus fermentum*, although less studied compared to the other strains, has shown promise in inhibiting the growth of pathogenic microorganisms, particularly by producing antimicrobial substances such as organic acids and bacteriocins.

Research suggests that *L. fermentum* may play a synergistic role when combined with other *Lactobacillus* species, enhancing their overall protective effect against vaginal infections. The synergistic interactions between these *Lactobacillus* species are essential for maintaining a healthy vaginal microbiota and preventing infections (4). When used in combination, these strains can enhance each

other's probiotic effects, creating a more robust defense against pathogenic bacteria. The combined use of multiple *Lactobacillus* strains, such as *L. acidophilus*, *L. rhamnosus*, *L. jensenii*, and *L. fermentum*, could provide a comprehensive approach to treating and preventing vaginal infections by maximizing the antimicrobial activity, immune modulation, and adherence properties of these bacteria. The synergistic effects of these strains are particularly relevant in addressing infections caused by multiple pathogens and in promoting long-term vaginal health.

Material and Methods

This study included 30 female participants aged 25 to 45 years diagnosed with vaginal infections, including bacterial vaginosis, vaginal candidiasis, and urinary tract infections caused by pathogens such as *Escherichia coli*, *Streptococcus agalactiae*, *Staphylococcus aureus*, *Candida albicans*, and *Gardnerella vaginalis*. Samples were collected using vaginal swabs, and pathogen identification was performed through classical culturing and PCR methods. Synergistic combinations of four *Lactobacillus* species (*Lactobacillus fermentum*, *Lactobacillus acidophilus*, *Lactobacillus jensenii*, and *Lactobacillus rhamnosus*) were tested, administered as vaginal capsules with a concentration of 10^8 CFU/ml, once daily for four weeks. Symptoms such as itching, discomfort, pain during sexual intercourse, pelvic pressure, unpleasant odor, irritation, increased discharge, and dryness were monitored using a standardized questionnaire before and after the treatment. Statistical data analysis was performed using SPSS software, with a significance threshold of $p < 0.05$.

Results

Table 1 presents the results of microbiological isolations in 30 participants for the presence of bacteria: *Escherichia coli*, *Streptococcus agalactiae*, *Staphylococcus aureus*, *Candida albicans*, and *Gardnerella vaginalis* before and after the use of *Lactobacillus*. The results are as follows: *Escherichia coli* was isolated in 10 participants, *Streptococcus agalactiae* in 7 participants, *Staphylococcus aureus* in 6 participants, *Candida albicans* in 8 participants, and *Gardnerella vaginalis* in 9 participants. Before the *Lactobacillus* treatment (*Lactobacillus fermentum*, *Lactobacillus acidophilus*, *Lactobacillus jensenii*, and *Lactobacillus rhamnosus*), the bacterial load was relatively high, with *Candida albicans* showing the highest colonization at 10^6 CFU/ml, followed by *Escherichia coli*, *Gardnerella vaginalis*, and *Staphylococcus aureus* at 10^5 CFU/ml, and *Streptococcus agalactiae* at 10^4 CFU/ml. After a four-week treatment with *Lactobacillus*, a significant reduction in infections was observed: *Escherichia coli*, *Streptococcus agalactiae*, *Gardnerella vaginalis*, and *Candida albicans* decreased to 10^2 CFU/ml, while *Staphylococcus aureus* decreased to 10^3 CFU/ml. The p-value was satisfactory and remained below $p < 0.05$.

Table 1: Microbiological isolation before and after treatment with Lactobacillus at 10⁸ CFU/ml.

Pathogen	Participants n=30	Value before treatment with Lactobacillus (X±SD) CFU/ml	Value before treatment with Lactobacillus (X±SD) CFU/ml	p- value	Significance (p<0.05)
Escherichia coli	10	105 ± 2.0 x 10 ⁴	102 ±1.2 x 10 ²	0.001	p<0.05
Streptococcus agalactiae	7	104 ± 1.5 x 10 ³	102 ± 1.0 x10 ¹	0.002	p<0.05
Staphylococcus aureus	6	105 ± 1.8 x 10 ⁴	103 ± 1.3 x10 ³	0.004	p<0.05
Candida albicans	8	106 ± 2.2 x 10 ⁵	102 ± 1.1 x10 ¹	0.001	p<0.05
Gardnerella vaginalis	9	105 ± 2.1 x 10 ⁴	102 ± 1.3 x 10 ²	0.002	p<0.05

Table 2: Symptoms before and after Lactobacillus 10⁸ CFU/ml treatment application.

Symptom	Participants n=30	Value before treatment with Lactobacillus (X±SD) CFU/ml	Value before treatment with Lactobacillus (X±SD) CFU/ml	value p-	Significance (p<0.05)
Itching	10	4.5 ± 0.8	1.2 ± 0.6	0.001	p<0.05
Discomfort	9	4.3 ± 0.7	1.5 ± 0.5	0.002	p<0.05
Pain during sexual intercourse	7	3.8 ± 0.9	2.0 ± 0.7	0.003	p<0.05
Pelvic pressure	8	4.0 ± .0	1.3 ± 0.4	0.002	p<0.05
Unpleasant odor	9	4.7 ± 0.6	2.0 ± 0.3	0.002	p<0.05
Increased discharge	8	4.2 ± 0.5	1.5 ± 0.8	0.002	p<0.05
Irritation	10	4.8 ± 0.7	1.3 ± 0.5	0.001	p<0.05
Increased discharge	7	3.5 ± 1.0	1.0 ± 0.6	0.002	p<0.05
Dryness	7	3.6 ± 0.9	1.5 ± 0.5	0.004	p<0.05

Conclusion

Based on the study results, it can be concluded that the application of Lactobacillus (*Lactobacillus fermentum*, *Lactobacillus acidophilus*, *Lactobacillus jensenii*, *Lactobacillus rhamnosus*) as a treatment for vaginal infections demonstrates a significant reduction in pathogen counts and alleviation of symptoms. Microbiological results show a significant decrease in the concentration of pathogens such as *Escherichia coli*, *Streptococcus agalactiae*, *Staphylococcus aureus*, *Candida albicans*, and *Gardnerella vaginalis* following a four-week treatment, with p-values indicating statistical significance (p<0.05). Symptomatic results also confirm the treatment's effectiveness, as symptoms such as itching, discomfort, irritation, unpleasant odor, pelvic pressure, increased discharge, and dryness were significantly reduced after the use of Lactobacillus. The p-values were within the bounds of statistical significance, confirming that the reduction in symptoms was a result of the treatment rather than random variation. Overall, the results suggest that Lactobacillus may be effective in reducing infections and symptoms associated with vaginal infections, providing a potential therapy for improving vaginal health and the quality of life for women experiencing these health issues.

Discussion

The findings of this study demonstrate that the application of Lactobacillus strains (*Lactobacillus fermentum*, *Lactobacillus*

acidophilus, *Lactobacillus jensenii*, and *Lactobacillus rhamnosus*) as a treatment for vaginal infections results in a significant reduction in pathogenic microorganisms and alleviation of associated symptoms. These results align with previous research highlighting the therapeutic potential of Lactobacillus in maintaining and restoring the balance of the vaginal microbiota. Lactobacillus species are known to play a critical role in maintaining vaginal microbiota homeostasis by producing lactic acid, hydrogen peroxide, and bacteriocins, which create an unfavorable environment for pathogenic microorganisms. For instance, studies by (5) and (6) confirm that Lactobacillus strains exhibit inhibitory effects against common pathogens, including *Escherichia coli*, *Gardnerella vaginalis*, and *Candida albicans*. These effects are attributed to their ability to lower vaginal pH and compete for adhesion sites on epithelial cells, which is consistent with the reduction of pathogenic load observed in this study. In this study, significant reductions were observed in pathogens such as *Escherichia coli* (10⁵ to 10² CFU/ml), *Streptococcus agalactiae* (10⁴ to 10² CFU/ml), and *Candida albicans* (10⁶ to 10² CFU/ml).

These findings are supported by the work of (7), who demonstrated that Lactobacillus supplementation reduces colonization by *Gardnerella vaginalis* and other pathogens associated with bacterial vaginosis. Similarly, studies by (8) emphasize the role of *Lactobacillus rhamnosus* in reducing the recurrence of bacterial vaginosis and candidiasis, further

corroborating the results of this study. The symptomatic relief reported in this study also aligns with existing literature. Symptoms such as itching, irritation, unpleasant odor, and increased discharge were significantly alleviated following *Lactobacillus* treatment. According to a study by Van (9), restoring *Lactobacillus* dominance in the vaginal microbiota leads to the resolution of symptoms commonly associated with bacterial vaginosis and vulvovaginal candidiasis. The reduction in irritation (mean 4.8 ± 0.7 to 1.5 ± 0.4) and unpleasant odor (mean 4.7 ± 0.6 to 1.3 ± 0.3) observed in this study mirrors findings in randomized controlled trials, such as those conducted by where probiotics improved both microbiological and clinical outcomes. One of the key strengths of this study is its focus on the synergistic effects of multiple *Lactobacillus* strains. Unlike single-strain approaches, multi-strain probiotics have been shown to exhibit broader antimicrobial activity and enhanced colonization ability.

For instance, (10) emphasized that combining strains such as *Lactobacillus rhamnosus* and *Lactobacillus acidophilus* enhances both antimicrobial properties and adhesion to epithelial cells, thereby increasing the effectiveness of probiotic therapy. While the results of this study are promising, it is essential to address potential limitations. The study included a relatively small sample size of 30 participants, which may limit the generalizability of the findings. Additionally, the study relied on self-reported symptom relief, which, although a common approach in clinical research, introduces a level of subjectivity. Future studies with larger sample sizes and more objective outcome measures, such as molecular quantification of *Lactobacillus* strains post-treatment, are recommended to further validate these findings. In conclusion, this study contributes to the growing body of evidence supporting the use of *Lactobacillus* as an effective treatment for vaginal infections. The significant reduction in pathogenic bacteria and relief of symptoms observed underscore the potential of multi-strain *Lactobacillus* formulations as a therapeutic strategy for improving vaginal health. These findings may have implications for developing targeted probiotic therapies aimed at reducing the prevalence and recurrence of vaginal infections, ultimately enhancing the quality of life for affected women.

Acknowledgement

None.

Conflict of Interest

No conflict of interest.

References

1. Chee WJY, Chew SY, Than LTL (2020) Vaginal microbiota and the potential of *Lactobacillus* derivatives in maintaining vaginal health. *Microbial Cell Factories*, 19(1): 203.
2. Choi SI, Won G., Kim Y, Kang CH, Kim GH (2022) *Lactobacilli* strain mixture alleviates bacterial vaginosis through antibacterial and antagonistic activity in *Gardnerella vaginalis*-infected C57BL/6 mice. *Microorganisms*, 10(2): 471.
3. Fuochi V, Cardile V, Petronio G, Furneri PM (2019) Biological properties and production of bacteriocins-like-inhibitory substances by *Lactobacillus* sp. strains from human vagina. *Journal of Applied Microbiology*, 126(5): 1541-1550.
4. Kumerová M, Veselá K, Kosová M, Mašata J, Horáčková Š, et al. (2021) Novel potential probiotic *Lactobacilli* for prevention and treatment of vulvovaginal infections. *Probiotics and Antimicrobial Proteins*, 13: 163-172.
5. Lin TC, Hsu IL, Tsai WH, Chu YC, Kuan LC, et al. (2021) Improvement of bacterial vaginosis by oral *Lactobacillus* supplement: A randomized, double-blinded trial. *Applied Sciences*, 11(3): 902.
6. Liu P, Lu Y, Li R, Chen X (2023) Use of probiotic *Lactobacilli* in the treatment of vaginal infections: In vitro and in vivo investigations. *Frontiers in Cellular and Infection Microbiology*, (13): 1153894.
7. Ouarabi L, Chait Y, Seddik HA, Drider D, Bendali F (2019) Newly isolated *Lactobacilli* strains from Algerian human vaginal microbiota: *Lactobacillus fermentum* strains relevant probiotic's candidates. *Probiotics and Antimicrobial Proteins*, 11: 43-54.
8. Singhal L, Gupta V, Gupta M, Goel P, Chander J (2020) Identification and Sensitivity of Vaginal and Probiotic *Lactobacillus* species to Urinary Antibiotics. *Journal of Laboratory Physicians*, 12(02): 111-114.
9. Superti F, De Seta F (2020) Warding off recurrent yeast and bacterial vaginal infections: Lactoferrin and *Lactobacilli*. *Microorganisms*, 8(1): 130.
10. van de Wijgert JH, Verwijs MC (2020) *Lactobacilli*-containing vaginal probiotics to cure or prevent bacterial or fungal vaginal dysbiosis: a systematic review and recommendations for future trial designs. *BJOG: An International Journal of Obstetrics and Gynaecology*, 127(2): 287-299.