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Research Article

Evaluate the Effectiveness and Safety of Probiotic Blend of Strains on the Clinical and Biological Parameters of Women with Urinary Tract Infection: A Randomized Controlled Study

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Abstract

Recurrent urinary tract infections (UTIs) are a significant therapeutic challenge, particularly for women, necessitating prolonged pharmacological treatment that contributes to antibiotic resistance. Probiotics have recently become more popular as a non-antibiotic choice because of its potential. In this randomized controlled trial, women with a history of recurrent urinary tract infections (rUTIs) were assigned to receive probiotic supplements or a placebo for 90 days. The outcomes measured were the number of symptomatic UTIs, vaginal pH, metabolic indicators, reproductive hormones, stress markers, and inflammatory markers. They also looked at how safe and tolerable it was. Probiotic medication significantly decreased recurrent UTIs (Δ –2.8, $p < 0.0001$) and vaginal pH (–0.9, $p < 0.0001$) in comparison to placebo. The lipid profile (total cholesterol, HDL-C), fasting glucose, and CRP all got better. Cortisol levels fell sharply, which means that the HPA axis was not working. There were small changes in the hormones FSH, LH, and estradiol. The adverse events in the probiotic group were less severe and less frequent than those in the placebo group. Probiotic supplementation is a safe and effective method for preventing recurrent urinary tract infections (rUTIs), while also offering additional systemic benefits for metabolism, inflammation, and stress physiology. There is a necessity for more extensive, longitudinal studies.

Keywords: Probiotics; Recurrent Urinary Tract Infections; Vaginal pH; Inflammation; Cortisol; Women's Health

Abbreviations

UTIs: Urinary Tract Infections; rUTIs: Recurrent Urinary Tract Infections; VHBAX: Vidya Herbs *Bacillus coagulans*; VHBCSI: Vidya Herbs *Bacillus clausii*; VHBSUB: Vidya Herbs *Bacillus subtilis*; NS: Not Significant; TEAEs: Treatment-Emergent Adverse Events; AEs: Adverse Events; SAEs: Serious Adverse Events; CTRI: Clinical Trials Registry of India; GCLP: Good Clinical Laboratory Practice; ICH: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; ICMR: Indian Council of Medical Research; USA: United States of America; SAS®: Statistical Analysis System; AFC: Antral Follicle Count; ENT: Ear, Nose, and Throat; pH: Potential of Hydrogen; HDL-C: High-Density Lipoprotein Cholesterol; CRP: C-Reactive Protein; HPA: Hypothalamic–Pituitary–Adrenal; FSH: Follicle-Stimulating

Hormone; LH: Luteinizing Hormone; WBC: White Blood Cell Count; RBC: Red Blood Cell Count; PCV: Packed Cell Volume; ESR: Erythrocyte Sedimentation Rate; SGOT: Serum Glutamic Oxaloacetic Transaminase; SGPT: Serum Glutamic Pyruvic Transaminase.

Introduction

Recurrent urinary tract infections (rUTIs) are prevalent bacterial infections in women, affecting 50–60% at least once in their lifetime, with recurrence rates of 25–30% within six months [1,2]. Recurrent urinary tract infections (rUTIs) not only reduce quality of life but also impose a substantial economic burden due to frequent healthcare visits, antibiotic prescriptions, and decreased work productivity [3]. Modern preventive and therapeutic strategies mostly rely on continuous or post-coital antibiotic prophylaxis. Long-term use of antibiotics, while effective, is associated with the emergence of antimicrobial resistance and the disturbance of the gastrointestinal and vaginal micro-biota [4,5]. This highlights the need for the creation of non-antibiotic treatments that are effective, long-lasting, and safe for long-term use.

The vaginal microbiome is playing crucial in maintain urogenital health of women. A healthy female body is rich source of Lactobacillus species, which synthesis lactic acid, hydrogen peroxide and bacteriocins that regulate the low pH of vagina and prevent the uropathogens like Escherichia coli from growing [6,7]. Dysbiosis, characterized by diminished lactobacilli and elevated pH, predisposes women to recurrent urinary tract infections (rUTIs) [8]. A multitude of clinical trials and systematic reviews validate Lactobacillus strain's prophylactic role in the restoration of vaginal microbial equilib-rium and prevention of recurrence urinary tract infections [9,10]. Probiotics confer systemic impact on the vaginal flora by helping in lipid metabolism [11], lower systemic inflammation, including C-reactive protein (CRP) [12], enhance glycaemia management [13], and control the activity of the hypothalamic-pituitary-adrenal (HPA) axis, which leads to lower cortisol and stress responses [14].

Additionally, new research suggests that probiotics may influence the regulation of reproductive hormones, namely the dynamics of follicle-stimulating hormone (FSH) and luteinising hormone (LH) [15]. Despite these promising findings, there is a lack of comprehensively evaluated clinical trials urogenital and systemic outcomes of probiotic therapy in women with recurrent urinary tract infections (rUTIs). Most of the studies done have focused on preventing recurrences and finding the specific systemic effects. This has underrated the total impact of probiotics therapy. Consequently, this ran-domised controlled study was designed to assess the efficacy of probiotics in preventing symptomatic recurrent urinary tract infections (rUTIs), while simultaneously evaluat-ing their effects on gynecological, metabolic, hormonal, and inflammatory outcomes in women predisposed to recurrence.

Study objectives

- To evaluate effectiveness of Probiotic Blend of strains VHBSUB, VHBCSI, VHBAX on the clinical and biological parameters of women with urinary tract infection.
- To evaluate safety of Probiotic Blend of strains VHBSUB, VHBCSI, VHBAX on the clinical and biological parameters of women with urinary tract infection.

Materials and Methods

Study design and population

An, randomized, double blind, placebo controlled, parallel, multicenter, phase 3 trial with 60 patients with history of recurrent UTIs was conducted at Rajalakshmi Hospital, Bangalore and Sunshine Hospital, Bangalore (Figure 1). The randomization schedule was generated with the SAS® software, Version 9.4 or higher version of SAS Institute Inc., USA. All patients were randomized (1:1) to receive either the Blend of strains VHBSUB, VHBCSI, VHBAX (treatment group) or the placebo (placebo group) for 60 days.

Ethical conduct of the study

The study was conducted as per the National Ethical Guidelines for Biomedical and Health Research involving Human participants ICMR (2017), ICH (Step 5) 'Guidance on Good Clinical Practice', New Drugs and Clinical Trials Rules 2019 G.S.R. 227(E) dated 19 Mar 2019, 'Good Laboratory Practice', 'Good Clinical Practices for Clinical Research in India' Guidelines, Good Clinical Laboratory Practice (GCLP) and Declaration of Helsinki (Fortaleza, October 2013). CTRI NUMBER: CTRI/2024/03/064088.

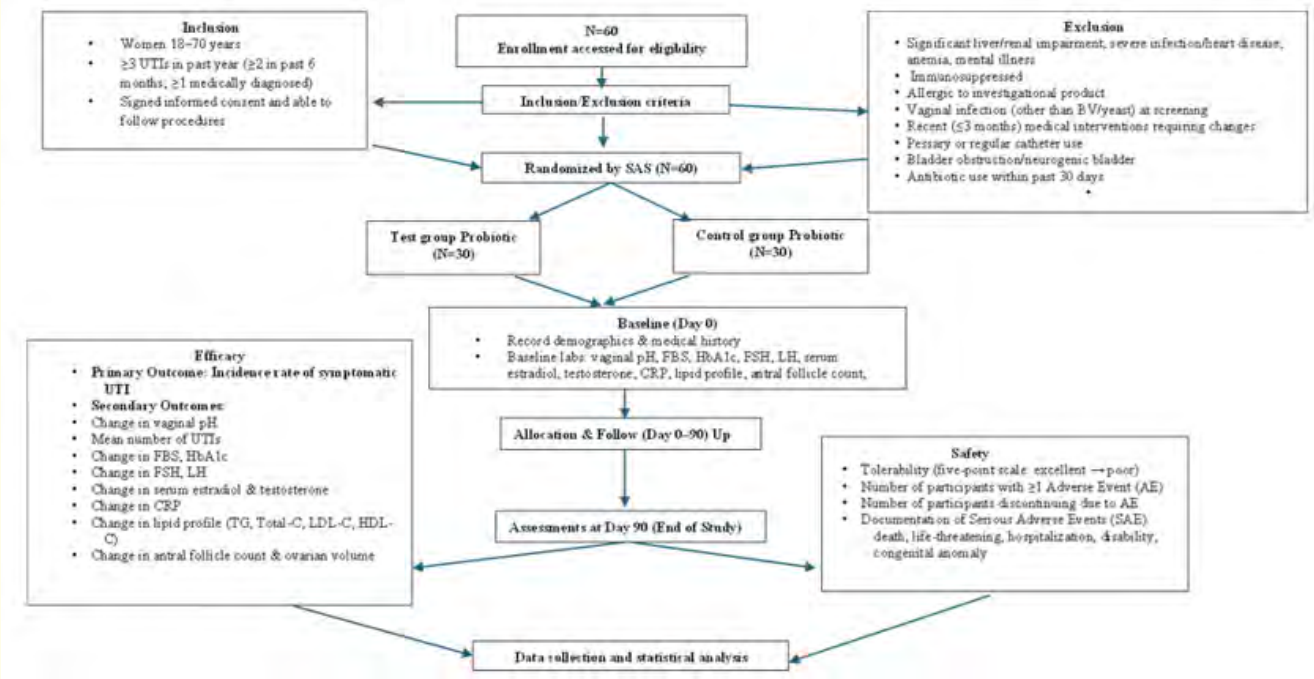

Figure 1: Investigational Plans.

Patient information and consent

All patients provided written informed consent to participate in the study before being screened. The original informed consent documents were kept in a confidential file in the Investigators' site record.

Outcomes

- Primary outcomes: number of symptomatic UTI episodes, vaginal pH.
- Secondary outcomes: ovarian volume, antral follicle count, metabolic markers, reproductive hormones, CRP, cortisol, and safety outcomes.

Statistical methods

Data was analyzed with 5% significance level (confidence interval 95%) and maintaining a minimum power of 80% for study using SAS software, version 9.1. The differences within the groups were assessed using t-test, whereas independent t-test were used for differences between groups. Efficacy analysis was performed for the intention-to-treat population. Separate analyses were performed for primary and secondary endpoints. The entire statistical analysis was performed as per the statistical analysis plan. Baseline and demographic characteristics were summarized. Continuous variables were summarized as mean ±Standard deviation, while categorical data were summarized as the frequency (or count) for that category. The number of AEs and number and percentage of patients experiencing AEs were summarized by severity, and relation to study medication. SAEs and AEs leading to premature study withdrawal were summarized.

Results and Discussion

The present study evaluated the efficacy and safety of the blend of VHBAX, VHCSI, VHBSUB probiotics and placebo in women with recurrent urinary tract infections (rU-TIs). Out of total 60 enrolled female participants, two randomized groups—a probiotic treatment group and a placebo group—were assigned. Both groups were comparable at baseline (day 0), with no statistically significant differences in demographics, medical history, or clinical findings across multiple systems, including general appearance, cardiovascular, gastrointestinal, neurological, and urogenital systems [16]. The overall mean age was 47.8 years, and the mean body mass index (BMI) was 27.0 ± 7.97 kg/m^2 (Table 1). No participants reported tobacco or alcohol use.

Parameter	Group	Baseline Mean (SD)	Day 90 Mean (SD)	Mean Change (Δ)	95% CI of Change	p-value
Mean No. of UTIs	Probiotic	3.5 (0.51)	0.7 (0.48)	−2.8	−3.1 to −2.5	<0.0001
	Placebo	3.7 (0.47)	3.6 (0.45)	−0.1	−0.3 to +0.1	0.108
Vaginal pH	Probiotic	6.6 (0.25)	5.7 (0.50)	−0.9	−1.1 to −0.7	<0.0001
	Placebo	6.8 (0.28)	6.6 (0.46)	−0.2	−0.5 to +0.1	0.076
Ovarian Volume (mL)	Probiotic	11.1 (0.96)	11.7 (1.68)	0.6	+0.02 to +1.2	0.0468
	Placebo	11.6 (1.22)	11.7 (1.44)	0.1	−0.4 to +0.6	0.5993
Antral Follicle Count	Probiotic	12.7 (0.84)	11.9 (1.54)	−0.8	−1.3 to −0.2	0.0095
	Placebo	12.5 (0.57)	12.4 (1.48)	−0.1	−0.4 to +0.2	0.1487

Table 1: Effect of Probiotics and placebo on gynecological factor from baseline to Day 90.

Clinical efficacy outcomes

At Day 90, the probiotic group demonstrated a highly significant reduction in UTI symptom scores compared with baseline (3.5 ± 0.51 to 0.7 ± 0.48; $p < 0.0001$). In contrast, the placebo group showed no statistically significant improvement (3.7 ± 0.47 to 3.6 ± 0.45; $p = 0.108$). Furthermore, the mean number of UTI episodes during the intervention period was markedly lower in the probiotic group, confirming the clinical efficacy of the VHBAX, VHCSI, and VHBSUB probiotic blend (Figure 2).

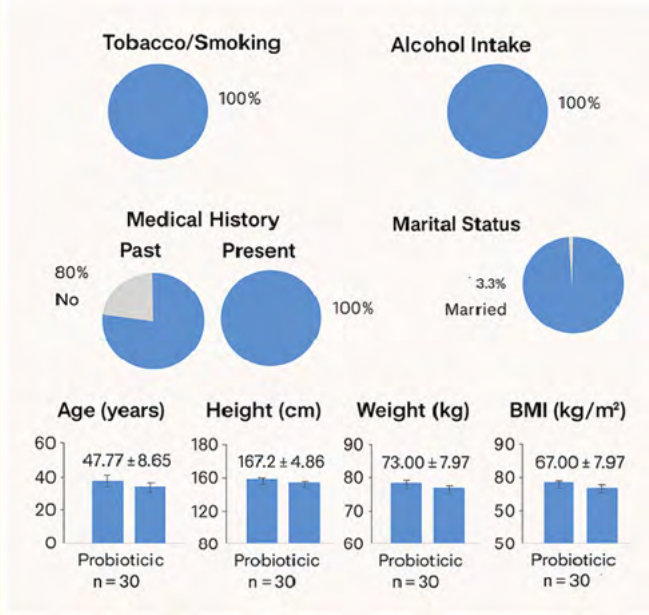

Figure 2: Demographics and Baseline Characteristics.

A significant reduction in vaginal pH (−0.9; $p < 0.0001$) was observed in the probiotic group, whereas no meaningful change occurred in the placebo group. The restoration of acidic vaginal pH likely reflects re-establishment of protective Lactobacillus-dominant microbiota, thereby limiting uropathogen colonization and recurrence. The probiotic group also exhibited notable improvements in reproductive health parameters, including a statistically significant increase in ovarian volume and a corresponding reduction in antral follicle count by the end of the 90-day study. The placebo group showed no substantial effect. These findings suggest potential of probiotics in regulating ovarian function; however, further larger studies are prerequisite.

Biochemical markers outcome

Metabolic and inflammatory markers

Triglycerides, total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol were compared between placebo and probiotic groups from baseline to trial conclusion (Table 2). Probiotics decreased total cholesterol ($p = 0.0198$) and enhanced HDL-C ($p < 0.0001$). The probiotic group also showed a significant decrease in fasting blood glucose levels ($p < 0.04$), while the placebo group showed no significant change ($p = 0.7202$).

Triglyceride and LDL levels remained stable throughout the study in both groups. The probiotic group demonstrated a highly significant reduction in C-reactive protein (CRP) levels, indicating an anti-inflammatory effect [17]. A statistically significant decrease in cortisol levels was also observed in the probiotic group, whereas

the placebo group experienced an increase in cortisol levels over the course of the study. Although lipid profile improvements were observed at certain time points, these were not sustained by the end of the study, suggesting that longer intervention periods may be required to fully realize probiotic benefits on lipid metabolism.

Parameter	Group	Baseline Mean ± SD (95% CI)	Day 90 Mean ± SD (95% CI)	p-value
Triglycerides (mmol/L)	Probiotic	135.2 ± 22.92 (126.7–143.7)	136.6 ± 27.21 (126.0–147.2)	0.7955
	Placebo	137.9 ± 18.24 (131.2–144.6)	132.4 ± 24.74 (123.2–141.6)	0.3776
Total Cholesterol (mmol/L)	Probiotic	185.0 ± 17.25 (178.6–191.4)	176.7 ± 11.25 (172.5–180.9)	0.0198
	Placebo	185.9 ± 13.57 (180.8–191.0)	180.7 ± 11.42 (176.4–185.0)	0.11
LDL-C (mmol/L)	Probiotic	107.2 ± 9.80 (103.6–110.8)	110.8 ± 17.10 (104.5–117.1)	0.2971
	Placebo	106.3 ± 9.71 (102.7–109.9)	109.9 ± 13.85 (104.7–115.1)	0.3999
HDL-C (mmol/L)	Probiotic	41.2 ± 6.72 (38.7–43.7)	47.7 ± 8.06 (44.7–50.7)	<0.0001
	Placebo	41.0 ± 6.05 (38.6–43.4)	45.5 ± 6.94 (42.8–48.2)	0.0001
Fasting Glucose (mg/dL)	Probiotic	112.2 ± 13.09 (107.4–117.0)	105.0 ± 11.07 (101.0–109.0)	0.04
	Placebo	106.6 ± 11.92 (102.4–110.8)	103.5 ± 9.75 (100.0–107.0)	0.27
FSH (mIU/L)	Probiotic	3.4 ± 0.69 (3.1–3.7)	4.0 ± 1.11 (3.6–4.4)	0.0385
	Placebo	3.2 ± 0.62 (3.0–3.4)	3.3 ± 1.00 (2.9–3.7)	0.6
LH (IU/L)	Probiotic	25.8 ± 2.60 (24.8–26.8)	27.5 ± 2.83 (26.4–28.6)	0.0158
	Placebo	24.7 ± 2.69 (23.6–25.8)	26.0 ± 3.63 (24.6–27.4)	0.0878
CRP (mg/L)	Probiotic	6.9 ± 5.23 (4.9–8.9)	4.3 ± 0.56 (4.1–4.5)	0.0002
	Placebo	6.5 ± 3.71 (5.1–7.9)	5.3 ± 2.68 (4.3–6.3)	0.25
Estradiol (pg/mL)	Probiotic	60.7 ± 14.88 (54.8–66.6)	55.0 ± 12.10 (50.4–59.6)	0.05
	Placebo	59.7 ± 16.10 (53.4–66.0)	59.3 ± 15.94 (53.0–65.6)	0.524
Cortisol (µg/dL)	Probiotic	15.3 ± 4.65 (13.5–17.1)	13.5 ± 4.38 (11.8–15.2)	<0.0001
	Placebo	15.3 ± 4.39 (13.6–17.0)	16.1 ± 2.33 (15.2–17.0)	0.38

Table 2: Evaluating the effect of Probiotics and placebo on Metabolism and hormones from baseline to Day 90.

Reproductive and endocrine parameters

The Probiotic group showed a substantial drop in serum estradiol levels, from 60.7±15.88 at baseline to 55.0±12.10 at the end of the investigation (p < Serum estradiol levels in the placebo group did not significantly alter, decreasing from 59.7±16.10 at baseline to 59.3±15.94 at the end of the study (p < 0.5240 From baseline to trial end, placebo and probiotic groups were compared for cortisol changes. In the Probiotic group, Cortisol levels changed by -1.8 ± 0.90, from 15.3 ± 4.65 at baseline to 13.5 ± 4.38 at the end of the trial. At the end of the research, the median Cortisol level was 14.8 (5.64, 20.89), down from 16.4 (8.21, 23.45). There was a significant decrease (p < 0.0001). For the placebo group, Cortisol levels climbed from 15.3 ± 4.39 at baseline to 16.1 ± 2.33 at the end of the research, with non-significant p-value of 0.38. The median baseline Cortisol level was 14.9 (8.35, 22.78) and the trial ended at 15.7 (9.40, 23.10). There was a significant rise (p < 0.0001).

These findings suggest a potential modulatory effect of probiotic supplementation on the hypothalamic–pituitary–ovarian axis. Emerging evidence indicates that gut microbiota contributes to estrogen metabolism and systemic hormonal regulation via the estrobolome pathway. The significant reduction in cortisol further supports involvement of the gut–brain axis, potentially mitigating stress-mediated immune suppression and inflammatory responses associated with recurrent infections.

Safety and tolerability

Biochemical and physical examination

Physical examination findings from baseline (Visit 0) to study completion (Visit 7) revealed no clinically significant differences between the probiotic and placebo groups across general, cardiovascular, respiratory, gastrointestinal, neurological, endocrine, and urogenital systems ($p > 0.05$), confirming overall clinical stability (Table 3).

Domain	Key Findings	Interpretation
UTI Outcomes	Probiotic: ↓; Placebo: NS	Significant ↓ UTIs with probiotic
Gynecological	Vaginal pH: ↓ with probiotics; Ovarian volume: ↑, NS; AFC: ↓, NS	Improved vaginal pH only
Metabolic	Probiotics: ↓ Total Cholesterol, ↑ HDL; Placebo: minor HDL rise only	Favorable effect of probiotics on lipids
Glucose	Probiotic: ↓; Placebo: slight, NS	Clinically meaningful reduction
Hormonal	FSH: ↑; LH: ↑; Estradiol: slight ↓, borderline	Mild reproductive axis modulation
Stress Marker	Cortisol: ↓ with probiotics; NS with placebo	Stress-modulating effect
Inflammatory	CRP: ↓ markedly with probiotics; NS with placebo	Strong anti-inflammatory effect
Safety	No adverse changes in labs, vitals, or organ function	Probiotics well tolerated

Table 3: Efficacy and Safety Outcomes.

Hematological parameters remained within physiological ranges in both groups. The probiotic group showed a slight increase in hemoglobin and RBC counts, whereas the placebo group demonstrated a mild decline. A more pronounced reduction in total WBC count and neutrophil percentage was observed in the probiotic group compared with placebo, accompanied by a modest increase in lymphocyte percentage. ESR decreased in both groups, with a relatively greater decline in the probiotic group, suggesting a potential reduction in systemic inflammatory burden. Other differential counts, including eosinophils and monocytes, showed minor fluctuations without clinical significance. Pregnancy tests remained negative throughout the study.

Biochemical parameters demonstrated good tolerability of the intervention. Liver enzymes (SGOT and SGPT) exhibited minimal, clinically insignificant increases in both groups and remained within normal reference limits. Serum creatinine levels slightly decreased in the probiotic group, indicating preserved renal function.

Vital signs including body temperature, pulse rate, systolic and diastolic blood pressure—remained stable in both groups, with only minor physiological fluctuations and no consistent adverse trends. Overall, the probiotic formulation was well tolerated and did not produce clinically meaningful alterations in hematological, hepatic, renal, or cardiovascular parameters. The observed reduction in WBC count and ESR in the probiotic group may reflect a modest anti-inflammatory effect, consistent with reductions in CRP reported elsewhere in the study. Importantly, no safety concerns emerged during the 90-day intervention period, supporting the favorable safety profile of the probiotic blend.

Changes in urine analysis

At screening, the probiotic group reported a marginally higher urine volume (24.17 ± 3.96 ml) than the placebo group (22.13 ±

3.62 ml). Both groups had shown similar specific gravity averages (1.02) with the minor variation in the probiotic group. Along with these, probiotic group exhibited a more stable urine pH (6.61 ± 0.27) than the placebo group (8.84 ± 11.46). Both the groups obeyed the healthy range color and clarity, and all of the biochemical markers—nitrate, glucose, protein, ketone bodies, bile salts, pigment, and urobilinogen. Microscopic examination revealed no abnormalities in either group. At Visit 7, the reported values for both groups: the mean volume was 25 ml, the specific gravity was 1.02, and the pH was a little lower (5.90 ± 0.61) in the probiotic group than in the placebo group (5.77 ± 0.54). The probiotic group had more clear samples, but the hue and transparency were the same. The biochemical and microscopic examinations did not reveal any significant differences between the groups during the investigation.

Adverse events

Mild adverse effects were reported in both the placebo and probiotic groups. In the placebo group, out of 60 female patients, 1 patient experienced symptoms of gastritis, while 1 patient reported a combination of bloating and gastritis (Figure 3). These symptoms were mild in nature. In the probiotic group, 1 female patient experienced nausea, and 1 reported symptom of gastritis, both of which were also mild. No patients in either group experienced severe treatment-emergent adverse events (TEAEs) or discontinued the study due to adverse effects. These findings confirm the favorable safety and tolerability profile of the probiotic formulation.

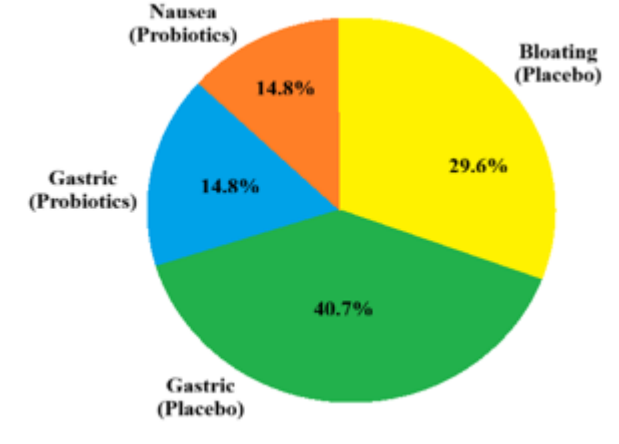

Figure 3: Adverse effect Distribution Probiotics vs Placebo.

The outcome of these is well aligning with existing literature on the safety and tolerability of probiotic interventions. In a 2024 randomized, double-blind, placebo-controlled trial found that oral and vaginal probiotic therapy in premenopausal women with recurrent UTIs reduced symptomatic UTIs more effectively than placebo [18]. Both mean recurrence and time to first recurrence improved significantly in probiotic groups. Similarly, in the Non-Antibiotic Prophylaxis for UTIs trial, postmenopausal women receiving Lactobacillus rhamnosus GR-1 and RC-14 experienced fewer UTIs (~3.3/year) compared to those receiving daily TMP-SMX (~2.9/year), without increased antibiotic resistance. A meta-analysis of nine clinical studies in-volving 726 women demonstrated that Lactobacilli products (oral or suppository) significantly reduced recurrent UTI episodes (pooled incidence ratio ~0.68; 95% CI 0.44–0.93; $p < 0.001$), though strains, durations, and follow-up periods varied [19]. In contrast, a systematic review of three RCTs (~284 premenopausal women) found no significant difference in UTI recurrence rates between probiotics and placebo (RR 0.59; 95% CI 0.26–1.33; $p = 0.20$), likely due to small sample size and protocol variability [20].

Conclusion

Probiotic treatment demonstrated exceptional efficacy in reducing symptomatic UTIs with improving inflammatory and stress biomarkers compared to placebo. Significant benefits were also observed in ovarian volume and antral follicle count, suggesting potential reproductive health advantages. Improvements in CRP and cortisol levels underscore the anti-inflammatory and stress-modulating effects of probiotics. While changes in lipid profiles were transient, the overall safety and tolerability of the intervention were confirmed. These findings highlight the therapeutic potential of probiotics as a safe and effective intervention for managing UTIs and improving systemic health parameters in women. Further studies with larger sample sizes and longer durations are warranted to validate these results and explore the underlying mechanisms of probiotic action in a broader population.

Conflict of Interest

This research received no external funding.

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