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ROLE OF *LACTOBACILLUS RHAMNOSUS* PROBIOTIC IN THE TREATMENT AND PREVENTION OF RECURRENCE OF BACTERIAL VAGINOSIS

[*1]Haider B. Al- Shammaa, [2]Reem Mohammed Badea Al-Badri, [3]Aminah Hilal Khallaf

[1]College of Medicine, University of Baghdad, Baghdad, Iraq.
[2]Al-Rusafa Health Directorate, Baghdad, Iraq.
[3]Al-Rusafa Health Directorate, Baghdad, Iraq.

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***Corresponding Author: Haider B. Al- Shammaa**

College of Medicine, University of Baghdad, Baghdad, Iraq.

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ABSTRACT

Background: Lactobacilli dominate the vaginal microbiota and are essential to its health. Various causes can reduce lactobacilli, allowing harmful bacteria to thrive and cause bacterial vaginosis. BV causes most vaginal discharge and odor in reproductive-age women and is associated with considerable morbidity. Several recent clinical trials have examined whether oral or intravaginal lactobacilli with or without antibiotics can enhance treatment outcomes and prevent BV recurrence. This study examined how Lactobacillus rhamnosus treats and prevents bacterial vaginosis. **Patients and Methods:** Using Amsel's criteria and Nugent score, 60 BV patients were included. Two groups of patients were formed. Group 1 got oral metronidazole 500 mg twice daily and vaginal capsules with L. rhamnosus (10^{10} CFU) twice daily for seven days. Only seven days of oral metronidazole 500 mg twice daily was given to Group 2. Patients were examined after 14 days using Amsel's criteria and Nugent score to reflect symptom improvement and satisfaction. After one month, patients were called to assess recurrence. **Results:** After two weeks, the probiotic plus metronidazole group exhibited higher improvement, with lower vaginal pH (−1.2 vs −0.5) and mean Amsel score (−2.5 vs −1.6). Post-treatment vaginal discharge did not differ between groups. Better patient satisfaction and symptom relief were in the probiotic group. After one month, group 1 (10.7%) had less recurrence than group 2 (70%). The higher cost diminished cost-effectiveness notwithstanding these gains. **Conclusion:** The findings suggest that *Lactobacillus rhamnosus* may enhance treatment response and reduce recurrence of bacterial vaginosis, although further studies are needed to confirm its cost-effectiveness.

KEYWORDS: *Lactobacillus, rhamnosus,* Probiotic, Recurrence, Bacterial, Vaginosis.

INTRODUCTION

The vagina is a distensible muscular tube extending from the introitus to the cervix and supported by the pelvic floor. It measures approximately 8 cm in length, with the uterine cervix projecting into its upper portion, forming the anterior, posterior, and lateral fornices. The vaginal wall consists mainly of muscular layers and is lined by non-keratinized stratified squamous epithelium. During the reproductive years, estrogen stimulates glycogen accumulation within the epithelial cells, which plays an essential role in maintaining the vaginal microenvironment and supporting the normal microbiota.[1,2] The vaginal microbiota, first described by Albert Döderlein in 1892, consists of diverse microorganisms that colonize the lower genital tract. In healthy women, the flora is dominated by *Lactobacillus* species, which are crucial for maintaining vaginal health.[3] These bacteria produce lactic acid through the metabolism of glycogen released from vaginal epithelial cells, thereby maintaining an acidic vaginal pH (approximately 3.5–4.5). This acidic environment inhibits the growth of pathogenic microorganisms and contributes to the natural defense of the female genital tract.[4,5] Several *Lactobacillus* species such as *L. crispatus*, *L. gasseri*, *L. iners*, and *L. jensenii* are commonly identified as dominant members of the

vaginal microbial community in reproductive-age women.[6,7] Lactobacilli protect the vaginal environment through multiple mechanisms. These include the production of lactic acid, hydrogen peroxide, and antimicrobial peptides such as bacteriocins, all of which contribute to inhibition of pathogenic bacteria. Lactic acid is particularly important in maintaining vaginal acidity and exerting antimicrobial activity against sexually transmitted pathogens. Some strains of lactobacilli also produce hydrogen peroxide, which has been proposed to further inhibit the growth of organisms associated with vaginal infections.[8,9] However, disruption of the balance between protective lactobacilli and other microorganisms can lead to vaginal dysbiosis and disease. Bacterial vaginosis (BV) is the most common cause of abnormal vaginal discharge in women of reproductive age. It is characterized by a reduction in lactobacilli and an overgrowth of anaerobic bacteria such as *Gardnerella vaginalis*, *Prevotella*, *Mobiluncus*, and *Atopobium* species.[10,11] This imbalance results in an elevated vaginal pH (>4.5), increased production of amines and short-chain fatty acids, and the characteristic fishy vaginal odor associated with BV. Microscopically, the presence of clue cells—vaginal epithelial cells covered with adherent bacteria—is a hallmark finding in BV.[12] The prevalence of BV varies globally, ranging from about 5% in asymptomatic populations to more than 50% in certain regions. It is associated with several risk factors including sexual behavior, smoking, vaginal douching, and intrauterine device use.[13] Diagnosis is commonly based on Amsel's criteria or Gram-stained smear scoring systems such as the Nugent score.[14] Although standard treatment with antibiotics such as metronidazole or clindamycin is generally effective, recurrence rates remain high. Consequently, interest has increased in alternative strategies such as probiotic therapy aimed at restoring normal vaginal flora and preventing recurrent BV.[15] The aim of the current study is to assess the role of the probiotic rhamnosus in the treatment and the prevention of recurrence of bacterial vaginosis.

METHOD

This study included sixty women attending the outpatient clinic of Baghdad Teaching Hospital who presented with complaints of vaginal discharge with or without unpleasant vaginal odor. The participants were married women of reproductive age. Women who were unmarried, divorced, or separated were excluded due to cultural considerations regarding sexual activity outside marriage. Additional exclusion criteria included pregnancy, postmenopausal status, known allergy to metronidazole, and the presence of any pathological condition involving the cervix or uterus. A detailed clinical history was obtained from all participants, including age, duration of marriage, past medical and surgical history, medication history, drug allergies, obstetric and gynecological history, sexual history, and the characteristics of vaginal discharge. Information about the onset of symptoms, whether the episode was the first or recurrent within the same year, duration of symptoms, color and quantity of discharge, presence of odor, and associated symptoms was also documented. Most patients belonged to the same ethnic background and had minimal or no smoking exposure. All patients underwent clinical examination to assess bacterial vaginosis using **Amsel's criteria**. The examination included assessment of thin greyish-white homogeneous vaginal discharge, measurement of vaginal pH using pH paper, and performance of the whiff test by adding 10% potassium hydroxide (KOH) to the vaginal discharge to detect a characteristic fishy odor. A high vaginal swab was collected for wet mount microscopy to identify clue cells. Each patient was assigned a score out of four based on Amsel's criteria. A second-high vaginal swab was obtained for Gram staining, and the **Nugent scoring system** was used to evaluate the vaginal flora, giving each patient a score out of ten. The sixty patients were then divided into two groups. Group 1 received oral metronidazole 500 mg twice daily for seven days along with intravaginal capsules containing *Lactobacillus rhamnosus* (10^{10} CFU) twice daily for seven days after receiving explanation about probiotic therapy and providing verbal consent. Group 2 received oral metronidazole 500 mg twice daily for seven days only. Patients were reassessed after 14 days using Amsel's criteria and Nugent scoring, and their symptom improvement and treatment satisfaction were recorded. Follow-up was conducted after 1–2 months by telephone to evaluate recurrence of symptoms and overall satisfaction with treatment. Each participant was assigned a serial identification number, and data were analyzed using SPSS version 20. Categorical variables were presented as frequencies and percentages, while continuous variables were expressed as mean ± standard deviation. Independent t-test was used to compare means between the two treatment groups, and paired t-test assessed changes within groups over time. Generalized Estimating Equations (GEE) evaluated the effects of time and treatment regimen, with $\mathbf{p < 0.05}$ considered statistically significant.

RESULTS

The baseline characteristics of patients in both groups were generally comparable. There were no statistically significant differences between the probiotic plus metronidazole group and the metronidazole-only group regarding age, duration of marriage, and parity ($p>0.05$). However, the number of abortions was significantly higher in group 2 compared with group 1 ($p=0.005$). as in table 1.

Table 1: Comparison of main criteria of the studied patients.

Variables	Group1 N=29 Mean±SD	Group2 N=30 Mean±SD	p-value
Age	33±6.9	32.8±6.8	0.896 *ns*
Duration of marriage	10.8±7	9±5.9	0.301 *ns*
Parity	3.6±2.5	2.7±2.1	0.121 *ns*
Abortion	1.5±0.6	4.5±0.7	0.005*
SD= Standard deviation, *ns*=not significant, *Significant at 0.05 level by Independent t-test			

Before starting treatment, most laboratory parameters of bacterial vaginosis were similar between the two groups. There were no significant differences in Amsel score or Gram stain results (p>0.05). However, vaginal pH was significantly higher in group 1 than group 2 (p=0.003). as in table 2.

Table 2: Comparison of vaginal discharge tests before starting treatment regimen, according to randomization in groups.

Parameters of vaginal discharge	Group1 N=29 Mean±SD	Group2 N=30 Mean±SD	p-value
PH level	5.3±0.7	4.7±0.7	0.003*
AMSEL score	3.8±0.4	3.6±0.5	0.096 *ns*
Gram stain	8.7±1.2	9±0.8	0.239 *ns*
SD= Standard deviation, ns=not significant, *Significant at 0.05 level by Independent t-test			

After two weeks of treatment, the probiotic plus metronidazole group showed significantly better improvement compared with the metronidazole-only group. Vaginal pH and Amsel score were significantly lower in group 1 (p=0.019 and p=0.009 respectively), while Gram stain scores showed no significant difference between the two groups. As in table 3.

Table 3: Comparison of vaginal discharge tests after 2 weeks of starting treatment regimen, according to medications.

Parameters of vaginal discharge	Group1 N=29 Mean±SD	Group2 N=29 Mean±SD	p-value
PH level	4±0.3	4.3±0.5	0.019*
AMSEL score	1.4±1	2.1±1	0.009*
Gram stain	3.5±1.7	3.7±2.2	0.698 *ns*
SD= Standard deviation, ns=not significant, *Significant at 0.05 level by Independent t-test			

Following treatment, there was no significant difference between the two groups in the persistence of vaginal discharge. However, bad vaginal odor, whiff test positivity, patient satisfaction, and recurrence of infection after one month were significantly lower in the probiotic-treated group (p<0.001). as in table 4.

Table 4: Comparison of signs and tests for bacterial vaginosis after starting of treatment regimen, according to medications.

Variables	Group1 N=29 No. (%)	Group2 N=29 No. (%)	Total N=58 No. (%)	p-value
Vaginal Discharge after 2 weeks [a]	14 (48.3%)	12 (40%)	26 (44.1%)	0.522 *ns*
Bad vaginal odor after 2 weeks [a]	5 (17.2%)	20 (66.7%)	25 (42.4%)	<0.001*
Whiff test after 2 weeks	6 (21.4%)	21 (70%)	27 (46.6%)	<0.001*
Wet test after 2 weeks	14 (50%)	20 (66.7%)	34 (58.6%)	0.198 *ns*
satisfaction after 2 weeks	26 (89.7%)	9 (30%)	35 (59.3%)	<0.001*
Recurrence of infection after one month	3 (10.7%)	21 (70%)	24 (41.4%)	<0.001*
Satisfaction after one month	25 (89.3%)	7 (23.3%)	32 (55.2%)	<0.001*
ns=not significant, *Significant at 0.05 level by Chi-square test [a] Counts of Group2 and total are 30 & 59 respectively				

Overall comparison before and after treatment demonstrated significant improvement in vaginal pH, Amsel score, and Gram stain score after therapy (p<0.001), indicating effective treatment of bacterial vaginosis. As in table 5.

Table 5: Comparison of vaginal discharge tests before and then after 2 weeks of starting treatment regimen.

Parameters of vaginal discharge	Visit 1 N=58 Mean±SD	Visit 2 N=58 Mean±SD	p-value
PH level	5 ± 0.8	4.1 ± 0.4	<0.001*
AMSEL score	3.7 ± 0.4	1.7 ± 1.1	<0.001*
Gram stain	8.8 ± 1	3.6 ± 2	<0.001*
SD= Standard deviation, *Significant at 0.05 level by paired t-test			

The Generalized Estimating Equations analysis showed that both treatment regimen and time had significant effects on several bacterial vaginosis outcomes, particularly bad vaginal odor, recurrence of infection, and patient satisfaction, confirming the beneficial impact of probiotic therapy combined with metronidazole. As in table 6.

Table 6: Generalized Estimating Equations, assessing the effects of time & treatment regimen on bacterial vaginosis prognosis.

Variables	Group1 No. (%)	Group2 No. (%)	p-value (Regimen) OR (95% CI)	p-value (time) OR (95% CI)
Vaginal Discharge[1]	27 (93.1%)	30 (100%)	0.645	<0.001*
Vaginal Discharge[2]	14 (48.3%)	12 (40%)	1.29 (0.44-3.74)	0.03 (0.01-0.13)
Bad vaginal odor[1]	28 (96.6%)	29 (96.7%)	0.001*	<0.001*
Bad vaginal odor[2]	5 (17.2%)	20 (66.7%)	0.13 (0.04-0.45)	0.02 (0-0.1)
Whiff test[1]	29 (100%)	30 (100%)	0.001*	-
Whiff test[2]	6 (21.4%)	21 (70%)	0.13 (0.04-0.43)	-
Wet test[1]	28 (96.6%)	29 (96.7%)	0.257	<0.001*
Wet test[2]	14 (50%)	20 (66.7%)	0.53 (0.18-1.58)	0.05 (0.01-0.2)
Recurrence of infection[1]	24 (82.8%)	21 (70%)	0.015*	0.001*
Recurrence of infection[2] (1 month)	3 (10.7%)	21 (70%)	0.33 (0.13-0.81)	0.2 (0.08-0.51)
Satisfaction[1] (2 weeks)	26 (89.7%)	9 (30%)	<0.001*	0.391
Satisfaction[2] (1 month)	25 (89.3%)	7 (23.3%)	24.77 (6.32-97.03)	0.8 (0.48-1.34)
Parameters	**Group1 Mean±SD**	**Group2 Mean±SD**	**p-value (Regimen) OR (95% CI)**	**p-value (time) OR (95% CI)**
PH[1]	5.3±0.7	4.7±0.7	0.203	<0.001*
PH[2]	4±0.3	4.3±0.5	1.17 (0.92-1.5)	0.42 (0.34-0.5)
AMSEL[1]	3.8±0.4	3.6±0.5	0.112	<0.001*
AMSEL[2]	1.4±1	2.1±1	0.78 (0.57-1.06)	0.14 (0.1-0.18)
Gram stain[1]	8.7±1.2	9±0.8	0.363	<0.001*
Gram stain[2]	3.5±1.7	3.7±2.2	0.77 (0.45-1.34)	0.01 (0-0.01)
[1] before treatment, [2]After 2 weeks of treatment OR= odds ratio, CI= Confidence interval *Significant at 0.05 level by Generalized Estimating Equations (GEE)				

DISCUSSION

Bacterial vaginosis (BV) is the most common cause of abnormal vaginal discharge and unpleasant vaginal odor among women of reproductive age. Although many cases are asymptomatic, recurrence rates remain high, reaching approximately 58% within 12 months after treatment, which represents a major challenge in clinical management.[16] In recent years, several clinical trials have investigated whether specific strains of *Lactobacillus* administered orally or intravaginally, either alone or in combination with antibiotics, could improve treatment outcomes and reduce recurrence of BV. Although many of these studies were relatively small, they demonstrated the potential of probiotic therapy to restore vaginal flora and improve clinical outcomes. In the present study, patients treated with probiotics plus metronidazole (group 1) demonstrated better therapeutic response compared with those treated with metronidazole alone (group 2). This improvement was reflected by a greater reduction in vaginal pH and Amsel's score after two weeks of treatment (−1.2 and −2.5 respectively in group 1 versus −0.5 and −1.6 in group 2). These findings are consistent with the results of a study conducted by Taheri in Shiraz University, which reported greater reduction in vaginal pH among women treated with probiotics compared with those receiving metronidazole alone, with pH <4.5 observed more frequently in the probiotic-treated group.[17] In the current study, no significant difference was observed between the two groups regarding persistence of vaginal discharge two weeks after treatment, with 48.3% of patients in group 1 and 40% in group 2 still reporting discharge. However, patients in the probiotic-treated group reported higher satisfaction and better overall symptom improvement, which was reflected by better Amsel's scores, although these differences did not reach

statistical significance. Furthermore, bad vaginal odor was less frequent in the probiotics plus metronidazole group (17.2%) compared with the metronidazole-only group (66.7%). The findings of the present study differ from those reported by Neri et al., who observed subjective clinical improvement in 100% of patients receiving probiotic therapy within two days of treatment initiation.[18] This discrepancy may be attributed to differences in the study population, as Neri et al. investigated pregnant women with BV during the first trimester, whereas pregnant women were excluded from the present study. Nevertheless, patient satisfaction in the probiotic-treated group remained high in the current study, with 89.7% reporting improvement within the first days of treatment and 89.3% remaining free of symptoms after one month. In contrast, only 30% of patients in the metronidazole-only group were satisfied with treatment after two weeks, and only 23.3% remained symptom-free after one month. Regarding treatment success based on Amsel's criteria, absence of BV after two weeks was observed in 62% of patients in the probiotic plus metronidazole group compared with 31% in the metronidazole-only group. These results are comparable with those reported by Parent et al., who found a BV cure rate of 63.3% in the lactobacilli-treated group compared with 10% in the placebo group.[19] Similarly, Anukam et al. reported a cure rate of 65% in patients treated with lactobacilli compared with 33% in those treated with metronidazole alone.[20] In contrast, the absence of BV according to Nugent scoring showed less difference between groups, with 65.5% in the probiotic-treated group and 58.6% in the metronidazole group. Similar findings were reported by Hallén et al., who found no significant difference between lactobacilli-treated and placebo groups using Nugent scoring.[21] This discrepancy may be explained by the fact that Amsel's criteria consider both clinical symptoms and vaginal environment, whereas Nugent scoring focuses primarily on bacterial morphotypes. Recurrence of BV symptoms occurred in only 10.7% of patients in the probiotics plus metronidazole group compared with 70% in the metronidazole-only group. These results are consistent with previous studies demonstrating lower recurrence rates among patients treated with probiotics.[18-20]

CONCLUSION

This study enrolled sixty outpatient department visitors of Baghdad Teaching Hospital who complained of vaginal discharge with or without bad vaginal odor and were diagnosed with bacterial vaginosis (according to AMSELS criteria and Nugents scoring system), divided into two groups, and received treatment to evaluate the role of probiotics (L.rhamnosus) in treating and preventing recurrences.

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