

Original Article

Inhibitory Effect of *Lactobacillus rhamnosus* on Pathogenic Bacteria Isolated from Women with Bacterial Vaginosis

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Abstract

Background: Considering the high prevalence of bacterial vaginosis and its association with urinary tract infection in women and treatment of gynecologic problems occur when a high recurrence of bacterial vaginosis is often treated with antibiotics. The purpose of this study is to investigate the inhibitory effect of *Lactobacillus rhamnosus* on pathogenic bacteria isolated from women with bacterial vaginosis, respectively.

Materials and Methods: 96 samples from women with bacterial vaginosis discharge referred to health centers dependent Shahid Beheshti University in 91-92 were taken by a gynecologist with a dacron swab and put in sterile tubes containing TSB broth and Thioglycollate broth and were immediately sent to the lab location in cold chain for the next stages of investigation. From Thioglycollate and TSB medium was cultured on blood agar and EMB and Palkam and Differential diagnosis environments, and then incubated for 24 h at 37°C. Strains of *Lactobacillus rhamnosus* were cultured in MRSA environment and were transferred to the lab. After purification of pathogenic bacteria, MIC methods and antibiogram, *Lactobacillus rhamnosus* inhibitory effect on pathogenic bacteria is checked. Statistical analysis was done by SPSS software v.16.

Results: The results of this study show the inhibitory effect of *Lactobacillus rhamnosus* on some pathogenic bacteria that cause bacterial vaginosis, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Streptococcus agalactiae*, *Enterococcus*, *Listeria monocytogenes* and *E.Coli*. Microscopic examination of stained smears of the large number of *Lactobacillus* and pathogenic bacteria showed reduced. The prevalence of abnormal vaginal discharge, history of drug use means of preventing pregnancy and douching, respectively, 61%, 55%, 42% and 13% respectively. Significant difference was observed between the use and non-use of IUD in women with bacterial vaginosis infection.

Conclusion: Our findings indicated the Inhibitory effect of *Lactobacillus rhamnosus* on the pathogenic bacteria that cause bacterial vaginosis. The results of this study confirm the hypothesis of inhibit of pathogens growth that cause bacterial vaginosis supported by probiotics and can have beneficial effects in the prevention and treatment of bacterial vaginosis.

Keywords: *Lactobacillus rhamnosus*, Probiotics, Bacterial vaginosis

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Introduction

Bacterial vaginosis infection is an inflammation of the vagina due to a decrease in lactobacilli natural vaginal NBM

overgrowth of anaerobic bacteria, Gram-negative, and in some cases created by cocci Gram-positive and the pH of the vagina increases for more than 4/5. Among the anaerobic bacteria which increase during bacterial

vaginosis are *staphylococcus aureus*, *Streptococcus Group B*, *staphylococcus epidermidis*, *staphylococcus saprophyticus*, *Gardnerella vaginalis*, *Listeria monocytogenes*, *E. coli*, *Enterococcus*^{1,3}.

In bacterial vaginosis recurrence rate is very high⁴ and risk for sexually transmitted diseases, including HIV, as well as urinary tract infections in postmenopausal women increases^{5,6}.

Bacterial vaginosis is usually treated by antibiotics metronidazole or clindamycin^{3,7}, but due to antibiotic resistance, side effects and recurrence of infection, the use of probiotics to replace the medication is taken^{6,8}.

The normal amount of lactobacilli in the vagina is 10⁷ and has recently been suggested when the balance of normal vaginal flora is disrupted and reduced levels of lactobacilli, probiotics can be used to re-establish balance in the vagina. In other words, lactobacilli are bacteria which support the host's health if they exist in sufficient amount⁹.

Due to the prevalence of bacterial vaginosis which is the major cause in women who visit to midwifery clinics and side effects of medicine or recurrence of infections which in cases has failed medication, currently probiotics methods including lactobacilli, due to their special effects, have given a good view to researchers in treatment of vaginosis. Various studies have been conducted to examine the effects of different forms of lactobacilli (douching, vaginal suppositories, tampons, etc.) in the treatment of vaginosis which have carried different results.

Methods

Lactobacillus rhamnosus strains (DMS 100271) which was frozen in the liquid MRS plus glycerol at -70°C fridge, were cultured in MRS solid and incubated in a CO₂ incubator and were incubated for 48h. The lactobacilli grown on solid MRS medium was inoculated in liquid MRS medium, and after 24 hours liquid MRS broth was removed and transferred to another environment, in order to strengthen lactobacilli.

96 samples of discharge from women with symptoms of bacterial vaginosis were removed by gynecologist with Dakron swab were inoculated on blood agar medium and kept in incubator. After 24 hours, due to the growth

of several types of colonies on blood agar medium, for each of the colonies on blood agar and EMB agar and Palkam an isolated culture was done.

The next day, on colonies just grown in blood agar, catalase test was done, also slide was prepared and Gram stain was carried and the findings were recorded.

For Gram-positive cocci and catalase-positive, diagnostic tests, including tests related to family Staphylococaceae coagulase, oxidase and susceptibility to bacitracin discs and Novobiocin were done and species, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* were recognized.

For Gram-positive and catalase-negative cocci Streptococaceae family includes diagnostic tests to check blood agar hemolysis and Bile esculin tests and PYR, growth in the presence of 40% bile, Hippurate hydrolysis test, Camp and sensitivity test discs Optochin and Bacitracin were done.

Enterococcus species, *Streptococcus agalactia* were detected in samples from patients. For bacteria grown on agar medium Palkam, catalase test, Gram slide preparation and camp were carried out and we can see that when the slide coccobacilli Gram-positive and catalase test and camp is positive, *L. monocytogenes* is detected.

To study the inhibitory effect of lactobacilli on bacterial pathogens isolated from genital specimens MIC method was first used. In this method, first isolated bacteria were cultured in liquid BHI medium. After 24 hours, a turbidity standard half McFarland was measured and their OD was checked like half McFarland reserment can build. 10 series sterile micro-tubes for each of the isolated bacteria were used and in all micro-tubes 500 λ BHI broth medium were poured and poured 500 Lambda of the bacteria into micro-tubes and the titration was done. It was incubated for 24h. Then 20 λ of lactobacilli was poured into strains of all micro-tubes. After 24 hours, some of each of the micro-tubes was removed and cultured in checked form on MH agar for the growth inhibitory effect.

Alternatively, 250 λ of the removed bacteria was poured into 750 λ of BHI medium containing *Lactobacillus rhamnosus* and incubated for 24h and then cultured on Mueller Hinton agar medium to give a raster. After 24 hours on the slides molar and gram stain method was done.

Alternatively, first blank discs were put in MRS broth containing *Lactobacillus Rhamnosus*, bacteria isolated from patients and then put by half a standard dilution McFarland had been created and on the surface of MH medium was cultured according to antibiogram disk diffusion method and treated lactobacilli and ciprofloxacin disks placed on them. After 24 hours of incubation inhibition zone around the discs impregnated with bacteria lactobacilli and antibiotic ciprofloxacin was observed.

The final method of lactobacillus cultures in BHI broth was removed and was cultured on the entire surface of Muller Hinton medium. We then used a sterile swab of bacteria that had been diluted to half McFarland standard and draw on Muller Hinton medium. After 24 hours we reviewed the results.

Data from this study were analyzed using SPSS software version 16 for statistical analysis. For qualitative variables we used the relative frequency (%) and to report the quantitative variables mean and median measures center were used.

Results

In this study, 96 women with symptoms of vaginal discharge bacterial vaginosis were examined to study the inhibitory effect of *Lactobacillus rhamnosus* on pathogenic bacteria causing BV. At the same time, common risk factors, including age, history of drug use, discharge abnormal vaginal douching, used of tools to prevent pregnancy were studied.

From the 96 patients examined who aged between 18-58 years the maximum age that had bacterial vaginosis was among 49 to 58 year old. 69 (72%) of patients had bacterial vaginosis. From the 69 patients examined in this study, 37 patients (54%) had a history of using drugs, 41 patients (59%) had abnormal vaginal discharge, 27 patients (39%) had a history of using the means of preventing pregnancy and 9 patients (13%) had a history of douching.

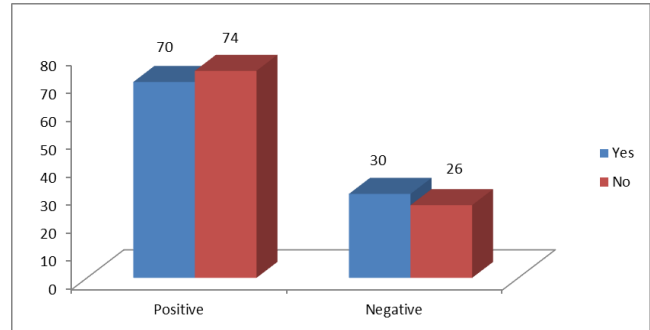


Diagram 1. Distribution of positive and negative results for bacterial vaginosis based on previous drug use.

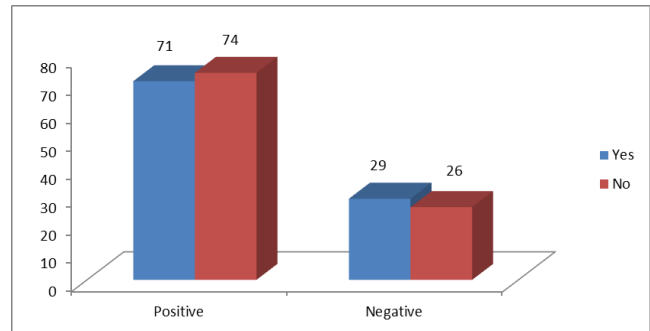


Diagram 2. Distribution of positive and negative abnormal vaginal discharge bacterial vaginosis times.

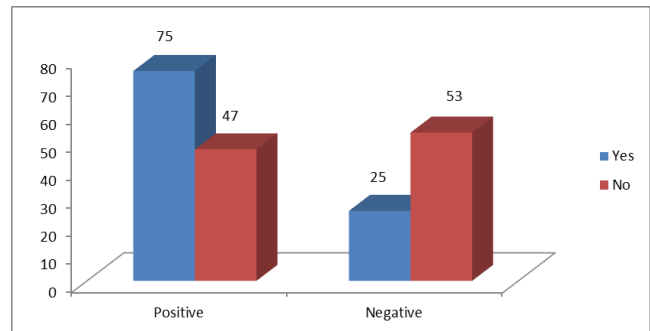


Diagram 3. Distribution of positive and negative results for bacterial vaginosis by means of pregnancy prevention.

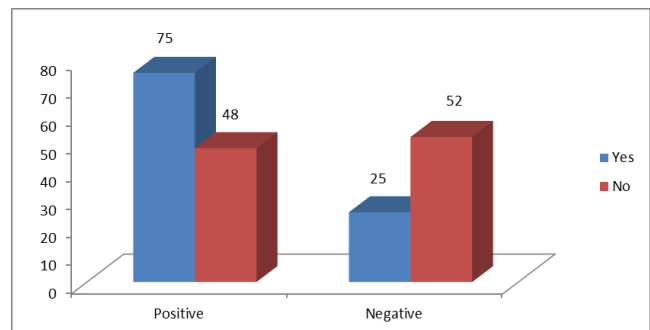


Diagram 4. Distribution of positive and negative results in terms of bacterial vaginosis and douching.

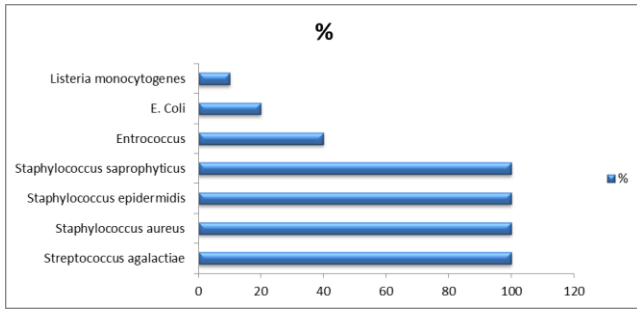


Diagram 5. Effect of *Lactobacillus Rhamnosus* inhibition on pathogenic bacteria isolated from patients with bacterial vaginosis.

Table1: Distribution of bacteria isolated from patients with bacterial vaginosis

Total (N=96)	Negative culture N (%)	Positive culture N (%)	The age range
12	4(33)	8(66)	18 -28
28	9(32)	19(68)	29 – 38
25	11(44)	14(56)	39 – 48
31	3(10)	28(90)	49 – 58
	0.033		P value

Table 2: Odds ratios of risk factors

P value	OR (95% CI)	Risk factors
0.786	0.79	History of drug use
0.93	0.86	Abnormal vaginal discharge
0.012	3.42	Means of preventing pregnancy
0.142	3.3	Douching

Discussion

Treatment with probiotics is based on the assumption of normal microbial flora. Data from various studies indicate clear evidence of the efficacy of probiotics in the treatment of human disease provide clinical profitability is yet to confirm and consolidate¹⁰. That's why we decided to investigate the inhibitory effects of probiotic lactobacilli on bacterial pathogens that cause bacterial vaginosis.

The results of this study demonstrate the inhibitory effect of *Lactobacillus rhamnosus* on some pathogenic bacteria that cause bacterial vaginosis, including

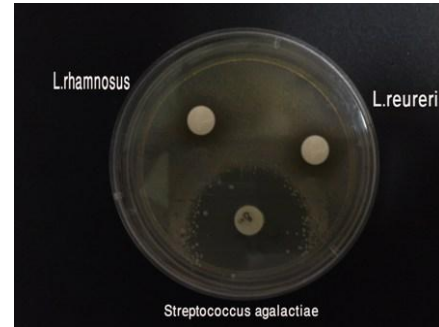


Figure 2. Growth inhibition of *Streptococcus agalactiae* by disk impregnated with lactobacilli.

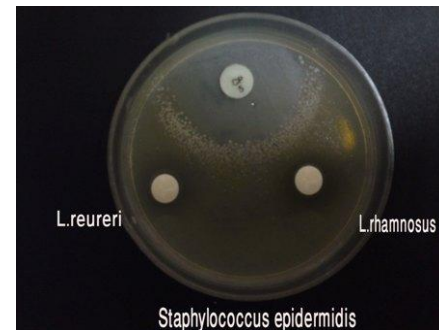


Figure 2. Growth inhibition of *Staphylococcus epidermidis* by discs impregnated with lactobacilli.

Staphylococcus aureus, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus* and *Streptococcus agalactiae*, *Escherichia coli* and *Listeria monocytogenes*. Microscopic examination of stained smears of the large number of *Lactobacillus* and pathogenic bacteria showed significant reduction.

We have not found similar studies. Other articles in the therapeutic effects of probiotics have in vivo conditions. The research was carried out in 2008 in Europe. 72 postmenopausal women between the ages of 55 and 65 years with asymptomatic bacterial vaginosis on a random day for 14 days received oral capsules containing *Lactobacillus* CFU *Lactobacillus rhamnosus* GR1 and RC14 *reuteri*. The results showed that the normal vaginal flora can be restored by oral administration of specific probiotic strains¹¹.

In a study in 2006, the effect of probiotic lactobacilli on in vitro adhesion of urogenital pathogens to vaginal epithelial cells was examined. The results showed that vaginal lactobacilli were able to inhibit the binding of *Staphylococcus aureus* and group B *Streptococcus* genito uropathogenic to vaginal epithelial cells. Results showed

potential protection of probiotic strains of lactobacilli in the vagina and further studies on their capacity to prevent and treat infections in women urogenital¹².

In a study done in UK in 2008, Rebecca et al showed that the prevalence of bacterial vaginosis was 40.2 and the bacterial vaginosis was associated with douching¹³.

In the present study, the prevalence of douching is 13 percent. A significant difference was observed between using and not-using douching in women with bacterial vaginosis infection.

A study in 2001 that was conducted on 357 patients showed IUD induces the risk of bacterial vaginitis by increasing the number of anaerobic bacteria¹⁴. In the present study, the prevalence of contraceptive use preventive tools including the IUD was 39 percent; there was a significant difference between using and not using IUD in women with bacterial vaginosis infection.

In a study in 2000 that was conducted on 956 women to assess the risk of bacterial vaginosis, 52% of women with bacterial vaginosis had a habit of smoking, which indicates the relationship between smoking and the risk of BV¹⁵. In our study, due to the presence of the low smoking women in this study, was not examined this risk factor.

According to the results of this study, the prevalence of abnormal vaginal discharge, history of drug use means of preventing pregnancy and douching, 61%, 55%, 42% and 13% respectively. No significant difference was observed between the use and non-use of douching.

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