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Investigate the Inhibitory Impression of *Lactobacillus Rhamnosus* and *Lactobacillus Reuteri* on *Escherichia Coli* isolated From Women with Bacterial Vaginosis

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Abstract

Introduction: With due attention to the relationship between bacterial vaginosis and urinary tract infection 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 inhibitory effect of Lactobacillus rhamnosus and Lactobacillus reuteri on E.coli isolated from women with bacterial vaginosis.

Methods: 96 samples from women with bacterial vaginosis discharge referred to health centers department Shahid Beheshti University in 91-92 were taken with a dacron swab and put in sterile tubes containing TSB and thioglycollate broth. Afterward, culture was transferred on blood agar, EMB, Palcam and Differential diagnosis environments. Strains of Lactobacillus rhamnosus and Lactobacillus reuteri were cultured in MRS environment and were transferred to the lab. After purification of E.coli, inhibitory effect of Lactobacillus rhamnosus and Lactobacillus reuteri on E.coli is checked. Statistical analysis was performed using SPSS software v.16.

Results: The results of this study demonstrate inhibitory effect of Lactobacillus rhamnosus and Lactobacillus reuteri on Ecoli that is one of the causes bacterial vaginosis is 20% and 30%, respectively.

Conclusions: Our findings indicated the little inhibitory effect of Lactobacillus rhamnosus and Lactobacillus reuteri on E.coli that is one of the cause bacterial vaginosis. The results of this study, the hypothesis that inhibit the growth of this Gram-negative pathogen causing bacterial vaginosis is not supported by probiotics.

INTRODUCTION

Bacterial vaginosis is a clinical syndrome with the increase in discharge of vaginal that has a fishy smell rotten, it is known that often after a bad sexual intercourse, will be reported. Bacterial vaginosis infection is a vaginal inflammation due to a decrease in lactobacilli normal flora accompanied by overgrowth of anaerobic bacteria, Gram-negative, and in some cases Gram-positive cocci pathogens 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 saprophyticus, Staphylococcus epidermidis, Gardnerella vaginalis, Listeria monocytogenes, Entrococcus, E. coli* [1-4]. 80 to 95% of vaginal flora consists predominantly of lactobacilli, including *L.gasseri, L.plantarum, L.brevis, L.casei, L.jensenii, L.acidophilus, L.ferentum, L.vaginalis, L.delbrueckii, L.salivarius, L. reuteri, L.rhamnosus* [3]. In bacterial vaginosis recurrence rate is very high [4] and risk for sexually transmitted diseases, including HIV, as well as urinary tract infections in postmenopausal women increases [5, 6]. Bacterial vaginosis is 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 10^7 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 maintain the host's health problems if they exist in sufficient amount [9]. Amsel criteria are used for diagnosing both, symptomatic and asymptomatic bacterial vaginosis. At least three of the four criteria should be met: (1) homogeneous, copious, milky vaginal discharge; (2) vaginal pH > 4.5; (3) bad-fish odor, due to the release of volatile amines (Whiff test); (4) observation of bacteria-covered epithelial cells (clue-cells) [10]. Due to the prevalence of bac-

terial 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. Mechanisms by which probiotics use healthy effects are defectively understood. Some authors contain competitive inhibition with pathogenic bacteria, antagonism through the production of antimicrobial substances (bacteriocins and hydrogen peroxide) and modulation of the immune system [11].

METHODS

Lactobacillus rhamnosus strains (DMS 100271) and *Lactobacillus reuteri* (DMS 20016) which were frozen in the liquid Man, Rogosa and Sharpe (MRS) plus glycerol at -70 fridge, were cultured in MRS solid and incubated in a CO2 incubator and were incubated for 48 h. 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 isolated culture was done. The next day, for bacteria grown in blood agar and the EMB we prepared slides and Gram stained to differentiate bacteria group of Enterobacteriaceae from other gram-negative bacteria tested oxidase and Gallery diagnostic testing Enterobacteriaceae family were laid, and bacteria *Ecoli* obtained.

To study the inhibitory effect of lactobacilli on bacterial pathogens isolated from genital specimens MIC (Minimum Inhibitory Concentration) 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 resentment can build. 10 series sterile micro-tubes for each of the isolated bacteria were used and in all micro-tubes500 Lambda BHI broth medium was poured and also poured 500 Lambda of the bacteria into micro-tubes and the titration was done. Incubated for 24h. Then 20 Landa 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 Muller Hinton agar for the growth inhibitory effect. Alternatively, first Blank discs were put in MRS broth containing *Lactobacillus Rhamnosus* and MRS broth medium containing *Lactobacillus reuteri* 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* and *Lactobacillus reuteri* on pathogenic bacteria causing BV (bacterial vaginosis). At the same time common risk factors, including age, history of drug use, discharge abnormal vaginal douching, and use 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. From 96 patients, 69 patients (72%) had bacterial vaginosis. Table 1 shows the distribution of bacteria isolated from patients with 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 (Table 2).

The inhibitory Effect of *Lactobacillus Reuteri and Lactobacillus Rhamnosus on E.Coli* isolated from patients with bacterial vaginosis is shown in Figs 1 and 2. The growth inhibition of E.Coli by discs impregnated with Lactobacilli is demonstrated in Fig 3.

Table1: Distribution of Bacteria Isolated from Patients with Bacterial Vaginosis

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

Table 2: Odds Ratios of Risk Factors		
Risk factors	**OR (95% Cl)**	**P value**
History of drug use	0.79	0.786
Abnormal vaginal discharge	0.86	0.93
Means of preventing pregnancy	3.42	0.012
Douching	3.3	0.142

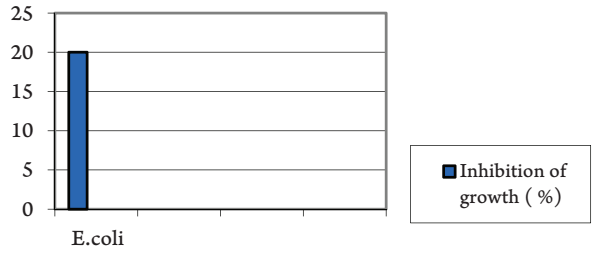

Figure 1: Inhibitory Effect of *Lactobacillus Reuteri* on *E.Coli* Isolated from Patients with Bacterial Vaginosis

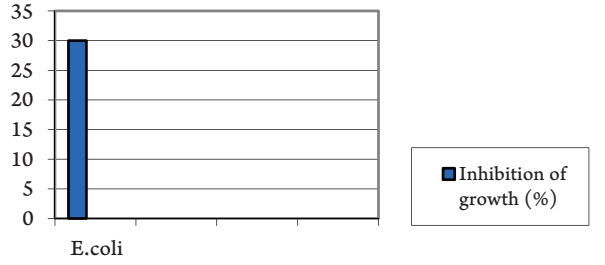

Figure 2: Inhibitory Effect of *Lactobacillus Rhamnosus* on *E.Coli* Isolated from Patients with Bacterial Vaginosis

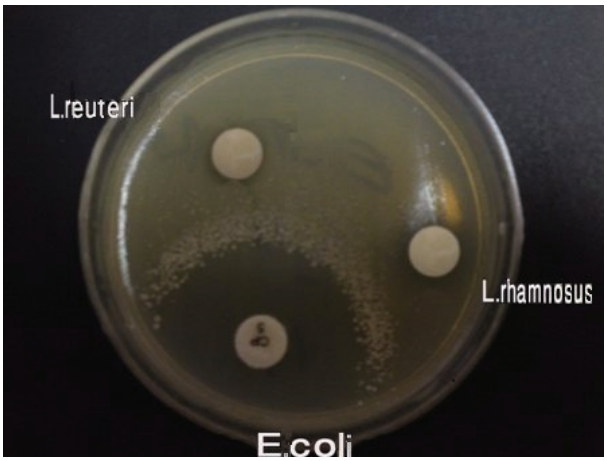

Figure 3: Growth Inhibition of *E.Coli* by Discs Impregnated with Lactobacilli

DISCUSSION

Treatment with probiotics is based on the assumption of normal microbial flora. Data from various studies indicated effect of probiotics in the treatment of human disease provide clinical profitability is yet to confirm and consolidate [12]. That's why we decided to investigate the inhibitory effects of probiotic lactobacilli on bacterial pathogens that cause bacterial vaginosis can review. The conclusions of this study demonstrate the inhibitory effect of *Lactobacillus rhamnosus* and *Lactobacillus reuteri* on *Escherichia coli*. Coman et al, Investigate the inhibitory impression of *Lactobacillus rhamnosus* IMC 501 and *lactobacillus paracasei* IMC 502 and their 1:1 combination on some gram negative and gram positive pathogenic bacteria and Candida strains. The results showed that more pathogenic bacteria and yeast were inhibited by probiotics tested [13]. Other articles in the therapeutic effects of probiotics have in vivo conditions. In a research performed in 2008 in Europe, 72 postmenopausal women between the ages of 55 to 65 years with asymptomatic bacterial vaginosis on a random day for 14 days oral capsules containing Lactobacillus CFU *Lactobacillus Rhamnosus* GR1 and RC14 Reuteri received. The results showed that more women are tested by probiotic treatment [14].

Rebecca et al, showed that the prevalence of bacterial vaginosis was 40.2, which was associated with douching [12]. 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. In a study conducted on 357 patients to assess bacterial vaginosis risk factors, use of IUD was found in 47.2% of subjects [13]. In our 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 on 956 women was conducted to assess the risk of bacterial vaginosis, 52% of 131 women with bacterial vaginosis had a habit of smoking, which indicates the relationship between smoking and the risk BV [14]. In our study due to the low smoking women in this study did not examine risk factors. According to the results of this study, douching, use of tools to prevent pregnancy, history of drug use, the prevalence of abnormal vaginal discharge, respectively, 13%, 42%, 55% and 61%. Mechanism of action of probiotics should be more clearly explained so based on the best species for probiotic use against a particular pathogen can be selected [12].

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AUTHORS' CONTRIBUTION

All authors collaborated in the preparation of this paper.

CONFLICTS OF INTERESTS

The authors declare no competing financial interests.

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