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Original Article

Oral *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 to reduce Group B *Streptococcus* colonization in pregnant women: A randomized controlled trial

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ABSTRACT

Objective: This study is to examine the effect of *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 taken orally before bedtime on Group B *Streptococcus* (GBS)-positive pregnant women with respect to becoming GBS negative.
Materials and Methods: In total, 110 pregnant women at 35–37 weeks of gestation who were diagnosed by GBS culture as being GBS positive for both vaginal and rectal GBS colonization were randomly assigned to be orally treated with two placebo capsules or two probiotic capsules (containing *L. rhamnosus* GR-1 and *L. reuteri* RC-14) before bedtime until delivery. All women were tested for vaginal and rectal GBS colonization again by GBS culture on admission for delivery.
Results: Of the 110 participants, 99 completed the study (49 in the probiotic group and 50 in the placebo group). The GBS colonization results changed from positive to negative in 21 women in the probiotic group (42.9%) and in nine women in the placebo group (18.0%) during this period (Chi-square $p = 0.007$).
Conclusion: Oral probiotic containing *L. rhamnosus* GR-1 and *L. reuteri* RC-14 could reduce the vaginal and rectal GBS colonization rate in pregnant women.

Introduction

Group B *Streptococcus* (GBS) is an encapsulated Gram-positive coccus that colonizes the gastrointestinal and genital tracts of 15–40% of pregnant women [1]. Although GBS colonization usually remains asymptomatic in these women, vertical transmission may occur when GBS ascends from the vagina to the amniotic fluid after the onset of labor or rupture of the membranes. In some cases, transmission can take place with intact membranes [2,3]. During the past 2 decades, GBS has been considered to be a leading cause of primary neonatal sepsis, pneumonia, and meningitis in the 1st week of life, which is known as early-onset GBS infection [4]. *Intrapartum* parenteral antibiotic prophylaxis for women with a positive GBS culture at 35–37 weeks of gestation is recommended by the Centers for Disease Control and Prevention (CDC). According to the 2010 CDC guidelines, a minimum of 4 hours of *intrapartum* antibiotic prophylaxis prior to delivery is recommended to prevent early-onset GBS infection because therapeutic drug level may not be achieved with < 4 hours of treatment [5]. Preliminary studies and data from a large health maintenance organization showed that 40–50% of GBS-colonized multiparous women are not able to receive antibiotics at least 4 hours before delivery due to the rapidity of their labor [6]. Newborns from GBS positive women with inadequate *intrapartum* antibiotic prophylaxis are deemed at risk. Low-risk infants are recommended to undergo 48 hours of observation. High-risk infants should additionally undergo blood cultures and a complete blood count [5]. GBS disease is not easily resolved by antibiotic treatment of the pathogen. Thus, such traditional approaches need to be re-evaluated.

Lactobacilli are the dominant bacteria of the vaginal flora. They possess antimicrobial properties that regulate other urogenital

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microbiota. The possible mechanisms of the probiotics acting in the vagina include modulation of host immunity, alteration of the microenvironment to be less receptive to pathogens (i.e. the production of lactic acid, bacteriocin, biosurfactants, hydrogen peroxide, and signaling compounds), and dislodging pathogen biofilms [7]. Using probiotic during pregnancy has an excellent safety record [8]. Taking oral probiotics containing lactobacilli daily has been shown to maintain normal lactobacilli vaginal flora [9]. Reid et al [10] showed that *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 are antagonistic to the growth and adhesion of various intestinal and urogenital pathogens, including GBS, *Gardnerella vaginalis*, and uropathogenic *Escherichia coli*. A study by Altoparlak et al [11] showed a negative correlation between the colonization rate of vaginal lactobacilli and GBS in pregnant women. The lactobacilli colonization rate was 21.3% in the GBS positive group, and 47.6% in the GBS negative group [11]. These results suggest that lactobacilli play a role in preventing vaginal colonization by GBS. The study of Velraeds et al [12] has also shown that certain lactobacilli can inhibit the growth and adhesion of streptococci *in vitro*. However, their ability to do so *in vivo* needs further testing.

Most research to date has focused on the potential of probiotics to prevent bacterial vaginosis and preterm labor. The effectiveness of probiotics as a surrogate or adjunctive therapy for *intrapartum* antibiotic prophylaxis in GBS colonized pregnant women has not been evaluated. To our knowledge, the present study is the first to investigate the role of probiotics in preventing and treating vaginal colonization by GBS in pregnant women. The purpose of this study was to examine whether oral *Lactobacillus*-containing probiotics can reduce the vaginal and rectal GBS colonization rate in GBS positive pregnant. Through the results of our study, we try to investigate the role of probiotics in preventing unnecessary tests, admission, and antibiotic treatments in newborns from GBS-positive mothers. We hope our study will have some impact on GBS sepsis protocols.

Materials and methods

This study was a prospective, double-blind randomized clinical trial that was performed in the Obstetric Department of the China Medical University Hospital, Taichung, Taiwan from March 1, 2011 to December 30, 2011. This research study was approved by the China Medical University Hospital Institutional Review Board (DMR99-IRB-309) and registered in the Clinical Trials.gov Protocol Registration and Results System (registration number NCT01577108). Written informed consent was obtained from each participant.

The inclusion criteria of our study were pregnant women, singleton pregnancy, with a positive GBS screening culture at 35–37 weeks of gestation. They agreed, throughout the trial period, to abstain from the use of any systemic or intravaginal antibiotic, antifungal agents, or any other intravaginal product (e.g., contraceptive creams, lubricants, and douches). The exclusion criteria included multiple gestations, pregnant women with impaired immunity, diabetes, or any other kind of significant disease or acute illness that could complicate the evaluation of the results. Pregnant women who received vaginal or systemic antibiotics and antifungal therapy within 2 weeks of the screening visit were also excluded.

Vaginal and rectal GBS screening cultures by swabbing both the lower vagina and rectum (through the anal sphincter) were performed for all pregnant women at 35–37 weeks of gestation in our outpatient department. The specimens were transported to laboratory as soon as possible and inoculated into the Lim broth. Women with vaginal and rectal GBS colonization were invited to participate in our study. Informed consent was obtained from each participant. The trial patients were doubled-blind computerized randomized by the hospital pharmacy. Each woman was assigned a number. Identical looking probiotic and placebo capsules were prepared and distributed in numbered containers by the pharmacy. The patients were provided two capsules of probiotics or placebos to be taken once daily at bedtime until delivery. The probiotic capsules contained dried viable *L. rhamnosus* GR-1 and *L. reuteri* RC-14, and each capsule contained 1×10^9 viable cells of both strains. The *L. rhamnosus* GR-1 and *L. reuteri* RC-14 strains were encapsulated in gelatin capsules, which were produced by Chr. Hansen (Horsholm, Denmark) using good manufacturing practices (U-relax). The placebo capsules contained the same composition (dextrose anhydrate, potato starch, microcrystalline cellulose, and magnesium stearate, gelatin, and titanium dioxide) except for the *L. rhamnosus* GR-1 and *L. reuteri* RC-14 strains. Vaginal and rectal GBS cultures were repeated for all participants at the time of admission for delivery. All the participants were treated according to the CDC's 2010 guidelines on GBS (GBS-positive mothers as assessed by culture at 35–37 weeks of gestation should receive at least 4 hours of *intrapartum* antibiotic prophylaxis) on admission for labor.

The primary outcome was the absence of vaginal and rectal GBS colonization in pregnant women who presented as GBS positive at 35–37 weeks of gestation after probiotics or placebo treatment. The secondary measures were the relationship between parity and newborn transfer units, and the cause of admittance to the neonatal unit. The Wilcoxon rank-sum test and Chi-square test were used to test for significant differences between the two groups in terms of maternal age, maternal weight, education level, parity, gestational week of delivery, duration of drug taking, neonatal birth weight, newborn transfer units, and Apgar scores. The Chi-square test was also used to evaluate the difference in the primary outcome between probiotics and placebo groups, the cause of admission to the neonatal unit, and the relationship between parity and newborn transfer units. Assuming GBS culture to become negative in $> 40\%$ of the participants in the study group and not $> 15\%$ in the placebo group, a sample size of 50 women per group was considered sufficient to reach 80% statistical power. All statistical analyses were calculated using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). A p value < 0.05 was considered to be statistically significant.

Results

The study was conducted in our hospital from March 2011 to December 2011. During the trial period, 1210 women, at 35–37 weeks of gestation, underwent vaginal and rectal GBS screening cultures in our outpatient department; 219 women had positive culture results. The GBS colonization rate was 18.1%. There were 110 pregnant women enrolled in the study. Overall, 99 participants completed the study. Three participants, two in the study group and one in the control group, dropped out of the study because of failure to undergo the GBS culture before delivery. Eight participants, four in the study group and four in the control group, withdrew from the study due to personal reason. A CONSORT flow diagram depicting information about the number of participants at the different stages of the trial is shown in Figure 1.

The demographic and clinical characteristics of the two groups are shown in Table 1. There were no significant differences between the two groups in terms of the following factors: maternal age, maternal weight, education level, parity, gestational week of delivery, duration of drug taking, neonatal birth weight, newborn transfer units, and Apgar score. There were no adverse treatment effects in terms of nausea, vomiting, diarrhea, abdominal pain, skin rash, or systemic infections after taking the capsules in any of the participants during the trial. In total, 24 neonates were transferred

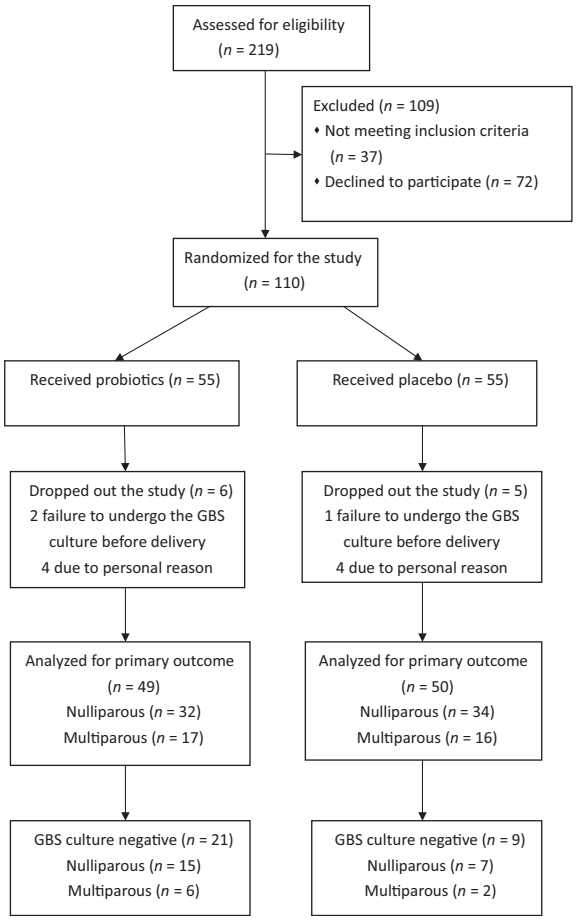

Figure 1. Flow diagram showing the number (*n*) of participants at different stages of the trial.

Table 1
Characteristics of the women and newborn in the study.

	Probiotic ($n = 49$)	Placebo ($n = 50$)	*p*
Maternal age (y)[a]	32.0 ± 4.0	32.0 ± 3.7	0.33
Maternal weight (kg)[a]	67.0 ± 4.7	68.0 ± 5.1	0.59
Gestational age (wk)[a]	39 ± 0.9	39 ± 1.1	0.19
Education level[b]			0.11
Below college	8	2	
College	41	48	
Parity[b]			0.99
Nulliparous	32	34	
Multiparous ≥ 1	17	16	
Duration of taking[a] (d)	21.2 ± 5.5	19.6 ± 4.8	0.76
Weight of newborn[a] (g)	3123 ± 398	3230 ± 358	0.07
Newborn transfer units[b]			0.62
Sick baby room	10	14	
Baby room	38	34	
Intensive care unit	1	2	
Apgar score < 7[b]			> 0.999
1 min	0	0	
5 min	0	0	

Data are expressed as mean ± standard deviation or *n*.
[a] Wilcoxon rank-sum test.
[b] Chi-square test.

to the sick baby room. With 20 of them (83.3%), six from nulliparous and 14 from multiparous mothers, had inadequate antibiotic treatment prior to delivery (< 4 hours before delivery). One neonate had hydronephrosis. One had *intrapartum* maternal fever. The remaining two had dyspnea after delivery. Results by the Chi-square test showed that the main cause of admission to sick baby room was due to inadequate antibiotic treatment ($p = 0.001$). Among our participants, 33 were multiparous and 66 nulliparous. Fifteen of the neonates from multiparous (45.5%), but only nine neonates from nulliparous women (13.6%) were transferred to the sick baby room. The Chi-square test showed a strong relationship between parity and the transfer rate to the sick baby room ($p < 0.001$). At the time of labor, nine of 50 participants in the placebo group (18.0%) and 21 of 49 participants in the probiotic group (42.9%) became GBS negative. The Chi-square statistical analysis showed a *p* value of 0.007, which suggests a significant difference in the negative GBS culture rate between the two groups.

Discussion

In our trial, 42.9% of the participants in the study group but only 18.0% in the placebo group achieved a negative GBS culture at the time of delivery. The results demonstrated that oral probiotic *L. rhamnosus* GR-1 and *L. reuteri* RC-14 could reduce vaginal and rectal GBS colonization in pregnant women. To our knowledge, this was the first clinical trial to use probiotics as a regimen for reducing the vaginal and rectal GBS colonization in pregnant women. A previous study showed that certain lactobacilli can inhibit the growth and adhesion of streptococci *in vitro* [12]. However, their ability *in vivo* was not tested. According to a study by Altoparlak et al [11], the *Lactobacillus* colonization rate was lower when combined with GBS colonization during pregnancy. A study by Kubota et al [13] also demonstrated that GBS inhibited *Lactobacillus in vitro*. These findings supported the idea that increasing the amount of *Lactobacillus* could reduce GBS colonization. However, the actual mechanism of probiotics in reducing GBS colonization was not investigated in our trial.

Even with the most valiant efforts, 40–50% of multiparous pregnant women colonized with GBS still have < 4 hours before delivery to be treated with preventive antibiotics [6]. In our study, 24 neonates were transferred to the sick baby room. Among these neonates, 45.5% came from multiparous women, but only 13.6% from nulliparous women. Owing to the rapidity of labor, *intrapartum* antibiotic prophylaxis to prevent early-onset GBS infection in multiparous patients is limited and ineffective. Thus, the current GBS prevention policy needs to be re-evaluated. In the absence of a plan to counteract the rapidity of labor in GBS-colonized pregnant women, particularly in multiparous patients, reducing the prenatal GBS colonization rate should be the touchstone in early-onset GBS disease prevention.

Reducing GBS colonization in pregnant women is the golden standard of the prevention of early-onset GBS disease in newborns. Our study results showed that nearly 43% of the GBS-positive pregnant women achieved negative GBS culture results after taking probiotics for ~3 weeks. A reduction in GBS colonization positivity at 35–37 weeks of gestation can reduce the early-onset GBS infection rate and the antibiotic usage during labor. Thus, probiotics have the potential to decrease the risk of early-onset GBS disease and the rate of antibiotic use during labor just by restoring normal vaginal flora and acidity without systemic effects. During pregnancy, treatment that can achieve the same goals but less invasive or with less adverse effects should be chosen. We propose that those in the high-risk group of GBS colonization during pregnancy (e.g., healthcare workers, black women, women with a high body mass index, and women with previous GBS infection or colonization) [14] and those who are multiparous should start oral probiotics from the beginning of the third trimester for the purpose of reducing prenatal GBS colonization. GBS screening and *intrapartum* management of these women should follow the CDC guidelines on GBS.

There are several limitations in our study. The sample size was small. All patients came from a single hospital and resided in the city of Taichung, Taiwan. Therefore, our findings cannot be generalized across different regions and races. Our study was not set up for long-term observation of neonatal outcomes. There was no cost-effectiveness analysis in our study. We also did not discuss the mechanisms of probiotics due to a lack of understanding on this aspect. In addition, our participants took probiotics for an average of 20 days. This might not be long enough for *Lactobacillus* to repopulate inside the vagina, displace pathogens, modulate host immunity, and alter the microenvironment of the vagina. Furthermore, our study lacked the information of the socioeconomic status, dietary habits, and the use of nutritional supplement of the participants. Therefore, the effectiveness of probiotic treatment in reducing the rate of GBS colonization remains speculative. In future, more research will be done on this subject to address our study limitations and corroborate our findings.

In conclusion, our study demonstrated that oral probiotics containing *L. rhamnosus* GR-1 and *L. reuteri* RC-14 could reduce the vaginal and rectal GBS colonization in pregnant women. We propose that oral probiotics should be administered early in pregnancy to reduce GBS colonization at 35–37 weeks of gestation. This could reduce early-onset GBS infection and the need for antibiotic treatment during labor. Moreover, it might help to overcome inadequate antibiotic treatment during labor in multiparous women and lead to a reduction of admission rate to the neonatal unit.

Conflicts of interest

The authors have no conflict of interest relevant to this article.

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