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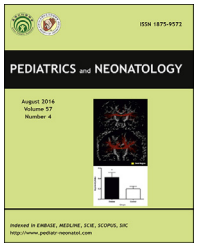

Original Article

Effect of probiotics on mother-to-neonate vertical transmission of group B streptococci: A prospective open-label randomized study

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Key Words
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vertical transmission

Background: Group B Streptococci (GBS) are common vaginal bacteria found in 20—30% of pregnant women and a significant cause of invasive infections in newborns. Recently, attention has been focused on the efficacy of probiotics during the perinatal period. However, the effect of probiotic intake on the mother-to-child transmission (MTCT) of GBS remains unknown.
Methods: Pregnant women with positive GBS results from vaginal and rectal swab cultures at 35—37 weeks of gestation were randomly assigned to the probiotic group or the control group in an open-label manner at the Department of Obstetrics and Gynecology, San-ikukai Hospital, Tokyo, Japan. The probiotic group received *Lactobacillus reuteri* during antenatal checkups from 35 to 37-week gestation to 1 month after delivery. Rectal swabs were obtained from the newborns at 5 days and at 1 month of age. Whole-genome sequencing was performed to test for GBS strains in the mother, whose newborn carried GBS at the 1-month checkup. Multi-locus sequence typing and single nucleotide polymorphism analyses were performed to identify MTCT.

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Results: Overall, 67 mother-infant pairs were included, with 31 in the probiotic group and 36 in the control group. The positivity rate of GBS in newborns at 1 month of age was 10% (n = 3) in the probiotic group and 28% (n = 10) in the control group. In newborns carrying GBS at 1 month of age, genetic analysis revealed that the MTCT rate was 6% in the probiotic group and 22% in the control group, although the difference was not statistically significant (p = 0.0927).
Conclusion: No statistically significant difference was found; however, the consumption of *L. reuteri* by women with GBS-positive pregnancies may inhibit the MTCT of GBS.
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1. Introduction

Approximately 20—30% of pregnant women carry Group B Streptococci (GBS) in their vagina, the primary causative agent of invasive infections in newborn infants. Such infections can be described as either early-onset disease (EOD) if they happen within 7 days of birth or late-onset disease (LOD) if they happen after 7 days of birth. With the inclusion of intrapartum antibiotic prophylaxis (IAP) in the Centers for Disease Control and Prevention guidelines and its widespread use, the rate of EOD has decreased due to vertical transmission from a GBS-carrying mother.[1] LOD often occurs as bacteremia, meningitis, or skin and soft tissue infections. The primary transmission route of GBS is maternal transmission; however, it can also be transmitted in a hospital setting or by a caregiver.[2] The risk of GBS carriage in late newborns is similar to the maternal carriage rate.[3]

If the mother-to-child transmission (MTCT) of GBS can be controlled, the risk of developing LOD can be reduced. While no confirmed method exists to prevent the MTCT of GBS during LOD, probiotic intake could prevent it.[4,5] Bacteria of the genus *Lactobacillus* belong to the family Lactobacillaceae and are used as probiotics. The vaginal microbiota of pregnant women with GBS has a lower proportion of *Lactobacillus* species compared to that of women without GBS.[6] *Lactobacillus reuteri* has an antimicrobial system called the reuterin system,[7] which has been reported to be associated with a reduction in bacterial vaginosis.[8] *L. reuteri* was reported to decrease LOD incidence during neonatal intensive care unit (NICU) stays in preterm infants and those with very low birth weights.[9] Maternal ingestion of a fixed-dose combination of *L. reuteri* and *Lactobacillus rhamnosus* containing 1×10^9 viable cells of both strains from 35 to 37 weeks of gestation to delivery has been shown to decrease GBS incidence.[10] *L. reuteri* is a probiotic widely used in the perinatal period; however, whether it affects GBS transmission in newborns remains unclear.

In this study, we conducted an open-label randomized observational study in which GBS-positive pregnant women were randomly assigned to either take *L. reuteri* or not from 35 to 37 weeks of gestation to the day of delivery. *L. reuteri* was selected based on previous studies that demonstrated its effectiveness as an oral probiotic in neonates[9] and because it was readily available in the form of commercial tablet foods of consistent quality. The period of probiotic administration was set to 1 month after birth, as roughly half of LOD cases in epidemiological studies conducted in Japan occurred within the first month of life.[11] The incidence of GBS in newborns at the 1-month follow-up was assessed as the primary outcome; the concordance between GBS serotypes in mothers and infants was also assessed. Finally, the GBS positivity rates in vaginal and rectal cultures at the time of delivery and 1 month after delivery were also measured as secondary outcomes.

2. Methods

Ethics approval

This study was conducted in accordance with the principles of the Declaration of Helsinki. Approval from the San-ikukai Hospital Ethics Committee was obtained prior to the start of the study (registration number: 169). The purpose of the study was explained to the mothers during their antenatal checkup at 35—37-week gestation, and informed consent was obtained in writing.

2.1. Study population and the clinical study protocol

Pregnant women with a positive GBS screening test at 35—37 weeks of gestation and their newborns at the Department of Obstetrics, San-ikukai Hospital, Tokyo, Japan, from September 1, 2020, to July 31, 2021, were included in the study. This study was an open-label, prospective, randomized controlled trial. Patients who consented to the study were randomly assigned to either the group administered with probiotic foods (the probiotic group) or the group without probiotic food intake (the control group) using a random number table. The clerk in the pediatrics department generated the random number sequences, the clerk in the obstetrics department enrolled the participants, and the pediatric physician, who was not involved in the number generation of patient enrollment, assigned participants to the interventions. Cases wherein a pregnant woman was transferred to another hospital, an infant was admitted to the NICU, or an infant was transferred to another hospital were excluded from the analysis.

Patient background and perinatal history were obtained from eligible pregnant women and newborns, respectively.

Mothers in the probiotic group were administered a single tablet of food containing *L. reuteri* DSM 17938 (Protectis® 10^8 CFU/tablet; BioGaia Co., Ltd., Stockholm, Sweden) once a day from the day of study entry until 1 month after delivery. Participating pregnant women were instructed to store the tablets at room temperature below 25 °C. In both the probiotic and control groups, vaginal and rectal swabs were collected from pregnant women at 35–37 weeks of gestation, at delivery, and at 1 month post-delivery. For newborns, rectal swabs were collected on day 5 and during the 1-month follow-up in both the probiotic and control groups. Given that the pregnant women participating in this study tested positive for GBS at 35–37 weeks of gestation, all participants who delivered vaginally were administered IAP, while those who underwent cesarean sections were administered surgical antibiotic prophylaxis. IAP with intravenous ampicillin (ABPC) was administered to all women who had a vaginal delivery, starting with an initial dose of 2 g, followed by 1 g every 4 h until delivery. Intravenous cefazolin (CEZ) at a dose of 1 g was administered as preoperative antibiotics to all women who underwent cesarean sections. In cases of emergency cesarean sections following the rupture of membranes, ABPC and CEZ were utilized for treatment. If postpartum antimicrobial therapy was deemed necessary, the attending obstetrician had the discretion to select the appropriate antimicrobial agent. As a primary outcome, we investigated the rate of GBS transmission in mothers and infants at 1 month of age with and without ingestion of *L. reuteri* from GBS-positive mothers. As a secondary outcome, we investigated the rate of MTCT of GBS at 1 month of age and further analyzed the rate of GBS mother-infant transmission when the mothers were persistent carriers at 1 month after delivery.

2.2. Specimen collection, isolation, and culture

A cotton tip swab (BBL Culture Swab Plus; Becton, Dickinson and Co., Ltd., Bergen, NJ) was used for sample collection. Specimens were collected from the mothers by scraping their vagina and rectum at 35–37 weeks of gestation, at delivery, and 1 month postpartum. Specimens were collected from newborns by scraping their rectum at 5 days and 1 month of age.

Maternal vaginal and rectal swabs were incubated in GBS medium F (Fuji Pharmaceutical Industries, Ltd., Toyama, Japan) at 37 °C for 24 h, according to the manufacturer's instructions. The strain was determined to be GBS-positive when the color of the medium changed from orange to reddish-orange. The strains were then axenically cultured on a 5% sheep blood agar medium (Becton, Dickinson and Co., Ltd.) and stored at −80 °C until further analyses.

GBS carriage in neonates was analyzed using the protocol described by Toyofuku et al.[12] where the newborns' rectums were swabbed for analysis. Briefly, the swabs were agitated with 500 μL of Todd Hewitt Broth (Becton, Dickinson and Co., Ltd.) and centrifuged at 2000×*g* for 5 min at 4 °C. Five microliters of the supernatant were incubated with GBS-selective Medipore medium (Eiken Chemical Co., Ltd., Tokyo, Japan) for 18–24 h at 37 °C. The colonies obtained were cultured on sheep blood agar medium (Becton, Dickinson and Co., Ltd.), and the strains were stored at −80 °C until further use.

2.3. DNA extraction

Using strains obtained from pure cultures as samples, DNA was extracted using a QIAGEN DNA Mini Kit (QIAGEN Co., Hilden, Germany).

2.4. Genetic identification methods

GBS detection was achieved using PCR primers targeting *dltS*, a gene encoding a histidine kinase that is unique to GBS, as previously described by Morozoumi et al.[13] Serotypes (Ia, Ib, II, III, IV, V, VI, VII, and VIII) were detected using DNA extracted from the strains and amplified using PCR, as described by Poyart et al.[14] Serotype IX was identified via PCR as reported by Imperi et al.[15]

2.5. Whole-genome sequencing for GBS strains

We performed whole-genome sequencing (WGS) of GBS strains from mothers whose 1-month-old newborns carried GBS and their children. A DNA Prep Library Kit (Illumina, Inc., San Diego, CA, USA) was used for WGS sample preparation. WGS was performed using a Miniseq Mid Output Kit (300 cycles) or an iSeq 100 i1 Reagent v2 (300 cycles) and a paired-end 2 × 150-bp cycle run on an Illumina Miniseq sequencing system or an Illumina iSeq 100 sequencing system.

2.6. Phylogenetic analysis of the strains using WGS

After sequencing, genome assembly and assignment of multi-locus sequence typing (MLST) *in silico* were carried out using MLST 2.0 (https://cge.cbs.dtu.dk/services/MLST/). Inter-strain whole-genome single nucleotide polymorphisms (SNPs) were detected using CSI Phylogeny 1.4 (https://cge.cbs.dtu.dk/services/CSIPhylogeny/) on the CGE website using reads 1 and 2 of the Miniseq or iSeq results.

To construct a whole-genome SNP tree including the GBS strains isolated in this study, we used the WGS of strain 09mas018883, a GBS strain of bovine origin,[16] as an outgroup strain. The strains were the same when the SNP difference was <50, and the strains were closely related if the combined result of the MLST was 50≤SNP differences<100.[17]

2.7. Statistical analysis

Chi-squared and Mann–Whitney *U* tests were performed for between-group comparisons using EZR ver1.53 statistical software.[18] The sample size was calculated using a 5% alpha error and an 80% detection rate.

3. Results

3.1. Characteristics of the subjects

A total of 108 pregnant women were included, of whom 106 at 35–37 weeks of gestation who provided informed

consent were randomly assigned to the various experimental groups. A total of 43 pregnant women were included in the probiotic group, and 63 pregnant women were included in the control group. Finally, 31 mother-child pairs in the probiotic group and 36 mother-child pairs in the control group were included in the final analysis after excluding those that did not complete the study or those who met the exclusion criteria (Fig. 1).

No statistically significant differences were found in maternal age, mode of delivery, social history, or infectious disease upon screening the included cases. All participating pregnant women had no history of using antimicrobials from enrollment until admission for delivery, and none of them exhibited any signs of infection. IAP and preoperative antibiotics were administered to all women. After delivery, cefaclor (750 mg) was administered for 3–5 days to 23 patients in the probiotic group and 29 patients in the control group to treat delivery wounds. One patient in the probiotic group received amoxicillin (AMPC, 1500 mg) and clavulanic acid (CVA, 350 mg) for 3 days, while one patient in the control group received cefcapene pivoxil (300 mg) for 3 days for mastitis. No statistically significant differences were found in the birth week or weight among the newborns. Blended feeding was more prevalent in the control cohort, while breastfeeding and formula feeding demonstrated no significant difference between the two groups. None of the neonates necessitated microbial culture examinations or antimicrobial therapy before the 1-month checkup, and none exhibited LOD (Table 1).

Pregnant women who participated in the study were asked to complete a questionnaire about their physical condition and the number of *L. reuteri* tablets they had taken. A total of 16 and 15 responses were recorded in the probiotic group and the control group, respectively. Vomiting, abdominal pain, diarrhea, pruritus, and rash were observed, but no symptoms were statistically significantly different between the two groups. No participant stopped consuming probiotics because of adverse events (Supplementary Table 1). The number of days of probiotic consumption was declared by 13 of the 16 people surveyed in the probiotic group. The median number of days they consumed *L. reuteri* was 47.5 (interquartile range, 42.75–56.25), with a median of 100% (interquartile range, 97–100%) of the number of days they were expected to consume *L. reuteri*.

3.2. GBS positivity rates and the MTCT of GBS

The positivity rates for GBS are shown in Table 2. Maternal GBS positivity in the probiotic group was 24% and 63% at delivery and 1-month follow-up post-delivery, respectively. In the control group, maternal GBS positivity was 24% and 52% at delivery and at 1-month follow-up post-delivery, respectively. Overall, the mean GBS positivity rates for all included patients at delivery and 1-month follow-up were 24% and 56%, respectively.

In the probiotic group, neonatal GBS positivity was 10% at both 5 days and 1 month of age. In the control group, GBS positivity at 5 days and 1 month of age was 19% and 28%, respectively. Details of 13 cases in which GBS was isolated in the mothers and neonates are shown in Table 3, including three mother-infant pairs in the probiotic group and ten in the control group, respectively.

The rates of MTCT were 6% and 22% in the probiotic and control groups, respectively. When the analysis was restricted to positive maternal postnatal cultures at 1 month post-delivery, the rates of MTCT were 33% in the

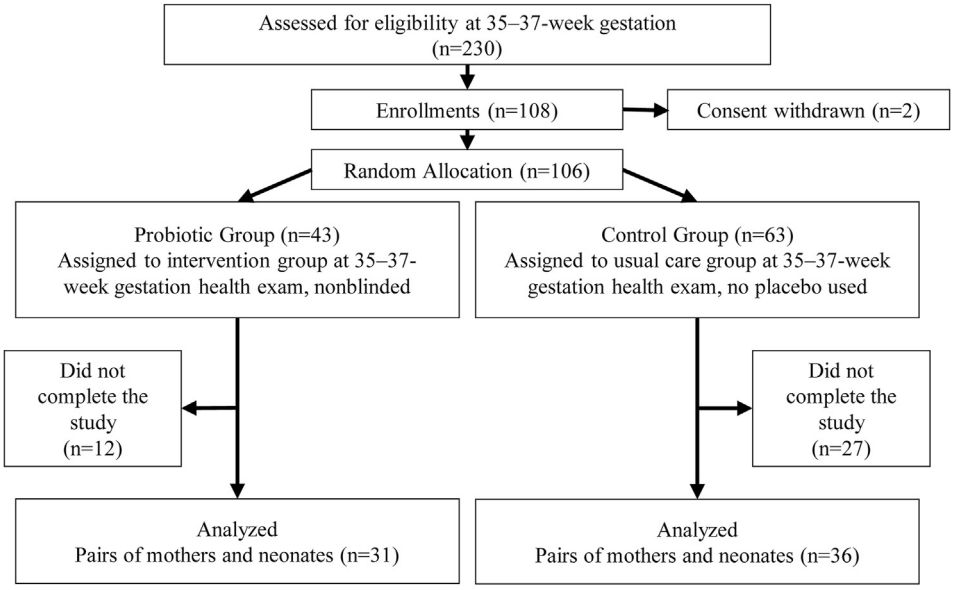

Figure 1 Study flowchart showing the number of participants at different stages of the trial. Of the 12 pairs of mothers and neonates in the probiotic group who could not complete the study, one pair was not indicated because the newborn was admitted to the NICU, and twelve pairs did not have maternal or neonatal specimens collected at the 1-month follow-up. Overall, 27 pairs of mothers and neonates were unable to complete the study in the control group, and 26 pairs did not have neonatal specimens collected at the 1-month follow-up. One pair was not indicated because of admission to the NICU. NICU, neonatal intensive care unit.

Table 1 Patient characteristics.

Patient background	Probiotics (n = 31)	Control (n = 36)	p-value
Pregnant women	n = 31	n = 36	
Maternal age (year)	34 (29–37)	35 (31–37.5)	0.459
Vaginal delivery	24	32	0.358
IAP			
ABPC	23	32	
ABPC + CEZ	1	0	
Cesarean section	7	4	
IAP or SAP			
ABPC + CEZ	2	2	
CEZ	5	2	
Nationality Japanese/Foreign	31/0	36/1	>0.99
Marital status	31	34	0.245
Previous smoker	3	5	0.719
Current smoker	0	1	>0.99
Previous drinking history	0	4	0.120
Current drinking history	0	1	>0.99
Infectious disease	0	1	>0.99
Screening[a] positivity			
Neonates	n = 31	n = 36	
Gestational age	39w4d (38w6d–40w2d)	39w5d (38w5d–40w2d)	0.777
Birth weight	3102 (2884–3254)	3055 (2847–3259)	0.622
Breastfeeding	7	3	0.168
Formula feeding	4	2	0.404
Mixed feeding	20	31	0.0482

Data are presented as the median (range).
Abbreviations: ABPC, ampicillin; CEZ, cefazolin; CS, cesarean section; IAP, intrapartum antibiotic prophylaxis; SAP, surgical antibiotic prophylaxis.
[a] Infectious disease screening; screening for syphilis, hepatitis B virus, hepatitis C virus, and chlamydia.

control and 0% in the probiotic group; the rate of MTCT in the probiotic group was statistically significantly lower than in the control group (p = 0.0191) (Table 2).

3.3. Cases of transient negativity at delivery

Of the 62 cases (29 in the probiotic group and 33 in the control group) involving the collection of vaginal-rectal cultures during delivery, 32 cases (15 in the probiotic group and 17 in the control group) tested positive for the vaginal-rectal culture of the mother during the 1-month checkup. Of these, 4 pairs (1 pair in the probiotic group and 3 pairs in the control group) were positive for the child, and 2 pairs in the control group were confirmed to have MTCT via SNP analysis (cases 9 and 11 in Table 3).

4. Discussion

4.1. GBS positivity rate in neonates

At 5 days and 1 month of age, GBS positivity in rectal swabs was 10% and 10% in the probiotic group and 19% and 28% in the control group, respectively. This was numerically higher than a previous Italian report, wherein the positivity rates for GBS at hospital discharge and at 8 weeks of age were 5.3% and 21.1%, respectively, among infants born to women with GBS who received IAP.[19] Although the control group in the current study had higher positivity rates than the previous study, the probiotic group had lower positivity rates at 1-month follow-up. We believe that the reason for the higher positivity rate at 1 month than at day 5 is that IAP suppressed early postnatal MTCT.

In this study, the GBS positivity rate in the probiotic group was lower than in the control group at both the 5-day and 1-month follow-ups, although the difference was not significant. Maternal vaginal and rectal cultures provide qualitative and not quantitative data, making it difficult to prove whether maternal GBS bacteria loads were reduced. However, the maternal ingestion of *L. reuteri* during late pregnancy may have decreased the number of GBS in the vaginal and intestinal flora and consequently suppressed GBS transmission to the infant. The lack of a significant difference in the overall positivity rate between the probiotic and control groups in this study may be due to the small number of cases; hence, a larger study could reveal a significant difference.

To the best of our knowledge, no studies to date have evaluated the effect of maternal probiotic administration on the rate of GBS retention in newborns. Although there have been reports of the effects of probiotic administration on infectious diseases in newborns, such as necrotizing enterocolitis (NEC) and fungal infections,[9,20–22] this study is unique owing to its focus on GBS incidence.

Table 2 Group B Streptococci (GBS) positivity rates and rates of mother-to-child transmission (MTCT).

Numbers of positive/total	Probiotics (n = 31) No. (%)			Control (n = 36) No. (%)		
	Maternal history of oral antibiotics	No maternal history of oral antibiotics	Total Probiotic group	Maternal history of oral antibiotics	No maternal history of oral antibiotics	Total Control group
Mothers						
GBS culture-positivity						
at delivery	5/23 (22%)	2/6 (33%)	7/29 (24%)	6/28 (21%)	2/5 (40%)	8/33 (24%)
at 1-month checkup	12/18 (67%)	3/6 (50%)	15/24 (63%)	15/28 (54%)	2/5 (40%)	17/33 (52%)
Newborns						
GBS culture-positivity						
at 5 days-old	2/16 (13%)	0/5 (0%)	2/21 (10%)	5/27 (19%)	1/4 (25%)	6/31 (19%)
at 1-month checkup	1/24 (4%)	2/7 (29%)	3/31 (10%)	8/30 (27%)	2/6 (33%)	10/36 (28%)
MTCT of GBS	1/24 (4%)	1/7 (14%)	2/31 (6%)	6/30 (20%)	2/6 (33%)	8/36 (22%)
Simultaneous GBS positivity between mother and child at the 1-month checkup						
MTCT of GBS	0/15 (0%)	0/2 (0%)	0/17 (0%)*	4/16 (25%)	2/2 (100%)	6/18 (33%)*

*p = 0.0191.

4.2. GBS positivity rate in the mothers

The GBS positivity rates in the mothers are shown in Table 2. Vaginal cultures were obtained from 62 to 57 GBS-positive pregnant women (at 35–37 weeks of gestation) at delivery and 1-month follow-up, respectively. At delivery, GBS positivity dropped to 24% in both groups. At the 1-month checkup, more than half of the women in both groups were recolonized with GBS: 63% in the probiotic group and 52% in the control group. This result indicates that eradicating GBS is difficult with a combination of IAP and probiotics. Moreover, no statistically significant difference was observed in the rate of GBS positivity in the vaginal and rectal cultures between mothers with and without *L. reuteri* consumption at the 1-month follow-up. Both groups exhibited lower retention rates than previously reported: 63% for the probiotic group and 52% for the control group.[19] We postulate that the transient reduction in GBS positivity rate during delivery was influenced by administering ABPC within 4 h before delivery in women with vaginal deliveries and CEZ as a preoperative antibacterial agent in women who underwent cesarean sections. Considering studies that reported a GBS positivity rate of 53% after 2 h of IAP, which dropped to 12% after 4 h, our findings align with a reasonable positive rate.[23]

Pregnant women who consumed a fixed-dose combination of 1×10^9 *L. reuteri* and *L. rhamnosus* from 35 to 37 weeks of gestation until the time of delivery had a significantly reduced incidence of GBS at delivery.[10] Table 4 summarizes previous studies that investigated the association between probiotic use and GBS retention in pregnant women. *Lactobacillus* spp. was the strain used in all studies, except for the study by Hanson et al., who used a combination of *Lactobacillus* spp. and *Bifidobacterium* spp.[10,24–29] The study of Martin et al. and our current study were single-strain studies, while other studies used combinations of two or more strains. Bacterial abundance ranged from 10^8 to less than 10^{10} in all studies, with storage methods of 4 °C or room temperature as listed, although this condition varied across studies. Our study utilized a commercial product, which was administered at room temperature below 25 °C according to the instruction manual, with confirmed probiotic intake duration ranging from 2 to 14 weeks. A unique point of our study was the use of 1 month postpartum as the endpoint, whereas other studies employed pregnancy or delivery as their endpoints. The absence of a decrease in the retention rate observed in our study, unlike the results of the study by Ho et al., which had a similar oral administration timing, was thought to be due to the lower bacterial count and the single-strain design of the probiotics in our study as compared to other studies.[10,24–29] A single strain, *Lactobacillus salivarius* CECT 9145 (10^9 CFU of *L. salivarius* CECT 9145), was reported to be effective in reducing GBS bacteria in a study by Martin et al.[25] However, our study did not reveal significant results, possibly because we used a different strain and had a qualitative nature, and the results may have varied during the quantitative analysis. The GBS positivity rates in newborns at 1 month of age are lower in the probiotic group than in the control group; however, the GBS positivity rates of mothers in the first month after delivery

Table 3 Information regarding the 13 mother-neonate pairs wherein GBS were isolated from the rectal swabs of neonates at 1 month of age.

	Study group	Type of delivery		Mother	Newborn	SNPs
1	Probiotics	VD	Serotype	III	III	1
			ST	17	17	
2	Probiotics	CS	Serotype	Ia	IV	4690
			ST	144	452	
3	Probiotics	VD	Serotype	Ia	Ia	38
			ST	144	144	
4	Control	VD	Serotype	NT	Ia	67
			ST	335	335	
5	Control	VD	Serotype	III	Ia	7253
			ST	1948	144	
6	Control	VD	Serotype	III	Ia	5292
			ST	335	626[a]	
7	Control	VD	Serotype	VIII	VIII	1
			ST	1	1	
8	Control	VD	Serotype	V	V	1
			ST	1	1	
9	Control	CS	Serotype	V	V	2
			ST	26	26	
10	Control	VD	Serotype	V	V	2
			ST	19	19	
11	Control	VD	Serotype	IV	IV	3
			ST	452	452	
12	Control	VD	Serotype	V	V	2
			ST	19	19	
13	Control	VD	Serotype	IV	IV	30
			ST	452	452	

Abbreviations: CS, cesarean section; N, negative; NI, positive but not isolated; NT, Nontypability; SNPs, single nucleotide polymorphisms; ST, sequence typing: VD, vaginal delivery; GBS, Group B Streptococci.

[a] As we could not determine the complete sequence type of this strain, we described the sequence type closest to this strain.

were higher. This difference in positivity rate may be related to the amount of GBS carried by the mothers. This study did not assess the bacterial load, but this may be influenced by the amount of GBS bacteria initially carried by the mothers. Another contributing factor was the postpartum prescription of oral antimicrobials to many participating mothers. The impact of such antibacterial medications on the gut microbiota may have constrained the efficacy of the probiotics used in this study.[30]

4.3. MTCT of GBS

Isolation cultures were possible in 13 neonates who tested positive for GBS on rectal swabs at 1 month of age. When WGS was conducted on 13 pairs of mothers and infants, the same strain was carried by the mother and child in 2 of 3 pairs in the probiotic group and 8 of 10 pairs in the control group. The same strain was carried by the mother and child in one case in the probiotic group and four cases in the control group when examined on day 5. These results also indicate that MTCT is an important transmission route for GBS in the early postnatal period. When limited to cases where the mother had been a continuous carrier in the first month after delivery, the probiotic group showed statistically significantly lower MTCT rates. Concurrent maternal carriage has previously been pointed out as a risk for LOD.[2] Our results suggested that probiotics may reduce MTCT in a child at risk of LOD.

In our study, the mother-infant GBS strain concordance rate at 1 month of age was 67% in the probiotic group and 80% in the control group, higher than a previously reported rate of 17%.[31] This may be due to the difference between cases of invasive infection and cases of carriage. Although the transmission of GBS to infants during LOD remains unclear, our results indicate that mother-infant transmission is one of the most important transmission routes for GBS. The neonatal and infantile stages represent a transition period in the diversity of the intestinal microbiota; administering antimicrobial drugs during delivery can have a notable impact on the child's composition of the intestinal microbiota.[30] Although the potential influence of IAPs on the effectiveness of probiotics cannot be dismissed, our analysis of GBS positivity rates within each group with and without maternal antimicrobial history did not reveal any statistically significant differences.

Regarding the three cases where the mothers and children carried different strains of GBS, horizontal transmission from the environment, potentially through contact with caregivers other than the mothers, may have played a role. Furthermore, some studies have reported that

Table 4 Previous reports on probiotics use and GBS carriage in pregnant women.

Reference	Species and daily dose of probiotics (CFUs)	Preservation method of probiotics	Administration duration	Study design	Number of cases enrolled	Result
M. Ho et al.[10]	2×10^9 *L. rhamnosus* GR-1 2×10^9 *L. reuteri* RC-14	NA	From weeks 35—37 of gestation to delivery	Randomized, double-blinded, placebo-controlled trial	55 in the probiotic group and 55 in the placebo group	The GBS retention rate at the time of admission for delivery was significantly reduced at delivery.
A. Farr et al.[24]	4×10^8 *L. jensenii* Lbv116 (DSM 22566) 2×10^9 *L. crispatus* Lbv88 (DSM 22566) 2×10^9 *L. rhamnosus* Lbv96 (DSM 22560) 6×10^9 *L. gasseri* Lbv150 (DSM 22583)	NA	14 days during weeks 33—37 of gestation	Randomized, double-blinded, placebo-controlled trial	41 in the probiotic group, 41 in the placebo group	No significant difference was found in GBS carriage in the vagina after 14 days of probiotic intake.
V. Martin et al.[25]	1×10^9 *L. salivarius* CECT 9145	4 °C	From weeks 26—38 of gestation	Randomized, double-blind, placebo-controlled trial	25 in the probiotic group and 32 in the placebo group	The mean GBS counts decreased significantly with the administration time of probiotics, from a mean value of 5.14 CFU/mL at 26 weeks to 3.80 CFU/mL at 38 weeks.
L. Hanson et al.[26]	$>7.5 \times 10^9$ *L. acidophilus* $>6.0 \times 10^9$ *B. lactis* $>1.5 \times 10^9$ *B. longum*	Room temperature	From week 28 ± 2 to 36 ± 2 of gestation	Open-label, two-group quasi-experiment	10 in the probiotic group and 10 in the control group	No significant differences were found in GBS colony counts between the probiotic and control group participants' vaginal or rectal swabs at 28 ± 2 weeks, 32 ± 2 weeks, and 36 ± 2 weeks gestation.
M. Sharpe et al.[27]	5×10^9 *L. Rhamnosus* GR-1 5×10^9 *L. Reuteri* RC-14	NA	from weeks 23—25 to 35—37 of gestation	Randomized, double-blind, placebo-controlled trial	73 in the probiotic group and 66 in the placebo group	The rates of GBS colonization did not differ significantly between groups at weeks 35—37 of gestation.
S. Husain et al.[28]	2.5×10^9 *Lactobacillus rhamnosus* GR-1 2.5×10^9 *Lactobacillus reuteri* RC-14	NA	from weeks 9—14 of gestation to delivery	Randomized, double-blind, placebo-controlled trial	123 in the probiotic group and 115 in the placebo group	No differences were found between the groups in the rates of vaginal colonization with GBS at weeks 18—20 of gestation.
P. Olsen et al.[29]	1×10^8 *Lactobacillus rhamnosus* GR-1 1×10^8 *Lactobacillus fermentum/reuteri* RC-14	NA	3 weeks or until delivery from week 36 of gestation	Randomized, open-label, controlled trial	21 in the probiotic group, 13 in the control group	No differences were found in the rates of vaginal colonization with GBS between the groups at three weeks post-consent or during labor.

Abbreviations: CFU, Colony-forming unit; NA, No data available; GBS, Group B Streptococci.

mothers can carry multiple strains of GBS, making it difficult to rule out the possibility that multiple strains were present but not identified in our samples.[32]

4.4. Limitations

Certain limitations of this study need to be acknowledged. The examination of neonatal GBS carriage was solely performed via rectal swabs. However, a previous study that investigated multiple samples obtained from the rectum, pharynx, and external ear revealed that the rectum had the highest GBS retention rate. Limiting the sampling to rectal swabs was considered to have the least impact on the retention rates.[19] Moreover, GBS carriage was determined qualitatively, not quantitatively. No other caregivers were investigated for GBS positivity; therefore, the source of horizontal transmission from non-mothers is unknown. We did not explore the possibility of horizontal transmission of GBS through breast milk as we did not culture breast milk samples in this study. However, this was a single-center study with a short follow-up period of only 1 month, the subjects were newborns, and the use of daycare facilities was low, so the differences in environmental factors between cases were considered limited. In addition, the smaller sample size compared to previous reports due to a decrease in the number of deliveries was a limitation.

Overall, mother-to-infant transmission is an important transmission pathway in GBS, even in late newborns. Maternal consumption of *L. reuteri* from late pregnancy to 1 month postpartum may reduce the incidence of GBS in newborns during the first month of life. However, as this was an open-label, small-scale study, larger, more accurate studies are necessary to verify our findings.

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Declaration of competing interest

None.

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References

1. Verani JR, McGee L, Schrag SJ. Prevention of perinatal group B streptococcal disease-revised guidelines from CDC, 2010. *MMMR Recomm Rep* 2010;**59**:1–36.
2. Berardi A, Rossi C, Lugli L, Creti R, Bacchi Reggiani ML, Lanari M, et al. Group B streptococcus late-onset disease: 2003-2010. *Pediatrics* 2013;**131**:e361–8.
3. Tazi A, Plainvert C, Anselem O, Ballon M, Marcou V, Seco A, et al. Risk factors for infant colonization by hypervirulent CC17 group B Streptococcus: toward the understanding of late-onset disease. *Clin Infect Dis* 2019;**69**:1740–8.
4. Buggio L, Somigliana E, Borghi A, Vercellini P. Probiotics and vaginal microecology: fact or fancy? *BMC Womens Health* 2019; **19**:25.
5. Jois RS, Tan JK, Silva D. Do probiotics in pregnancy reduce the risk of group B streptococcal colonisation? *J Paediatr Child Health* 2020;**56**:1468–72.
6. Kubota T, Nojima M, Itoh S. Vaginal bacterial flora of pregnant women colonized with group B streptococcus. *J Infect Chemother* 2002;**8**:326–30.
7. Engels C, Schwab C, Zhang J, Stevens MJ, Bieri C, Ebert MO, et al. Acrolein contributes strongly to antimicrobial and heterocyclic amine transformation activities of reuterin. *Sci Rep* 2016;**6**:36246.
8. Mastromarino P, Vitali B, Mosca L. Bacterial vaginosis: a review on clinical trials with probiotics. *New Microbiol* 2013;**36**: 229–38.
9. Oncel MY, Sari FN, Arayici S, Guzoglu N, Erdeve O, Uras N, et al. Lactobacillus Reuteri for the prevention of necrotising enterocolitis in very low birthweight infants: a randomised controlled trial. *Arch Dis Child Fetal Neonatal Ed* 2014;**99**: F110–5.
10. Ho M, Chang YY, Chang WC, Lin HC, Wang MH, Lin WC, et al. Oral Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 to reduce Group B Streptococcus colonization in pregnant women: a randomized controlled trial. *Taiwan J Obstet Gynecol* 2016;**55**:515–8.
11. Matsubara K, Hoshina K, Suzuki Y. Early-onset and late-onset group B streptococcal disease in Japan: a nationwide surveillance study, 2004-2010. *Int J Infect Dis* 2013;**17**: e379–84.
12. Toyofuku M, Morozumi M, Hida M, Satoh Y, Sakata H, Shiro H, et al. Effects of intrapartum antibiotic prophylaxis on neonatal acquisition of group B streptococci. *J Pediatr* 2017;**190**: 169–73. e1.
13. Morozumi M, Chiba N, Igarashi Y, Mitsuhashi N, Wajima T, Iwata S, et al. Direct identification of Streptococcus agalactiae and capsular type by real-time PCR in vaginal swabs from pregnant women. *J Infect Chemother* 2015;**21**:34–8.
14. Poyart C, Tazi A, Réglier-Poupet H, Billoët A, Tavares N, Raymond J, et al. Multiplex PCR assay for rapid and accurate capsular typing of group B streptococci. *J Clin Microbiol* 2007; **45**:1985–8.
15. Imperi M, Pataracchia M, Alfarone G, Baldassarri L, Orefici G, Creti R. A multiplex PCR assay for the direct identification of the capsular type (Ia to IX) of Streptococcus agalactiae. *J Microbiol Methods* 2010;**80**:212–4.
16. Zubair S, de Villiers EP, Fuxelius HH, Andersson G, Johansson KE, Bishop RP, et al. Genome sequence of Streptococcus agalactiae strain 09mas018883, isolated from a Swedish cow. *Genome Announc* 2013;**1**:e00456-13.
17. Toyama Y, Hisata K, Kasai Y, Nakano S, Komatsu M, Shimizu T. Molecular epidemiology of meticillin-susceptible Staphylococcus aureus in the neonatal intensive care unit. *J Hosp Infect* 2022;**129**:75–81.
18. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant* 2013;**48**:452–8.
19. Berardi A, Rossi C, Creti R, China M, Gherardi G, Venturelli C, et al. Group B streptococcal colonization in 160 mother-baby pairs: a prospective cohort study. *J Pediatr* 2013;**163**: 1099–104.
20. Romeo MG, Romeo DM, Trovato L, Oliveri S, Palermo F, Cota F, et al. Role of probiotics in the prevention of the enteric colonization by Candida in preterm newborns: incidence of late-onset sepsis and neurological outcome. *J Perinatol* 2011; **31**:63–9.

21. Cui X, Shi Y, Gao S, Xue X, Fu J. Effects of Lactobacillus reuteri DSM 17938 in preterm infants: a double-blinded randomized controlled study. *Ital J Pediatr* 2019;**45**:140.
22. Oncel MY, Arayici S, Sari FN, Simsek GK, Yurttutan S, Erdeve O, et al. Comparison of Lactobacillus reuteri and nystatin prophylaxis on Candida colonization and infection in very low birth weight infants. *J Matern Fetal Neonatal Med* 2015;**28**:1790–4.
23. Scasso S, Laufer J, Rodriguez G, Alonso JG, Sosa CG. Vaginal group B streptococcus status during intrapartum antibiotic prophylaxis. *Int J Gynaecol Obstet* 2015;**129**:9–12.
24. Farr A, Sustr V, Kiss H, Rosicky I, Graf A, Makristathis A, et al. Oral probiotics to reduce vaginal group B streptococcal colonization in late pregnancy. *Sci Rep* 2020;**10**:19745.
25. Martín V, Cárdenas N, Ocaña S, Marín M, Arroyo R, Beltrán D, et al. Rectal and vaginal eradication of Streptococcus agalactiae (GBS) in pregnant women by using Lactobacillus salivarius CECT 9145, A target-specific probiotic strain. *Nutrients* 2019;**11**:810.
26. Hanson L, Vandevusse L, Duster M, Warrack S, Safdar N. Feasibility of oral prenatal probiotics against maternal group B Streptococcus vaginal and rectal colonization. *J Obstet Gynecol Neonatal Nurs* 2014;**43**:294–304.
27. Sharpe M, Shah V, Freire-Lizama T, Cates EC, McGrath K, David I, et al. Effectiveness of oral intake of Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 on Group B Streptococcus colonization during pregnancy: a midwifery-led double-blind randomized controlled pilot trial. *J Matern Fetal Neonatal Med* 2021;**34**:1814–21.
28. Husain S, Allotey J, Drymoussi Z, Wilks M, Fernandez-Felix BM, Whiley A, et al. Effects of oral probiotic supplements on vaginal microbiota during pregnancy: a randomised, double-blind, placebo-controlled trial with microbiome analysis. *BJOG* 2020;**127**:275–84.
29. Olsen P, Williamson M, Traynor V, Georgiou C. The impact of oral probiotics on vaginal Group B Streptococcal colonisation rates in pregnant women: a pilot randomised control study. *Women Birth* 2018;**31**:31–7.
30. Schwartz DJ, Langdon AE, Dantas G. Understanding the impact of antibiotic perturbation on the human microbiome. *Genome Med* 2020;**12**:82.
31. Almeida A, Villain A, Joubrel C, Touak G, Sauvage E, Rosinski-Chupin I, et al. Whole-genome comparison uncovers genomic mutations between group B streptococci sampled from infected newborns and their mothers. *J Bacteriol* 2015;**197**:3354–66.
32. Beauruelle C, Pastuszka A, Mereghetti L, Lanotte P. Group B Streptococcus vaginal carriage in pregnant women as deciphered by clustered regularly interspaced short palindromic repeat analysis. *J Clin Microbiol* 2018;**56**:e01949–17.

Appendix A. Supplementary data

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