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Lisa Hanson
Marquette University

Leona VandeVusse
Marquette University

Marie Forgie
Advocate Aurora Health

Emily Malloy
Marquette University

Maharaj Singh
Marquette University

See next page for additional authors

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Lisa Hanson
Marquette University College of Nursing, Milwaukee, WI
Leona VandeVusse
Marquette University College of Nursing, Milwaukee, WI
Marie Forgie
Advocate Aurora UW Medical Group, Aurora Sinai Medical Center, Milwaukee, WI
Emily Malloy
Advocate Aurora UW Medical Group, Aurora Sinai Medical Center, Milwaukee, WI
Maharaj Singh
Marquette University College of Nursing, Milwaukee, WI
Advocate Aurora Research Institute, Milwaukee, WI
MaryAnne Scherer
Advocate Aurora Sinai Midwifery and Wellness Center, Milwaukee, WI
Diana Kleber

Advocate Aurora Research Institute, Milwaukee, WI
Jonah Dixon
University of Wisconsin School of Medicine and Public Health, Madison, WI
Andrew J. Hryckowian
Division of Gastroenterology and Hepatology, Department of Medicine, University of Wisconsin–Madison, Madison, WI
Department of Medical Microbiology and Immunology, University of Wisconsin–Madison, Madison, WI
Nasia Safdar
University of Wisconsin School of Medicine and Public Health, Madison, WI

Abstract

Background

Probiotics have been suggested as a strategy to reduce antenatal group B *Streptococcus* colonization. Although probiotics are known to improve gastrointestinal symptoms, this has not been studied during pregnancy.

Objective

This study aimed to evaluate the efficacy of a probiotic to reduce: (1) standard-of-care antenatal group B *Streptococcus* colonization and colony counts and (2) gastrointestinal symptoms of pregnancy.

Study Design

In a double-blind fashion, 109 healthy adult pregnant people were randomized to Florajen3 probiotic or placebo capsules once daily from 28 weeks' gestation until labor onset. Baseline vaginal and rectal study swabs for group B *Streptococcus* colony-forming units and microbiome analysis were collected at 28 and 36 weeks' gestation. Standard-of-care vaginal to rectal group B *Streptococcus* swabs were collected from all participants at 36 weeks' gestation and determined the need for intrapartum antibiotic prophylaxis. Data collection included solicitation of adverse events, demographic information, Antepartum Gastrointestinal Symptom Assessment score, yogurt ingestion, sexual activity, and vaginal cleaning practices.

Results

A total of 83 participants completed the study to 36 weeks' gestation with no adverse events. Standard-of-care group B *Streptococcus* colonization was 20.4% in the control group and 15.4% in probiotic group participants (−5%; *P*=.73). The relative risk for positive standard-of-care vaginal–rectal group B *Streptococcus* colonization was 1.33 (95% confidence interval, 0.5–3.40) times higher in the control group than in the probiotic group (*P*=.55). There were no differences in median vaginal (*P*=.16) or rectal (*P*=.20) group B streptococcus colony-forming units at baseline or at 36 weeks (vaginal *P*>.999; rectal *P*=.56). Antepartum Gastrointestinal Symptom Assessment scores were similar at baseline (*P*=.19), but significantly decreased in probiotic group participants at 36 weeks (*P*=.02). No covariates significantly altered group B *Streptococcus* colonization. Significantly more Florajen3 bacteria components were recovered from the vaginal–rectal samples of probiotic group participants (32%; *P*=.04) compared with controls.

Conclusion
The findings of this study provided insufficient evidence for the clinical application of the Florajen3 probiotic intervention to reduce standard-of-care vaginal–rectal group B *Streptococcus* colonization. The prevalence of group B *Streptococcus* was lower than expected in the study population, and intervention adherence was poor. Probiotic bacteria colonization of the genitourinary tract occurred more in intervention group participants than in controls and significantly reduced gastrointestinal symptoms of pregnancy.

Key words
Antenatal, gastrointestinal symptoms, group B *Streptococcus,* probiotics

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Why was this study conducted?
Currently 20-30% of pregnant people are exposed to intrapartum antibiotics for GBS prophylaxis. Probiotic interventions have been suggested as a primary prevention strategy for antenatal GBS colonization. The efficacy of probiotics to reduce antenatal GI symptoms has not been studied.

Key findings
GBS colonization was reduced in the probiotics group by 5% (*P*=.73). The relative risk for positive standard-of-care vaginal–rectal GBS colonization (SOC GBS) was 1.33 (95% confidence interval, 0.5–3.40) times higher in the control group than in the probiotic group (*P*=.55). There was no significant difference in GBS colony-forming units or intrapartum antibiotic prophylaxis doses between the groups. Eight weeks of the probiotic intervention significantly reduced GI symptoms of pregnancy compared with placebo (*P*=.02).

What does this add to what is known?
The findings of this study provided insufficient evidence for the clinical application of the Florajen3 probiotic intervention to reduce SOC GBS. Probiotics may be suggested to address GI symptoms of pregnancy. No adverse events occurred.

Introduction
Streptococcus agalactiae (or group B *Streptococcus* [GBS]) is an encapsulated, gram-positive, beta-hemolytic anaerobe that asymptomatically colonizes the genitourinary tract of 20% to 30% of pregnant people in the United States.[1,2] GBS colonization has been associated with employment in health care, African descent, low vitamin D levels, poor vaginal hygiene, being overweight or obese, engaging in oral sex, and frequent sexual intercourse.[3, 4, 5, 6, 7]

Vertical transmission of GBS during normal vaginal birth can lead to neonatal colonization and risk for early-onset GBS disease (EOGBSD). Since 2010, universal GBS third-trimester antenatal screening has been recommended,[8] more recently at 36 0/7 to 37 6/7 weeks' gestation[9] to reduce EOGBSD. This universal screening approach has been adopted in the United States and is recommended in countries and regions with high GBS prevalence. Although highly effective at reducing EOGBSD, perinatal exposure to antibiotics may have unintended consequences for laboring people and neonates.[10]

Antenatal probiotic interventions have been suggested as a strategy to reduce antenatal GBS colonization and the need for intrapartum antibiotic prophylaxis.[8,11] According to in vitro studies, the mechanisms of action of probiotics in reducing GBS are acidification, adherence to vaginal epithelial cells, and immune modulation.[12] To date, 7 clinical trials of probiotics to reduce GBS have been published.[13, 14, 15, 16, 17, 18, 19] Probiotic interventions varied between studies in terms of species and strain, dosage, and gestational age at initiation. Many of these studies were pilot and/or feasibility studies and lacked the statistical power to demonstrate efficacy of the probiotic intervention.[12] The purpose of this paper is to present the findings of a phase II, double-blind, randomized, placebo-controlled trial of an antenatal probiotic intervention to reduce GBS colonization. A secondary purpose was to determine if the probiotic intervention reduces gastrointestinal (GI) symptoms of pregnancy.

Materials and Methods

The US Food and Drug Administration (FDA) determined that a full Investigation of New Drug Application (IND) was required before this study could be initiated. This was because of the special population of pregnant participants and the "drug use" of the probiotic to reduce GBS.[20] This study was approved by 3 institutional review boards (IRBs), including those of the study setting (a large Midwestern tertiary-care hospital) and the universities of the principal investigator and coinvestigators.

On the basis of the knowledge that 30% of the prenatal population of the study setting was colonized with GBS in the previous year, a power analysis determined that a sample size of 80 (40 probiotic, 40 placebo) was required to show a 22% decrease in the proportion of standard-of-care vaginal–rectal GBS colonization (SOC GBS) between groups with 80% power (alpha=0.05). In a pilot and feasibility study, 30% of healthy pregnant participants were withdrawn or dropped out[13]; therefore, up to 116 participants would be enrolled to achieve the desired sample size. Enrollment and data collection took place between February 2019 and June 2021, with an IRB-required COVID-19 recruitment pause from May to August 2020. Inclusion and exclusion criteria appear in Table 1. Study enrollment was from 28±2 weeks' gestation through 2 months after birth when data collection was completed. A schema of the study is presented in Figure 1.

Table 1. Study inclusion and exclusion criteria

Inclusion criteria
Healthy adult (aged ≥18 y) pregnant women who are at 28±2 weeks' gestation at enrollment (calculated from the first day of last normal menstrual period and/or ultrasound)
With: no obstetrical complications[a] (eg, preeclampsia, gestational diabetes mellitus, multiple gestation)
No fetal complications (eg, birth defect, intrauterine growth restriction)
No medical complications (eg, hypertension, diabetes mellitus)
Who do not currently ingest an over-the-counter probiotic supplement (not including yogurt)
Who can both speak and read English
Who regularly attend prenatal care appointments (defined as not >1 previous missed appointment during this pregnancy)
Exclusion criteria

Pregnant women who have a history of GBS bacteriuria during the current pregnancy or have previously given birth to a GBS-affected child
Women who are planning an elective repeated cesarean delivery

GBS, group B Streptococcus.

[a]Multigravidas with uncomplicated GBS colonization in a previous pregnancy were eligible for participation in the study.

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STUDY VISITS	1	2		3	4	5
	28±2 weeks	36±2 weeks	Birth	1-2 days post birth	6 weeks post birth	12 weeks post birth
	Probiotic Capsules or Placebo Capsules					
		Screened for Participant Adverse Events*				
		Screened for Newborn Adverse Events*				
	Enrollment Consent Allocation Study bottle			Returned study bottle		
LABS	Study GBS vaginal and rectal swabs (GBS CFUs and Microbiome)	Standard of Care (SOC) GBS vaginal to rectal screening & Study GBS vaginal and rectal swabs (GBS CFUs and Microbiome)	IAP as indicated by SOC GBS			
IPAD QUESTIONNAIRES	• Demographics • AP-GI-SA • Yogurt ingestion • Sexual activity	•AP-GI-SA •Yogurt ingestion •Sexual activity		•Yogurt ingestion •Sexual activity		

Note: *Required by the FDA

Figure 1. Study schema

The *superscript letter a* denotes required by the US Food and Drug Administration.

AP-GI-SA, Antepartum Gastrointestinal Symptom Assessment; *CFU*, colony-forming units; *GBS*, group B *Streptococcus; IAP*, Intrapartum Antibiotic Prophylaxis; *SOC GBS*, standard-of-care vaginal–rectal GBS colonization.

The study capsules were initiated at 28±2 weeks' gestation. Each Florajen3 capsule (American Lifeline, Inc. 138 First Street, Baraboo, WI.) contained 15×10^9 colony-forming units (CFUs) of freeze-dried probiotic bacteria (*Lactobacillus acidophilus, Bifidobacterium lactis*, and *Bifidobacterium longum*) combined with a carrier of microcrystalline cellulose (MCC). The placebo capsules were identical in appearance and taste and composed of MCC. Both probiotic and placebo capsules were tested for additional standards required by the FDA IND (available in online supplemental materials). Randomization to probiotic and placebo groups was determined by a random numbers table used by an investigational pharmacist who: (1) bottled and labeled the study capsules with a participant's study number, blind to the rest of the investigative team, (2) kept the list of group assignment by study number, and (3) applied a MEMS cap counter (AARDEX Group, Seraing, Belgium) to each study bottle. Each opening of the bottle was recorded by the related adherence software. The bottles were refrigerated and distributed by the research coordinator, sequentially by study number as participants were enrolled. During the required COVID-19 enrollment pause, probiotic and placebo capsules were

retested for potency and purity at a third-party laboratory and then placed in a deep freezer. Probiotic potency testing was completed every 6 months throughout the study in the infectious disease laboratory of the seventh author. Participants were asked to keep the study bottle refrigerated and were assisted in setting up a mobile phone daily reminder to take their capsule and were reminded about pill adherence at each study visit.

The certified research coordinators (CRCs) screened participants, completed the informed consent, enrolled participants, and initiated the study capsules. They monitored data collection through all study visits and entered data into the REDCap system (Vanderbilt University, Nashville, TN). The CRCs assisted the principal investigator in preparing reports for Data Safety Monitoring Board meetings, IRB reviews, and required FDA IND reports. After training in data collection and meeting the IRB requirements for study participation, prenatal providers (certified nurse–midwives, nurse practitioners, and obstetrician–gynecologists) completed the state-required portions of the informed consent process (discussion of potential risks and benefits) and facilitated study swab collection.

A series of iPad questionnaires were used, including a brief demographic survey that was self-administered only at study enrollment. At baseline (28±2 weeks) and 36 weeks' gestation, participants completed the Antepartum Gastrointestinal Symptom Assessment (AP-GI-SA),[21] in which each of the 10 GI symptoms on the AP-GI-SA is scored from 1 (no problem) to 5 (very severe problem), leading to a composite score between 10 and 50. A questionnaire (presented in online supplemental materials) about dietary yogurt ingestion, sexual activities, and vaginal cleaning practices during the past week (all considered potential confounding variables) was also administered at baseline and at 36 weeks' gestation. All these questionnaires had been pilot-tested in a feasibility study.[13] Potential intervention side effects were also solicited at each prenatal and study visit.

Study visits were planned to coincide with scheduled prenatal visits. All participants had the option of self- or provider-collected swabs and had the SOC GBS swab at 35 to 37 weeks (changed to 36 0/7 to 37 6/7 weeks in 2019, on the basis of updated guidelines).[9] Positive SOC GBS swabs reflect the detection of any GBS on the agar plate.[22] SOC GBS swabs were analyzed by the hospital laboratory; results were a part of the electronic medical record and thus determined the need for intrapartum antibiotic prophylaxis. At study enrollment and 36 weeks' gestation, participants had separate vaginal and rectal study swabs for 2 purposes: (1) GBS colony counts in CFUs and (2) to examine the microbiome. These study swabs were labeled with the participant study number and kept refrigerated, and were shipped overnight to the infectious disease laboratory for plating and incubation. Each swab was vortexed in 1 mL of 1X phosphate-buffered saline (PBS) for 15 seconds. The solution was serially diluted in PBS, plated on Granada agar, and incubated at 36°C for 24 hours in a candle jar. The number of discrete GBS colonies on each plate was counted, recorded, and used to calculate the CFU/mL value, accounting for dilution factor. Microbiome swabs were used to identify if the probiotic bacteria were present in the vagina and rectum of study participants at 36 weeks' gestation. DNA extracted from microbiome swabs was analyzed for bacterial composition using 16S ribosomal RNA sequencing of the v4 region on the Illumina MiSeq (Illumina, Inc, San Diego, CA) using 2 × 250 paired-end reads.

Data were imported into SAS, version 9.4 (SAS Institute, Cary, NC) and cleaned and double-checked for accuracy. Intention-to-treat analysis was used. All categorical variables including demographics and

outcome variables were reported as frequencies and percentages and were compared using chi-square testing and/or Fisher exact tests for the 2 groups (probiotics and control), whereas for numeric continuous variables, the means for the 2 groups were compared using the *t*-test for independent samples. Logistic regression models were used to predict positive GBS and to predict probiotic group membership. For all statistical tests, an alpha of 0.05 was used, and all statistical analysis was done using SAS version 9.4.[23]

Results

Data collection was completed in August 2021, after which unblinding occurred. Probiotic potency was maintained throughout the study period during all 5 testing periods in a range of 15×10^9 to 21.3×10^9 CFU. Purity testing before and after freezing for the COVID-19 recruitment pause demonstrated that the FDA standards for purity were maintained.

A Consolidated Standards of Reporting Trials (CONSORT) flow diagram of recruitment, enrollment, study completion, and withdrawals is presented in Figure 2. Eighty-three participants completed the study to the 36-week time point—39 in the probiotics group and 44 in the control group. Participants were withdrawn from the study if they developed pregnancy complications at any point in the study. Of the 6 participants who withdrew from the probiotic group, one did so because of "loose stools" that she attributed to the study capsules.

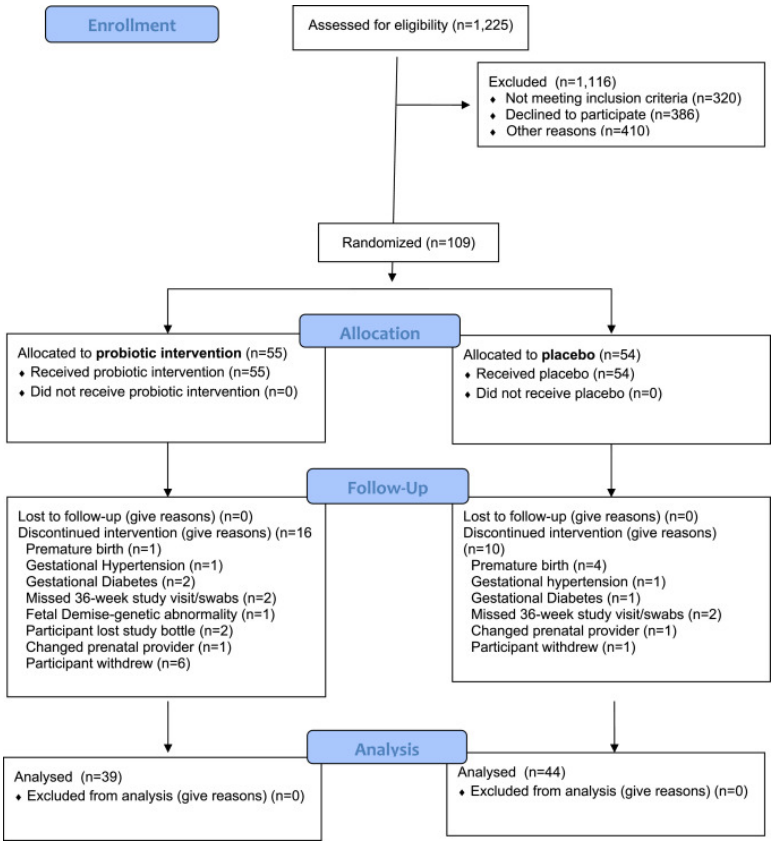

Figure 2. CONSORT flow diagram

CONSORT, Consolidated Standards of Reporting Trials.

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A comparison of probiotic and placebo participants' demographic characteristics, perinatal and neonatal outcomes, AP-GI-SA scores, intrapartum antibiotic prophylaxis doses, and adverse events is presented in Table 2. There were no significant differences between the groups in demographic characteristics, perinatal or neonatal outcomes, or intrapartum antibiotic prophylaxis doses. At baseline (28±2 weeks' gestation), the average AP-GI-SA scores were similar between groups (control group=16.2±4.9; probiotic group=14.9±3.4; Cohen's d=0.29; 95% confidence interval [CI], −0.145 to 0.722; *P*=.19). At 36 weeks, probiotic group participants had significantly lower scores (control group=15.6±3.9; probiotic group=13.7±2.9; Cohen's d=0.528; 95% CI, 0.087–1.088; *P*=.2).

Table 2. Demographic and perinatal and neonatal outcomes of study participants

Variable	**Total (N=83)**	**Probiotics (N=39)**	**Placebo (N=44)**	***P* value**
Age (y), mean±SD	28.5±5.5	28.6±5.4	28.4±5.5	.88
Race, n (%)				
Asian	4 (4.8)	2 (5.1)	2 (4.6)	.77
Black	33 (39.8)	17 (43.6)	16 (36.4)	
White	42 (50.6)	19 48.7)	23 (52.3)	
Other[a]	4 (4.8)	1 (2.6)	3 (6.8)	
Ethnicity, n (%)				
Hispanic	6 (7.2)	2 (5.1)	4 (9.1)	.49
Non-Hispanic	77 (92.8)	37 (94.9)	40 (90.9)	
Gestational age at birth		39.0 (1.4)	39.28 (1.1)	.48
Parity, n (%)				
Nulliparous	36 (43.9)	16 (41.0)	20 (46.5)	.62
Multiparous	46 (56.1)	23 (59.0)	23 (53.5)	
Mode of birth, n (%)				
Vaginal	67 (80.7)	32 (82.1)	35 (79.6)	>.99 (Fisher test)
Cesarean	15 (18.1)	7 (17.9)	8 (18.2)	
Vacuum	1 (1.2)	0 (0.0)	1 (2.3)	
Apgar scores (mean±SD)				
1-min	7.7±1.2	7.6±1.1	7.6±1.2	.95
5-min	8.8±0.6	8.8±0.5	8.8±0.6	.84
Neonatal resuscitation, n (%)				
Yes	18 (21.7)	7 (18.0)	11 (25.0)	.44
No	65 (78.3)	32 (82.0)	33 (75.0)	
Neonate (mean±SD)				
Birthweight	3361.2±461.6	3368.3±479.3	3355.0±450.8	.89
Length	51.8±2.8	51.5±2.8	52.1±2.8	.41
Head circumference	34.1±1.7	34.2±1.5	34.0±1.8	.57
AP-GI-SA score (mean± SD)				
Baseline 28 wk	15.6±4.3	14.7 (3.2)	16.2 (4.8)	.99
36 wk	14.7±3.6	13.58 (2.77)	15.64 (3.96)	.01

Intraparutm antibiotic prophylaxis, n (%)				
0 doses	77 (97.8)	34 (87.2)	43 (97.7)	.09
≥1 doses	6 (7.2)	5 (12.8)	1 (2.3)	(Fisher test)
Education				
Less than HS		1 (2.6)	3 (6.8)	.94
HS/GED		12 (30.8)	13 (29.6)	
Some college		7 (18.0)	8 18.2)	
2-y college		3 (7.7)	3 (6.8)	
4-y college		12 (30.8)	11 (25.0)	
Master's degree		4 (10.3)	6 (13.6)	
Marital status, n (%)				
Single/divorced	42 (50.6)	19 (48.7)	23 52.3)	.74
Partnered/married/domestic partner	41 (49.4)	20 (51.3)	21 (47.7)	
Living arrangement, n (%)				
Live alone	9 (11.0)	4 (10.3)	5 (11.6)	>.99 (Fisher test)
Living with others	82 (89.0)	35(89.7)	38 (88.4)	
Income level, n (%)				
<$40,000	40 (49.4)	20 (51.3)	20 (47.6)	.78
$40,000–69,999	14(17.3)	8 (20.5)	6 (14.3)	
$70,000–99,999	12 (14.8)	5 (12.8)	7 (16.7)	
>$100,000	15 (18.5)	6 (14.4)	9 (21.4)	

AP-GI-SA, Antepartum Gastrointestinal Symptom Assessment; *GED*, General Educational Development; *HS*, high school; *SD*, standard deviation.

[a]Did not answer or indicated >1 race.

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Baseline and 36-week GBS CFUs were compared between groups and with SOC GBS culture results. These findings are presented in Table 3. Participants in the probiotic group had 5.075% reduction in SOC GBS. In the analysis of group differences in only positive results at 36 weeks' gestation, the probiotic group participants had 20% less positive SOC GBS compared with the control group (*P*=.55). The relative risk for positive SOC GBS was 1.33 (95% CI, 0.5–3.40) times higher in the control group than in the probiotic group (*P*=.55). No significant differences were found in average GBS CFUs between probiotic and control group participants (*P*=.18), over time (baseline and 36 weeks; *P*=.36) or interaction effect (group × time; *P*=.36). Similarly, no significant differences were found in median GBS CFUs between probiotic and control group participants at either time point. Figure 3 shows the median vaginal and rectal CFUs among participants who had any detectable GBS CFUs at baseline (28 weeks) and 36 weeks. Three cases of GBS were identified on the 36-week vaginal or rectal quantitative study swabs (CFUs) but not on the SOC GBS swabs.

Table 3. Comparison of participant group B *Streptococcus* findings

CFU	GBS source	Probiotic (N=39)	Placebo (N=44)	*P* value
Quantitative CFU				
Baseline 28-wk, mean (SD)	Vaginal	10.4 (2.4)	991.8 (4848.6)	.19
	Rectal	584.6 (3560.6)	992.9 (3588.5)	.67
Baseline 28-wk, median (range)	Vaginal	10 (10–25)	10 (10–29,700)	.16[a]
	Rectal	12.5 (10–2250)	10 (10–22,740)	.20[a]
36-wk, mean (SD)	Vaginal	11.32 (6.65)	317.1 (1957.1)	.31
	Rectal	604.2 (2684.2)	2046.6 (12,805.4)	.47
36-wk, median (range)	Vaginal	10 (10–3,225,000)	10 (10–13,000)	>.99[a]
	Rectal	755 (10–16,500)	62.5 (10–85,000)	.56[a]
Standard-of-care vaginal-rectal GBS colonization				
36-wk, n (%)	Vaginal to rectal	6/39 (15.38%)	9/44 (20.45%)	.73

CFU, colony-forming unit; *GBS*, group B *Streptococcus; SD*, standard deviation.

[a]*P* value using Mann–Whitney test.

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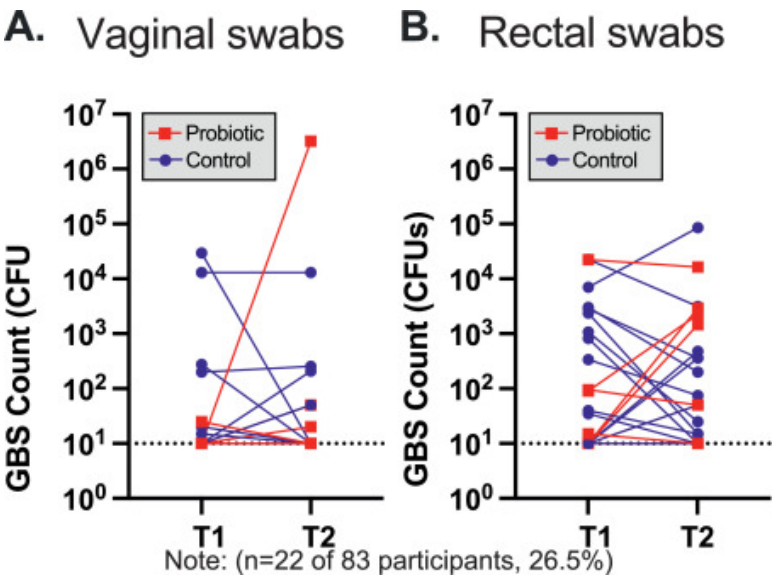

Figure 3. Median GBS CFUs among participants with any bacteria detected at T1 and/or T2

CFU, colony-forming unit; *GBS*, group B *Streptococcus*.

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Polymerase chain reaction (PCR) testing was used to identify the presence of the 3 probiotic bacteria contained in the Florajen3 capsules (*B longum, B lactis, L acidophilus*) in the vaginal and rectal microbiomes of study participants. There was no significant difference between groups in the presence of the probiotic bacteria on the rectal swabs (*P*=.15), whereas the vaginal swabs showed a trend toward greater presence in probiotic group participants (*P*=.07). When the positive vaginal and rectal PCR findings were combined, significantly more of the probiotic group participants had at least 1 of the 3 probiotic bacteria in vaginal and/or rectal swabs compared with those in the control group (68% vs 32%; *P*=.04).

MEMS counts and manual capsule counts provided identical adherence data. The MEMS cap software indicated that the average adherence rate was 0.51±0.5 (probiotic group) and 0.60±0.3 (control group) and was not significantly different between groups (Cohen's d=0.230; 95% CI, −0.203 to 0.661; *P*=.3).

More probiotic participants took antenatal antibiotics (5/39) compared with controls (1/44; *P*=.1). Regression analysis was used to identify individual characteristics of participants who were previously shown to be associated with GBS carrier status or to detect alterations in the microbiome. Predictor variables used in the regression model were African American race, types of sexual activity, antibiotic use, and vaginal cleansing practices. When all covariates were entered into the regression model, they predicted 61% of cases of positive SOC GBS. However, none of the individual covariates adjusted for the remaining covariates in the regression model significantly predicted positive GBS (*P*>.05).

Comments

Principal findings

Probiotic group participants had a small decrease in SOC GBS compared with controls, which was not statistically significant. Subsequently, there was no significant difference in GBS CFUs or intrapartum antibiotic prophylaxis doses between groups. Although not significantly different at baseline, AP-GI-SA scores were significantly reduced in probiotic group participants at 36 weeks' gestation. The study participants had no adverse events.

Results in the context of what is known

The 20% reduction in GBS prevalence in the probiotic group allowed comparison with a meta-analysis of 6 clinical trials (N=709).[13, 14, 15, 16, 17, 18] An antenatal probiotic intervention reduced the probability of a positive GBS result by 44% (odds ratio, 0.56; 95% CI, 8.7–194.1; *P*=.02) (N=709).[12] The small effect size in this study did not reach statistical significance and therefore did not provide convincing evidence against the hypothesis that the treatment effect was 0. A phase-3 study is warranted to determine the efficacy of the intervention in a larger population.

One of the critiques of probiotic interventions are the uncertainties of probiotic manufacturing, dosage, and potency. Probiotic dosages in clinical trials to reduce antenatal GBS colonization ranged from 1×10^8 to 15×10,[13, 14, 15, 16, 17, 18, 19] with efficacy shown at a dosage of 2×10^9 CFU for an average of only 20 days.[15] This study used a high-potency commercially available multispecies probiotic intervention under an FDA IND. The probiotic intervention maintained its potency and purity throughout the study despite the need for freezing during the COVID-19 recruitment pause.

Previous clinical trials did not report demographic variables and covariates that can contribute to increased or decreased GBS colonization. In this study, the sample was diverse in terms of race, ethnicity, relationship status, education level, and socioeconomic status. We collected participant demographics, sexual practices, vaginal cleansing practices, antibiotic use, and yogurt ingestion to verify GBS risk factors, in accordance with the literature. Although these factors explained 61% of the variability in SOC GBS, none of the individual variables significantly predicted GBS colonization.

A reduction in GI symptoms was associated with probiotic interventions in 2 previous studies of probiotics to reduce antenatal GBS colonization.[13,14] This study provided a quantitative measure for this outcome: the AP-GI-SA. The Cronbach alpha for the AP-GI-SA in this study was 0.77 vs 0.67 in preliminary testing.[21]

Participants who developed pregnancy complications after enrollment were withdrawn. The FDA required solicitation of potential adverse events at each study visit and prenatal visit and for 2 months following birth for both birth givers and infants. The study Data Safety Monitoring Board determined that the report of loose stools by one participant was not an adverse event; therefore, no adverse events were reported in this study. There were no significant differences in antenatal complications leading to study withdrawal between groups, and there were fewer premature births in the probiotic group participants. In >2 decades of research, there has been no evidence of serious adverse events in studies of antenatal probiotic interventions.[24] A recent Cochrane review aimed at determining the evidence for antenatal probiotic interventions to prevent gestational diabetes mellitus, including 7 trials with 1647 participants.[25] Two of the studies included only overweight and/or obese participants. On the basis of a subanalysis/sensitivity analysis (4 studies of 955 participants),[26, 27, 28, 29] the authors concluded that probiotic interventions significantly increased the risk of preeclampsia (relative risk, 1.85; 95% CI, 1.04–3.29). In contrast, Sheyholislami et al[30] systematically reviewed and meta-analyzed 95 randomized controlled trials where probiotic interventions were used during pregnancy and lactation. The authors found 11 trials in which minor adverse events were reported, including primarily GI side effects (flatulence and intestinal cramping, nausea, softer stool consistency, and altered taste). No serious adverse events were associated with antenatal probiotic interventions for the pregnant person or neonate. The safety of probiotic interventions in low-risk pregnant populations is well-supported,[24,30] whereas cautious use in higher-risk pregnant populations may be warranted.

Clinical implications

Currently, there are no evidence-based primary prevention strategies for antenatal GBS colonization. The findings of this study provide insufficient evidence for the clinical application of the Florajen3 probiotic intervention to reduce SOC GBS.

The management of GI symptoms of pregnancy presents challenges for pregnant people and providers.[31] Multispecies probiotic interventions have been shown to significantly increase gut transit time and stool frequency and enhance consistency in constipated (nonpregnant) adults.[32] Probiotic group participants had reduced GI symptoms of pregnancy after 8 weeks of the intervention. Prenatal providers could recommend a trial of an over-the-counter probiotic supplementation such as Florajen3 (now marketed as Florajen Digestion) to address GI symptoms of pregnancy.

Research implications

On the basis of the findings of this study, larger well-controlled trials of probiotics to reduce GI symptoms of pregnancy are recommended. Because nutrition seems to be a significant factor in the prenatal microbiome,[33] the addition of a comprehensive dietary inventory such the Automated Self-Administered 24-Hour (ASA24) Dietary Assessment Tool[34] would provide more detail about dietary habits of participants.

GBS CFUs did not significantly differ between groups at baseline or 36 weeks' gestation. Although the GBS CFUs identified participants who were missed on the SOC GBS screening, this measure did not contribute to an enhanced understanding of antenatal GBS colonization in relation to the probiotic intervention. Current Centers for Disease Control and Prevention (CDC)–recommended laboratory analysis techniques for SOC GBS do not use CFU counts (CDC for laboratory clinicians).[22] Although not useful for group comparisons in this study, GBS CFU colony counts may be valuable in future probiotic intervention studies to explore GBS serotyping. According to a systematic review of in vitro studies of probiotics to reduce GBS, certain GBS serotypes may be more responsive to probiotic interventions in vivo.[12]

The microbiome analysis results verified adherence to the study intervention given that the bacterial species in the probiotic intervention were present more frequently in the vaginal and rectal microbiomes of study participants than in those of controls. MEMS cap counts provided data identical to manual pill counts. Combined adherence monitoring and enhancement systems are now available for clinical trials including bottle caps that provide visual and auditory reminder signals and software allowing smart phone reminders and messaging.

Strengths and limitations

This study had several strengths and limitations. Despite the 4-month recruitment pause, the desired sample size was achieved. The dropout rate was 24%, lower than the 30% in the preliminary and feasibility study.[13] The rates of SOC GBS in the control group reflected a lower population rate of GBS than what was used to calculate sample size. The low adherence to the study capsules and loss to follow-up were additional limitations of the study. The average intake of study capsules was half, despite efforts by the research coordinator to set alarms on participants' phones and verbal reminders to take the study capsules daily. The study capsules were refrigerated, which may have negatively influenced adherence. Low adherence to the intervention may have contributed to the lack of statistically significant findings in this study.[35]

Conclusions

GBS colonization was reduced in the probiotics group. The prevalence of GBS was lower than expected in the study population, and intervention adherence was poor. Probiotic bacteria colonization of the genitourinary tract occurred more in the intervention group than in the control group and significantly reduced GI symptoms of pregnancy.

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