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Abstract

Objective

To examine the effect of an oral prenatal probiotic on group B *Streptococcus* (GBS) colonization and to demonstrate the feasibility of a larger randomized controlled trial.

Design

This pilot study was an open-label, two-group quasi-experiment.

Setting

An urban central city nurse-midwifery and wellness center serving a diverse population.

Participants

Ten pregnant participants received the oral probiotic (Florajen3) taken once daily, and 10 participants served as controls.

Methods

A questionnaire on dietary practices, vaginal cleansing, sexual history, and symptoms and GBS colony count samples were taken at 28-, 32-, and 36-weeks gestation.

Results

Participants in the probiotic group reported no adverse events or minor side effects; one half reported improved gastrointestinal symptoms. Although two women in each group had positive qualitative prenatal GBS cultures at 36 weeks, the probiotic group participants had lower quantitative GBS colony counts. The eight GBS negative averaged 90% probiotic adherence compared with two GBS positive women who averaged 68%. Yogurt ingestion was inversely related (p= .02) to GBS colonization.

Conclusions

Prenatal probiotic therapy has the potential to reduce GBS colonization. The potential of the probiotic intervention appears to be linked to daily adherence. A controlled clinical trial with a larger, adequately powered sample is feasible and justified.

Keywords

Pregnancy, probiotics, group B *Streptococcus*

Introduction

Normal bacterial flora such as *Bifidobacterium* species make up more than 90% of normal colon microflora and are widely regarded as markers of gut and immune health (Centers for Disease Control and Prevention [CDC], 2002, 2010; Fooks & Gibson, 2002). The bacterial environment can be enhanced through probiotic bacteria supplementation. Probiotics are considered a food supplement and are generally regarded as safe because they are not systemically absorbed in healthy individuals (Elias, Bozzo, & Einarson, 2011). They confer health benefits on the host through a number of mechanisms, including maintaining homeostasis of gut bacteria, acidifying mucosal surfaces, and preventing pathogen adherence (Food and Agriculture Organization of the United Nations and World Health Organization [FAO/WHO], 2001). Probiotics must survive the acidic human gastrointestinal

tract and adhere to the gut mucosa (FAO/WHO, 2002). Multispecies probiotic supplements may be beneficial due to synergies that enhance healthy colonization on mucosal surfaces (Kim et al., 2006).

Vaginal Flora and Probiotic Interventions

Independent of hygiene, most vaginal bacteria originate and ascend from intestinal microbes (Bolton, Van Der Straten, & Cohen, 2008). The predominance of *Lactobacillus* is the marker for healthy vaginal flora and can be identified on microscopic examination of vaginal secretions (Donders, 1999). There have been a number of studies of women's health applications of probiotics because vaginal flora can be modified through the use of probiotic supplements (Abad & Safdar, 2009). The authors of a meta-analysis concluded that oral probiotics can treat bacterial vaginosis, though efficacy against candidiasis or cystitis was not demonstrated (Abad & Safdar, 2009).

Midwives suggest probiotics as a safe, nonpharmacologic strategy to prevent group B *Streptococcus* infection, although this clinical approach had not been scientifically studied previously.

Prenatal Probiotics

The authors of an integrative review of the literature documented that a variety of probiotics have been administered during clinical trials that included more than 2,000 pregnant experimental group participants who resided in mostly European countries with no reports of side effects or negative sequelae (VandeVusse, Hanson, & Safdar, 2013). Prenatal probiotics significantly reduce the risk of atopy in offspring (West & Prescott, 2013); however, more research specific to maternal and neonatal outcomes is needed.

Probiotic Actions against Group B *Streptococcus (GBS)*

Midwives have suggested probiotics as a safe, nonpharmacologic strategy to prevent GBS infection (Singleton, 2007), though this clinical approach had not yet been examined scientifically at the time of our study. *Lactobacillus* predominance in vaginal flora was associated with reduced GBS colonization in two studies. In a randomized controlled trial (RCT) of 191 nonpregnant women, a perineal topical probiotic intervention decreased GBS in vaginal flora (Ronnqvist, Forsgren-Brusk, & Hakansson, 2006). An analysis of the vaginal flora of 201 pregnant Iranian women (who did not receive a probiotic intervention) revealed that 12% were GBS colonized and that *Lactobacillus* was inversely related to GBS colonization (Moghaddam, 2010).

In vitro testing demonstrated that the live culture, freeze-dried, probiotic combination Florajen3 (> 7.5 x10^9 *L. acidophillus*, > 6.0 x10^9 *B. lactis*, and > 1.5 x10^9 *B. longum*) inhibits GBS when they are cultured together independent of lactic acid production (Ephraim et al., 2012). Florajen3 had good adherence to epithelial cells in cell culture and resulted in a significant drop in the pH (Ephraim et al., 2012). These findings suggested that Florajen3 administered prenatally has the potential to reduce GBS colonization in vivo.

Prenatal GBS Colonization

GBS causes the most prevalent perinatal infection, and GBS colonization of the vaginal and gastrointestinal tracts is transient (Kubota, Nojima, & Itoh, 2002), with rates in the range of 10% to 30% with higher percentages among African Americans (Hickmann, Rench, Ferrieri, & Baker, 1999). GBS is transferred to the neonate during the birth process through contact with the birth canal (CDC, 2010). Neonates exposed to GBS are at risk for acquiring early-onset group B *Streptococcus* disease (EOGBSD), which has a significant mortality rate of 5% to 10% (CDC 2010). The rates of EOGBSD vary by continent according to the United Kingdom National Screening Committee (UK NSC; 2012). The lowest average rate of EOGBSD per 1,000 live births was reported in Southeast Asia (0.11) with higher rates reported in Africa (0.53), Europe (0.45), and the Americas (0.50). Internationally, there is variation in approaches to EOGBSD prevention. In some countries such as the United Kingdom, a risk-based approach is used to determine candidates for intrapartum antibiotic prophylaxis (IAP) (UK NSC, 2012),

whereas in other countries, prenatal screening and risk factors are combined to determine the need for IAP (Association of Ontario Midwives, 2010).

Prevention Guidelines for EOGBSD

GBS causes the most prevalent perinatal infection, and GBS colonization of the vaginal and gastrointestinal tracts is transient (Kubota, Nojima, & Itoh, 2002), with rates in the range of 10% to 30% with higher percentages among African Americans (Hickmann, Rench, Ferrieri, & Baker, 1999). GBS is transferred to the neonate during the birth process through contact with the birth canal (CDC, 2010). Neonates exposed to GBS are at risk for acquiring early-onset group B *Streptococcus* disease (EOGBSD), which has a significant mortality rate of 5% to 10% (CDC 2010). The rates of EOGBSD vary by continent according to the United Kingdom National Screening Committee (UK NSC; 2012). The lowest average rate of EOGBSD per 1,000 live births was reported in Southeast Asia (0.11) with higher rates reported in Africa (0.53), Europe (0.45), and the Americas (0.50). Internationally, there is variation in approaches to EOGBSD prevention. In some countries such as the United Kingdom, a risk-based approach is used to determine candidates for intrapartum antibiotic prophylaxis (IAP) (UK NSC, 2012), whereas in other countries, prenatal screening and risk factors are combined to determine the need for IAP (Association of Ontario Midwives, 2010).

Unintended Consequences of IAP

Up to 30% of women in labor are exposed to IAP (Glasgow et al., 2005; Ohlsson & Shah, 2009), which changes their experiences of otherwise normal labor and birth (Hanson & VandeVusse, 2010). Intrapartum antibiotic prophylaxis is associated with unintended consequences for the woman, including an increased incidence of antibiotic resistance (Glasgow et al., 2005), allergic sensitization (McKeever et al., 2002), diarrhea (including *Clostridium difficile*) (de Vrese, 2009), and fungal infections (de Vrese, 2009). Neonates exposed to IAP have an increased incidence of *Escherichia coli* infections (Bizzarro, Dembry, Baltimore, & Gallagher, 2008; Glasgow et al., 2005), a greater risk of allergic sensitivity (Bedford Russell & Murch, 2006), and thrush (Ohlsson & Shah, 2009). Further, neonates born to GBS positive mothers must be observed for 48 hours for signs of EOGBSD, resulting in longer lengths of stay (Balter et al., 2003; Buckler et al., 2010; Glasgow et al., 2007) with increased hospital costs (Buckler et al., 2010; Glasgow et al., 2007).

The purpose of our quasi-experimental pilot study was to test the effect of oral prenatal probiotics against GBS colonization in pregnancy and evaluate the feasibility of a larger RCT. If prenatal probiotic exposure can be shown to reduce GBS colonization, then fewer women would require IAP and fewer fetuses would be exposed to adult doses of antibiotics shortly before birth.

Methods

A nonblinded, open-label, quasi-experimental design was approved by the Intitutional Review Boards of three institutions (two universities and the clinical research site). The study setting was a large clinical practice of certified nurse-midwives (subsequently referred to as midwife) serving a culturally and economically diverse urban population in the Midwest region of the United States.

Participants

Women self-selected to participate in screening and informed consent procedures done by the first two authors. The following inclusion criteria were used at study enrollment: low risk (no obstetric, fetal, medical or genetic risk factors), adult (≥18 years of age), pregnant at 28 ± 2 weeks gestation, able to speak and write English, and expressing willingness to particate in the study invervention (oral probiotic) and data collecion (vaginal and rectal swabs, questionnaires).

A convenience sample of 20 healthy pregnant participants was sought. Following informed consent, the first 10 participants were assigned to the experimental group. Nonrandom assignment was used to assure that the 10 experimental group participants were enrolled. After enrolling the experimental group, the next 10 women were assigned to the control group.

Intervention

The study intervention consisted of one capsule of Florajen3 (previously described) orally each day. Florajen3 meets the internationally established probiotic criteria (FAO/WHO, 2001). The probiotics contained in Florajen3 are non-spore-forming, lactose- and hydrogen-peroxide-producing bacteria. The manufacturer recommends refrigeration to maintain maximum potency. As part of the preparation for this study, a sample of Florajen3 was left unrefrigerated for a period of 6 weeks and appropriate colony counts were sustained. Therefore for the purpose of the study, the participants in the experimental group were allowed to leave the probiotic unrefrigerated in an effort to improve daily compliance. Experimental group study participants were made aware of the probiotic brand used (open label) as the intervention. Further, the current state of knowledge about prenatal probiotics, including potentially rare side effects, were discussed at length using a 10-page informed consent document. The Florajen3 was placed in a study bottle equiped with an electronic cap monitoring sytem (MEMS) designed to record each time the bottle was opened by the participant. Each opening of the cap bottle equipped with this system is recorded on a microchip. The number of openings are retreivable via computer software when the bottle caps are returned. Researchers, midwives, and participants were aware of group assignment. The control group participants did not receive a placebo.

Procedures

Midwife prenatal care providers and staff were oriented to the study data collection procedures. Study data were collected at three points during routine prenatal visits with each participant's midwife (28 ± 2 weeks, 32 ± 2 weeks, and 36 ± 2 weeks gestation). The study was considered completed at the 36 ± 2 weeks gestation visit. All participants received compensation at the completion of two study visits: $25 USD at 32 ± 2 weeks and $75 USD at 36 ± 2 weeks gestation. Probiotic group participants were offered a supply of probiotics for the remainder of pregnancy.

Data collection measures and timing are summarized in Table 1. The midwife prenatal provider collected demographic information (birth date, race, and gestational age), and the vaginal and rectal swabs for GBS and wet mount, using accepted criteria to identify the vaginal pH and the presence of normal flora and/or pathogens (Donders, 1999). The wet mounts were examined microscopically by each midwife using standardized wet mount diagnostic procedures with normal saline and potassium hydroxide (Donders, 1999) and documented in a standard format. Participants completed a brief questionnarie (Table 2) at each study visit that asked them to describe yogurt ingestion, sexual activity (frequency and type), and vaginal cleansing practices (if used) in the past week; these were considered potential confounding variables. The participants were also asked if these practices were typical for them, and if not, how they were atypical. Women in the experimental group were asked to report any side effects that they attributed to the probiotics by notifying their midwife immediately, if necsessary, and then also noting the information on the study questionnaire. As part of routine prenatal care, the CDC-required GBS vaginal to rectal swab was collected by each participant's midwife and sent to the hospital laboratory between the 35- and 37-week visit. This was the only result that determined the need for IAP.

Table 1. Data Collection Measures and Timing

		Timing		
Variable	**Measure**	***28±2 weeks***	***32±2 weeks***	***36±2 weeks***
Collection as part of routine prenatal care				
GBS colonization	Vaginal to rectal swab[a]	n/a[b]	n/a	X[c]

Study-specific collection				
GBS colony counts	Vaginal and rectal swabs	X	X	X
Vaginal flora and pH	Wet mount	X	X	X
	pH			
	Consistency			
	Color			
	Odor			
	Lactobacillus to bacteria ratio			
	Epithelial/leukocyte ratio			
	Clue cells			
	Bacterial vaginosis			
	Trichomonas			
	Yeast			
Intervention adherence	Capsule count	X	X	X
	MEMS® AARDEX® cap			

Note GBS = group B *Streptococcus*.

a. This prenatal GBS screen was only done at 36± 2 weeks per CDC guidelines
b. n/a = not applicable
c. X = sample collection.

Table 2. Dietary, Sexual, and Vaginal Hygiene Questionnaire

	Circle frequency							
Practices	(of days during last week)							
Ate yogurt [brand_____]	0	1	2	3	4	5	6	7
Took antibiotic	0	1	2	3	4	5	6	7
Took a probiotic supplement besides “Florajen3” for study	0	1	2	3	4	5	6	7
Had sexual activity								
Penis to vagina intercourse	0	1	2	3	4	5	6	7
Penis to anus intercourse	0	1	2	3	4	5	6	7
Oral sex given to partner	0	1	2	3	4	5	6	7
Oral sex received from partner	0	1	2	3	4	5	6	7
Used a douche	0	1	2	3	4	5	6	7
Cleaned inside your vagina with soap	0	1	2	3	4	5	6	7

Researchers oversaw data collection and sample packaging. Participants were asked to return their probiotic bottles at each study visit and the Florajen3 capsules were replaced with a fresh supply. This gave the researchers the opportunity to notify the laboratory to record the date for later evaluation of the electronic cap monitoring results and conduct pill counts as an additional means to monitor women's responses to study participation, as well as probiotic adherence at each study visit. The vaginal and rectal swabs were placed on ice, packaged according to accepted procedures for human specimen handling, and shipped overnight to the laboratory of the final author, located 80 miles from the study site, for processing and analysis.

Laboratory Analysis

Upon receipt at the laboratory, the vaginal and rectal swabs were processed for quantitative colony counts of GBS. Polymerase chain reaction (PCR) was performed to determine whether bacteria isolated were the same as those in Florajen3. If positive for PCR, pulsed field gel electrophoresis (PFGE) would be performed to confirm the strain identity. These results were for research purposes only and were not available to the practicing midwives.

The routine CDC-recommended prenatal GBS vaginal to rectal swabs were analyzed at the hospital laboratory, using a culture based method in which a threshold for GBS positive results is considered greater than 10^2 colony forming units (CFU) per swab. These results determined the need for IAP and were available to the midwives for management during labor.

Statistical Analysis

Demographics, the study variables, and laboratory data were entered into and analyzed with a statistical software package. Descriptive statistics, confidence intervals, chi-squareds, *t* tests, and correlations were used for analysis. Data were compared between participants at each study visit and between groups at each data collection point. The qualitative prenatal GBS culture results (positive or negative) from the hospital laboratory were compared to the quantitative vaginal and rectal GBS study results, adherence data, and confounding variables (yogurt ingestion, sexual activity, and vaginal cleansing practices).

Results

A flow diagram of study participants is presented in Figure 1, including enrollment, nonrandom allocation, group assignment, and those included in the final analysis. There were no screening failures from the healthy, low-risk prenatal midwifery caseload. Following enrollment, one experiemental group participant declined to continue because she said her family was not comfortable with her participation, two moved, and two were transferred to physician care. One of the transferees was diagnosed with gestational diabetes within a week of study enrollment and the other spontaneously delivered an otherwise healthy late preterm infant at 34-weeks gestation; therefore, neither completed the study. Only one woman in the control group declined to continue the study because she did not want to experience the required rectal swabs.

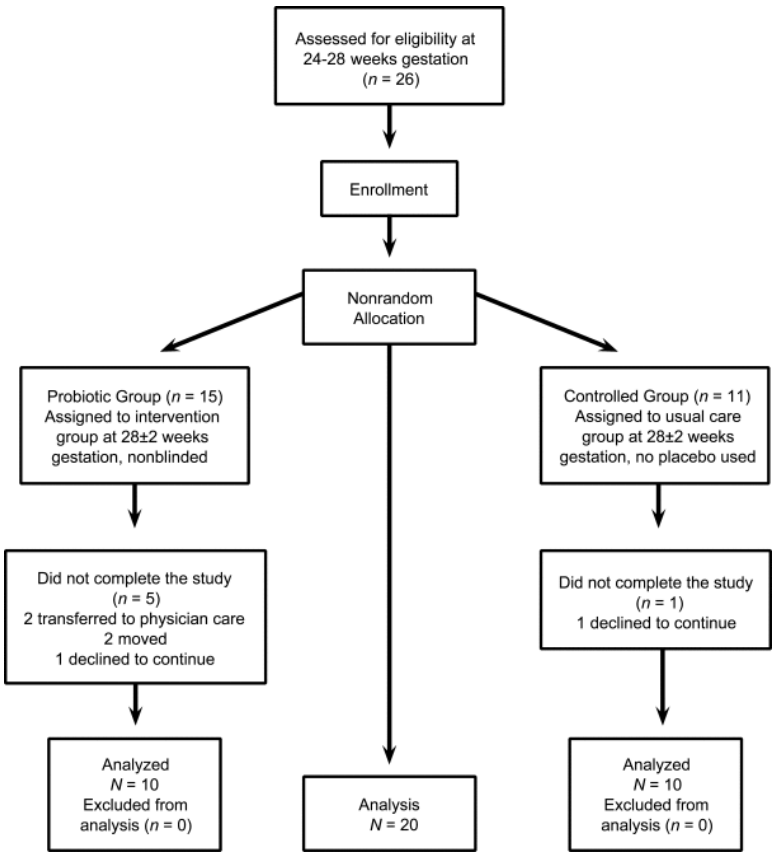

Figure 1. Participant flow diagram.

The potential of the probiotic intervention appears to be linked to daily adherence.

Twenty cases were completed and analyzed (10 probiotic group and 10 controls). There were no adverse outcomes or negative side effects reported by any probiotic group participants. One half of the women who took probiotics spontaneously reported improved gastrointestinal symptoms. One woman reported that she did not have bacterial vaginoisis while taking the probiotic, which she stated had been a persistent problem for her. Participant demographic findings are presented in Table 3. There were no significant differences between groups for maternal age, gestational age at enrollment, or parity.

Table 3. Participant Demographics

	Intervention group	**Control group**		
Variable	**(*n* = 10)**	**(*n* = 10)**	***p***	**Statistical test**
	Mean (*SD*)	**Mean (*SD*)**		
Age (years)	25.8 (3.8)	25.9 (5.1)	.961	*t* test
Gestational age (weeks) at enrollment	28.1 (1.6)	27.9 (0.8)	.668	*t* test
	Number (%)	**Number (%)**		
Race (*n* = 20)			.165	*t* test
African American (*n* = 10)	7 (35)	3 (15)		
Hispanic (*n* = 1)	0	1 (0.5)		
White (*n* = 9)	3 (15)	6 (30)		
Living Children (*n* = 20)			.424	Pearson's
Nulliparous (*n* = 6)	2 (10)	4 (20)		Chi-squared
Multiparous (*n* = 14)	8 (40)	6 (30)		

Vaginal and rectal GBS colony counts for each participant at each study visit are presented in Table 4. The study rectal cultures were positive in 11 women in both groups, whereas their vaginal swabs were negative. There were no significant differences (*p* .05) in GBS colony counts between probiotic and control group participants' vaginal or rectal swabs at any of the three data collection points. Because GBS colonization at 35 to 37 weeks is clinically relevant, the qualitative prenatal and the quantitative study laboratory results at this gestation are also presented in Table 4 for comparison. Two participants in each group had positive prenatal GBS culture results. The two probiotic group participants had lower colony counts (2×10^2 CFU) on the quantitative cultures than the two control group participants (7×10^2 CFU and 2.07×10^5 CFU). One participant in each group was negative on the prenatal culture despite measurable GBS on a study swab. There were no significant differences (*p* .05) in GBS results based on group assignment, vaginal pH, or wet mount results.

Table 4. Quantitative GBS Results in Colony Counts at Three Study Visits Compared with 36±2 Weeks Qualitative GBS Prenatal Culture

		28±2 weeks (study baseline)		**32 ±2 weeks**		**36 ±2 weeks**		
Study group	**Subject number**	**Vaginal**	**Rectal**	**Vaginal**	**Rectal**	**Vaginal**	**Rectal**	**36 ±2 weeks qualitative prenatal culture**
Probiotic	1							*
	2							
	3							
	4	1.0×10^3	2.0×10^4		2.0×10^2		2.0×10^2	Positive
	5							
	6			2.0×10^5		2.0×10^5		Positive

	7							
	8		1.63 x10^5		3.5 x10^5		3.5 x10^5	
	9							
	10							
Control	11							
	12	5.5 x10^4	3.3 x10^4		7.0 x10^2		7.0 x10^2	Positive
	13							
	14							
	15							
	16							
	17	3.4 x10^4	9.0 x10^2		2.07 x10^5		2.07 x10^5	Positive
	18			7.0 x10^2		7.0 x10^2		
	19				1.6 x10^4		1.6 x10^4	
	20							

Note: * Blank cell = Negative at 10^2 Colony Forming Units/Swab

Analyses using PCR were performed on 117 isolates from the 10 participants who took the probiotic. Of those, four tested positive for at least one of the bacteria contained in Florajen3. One of the bacterial strains contained in Florajen3 (*B. lactis*) was identified by PCR and PFGE in the rectal specimen of one experimental group participant, indicating that the probiotic survived the gastrointestinal tract in this case. None of the Florajen3 probiotic strains was detected in the vaginal specimens. Details of laboratory methods are presented online as supplemental materials in an appendix.

The MEMS cap data were not statistically analyzed due to multiple problems with data retrieval, including lost or destroyed caps. Therefore, pill counts were the only usable adherence measure. Pill counts were compared between GBS positive (n = 2) and negative (n = 8) participants in the probiotic group. The two participants who were GBS positive averaged 68% probiotic adherence based on pill counts, whereas the eight who remained GBS negative had a 90% adherence rate (p .05).

Confounding variables were examined for their effect on GBS colonization independent of study group assignment. There were no significant differences (p .05) in GBS results on any confounding variables collected on the questionnaire (Table 2), except yogurt ingestion. Women who consumed yogurt were significantly more likely to be GBS negative (p = .02). Yogurt ingestion ranged from 0 to 9 times per week at the three data collection points. One half of the participants indicated that they typically consumed no yogurt. Of those who ingested yogurt, the average frequency was 2.3 times per week. There were no patterns in specific brands consumed.

Several nonsignificant trends (p .05) were noted. Women who had higher rates of GBS also more frequently reported participating in oral sex (given or received) and ingesting prenatal antibiotics. Vaginal cleansing practices were unrelated to GBS findings. Only a few women in either group reported douching (none in the experimental group and two in the control). However, a total of five women in each group reported at one or more study visits cleaning inside the vagina with soap during the prior week. This practice increased in frequency among the participants in the experimental group whereas it was reported less often in the control group over time.

Discussion

Florajen3 inhibited GBS in vitro (Ephraim et al., 2012), and our findings suggest that it has a suppresive effect in vivo. Seven participants in the probiotic group and six controls remained negative for GBS at all three study visits. Three of the four participants who were positive on the qualitative 36-week prenatal GBS culture had detectable GBS colony counts at baseline and the two subsequent data collection points. Due to financial constraints and the small sample size, this study was not statistically powered to demonstrate a significant reduction in prenatal GBS infections. However, in this small sample, probiotic group participants had lower GBS colony counts compared to controls. Although these findings are hopeful, a RCT with a larger sample is needed to demonstrate that third trimester probiotics inhibit GBS colonization.

The findings of a recent integrative review indicated that in 23 of 35 studies (66%) of prenatal probiotics, the intervention was initiated during the third trimester (VandeVusse et al., 2013). It is unknown if earlier probiotic administration is necessary to inhibit GBS colonization at the clinically important testing point of 35- to 37-weeks gestation (CDC, 2010). This study demonstrated the feasibility of a larger RCT using the same population and study procedures. In a larger trial, more detailed analysis of *Lactobacillus* species would allow for better comparisons of between group differences to aid in clarifying the mechanisms of action of probiotics against GBS *in vivo*.

Confounding Variables

The effect of vaginal cleansing practices on GBS is unknown. There is a direct association between douching and vaginitis (Cottrell, 2010). However, consistent with the collective advice of the midwife providers against douching, this practice was reported by only 10% of the participants. In addition, more then one half of the women in each group reported at one or more study visits that they cleaned inside the vagina with soap during the prior week. This practice is not well understood and needs further study for its possible impact on vaginal flora and GBS.

The association between sexual activity and vaginal flora has been studied with mixed results. Antonio, Meyn, Murray, Busse, and Hillier (2009) found that nonpregnant women who reported participating in unprotected sexual intercourse during the past week were less likely to be colonized with vaginal *Lactobacillus*. Newton, Butler, and Shain (1996) found that specific sexual practices were not predictors of GBS colonization. In fact, women who reported anal intercourse were found to have lower rates of GBS. However, women who reported more than one sexual partner in the prior month had higher rates of heavy GBS colonization. The relationship between sexual practices on *Lactobacillus* and GBS colonization needs more study.

A significant inverse relationship between yogurt ingestion and GBS colonization at 36-weeks gestation was identified. The ingestion of cultured milk products in the United States of America is increasing. However, the diversity of brands, the variability of probiotic bacterial species and colony counts, and the limits of dietary recall make controlling this confounder a challenge in future research.

The amount of active probiotic bacteria ingested daily appears to be an important consideration (VandeVusse et al., 2013). Two prospective cohort studies were conducted in Norway to explore the impact of prenatal consumption of probiotic cultured milk products (Biola and Cultura) on two perinatal complications. The intake of probiotic milk products was associated with reduced risks of preeclampsia and spontaneous preterm delivery, respectively (Brantsaeter et al., 2011; Myhre et al., 2011). Dosage was estimated by food frequency questionnaires and categorized (differently in each study) as zero, low, and high intake. The mean high intake combined from the two studies resulted in an average consumption of 138 ml per day, equivalent to 10^9 CFU of *Lactobacillus* and *Bifidobacterium*. In comparison, the probiotic supplement used in this study (Florajen3) represented a 15 times higher dose of active probiotics. The optimal dosing of prenatal probiotics has not been determined and needs further scientific investigation.

Adherence

The potential of the probiotic interventon appears to be linked to daily ingestion. The MEMS caps were used with the participants in the probiotics group to record adherence. Unfortunately, data from one half of the MEMS caps were not usable and therefore were not analyzed (two were never returned by participants who moved away). Therefore, adherence was measured indirectly by the use of pill counts. This was a less accurate means of determining how much probiotic was injested between study visits. Overall, probiotic adherence averaged 86%, indicating that prenatal participants can be expected to follow the study protocol.

Limitations

This study has additional limitations. The findings are not generalizable due to the small sample size that did not allow for the detection of differences between groups on the dependent variable. The prenatal GBS results were analyzed using the culture based method and the study swabs were analyzed using the more sensitive PCR method. Laboratory recovery of bacterial species contained in the probiotic could have been limited by variations in probiotic adherence between subjects, overnight shipping of the samples, and the amount of fecal material present on the swabs. It was difficult to demonstrate that probiotic ingestion could be verified by culture and molecular methods. Sample collection was done by the midwife prenatal care providers and there may have been differences in swab techniques despite study protocol orientation.

A larger, prospective, double blind, placebo controlled trial is feasible in a midwifery practice that serves a diverse population.

Absence of Side Effects

Women in the intervention group reported no side effects related to the Florajen3 intervention. The finding that one half of the women who consumed the probiotic reported improved gastrointestinal symptoms stimulated pilot testing of a revised symptom tool for future research.

Future Research

The study was not powered to examine the efficacy of the probiotic intervention against GBS colonization. Given that a 20% GBS rate was documented in this study, a sample size of 440 would be needed to demonstrate a reduction in GBS colonization from 20% to 10% at 36-weeks gestation. Patient-collected GBS swabs yield similar colonization rates compared to those collected by providers, but self collection may be more acceptable to women (Arya, Cryan, O'Sullivan, Greene, & Higgins, 2008; Mercer, Taylor, Fricke, Baselski, & Sibai, 1995). Self-collection of vaginal and rectal samples and wet mounts could possibly improve participation and study continuation in future research. Because adherence is an important issue, the use of MEMs caps should be considered in future studies. However more efforts to assure return of the caps should be instituted. In a larger RCT of prenatal probiotics, any impact of potential confounding variables (yogurt ingestion, sexual activity, and vaginal cleansing practices) on GBS colonization would be expected to be equivalent between groups.

Conclusion

This is the first published study of prenatal probiotics conducted in the United States. Because GBS is an issue for childbearing women worldwide, more research on clinical interventions to prevent colonization before birth is needed. This study demonstrates that a larger prospective double blind placebo controlled trial is feasible in a midwifery practice that serves a diverse population.

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References

Abad and Safdar, 2009 C.L. Abad, N. Safdar **The role of lactobacillus probiotics in the treatment or prevention of urogenital infections: A systematic review** Journal of Chemotherapy, 21 (3) (2009), pp. 243-252

Antonio et al., 2009 M.A.D. Antonio, L. Meyn, P.L. Murray, B. Busse, S.L. Hillier **Vaginal colonization by probiotic L. bacillus crispatus CTV-05 is decreased by sexual activity and endogenous lactobacilli** Journal of Infectious Diseases, 199 (2009), pp. 1506-1513

Arya et al., 2008 A. Arya, B. Cryan, K. O'Sullivan, R.A. Greene, J.R. Higgins **Self-collected versus health professional-collected genital swabs to identify the prevalence of group B Streptococcus: a comparison of patient preference and efficacy** European Journal of Obstetrics, Gynecology, & Reproductive Biology, 139 (1) (2008), pp. 43-45

Association of Ontario Midwives, 2010 Association of Ontario Midwives **Group B Streptococcus: Prevention and management in labour** Author, Toronto, Canada (2010)

Balter et al., 2003 S. Balter, E.R. Zell, K.L. O'Brien, A. Roome, H. Noga, M. Thayu, A. Schuchat **Impact of intrapartum antibiotics on the care and evaluation of the neonate** Pediatric Infectious Disease Journal, 22 (2003), pp. 853-857

Bedford Russell and Murch, 2006 A.R. Bedford Russell, S.H. Murch **Could peripartum antibiotics have delayed health consequences for the infant** BJOG: An International Journal of Obstetrics and Gynaecology, 113 (2006), pp. 758-765

Bizzarro et al., 2008 M.J. Bizzarro, L.M. Dembry, R.S. Baltimore, P.G. Gallagher **Changing patterns in neonatal Escherichia coli sepsis and ampicillin resistance in the era of intrapartum antibiotic prophylaxis** Pediatrics, 121 (2008), pp. 689-696

Bolton et al., 2008 M. Bolton, A. Van Der Straten, C.R. Cohen **Probiotics: Potential to prevent HIV and sexually transmitted infections in women** Sexually Transmitted Infections, 35 (3) (2008), pp. 214-225

Brantsaeter et al., 2011 A.L. Brantsaeter, R. Myhre, M. Haugen, S. Myjing, V. Sengpiel, P. Magnus, H.M. Meltzer **Intake of probiotic food and risk of preeclampsia in primiparous women: The Norwegian mother and child cohort study** American Journal of Epidemiology, 174 (7) (2011), pp. 807-815

Buckler et al., 2010 B. Buckler, J. Bell, R. Sams, W. Cagle, S.A. Bell, C. Allen, J. Bhatia **Unnecessary workup of asymptomatic neonates in the era of Group B Streptococcus prophylaxis. Infectious Diseases in Obstetrics and Gynecology, Epub 2010 August 22. Article ID 369654** (2010) http://www.researchgate.net/publication/46181778_Unnecessary_workup_of_asymptomatic_neonates_in_the_era_of_group_B_streptococcus_prophylaxis

Centers for Disease Control and Prevention (CDC), 2002 Centers for Disease Control and Prevention (CDC) **Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC** MMWR Morbidity and Mortality Weekly Report, 51 (11) (2002), pp. 1-22

Centers for Disease Control and Prevention (CDC), 2010 Centers for Disease Control and Prevention (CDC) **Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC, 2010** MMWR Morbidity and Mortality Weekly Report, 59 (10) (2010), pp. 1-32

Cottrell, 2010 B.H. Cottrell **An updated review of evidence to discourage douching** MCN: American Journal of Maternal/Child Nursing, 35 (2) (2010), pp. 102-107

de Vrese, 2009 M. de Vrese **Health benefits of probiotics and prebiotics in women** Menopause International, 15 (2009), pp. 35-40

Donders, 1999 G.G. Donders **Microscopy of the bacterial flora on fresh vaginal smears** Infectious Diseases in Obstetrics and Gynecology, 7 (1999), pp. 177-179

Elias et al., 2011 J. Elias, P. Bozzo, A. Einarson **Are probiotics safe for use during pregnancy and lactation** Canadian Family Physician, 57 (2011), pp. 299-301

Ephraim et al., 2012 E. Ephraim, R.D. Schultz, M. Duster, S. Warrack, C.A. Spiegel, N. Safdar **In-vitro evaluation of the antagonistic effects of the probiotics Lactobacillus Rhamnosus Hn001 and Florajen 3 against Group B Streptococci** International Journal of Probiotics and Prebiotics, 7 (3/4) (2012), pp. 113-120

Food and Agriculture Organization of the United Nations & World Health Organization, 2001 Food and Agriculture Organization of the United Nations & World Health Organization **Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria: Report of a joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria** (2001) http://search.who.int/search?q=Probiotics+definition&ie=utf8&site= defaultlection&client=-en&proxystylesheet=-en&output=xml-no-dtd&oe=utf8

Food and Agriculture Organization of the United Nations & World Health Organization, 2002 Food and Agriculture Organization of the United Nations & World Health Organization **Guidelines for the evaluation of probiotics in food: Report of a joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food** (2002) http://www.fda.gov/ohrms/dockets/dockets/95s0316/95s-0316-rpt0282-tab-03-ref-19-joint-faowho-vol219.pdf

Fooks and Gibson, 2002 L.J. Fooks, G.R. Gibson **Probiotics as modulators of the gut flora** British Journal of Nutrition, 88 (1 Suppl) (2002), pp. S39-S49

Glasgow et al., 2007 T.S. Glasgow, M. Speakman, S. Firth, B. James, C.L. Byington, P.C. Young **Clinical and economic outcomes for term infants associated with increasing administration of antibiotics to their mothers** Paediatric and Perinatal Epidemiology, 21 (2007), pp. 338-346

Glasgow et al., 2005 T.S. Glasgow, P.C. Young, J. Wallin, C. Kwok, G. Stoddard, S. Firth, C.L. Byington **Association of intrapartum antibiotic exposure and late-onset serious bacterial infections in infants** Pediatrics, 116 (2005), pp. 696-702

Hanson and VandeVusse, 2010 L. Hanson, L. VandeVusse **Group B streptococcus intrapartum prophylaxis guidelines adherence: A perinatal risk management issue** Journal of Perinatal and Neonatal Nursing, 24 (2) (2010), pp. 100-103

Hickmann et al., 1999 M.E. Hickmann, M.A. Rench, P. Ferrieri, C.J. Baker **Changing epidemiology of group B streptococcal colonization** Pediatrics, 104 (1999), pp. 203-209

Kim et al., 2006 L.S. Kim, L. Hilli, J. Orlowski, J.L. Kupperman, M. Baral, R.F. Waters **Efficacy of probiotics and nutrients in functional gastrointestinal disorders: A preliminary clinical trial** Digestive Diseases and Sciences, 51 (2006), pp. 2134-2144

Kubota et al., 2002 T. Kubota, M. Nojima, S. Itoh **Vaginal bacterial flora of pregnant women colonized with group B streptococcus** Journal of Infection and Chemotherapy, 8 (2002), pp. 326-330

McKeever et al., 2002 T.M. McKeever, S.A. Lewis, C. Smith, J. Collins, H. Heatlie, M. Frischer, R. Hubbard **Early exposure to infections and antibiotics and the incidence of allergic disease: A birth cohort study with the West Midlands General Practice Research Database** Journal of Allergy and Clinical Immunology, 109 (1) (2002), pp. 43-50

Mercer et al., 1995 B.M. Mercer, M.C. Taylor, J.L. Fricke, V.S. Baselski, B.M. Sibai **The accuracy and patient preference for self-collected group B Streptococcus cultures** American Journal of Obstetrics and Gynecology, 173 (1995), pp. 1325-1328

Moghaddam, 2010 M.N. Moghaddam **Recto-vaginal colonization of Group B Streptococcus in pregnant women referred to a hospital in Iran and its effect on Lactobacillus normal flora** International Journal of Biological Sciences, 10 (2) (2010), pp. 166-169

Myhre et al., 2011 R. Myhre, A.L. Brantsaeter, S. Myking, H.K. Gjessing, V. Sengpiel, H.M. Meltzer, B. Jacobsson **Intake of probiotic food and risk of spontaneous preterm delivery** American Journal of Clinical Nutrition, 93 (2011), pp. 151-157

Newton et al., 1996 E.R. Newton, M.C. Butler, R.N. Shain **Sexual behavior and vaginal colonization by Group B Streptococcus among minority women** Obstetrics and Gynecology, 88 (4) (1996), pp. 577-582

Ohlsson and Shah, 2009 A. Ohlsson, V.S. Shah **Intrapartum antibiotics for known maternal Group B streptococcal colonization. Cochrane Database of Systematic Reviews 2009, Issue 3. Art. No.: CD007467** (2009)

Ronnqvist et al., 2006 P.D.J. Ronnqvist, U.B. Forsgren-Brusk, E.E.G. Hakansson **Lactobacilli in the female genital tract in relation to other genital microbes and vaginal pH** Acta Obstetricia et Gynecologica Scandinavica, 85 (2006), pp. 726-735

Singleton, 2007 M.L. Singleton **Group B Strep prophylaxis: What are we creating** Midwifery Today, 81 (2007), pp. 18-20

United Kingdom National Screening Committee (UK NSC), 2012 United Kingdom National Screening Committee (UK NSC) **Screening for Group B Streptococcal infection in pregnancy: External review against programme appraisal criteria for the UK National Screening Committee** Bazian Limited, London, UK (2012)

VandeVusse et al., 2013 L. VandeVusse, L. Hanson, N. Safdar **Perinatal outcomes of prenatal probiotic and prebiotic administration: An integrative review** Journal of Perinatal & Neonatal Nursing, 27 (4) (2013), pp. 288-301

West and Prescott, 2013 C. West, S. Prescott **Prebiotics and probiotics for prevention of allergic disease** (2013) http://www.uptodate.com/contents/prebiotics-and-probiotics-for-prevention-of-allergic-disease

Supporting Information

Appendix: Molecular Methods

Polymerase Chain Reaction (PCR) Methods

Polymerase chain reaction was performed using methods developed by DuPont Nutrition and Health group. DNA was extracted using heat lysis (1-2 colonies picked to 50 µL nuclease free water and boiled for 10 minutes). PCR reactions specific for each species of bacteria were run.

Primers were obtained from IDT DNA. The reaction mixture consisted of 17.4 µL nuclease free water, 2.5 µL buffer, 2 µL dNTPs, and 0.125 µL Taq. Then 1 µL each primer (20 µM) and 1µL DNA was added. The running conditions were: 7 min at 95ºC, followed by 30 cycles of 95ºC for 30 seconds, annealing (see Table 1) for 30 seconds and 72ºC for 30 seconds, followed by a 5 minute 72ºC cycle for final extension. Product DNA was amplified using the Applied Biosystems 2720 Thermal Cycler and detected on a 1.2% FlashGel.

Table 1. Primers and running conditions

Organism	Primers (forward and reverse)	Annealing temperature (˚C)
L. acidophilus	5'-GGTTGGGGAAATGCAAACTAAAGA-3' 5'-AAAGTGCACAAAACTAGCACCTTT-3'	56
B. lactis	5'-GCACCGCGGCGTGGAAGAA-3' 5'-AGGTTGACCTCATCGGCGAGCTCT-3'	63
B. longum	5'-TACGAAGCTCTGAAGCCGTACGCT-3' 5'-CCTTCTGAGCCTCGTCGCCCT-3'	63

Pulsed Field Gel Electrophoresis (PFGE) Methods

Isolates were grown for 48 - 72 hours in MRS broth supplemented with 0.05% cysteine or pre-reduced BHI broth. 400 µL of cell suspension was transferred to a 1.5 mL microcentrifuge tube which was centrifuged at approximately 13000 x g for 3 minutes. The supernatant was removed and the pellet was resuspended in 300 µL TE. PFGE plugs were then prepared according to a previously published method (Halpin, Garrett, Ribot, Graves, & Cooper, 2010) with the following alterations: 15 µL of proteinase K and 300 µL 1.8% SeaKem Gold agarose without 1% sodium dodecyl sulfate. Plugs were incubated overnight in a 55˚C water bath. The restriction digest was performed as previously described (Ribot et al, 2006). PFGE was performed on the

BioRad Genepath, program 13, with switch times of 1-23 seconds, non-linear, 6V/cm, run angle of 120º for 18.5 hours.

References

Halpin, J., Garrett, N., Ribot, E., Graves, L., & Cooper, K. (2010). Re-evaluation, optimization, and multilaboratory validation of the PulseNet-standardized pulsed-field Gel electrophoresis protocol for Listeria monocytogenes. Foodborne Pathology and Disease, 7(3), 293-298.

Ribot, M., Fair, M., Gautom, R., Cameron, D.N., Hunter, S.B., Swaminathan, B., & Barrett, T.J.(2006). Standardization of pulsed-field gel electrophoresis protocols for the subtyping of Escherichia coli O157:H7, Salmonella, and Shigella for PulseNet. Foodborne Pathology Disease, 3(1), 59-67.