

Oral Probiotic Supplementation and Group B
Streptococcus Rectovaginal Colonization in
Pregnant Women: a Randomized Double-blind
Placebo-controlled Trial

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Title

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Introduction and Specific Aims

Group B Streptococcus (GBS) is a leading cause of perinatal morbidity and mortality, including neonatal sepsis and maternal puerperium infections. In light of significant consequences of GBS colonization in pregnancy, GBS screening at 35-37 weeks' gestational age and prophylaxis at time of labor is recommended in the United States. Although intrapartum antibiotic prophylaxis effectively prevents perinatal transmission in colonized pregnant women, numerous challenges in this management remain, including allergic reactions to antibiotic use, antimicrobial resistance development, potential unpredictability of transient colonization, as well as drug and administration costs. Identifying strategies that decrease the rate of GBS colonization would be beneficial in reducing such obstacles.

Probiotics are live microorganisms that are thought to be healthy for the host organism. One of the mechanisms proposed for the potential benefit of probiotics includes balance of intestinal microbial environment and inhibition of pathogenic bacterial growth. Recently, lactobacilli-containing probiotics have been studied for use in intestinal and non-intestinal conditions, including urogenital infections. Several studies have demonstrated that use of probiotics may reduce the incidence of bacterial vaginosis (BV). Given the potential benefits of probiotics in treatment of bacterial vaginosis, a similar beneficial effect of increased balance in normal vaginal microflora may reduce GBS colonization in pregnant women. We hypothesize that supplementation with oral probiotics may reduce the rate of GBS colonization in term pregnant women.

We propose the following specific aims to examine the aforementioned hypothesis:

1. Compare GBS rectovaginal colonization rate in pregnant women at 35-37 weeks' gestational age after oral supplementation with probiotics during the second and third trimesters with the rate in pregnant women who receive placebo.
2. Compare perinatal outcomes related to infectious morbidity between the two groups.

Background

Group B streptococcus (GBS) is an encapsulated gram-positive diplococcus that frequently colonizes the human genital and gastrointestinal tracts, as well as the upper respiratory tract of young infants. Approximately 10-30% of pregnant women in the United States are colonized with GBS in the vaginal or rectal area.(1) Colonization prevalence in pregnant women differs by ethnic groups, geographic locales, and age.(1)

GBS is a leading cause of morbidity and mortality among newborn infants; is a common cause of maternal peripartum infections; and has been associated with adverse obstetric

events. (2-8) GBS colonization of the mucous membrane is typically asymptomatic. Vertical transmission of GBS at the time of birth occurs in 50-70% of infants born to colonized mothers, and of these, 1-2% will develop early-onset invasive disease. (2) Transmission may also occur per an ascending route and cause intrauterine fetal infection. Maternal colonization is the primary risk factor for GBS infection in neonates and young infants (younger than 90 days of age).(4,9) Other risk factors for neonatal infection include maternal GBS colonization density, with approximately 2.5 times greater risk of infant infection in pregnant women with heavy versus light colonization.(10)

Neonatal infectious complications of GBS infection include bacteremia, sepsis, pneumonia, and meningitis, sometimes with permanent neurologic sequelae, as well as neonatal death.(2-4) Maternal infections attributable to GBS in pregnancy include asymptomatic bacteriuria, urinary tract infections, intraamniotic infection or chorioamnionitis, and postpartum endometritis. Less commonly, pneumonia, puerperal sepsis, and bacteremia can occur. GBS colonization has also been associated with obstetrical complications, including fetal demise, midgestation pregnancy loss, preterm labor, preterm premature rupture of membranes, and preterm delivery. (2-8)

Consensus guidelines for prevention of perinatal GBS disease recommending either risk-based or screening-based strategies have been issued since the early 1990's by the American College of Obstetricians and Gynecologists (ACOG), American Academy of Pediatrics (AAP), and Centers for Disease Control and Prevention (CDC). In 2002 Schrag *et al* demonstrated that antenatal screening for GBS prevented approximately 55% more cases of early-onset sepsis than the risk-based approach.(11) In August of 2002 the CDC recommended universal prenatal screening for vaginal and rectal GBS colonization of all pregnant women at 35-37 weeks' gestation for the prevention of perinatal GBS disease.(4)

Universal prenatal screening at 35-37 weeks' gestation with intrapartum antibiotic prophylaxis of colonized women during labor is thus far the most successful strategy for prevention of perinatal GBS disease. However, there remain several challenges pertaining to screening and treatment considerations. Although the accuracy of late antenatal screening cultures performed within 5 weeks of delivery in predicting genital GBS colonization at time delivery have relatively high positive (87%) and negative (96%) predictive values, this identification is not perfect. (12) In reality, greater than 10% of women may receive unnecessary treatment, while a small minority may not receive appropriate intrapartum antibiotic prophylaxis.

Overuse of antibiotics, which may lead to organism resistance and potential allergic reactions to antimicrobials, is another potential consequence of our current management strategy. In women with rectovaginal GBS colonization at term, without use of intrapartum antibiotics, the incidence of invasive neonatal GBS disease ranges from 2 to 3 cases per 1,000 live births. (3, 13) Thereby, large numbers of mothers and infants may be exposed to unnecessary antibiotics. Based on a 2002 Cochrane Database Review, it is estimated that 1,000 women must be treated with antibiotics to prevent 1.4 cases of

disease. (14) While administration of antibiotics does decrease GBS neonatal early-onset disease, in general the cost of large scale antibiotics use is out of proportion to the improvement in public health. Intravenous antibiotic therapy is costly and utilizes hospital personnel time. Also, up to 10% of women administered antibiotics in labor will experience some form of allergic reaction; 1 in 100,000 will die from anaphylactic shock.(4, 15) Women who are allergic to penicillin and not candidates for cephalosporin therapy are treated with alternative antibiotic therapies such as erythromycin, clindamycin, and vancomycin. However, these are not well studied and may not achieve therapeutic levels in cord blood. These alternative antibiotics have very limited human transplacental data and also may result in greater maternal adverse events. (16, 17) Widespread exposure to antibiotics results in selection of resistant organisms as recently demonstrated by reports of increasing resistance to clindamycin (>20%) and erythromycin (>30%); this resistance may lead to reduction of antimicrobial effectiveness.(18-20) Finally, risk of maternal candidiasis may increase with use of antibiotics, which may in turn lead to breastfeeding difficulties.(21) Therefore, given these limitations to our current management of GBS during pregnancy and labor, strategies to decrease maternal colonization would be of tremendous benefit.

According to the currently adopted definition by the Food and Agricultural Organization of the United Nations and World Health Organization, probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host.(22) Numerous recent studies have been conducted investigating the potential therapeutic benefits of probiotics. It has been proposed that the potential beneficial effect of probiotics involves improvement of intestinal microbial balance and inhibition of the growth of pathogenic bacteria. Recently, lactobacilli-containing probiotics have been studied for use in a number of intestinal and nonintestinal conditions, including Clostridium difficile infection, inflammatory bowel diseases, and urogenital infections.http://www.femalepatient.com/html/arc/sig/urog/articles/035_08_042.asp - 2#2 (23) Investigators suggest that imbalance in the normal vaginal bacterial flora may contribute to the pathogenesis of urogenital infections, especially BV and UTI. (24) Thereby, restoration of the normal vaginal flora with use of lactobacilli-containing probiotics has been proposed as a novel approach for the prevention and treatment of urogenital infections. (23)

Genitourinary infections in women are often characterized by an alteration in the local flora, with a transformation from a predominance of lactobacilli to coliform pathogens. This can occur as a result of multiple factors, including hormone deficiency, sexual activity, and contraception.(25) In order for probiotics to be effective in the prevention or treatment of urogenital infections, lactobacilli must exhibit antibacterial activity.(26) One antibacterial property of lactobacilli is the ability to maintain an acidic pH <4.5, which is conducive to lactobacilli replication and their subsequent production of antibacterial metabolites such as bacteriocin and hydrogen peroxide. It has been noted that different strains of lactobacilli produce varying amounts of these substances.(27) Lactobacilli-containing probiotics also offer protection by producing biosurfactants, which interfere with growth and adhesion of organisms such as Escherichia coli and Enterococcus

faecalis to uroepithelial cells.(28) Finally, lactobacilli may also block adhesion or displace uropathogens that are adherent to vaginal epithelial cells.(29)

Recent studies and reviews have demonstrated successful treatment of BV with probiotics in majority of trials.(30-39) These trials generally included young, healthy, premenopausal women who were treated with lactobacilli-containing probiotics. Several studies included pregnant women, as well.(31,32) Most protocols included probiotics administered intravaginally, however oral route has also proven successful for treatment of BV.(37) Studies differed by strains, concentrations, and preparations of lactobacilli used, including *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus reuteri*, and *Lactobacillus crispatus*. The utility of probiotics for either prevention or adjunctive treatment of vulvovaginal candidiasis and urinary tract infections has yet to be proven, since most studies included in recent reviews showed no benefit and conflicting results.http://www.femalepatient.com/html/arc/sig/urog/articles/035_08_042.asp - 5#5 (26,30) The conflicting data may be due to heterogeneity of the strains, preparations of lactobacilli used, route of administration, and duration of treatment.

Several recent studies have also reported probiotics to be safe for use during pregnancy without demonstrated adverse perinatal outcomes. (40, 41) However, a recent review of risks associated with probiotic use identified major and minor risk factors for associated infectious morbidities, including immunocompromised status, premature infants, central venous catheter, impaired intestinal epithelial barrier, administration of probiotic by jejunostomy, concomitant use of broad spectrum antibiotics, probiotics with high mucosal adhesion or known pathogenicity, and cardiac valvular disease. (42) Specifically, pregnancy was not included as a risk factor. While caution for adverse effects must be maintained with the growing use of probiotics, the majority of the available evidence to date suggests that probiotics are safe, except in limited circumstances. Larger studies have not yet reproduced these findings. Furthermore, no major side effects from the use of probiotics were reported in the studies on genitourinary infections. All preparations, regardless of strain and route of administration, were tolerable and caused minimal adverse effects in healthy, young, premenopausal women.

Review of the literature does not demonstrate any specific strains of probiotic organisms that are known to be effective in displacing GBS either in the gut or in the reproductive tract and no study has investigated whether probiotics may effect rectovaginal colonization in pregnant women. However, the biological plausibility exists that there may be a beneficial effect of decreased GBS colonization given observed associations of reduction of other genital infections with probiotic therapy.

Preliminary Studies

In an empiric observational case series in two midwifery practices, 80 pregnant women were supplemented with oral probiotics. Given the average colonization rates, we would expect 10-30% GBS colonization rate or 8-24 cases in this cohort. However, of the 80 women who took oral probiotics throughout their pregnancies, only 1 woman had GBS

colonization (1.25%) at time of screening between 35-37 weeks. (personal communication)

Methods

A. Design: prospective randomized double-blind placebo-controlled trial.

B. Site: A collaborative trial including Stanford University School of Medicine/Lucile Packard Children's Hospital (LPCH) Obstetric Clinic, Dominican Hospital/ Aptos Women's Health (AWH) private Obstetrics-Gynecology practice in Santa Cruz, and private Midwifery Practice of Karen Ehrlich, a certified midwife and co-investigator of this study. We plan to enroll the majority of subjects (approximately 2/3) at Stanford University School of Medicine/Lucile Packard Children's Hospital Obstetric Clinic.

C. Inclusion criteria:

1. Pregnant women < 28 weeks' gestation.
2. 18 years of age or older.
3. Singleton gestation.

D. Exclusion criteria:

1. Preexisting morbidity: Immunocompromised status (HIV +; malignancy; history of organ transplant; chronic steroid therapy; autoimmune disease requiring treatment during pregnancy, and other immunocompromised states); Type 1 diabetes and type 2 diabetes; congenital cardiac disease and cardiac valvular disease requiring antibiotic prophylaxis during procedure/labor; pulmonary disease (except mild asthma); renal disease; chronic hepatic disease (Hepatitis B, C); inflammatory bowel disease (Crohn's disease or ulcerative colitis); stomach or duodenal ulcer; bowel resection, gastric bypass, and chronic indwelling venous, bladder, or gastric catheter.
2. Multi-fetal gestation.
3. Regular use of probiotics preparations in the 3 months prior to beginning the study treatment or use of any additional probiotics preparations (other than study treatment) at any time during the study period (including over the counter food supplements such as Activia, BioK, other oral or vaginal probiotics products (BUT not including other common forms of yogurt).
4. Chronic (daily) use of broad spectrum antibiotics.
5. History of infant with GBS sepsis.
6. IUGR, Fetal Anomalies-major diagnosed at time of second trimester anatomy ultrasound
7. Anticipated delivery <35 weeks for maternal/fetal indication
8. Placenta previa or accreta (with anticipated delivery prior to 35 weeks)

E. Outcomes:

1. **Primary:** GBS colonization at 35-37 weeks

2. Secondary: maternal ante- intra- and postpartum outcomes (urinary tract infections, chorioamnionitis, endometritis, cellulitis, bacteremia, sepsis, and other infectious morbidity) and neonatal outcomes (gestational age at delivery, APGAR scores, bilirubin levels, C-reactive protein, rule out sepsis evaluation, sepsis, pneumonia, meningitis, neonatal ICU admission, and length of hospital stay).

F. Agents:

1. Treatment arm: Study probiotic dietary supplement arm will consist of 1 capsule (>1 billion CFU/capsule) daily including two strains, Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14, which have been shown to reduce the incidence of bacterial vaginosis and candidiasis. (43) Those two strains are present in Jarrow's Femdophilus, which is produced by Chr Hansen in Denmark under the brand name Urex. Shelf life is two years when stored at room temperature (max 25 degrees Celsius).

2. Control arm: Placebo arm will consist of one inert (heat inactivated) capsule daily.

G. Study Drug and Placebo Supply, Transport, and Storage:

1. Chr Hansen A/S will provide the active probiotic dietary supplement and placebo oral tablets for 372 patients (186 per arm of the trial.) However, the company providing the study probiotic dietary supplement and placebo will not have access to preliminary data during the conduct of the study and will not have the right of review of the results prior to publication. In summary, 27,900 doses of product and 27,900 doses of placebo will be provided. (Appendix 1) Capsules will be packed in aluminum tubes and these will then be packed in cardboard boxes and sent by courier from manufacturer to study site. The tubes containing the capsules will be packed such that no physical damage will occur to the individual capsules. Refrigeration is not necessary since bacteria count of minimum 1 billion is guaranteed for 2 years when stored at room temperature up to 25 degrees Celsius. Capsules should not be exposed to direct sunlight when stored. The manufacturer recommends 5 degrees Celsius storage to preserve bacterial potency if more than 6 months of storage is expected. Study participants will be instructed to store capsules outside of children's reach and at room temperature and to avoid direct sun exposure to capsules.

H. Patient Recruitment:

1. Pregnant study subjects who meet inclusion criteria will be recruited prior to 28 weeks' gestational age at one of the following collaborative sites by either study co-investigator or study staff: LPCH Obstetrics Clinic and the collaborating sites of AWH Obstetrics and Gynecology Clinic, other private obstetric-gynecology offices in Santa Cruz, and

homes of patients who have elected to receive prenatal care and delivery at home by Midwife Karen Ehrlich, one of the co-investigators of this study.

I. Randomization Strategy:

1. Manufacturer will send identical probiotic and placebo capsules. The probiotic and placebo capsules will be originally packed in separate containers, labeled as designated probiotic versus placebo for each specific study enrollee (with study material information only available to the designated pharmacist at LPCH). The designated pharmacist at LPCH will be responsible for randomization assignment based on our pre-generated randomization table and distribution of study material to research coordinator for disbursement to enrollees at all sites.
 - a. Investigators will receive individual DEIDENTIFIED study material (patient probiotic versus placebo tubes) coded by a unique random number.
 - b. Each enrolled patient will receive supplies at study visit..
 - c. At conclusion of study, the participant randomization arm will be identified by the designated pharmacist.

J. Compliance Assessment:

1. Compliance with study drug (probiotic versus placebo) will be assessed by self-reported and provider mechanisms at monthly visits.
2. Subjects will be asked by investigators how many capsules they have missed since the last monthly visit.
3. Investigators will perform capsule count of the subject's monthly distributed study drug.
4. Investigators will provide subjects with a single aluminum tube of 30 capsules for the next monthly period.

I. Clinical Protocol:

1. Investigators will recruit study participants up to 28 weeks' gestational age.
2. Following informed patient consent, patients will be blindly randomized to either study probiotic dietary supplement or placebo per computer generated randomization table.
3. A hard-copy questionnaire/data sheet will be generated for each patient and will be labeled with patient's unique number, name and other data points that we intend to collect prospectively.
4. Subjects will be initiated on probiotic or placebo consisting of one capsule daily between 20-28 weeks' gestation and continued until delivery. Once enrolled, patients will receive a study questionnaire to complete with study investigator(s) or study coordinator(s) and a monthly supply of probiotic dietary supplement vs. placebo either at enrollment or subsequent study visit. Subjects will be instructed to store capsules away

from sun and consume 1 capsule with food every day at the same time (as determined to be the most convenient time for that patient).

5. Women diagnosed with GBS bacteriuria during the index pregnancy will be enrolled/maintained in the study, will have routine GBS cultures at 35-37 weeks for study purposes, and will receive intrapartum intravenous antibiotics for GBS prophylaxis per routine standard of care.

6. As is the current standard of care, subjects will undergo rectovaginal culture for GBS at 35-37 weeks' gestation. Any subject who tests positive will be treated with intrapartum intravenous antibiotic prophylaxis per routine GBS management protocol.

7. Women who deliver preterm will receive chemoprophylaxis unless their GBS status within the most proximate 4 weeks is known per routine GBS management protocol. We will obtain a culture prior to initiation of antibiotics in those women delivering preterm without known group B strep status.

8. Study visits will be conducted during routine monthly prenatal appointments to assess adverse events, study treatment compliance, and to dispense subsequent 30-day supply of probiotic or placebo capsules.

Patients will complete a study visit questionnaire with the investigator(s) or study coordinator(s). Patients will also receive an additional monthly allotment of 30 capsules. The capsule bottle from the previous cycle will be collected and dated if there are capsules remaining in the bottle. Remaining capsules will be counted and refrigerated for future use.

9. Patient questionnaire/database assessing adverse events will be amended at each study visit, which will occur monthly. The questionnaire/database will assess adverse events, including rash, significant nausea/vomiting, significant diarrhea, persistent fever, and compliance data. The questionnaire will also remind patients that all adverse events must be reported to the Protocol Director immediately at all times.

10. Additionally, subjects may opt to have serial vaginal swabs collected to assess potential beneficial effects of probiotics on the vaginal microbiota and BV status. Vaginal swabs will be collected (either by study personnel or self-collected by the study participant). Swabs will be inserted 1-2 inches into the vaginal introitus and spun for 20 seconds and then withdrawn. Swabs will be collected at the following time points: prior to probiotic/placebo initiation, every 1-4 weeks from time of enrollment to time of delivery, and postpartum serially up to 12 months. These swabs will be stored at -20 degrees Celsius or colder for additional microbiologic analyses.

11. Additionally, placental tissue may be collected at time of delivery for possible future microbiome and/or other analyses.

12. Labor: The patient will receive standard delivery and newborn care. Patients with a positive GBS culture will be treated with standard antibiotics in labor.

13. Postpartum and neonatal care: The patient will receive routine postpartum care per the obstetric team. Data regarding her postpartum course and neonatal outcomes will be collected.

G. Sample size calculation:

1. The baseline rectovaginal GBS colonization is approximately 25% in recent years in our practice, we have powered the study to identify a 40% reduction of GBS colonization to 15%. Using a two-tailed alpha of 0.05, 133 patients per arm (266 patients total) are needed to fulfill a power of 80% to detect a difference of 40% from current baseline GBS colonization of 25%.

2. Given anticipated 30% drop out/lost to follow-up and 10% preterm delivery prior to 35-37 weeks' gestation, we propose increasing our sample size by 40% to account for this subject loss. Therefore, we propose recruitment of 372 patients total (186 in each arm).

H. Statistics:

1. Analysis will be by intention to treat. Demographic data, specific information regarding the course of labor, and outcome variables will be collected on printed and numbered data sheets and entered into a relational database (Access; Microsoft Corporation, Redmond, WA). All data management and analysis will be performed using STATA version 7 software (StataCorp – College Station, TX). Descriptive statistics will be performed. All tests of significance will be 2-tailed and an alpha level of 0.05 will be used. Categorical data will be analyzed by the uncorrected χ^2 and Fisher exact test, as appropriate. Continuous data will be analyzed by the unpaired Student *t* test.

I. Data safety and monitoring plans:

1. The Protocol Director (PD) will be responsible for monitoring data collected.

2. Patient questionnaire/database assessing adverse events will be completed at each study visits, approximately every month. The questionnaire will assess adverse events, including rash, significant nausea/vomiting, significant diarrhea, persistent fevers. The questionnaire/database will also remind patients that such events must be reported to the Protocol Director immediately at all times.

3. Serious adverse events will be reported to the PD within one week. An interim analysis will be conducted to when primary outcome data is available for 150 subjects.

4. Data or events captured by the monitoring plan will be assessed monthly.

5. The following will be monitored:

- a. Side effects related to consumption of the probiotic or placebo capsules
- b. Rates of compliance

- c. GBS colonization culture results
- d. Neonatal morbidity or mortality, including but not limited to infection and duration of hospitalization
- e. Maternal morbidity and mortality, including but not limited to postpartum infection, complications, and duration of hospitalization

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Appendix A: Letter from Manufacturer confirming their agreement to supply of probiotic dietary supplement and placebo free of charge for this investigator-initiated study.

Appendix B: Patient Questionnaire to be completed at monthly study visits.