The Effects of Prophylactic Probiotic use on Reducing Group B *Streptococcus* Colonization in Pregnant People

Effie Karriker and Melissa Tomback

University of New England School of Nursing and Population Health

NSG 332: Evidence Based Practice

Professor Nickerson

November 3rd, 2023

The Effects of Prophylactic Probiotic use on Reducing Group B *Streptococcus* Colonization in Pregnant People

Group B *Streptococcus* (GBS) is a gram-positive coccus that normally colonizes the digestive and genital tract (Puopolo & Madoff, 2023). Worldwide, approximately 18% of pregnant people[1] carry GBS, and in the United States of America (USA), approximately 25% of pregnant people are GBS positive (Center for Disease Control and Prevention, 2022). GBS can be passed onto an infant during birth when it travels through the vaginal canal, a process called vertical transmission. Unfortunately, for newborns who have an immature immune system, GBS can result in early onset GBS disease requiring a lengthy stay in the NICU or even death. Currently, in the USA, the treatment for pregnant people positive for GBS is intravenous antibiotics during labor, which reduces the risk of vertical transmission (Baker, 2023). While effective at decreasing GBS, the systemic antibiotics wipe out beneficial bacteria throughout the body and, most importantly for pregnant people, the genital tract. As a result, both the pregnant person's and infant's microbiome are negatively impacted, thereby subjecting them to other infections. Because of the negative effects of antibiotics, researchers have explored alternative treatments to reduce GBS colonization. One such alternative is using prophylactic probiotics during pregnancy, which is the question discussed here.

Problem Statement and Research Question

In the USA, between 20% and 30% of all pregnant people receive antibiotics during labor to prevent transmission of GBS to the newborn (Hanson et al., 2023). The reason to prevent vertical transmission to the newborn is to prevent early onset GBS disease which has a 1-3% mortality rate in term infants and a 20-30% mortality rate in preterm infants (Baker, 2023). Early

[1] For the purpose of this paper, pregnant persons/people are those who are assigned female at birth regardless of gender presentation.

onset GBS disease occurs within the first week of life and manifests through bloodstream infections, sepsis, pneumonia, and meningitis (Center for Disease Control, 2022). If a pregnant person positive with GBS is not treated with antibiotics in labor, the baby has a 1-2% chance of early onset GBS disease, in contrast to 0.2% risk for those treated with antibiotics during labor (Boyer & Gotoff, 1985; Ohlsson, 2013; Dekker, 2023). This is a reduction of 83% (Ohlsson & Shah, 2013). While the percentage of babies contracting early onset GBS disease may seem low, approximately 6.9% of these full-term infants will die from their infection (Nanduri et al., 2019). Furthermore, consequences of GBS infection can lead to late onset GBS disease and resulting death, stillbirths, and disabilities (Seale et al., 2017; Dekker, 2023). Worldwide, "conservative estimates suggest that GBS is a leading contributor to adverse maternal and newborn outcomes" (Seale et al., 2017, p. S200).

Currently, there are two primary approaches used to prevent early onset GBS. The universal screening approach, practiced in the USA, screens all pregnant people between 35-37 weeks' gestation for GBS. If positive, the pregnant person is treated with prophylactic antibiotics in labor (Baker, 2023; Dekker, 2023). Alternatively, the risk-based screening approach forgoes GBS screening in pregnant people. Instead, it uses risk factors for early onset GBS, such as preterm labor or prolonged rupture of membranes, to determine prophylactic antibiotic use during labor (Baker, 2023; Dekker, 2023). Regardless of the approach, pregnant people received antibiotics approximately 33% of the time (Dekker, 2023).

While antibiotics prevent bacterial infections, they do have their drawbacks. Not only do antibiotics target the bad bacteria, but they also kill off good bacteria, destroying the gut microbiome. When the good bacteria are wiped out, other microbes within the ecosystem, like *Clotridoides difficile (C. diff)* or *Candida,* can multiply and overtake the microbiome, causing

dangerous infections. *C. diff,* a largely antibiotic resistant bacterium, can cause life-threatening diarrhea (Center for Disease Control and Prevention, 2021). *Candida*, a fungus, can cause Candidiasis, a yeast infection, in areas such as the vagina or mouth (Center for Disease Control and Prevention, 2022). This is especially pertinent to the pregnant population as the use of antibiotics during labor can result in a vaginal yeast infection. As the baby passes through the vaginal canal, these infections can then be passed on to its newborn's mouth, called thrush, which can impact breastfeeding. Additionally, infants exposed to perinatal antibiotics have higher incidences of allergy, type 1 diabetes, and obesity later in life (Walker, 2017).

Probiotics are the antithesis of antibiotics: they are live microorganisms, beneficial bacteria and yeasts, that help create a healthy microbiome in the gut. Probiotics can be found in over-the-counter supplements containing bacteria, like *Lactobacillus* and *Bifidobacterium,* or in fermented foods such as yogurt, kefir, miso, and lacto-fermented vegetables (Ito et al., 2019; Wang & Shurtleff, 2019). Theoretically, increasing the good bacteria in the body by taking probiotics should decrease the likelihood that GBS will colonize during pregnancy. The rationale for this is that supplemented bacteria such as *Lactobacilli* colonize and acidify the vaginal environment, which inhibits the growth of GBS (Hanson et al., 2022). This paper explores whether the use of prophylactic probiotics reduces the infection rates of GBS in pregnant persons.

Theoretical Framework

This study will be guided by Florence Nightingale's Environmental Theory. Nightingale's metaparadigm includes person, environment, health, and nursing. Her theory states that manipulation of the environment, through means such as cleanliness or food choices, enhances patient recovery and health. The PICOT question, "In pregnant persons, does the use of

prophylactic probiotics reduce the infection rates of GBS during pregnancy" relates directly to a patient's environment by looking at probiotic usage. Because a patient's food, water, and medication are included in the internal environment in Nightingale's Environmental Theory, a patient's supplementation of probiotics though over-the-counter supplements or fermented foods can be seen as a manipulation of this environment. Furthermore, the impact that probiotics, beneficial microorganisms, have on the microbiome and overall intestinal ecosystem of a patient cannot be understated. By focusing on probiotics, the intent of this paper is to study how the alteration of environment will improve patient outcomes, as evidenced by a decreased instance of GBS during pregnancy.

Synthesis of Literature

A PICOT- guided literature review and appraisal generated four recent studies that examine the use of prophylactic probiotic use on the incidence of GBS (Appendix A and B).

Ho et al. (2016) conducted a double-blind randomized control trial on the effect of oral supplementation of *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 on 110 GBS-positive pregnant people. The study found clinically significant data indicating that probiotics containing *L. rhamnosus* GR-1 and *L. reuteri* RC-14 reduce the colonization of GBS in pregnant people. Two additional randomized control trials studying different *Lactobacillus* strains (Farr et al., 2020; Hanson et al., 2023) provided evidence correlating probiotic usage to a decrease in GBS in pregnant people; however, neither of these studies produced statistically significant findings.

Evidence suggests that *Lactobacillus* makes up 90% of the vaginal microbiome and plays a vital role in maintaining the appropriate vaginal pH to protect against infection (Chen et al., 2017). Promoting the colonization of *Lactobacillus* through a probiotic supplement may be the

most promising area of probiotic study at this time. This is supported by the in vitro findings discussed in Hanson et al.'s systematic review analyzing the use of probiotics to reduce GBS (2022). In this review, four in vitro studies tested differing strains of *Lactobacillus* against GBS isolates from vaginal swabs, rectal swabs, blood stream samples, and the American Type Culture Collection (ATCC) on human vaginal epithelial cells (Ephraim et al., 2012; Martin et al., 2019; Marziali et al., 2019; Zárate & Nader-Macias, et al., 2006, cited in Hanson et al., 2022). Significant data found that probiotics, specifically *Lactobacillus*, can reduce GBS through acidification (Ephraim et al., 2012; Martin et al., 2019; Marziali et al., 2019, cited in Hanson et al., 2022) and adhesion (Martin et al., 2019; Zárate & Nader-Macias, et al., 2006, cited in Hanson et al., 2022). While this in vitro data demonstrates promising evidence for GBS reduction in clinical trials, this data could not be replicated in all the clinical studies reviewed. The lack of significant data produced from Hanson et al. (2023) and Farr et al. (2020) as opposed to the clinically significant data found by Ho et al. (2016), may suggest that different species and strains of *Lactobacillus* play a role in the inhibition of GBS. Additionally, the incongruencies between the in vitro and clinical trials may also be a biproduct of the unique microbiome of each vaginal tract. Chen et al. (2017) estimates that the vagina contains 10^{10}–10^{11} bacteria. The number and type of bacteria in the vaginal tract changes throughout the menstrual cycle and pregnancy (Chen et al., 2021). It is impossible to recreate the diverse microbiome or the fluctuating conditions of the vaginal tract in in vitro studies.

Additionally, the duration of treatment and potency of the probiotic may have played a role in the studies' results. Ho et al. (2016) tested pregnant people with a gestation of 35-37 weeks for GBS. Those who were positive and accepted into the study were instructed to take 2 x 10^9 colony forming units (CFU) up until delivery. The participants took the probiotics for an

average of 19 days. Farr et al. (2020) included a similar demographic of people in their study and began their trial between 32-36 weeks' gestation. Unlike Ho et al., Farr administered double the probiotics at 4 x 10^9 CFU and for 14 days (2020). Even with a much higher potency of probiotic, Farr et al.'s probiotic group had no significant difference in GBS colonization when compared to the placebo (2020). This alludes to the idea that dosage and potency alone may not influence efficacy of probiotic treatment.

To address this issue, Hanson et al. (2023) evaluates the use of probiotics with a higher CFU and longer period of time. This robust study measured the GBS colonization in pregnant people given a probiotic capsule, *Florajen3*, containing 15 x 10^9 CFUs from 28 weeks' gestation to 36 weeks' gestation (a total of 8 weeks). While the study concluded that patients in the control group had less probiotic colonization in their genitourinary tract and were at a 1.33% higher risk for GBS colonization, the study was unable to provide significant data to link probiotic usage to decreased GBS colonization in pregnant people (Hanson et al., 2023). It is important to note, however, Hanson et al. (2023) did find statistically significant data linking probiotics to decreased gastrointestinal symptoms in pregnancy.

Timing of the probiotic intervention may also play a role in the decreased GBS colonization outcome. Through the universal screening approach for GBS, pregnant persons are tested for GBS colonization between 35-37 weeks; during this time period, GBS culture results are the most accurate (Baker, 2023). The studies assessed probiotics before and after this 35–37 week timeframe. Hanson et al. (2023) and Sharpe et al. (2019), found in Hanson et al.'s systematic review (2022), both trialed probiotics during the second and early third trimesters before GBS screening was completed. These trials hypothesized that probiotics could improve colonization in the genitourinary tract, thus protecting the patient from overgrowth or

colonization of GBS. Both studies could not provide significant data that probiotics reduce the rate of GBS (Hanson et al., 2023; Sharpe et al., 2019 as cited in Hanson et al., 2022). Contrarily, Ho et al. (2016) and Farr et al. (2020) studied probiotics on GBS positive people after the GBS screening at 35-37 weeks. While Farr et al. (2020) was unable to produce significant data, Ho et al.'s (2016) significant data suggests that a reduction of GBS colonization is possible with probiotics. This may indicate that probiotics are a more successful prophylactic tool in later pregnancy.

However, it is important to note that a huge limitation comes from including pregnant people who are negative with GBS. Because Hanson et al. (2023) and Sharpe et al. (2019) included all participants regardless of their GBS status, the efficiency of the desired outcome was minimized. This is due to the fact that only 75% of pregnant people are GBS negative, which reduces the number of participants where the probiotic intervention would be most effective. "A more efficient design would involve allocating participants to the intervention… as the intervention would be limited to the population of interest" (Sharpe et al., 2019, p.1817).

Conclusions and Implications for Practice

As of this date, more research is needed to evaluate the prophylactic use of probiotics to reduce GBS colonization in pregnant people. All the studies so far have had a small sample size and limited demographic. Each study was limited to specific cities in the following countries: Australia (Farr et al., 2020), China (Ho et al., 2016), Canada (Sharpe et al., 2019), United States (Hanson et al., 2023). Not only is data from limited demographics hard to generalize to a global population, but it also calls into question the roll that diet has on baseline GBS colonization, which none of the trials evaluated. Each participant's diet will change based on their culture and socioeconomic status. Additionally, the various studies analyzed specific probiotic strains, and

there is a plethora of probiotics on the market. The studies also had participants taking the probiotics for different lengths of time. Adherence rates for participants were all over the map. For instance, in Hanson et al.'s (2023) study, the average intake of study capsules was half of the preferred amount even though participants received daily reminders to take their pills. Hanson et al. (2023) suggested that "low adherence to intervention(s) may have contributed to the lack of statistically significant findings", which can be applied to all the studies reviewed (p. 9).

Because the number of people that are positive for GBS is so high compared to the number of newborns who get early onset GBS disease, even a small reduction of positive GBS colonization rates would be meaningful: "A total of 1029 pregnant people would need to be treated with IAP [intrapartum antibiotic prophylaxis] to prevent one additional EOGBSD [early onset GBS disease]" (Sharpe et al., 2021). Because of the negative consequences that antibiotics pose to both mother and baby, it is imperative that further interventions for GBS prevention be studied. Given that none of the studies reviewed found adverse side effects from taking probiotics during pregnancy, the overall health of the USA and the world compels further study of this issue.

References

Baker, Carol. (2023). *Prevention of early-onset group B streptococcal disease in neonates.* Up to Date. Retrieved November 29, 2023, from: https://www-uptodate.com.une.idm.oclc.org/contents/prevention-of-early-onset-group-b-streptococcal-disease-in-neonates/abstract/1

Center for Disease Control and Prevention. (2022, June 28). *Candidiasis*. Center for Disease Control and Prevention. Retrieved November 29, 2023 from: https://www.cdc.gov/fungal/diseases/candidiasis/index.html

Center for Disease Control and Prevention. (2021, July 12). *C. diff (Clotridoides difficile)*. Center for Disease Control and Prevention. Retrieved November 29, 2023 from https://www.cdc.gov/cdiff/index.html

Center for Disease Control and Prevention. (2022, October 18). *Group B Strep*. Center for Disease Control and Prevention Retrieved November 23, 2023 from https://www.cdc.gov/groupbstrep/index.html

Chen, C., Song, X., Wei, W., Zhong, H., Dai, J., Lan, Z., Li, F., Yu, X., Feng, Q., Wang, Z., Xie, H., Chen, X., Zeng, C., Wen, B., Zeng, L., Du, H., Tang, H., Xu, C., Xia, Y., Xia, H., … Jia, H. (2017). The microbiota continuum along the female reproductive tract and its relation to uterine-related diseases. *Nature communications*, *8*(1), 875. https://doi.org/10.1038/s41467-017-00901-0

Chen, X., Lu, Y., Chen, T., & Li, R. (2021). The Female Vaginal Microbiome in Health and Bacterial Vaginosis. *Frontiers in cellular and infection microbiology*, *11*, 631972. https://doi.org/10.3389/fcimb.2021.631972

Farr, A., Sustr, V., Kiss, H., Rosicky, I., Graf, A., Makristathis, A., Foessleitner, P., & Petricevic, L. (2020). Oral probiotics to reduce vaginal group B streptococcal colonization in late pregnancy. *Scientific reports*, *10*(1), 19745.

Dekker, Rebecca. (2023, February 6). *The Evidence on: Group B Strep*. Evidence Based Birth. https://evidencebasedbirth.com/groupbstrep/

Hanson, L., VandeVusse, L., Forgie, M., Malloy, E., Singh, M., Scherer, M., Kleber, D., Dixon, J., Hryckowian, A. J., & Safdar, N. (2023). A randomized controlled trial of an oral probiotic to reduce antepartum group B Streptococcus colonization and gastrointestinal symptoms. *American journal of obstetrics & gynecology MFM*, *5*(1), 100748.

Hanson, L., VandeVusse, L., Malloy, E., Garnier-Villarreal, M., Watson, L., Fial, A., Forgie, M., Nardini, K., & Safdar, N. (2022). Probiotic interventions to reduce antepartum Group B streptococcus colonization: A systematic review and meta-analysis. *Midwifery*, *105*, 103208.

Ho, M., Chang, Y. Y., Chang, W. C., Lin, H. C., Wang, M. H., Lin, W. C., & Chiu, T. H. (2016). Oral Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 to reduce Group B Streptococcus colonization in pregnant women: A randomized controlled trial. *Taiwanese journal of obstetrics & gynecology*, *55*(4), 515–518.

Ito, M., Takamori, A., Yoneda, S., Shiozaki, A., Tsuchida, A., Matsumura, K., Hamazaki, K., Yoneda, N., Origasa, H., Inadera, H., Saito, S., & Japan Environment and Children's Study (JECS) Group (2019). Fermented foods and preterm birth risk from a prospective large cohort study: the Japan Environment and Children's study. *Environmental health and preventive medicine*, *24*(1), 25. https://doi.org/10.1186/s12199-019-0782-z

Nanduri, S. A., Petit, S., Smelser, C., Apostol, M., Alden, N. B., Harrison, L. H., Lynfield, R., Vagnone, P. S., Burzlaff, K., Spina, N. L., Dufort, E. M., Schaffner, W., Thomas, A. R., Farley, M. M., Jain, J. H., Pondo, T., McGee, L., Beall, B. W., & Schrag, S. J. (2019). Epidemiology of Invasive Early-Onset and Late-Onset Group B Streptococcal Disease in the United States, 2006 to 2015: Multistate Laboratory and Population-Based Surveillance. *JAMA pediatrics*, *173*(3), 224–233.

Puopolo, K. M., & Madoff, L. C. (2022). *Group B streptococcal infection in pregnant individuals.* Up to Date. Retrieved November 24, 2023, from https://www-uptodate-com.une.idm.oclc.org/contents/group-b-streptococcal-infection-in-pregnant-individuals/print?search=group%20b%20strep%20in%20pregnancy&source=search_result&selectedTitle=1~150&usage_type=default&display_rank=1

Seale, A. C., Bianchi-Jassir, F., Russell, N. J., Kohli-Lynch, M., Tann, C. J., Hall, J., Madrid, L., Blencowe, H., Cousens, S., Baker, C. J., Bartlett, L., Cutland, C., Gravett, M. G., Heath, P. T., Ip, M., Le Doare, K., Madhi, S. A., Rubens, C. E., Saha, S. K., Schrag, S. J., … Lawn, J. E. (2017). Estimates of the Burden of Group B Streptococcal Disease Worldwide for Pregnant Women, Stillbirths, and Children. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*, *65*(suppl_2), S200–S219.

Sharpe, M., Shah, V., Freire-Lizama, T., Cates, E. C., McGrath, K., David, I., Cowan, S., Letkeman, J., & Stewart-Wilson, E. (2021). Effectiveness of oral intake of *Lactobacillus rhamnosus GR-1* and *Lactobacillus reuteri RC-14* on Group B *Streptococcus* colonization during pregnancy: a midwifery-led double-blind randomized controlled pilot trial. *The journal of maternal-fetal & neonatal medicine: the official journal of the European*

Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians, *34*(11), 1814–1821.

Walker W. A. (2017). The importance of appropriate initial bacterial colonization of the intestine in newborn, child, and adult health. *Pediatric research*, *82*(3), 387–395.

Wang, Y., & Shurtleff, D. (2019, August). *Probiotics: What you need to know*. National Center for Complementary and Integrative Health. https://www.nccih.nih.gov/health/probiotics-what-you-need-to-know

Appendix A
Annotated Bibliography

Hanson, L., VandeVusse, L., Malloy, E., Garnier-Villarreal, M., Watson, L., Fial, A., Forgie, M., Nardini, K., & Safdar, N. (2022). Probiotic interventions to reduce antepartum Group B streptococcus colonization: A systematic review and meta-analysis. *Midwifery*, *105*, 103208.

Using data collected from PubMed, CINAHL, and Cochrane Library, this systematic review analyzes the use of probiotics to reduce Group B *streptococcus* (GBS) from data in five in vitro studies and six clinical trials. Of the in vitro research examined, the meta-analysis found that the use of probiotics antagonizes GBS through acidification, adherence, and immune modulation. The meta-analysis of the clinical trials was less conclusive with two studies reporting no statistical significance, two studies reporting statistical significance for a decrease in GBS, and one study reporting a 70% decrease in GBS with no statistical significance reported. In conclusion, the researchers found that more large, well-controlled trials are necessary for considering probiotic effects on GBS colonization.

The objective of this systematic review directly relates to our PICOT question studying the efficacy of probiotic use on the reduction of GBS colonization. While our goal is to focus on data collected through evidence-based practice and clinical trials, the addition of in vitro studies in this review helped to remove many of the variables that are hard to control in clinical trials. These include things like diet, duration of probiotic treatment, and gestation at which probiotic treatment is started and stopped. The in vitro studies also produced much clearer results with less bias. The bulk of the limitations from this article come from the data in the six clinical trials. These limitations included: a high risk of bias

for five out of six of the clinical trials, large variations of probiotic species tested, and various testing methods for colonization of GBS in the pregnant participants. There is a scarcity of clinical trials studying probiotics' effect on reduced GBS colonization. This is largely due to the surplus of limitations. While the article came from a credible journal and used the Cochrane data form to extract data, I would hesitate to use a few of the clinical trials mention in our paper due to poor research practice and high risks of bias.

Ho, M., Chang, Y. Y., Chang, W. C., Lin, H. C., Wang, M. H., Lin, W. C., & Chiu, T. H. (2016). Oral Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 to reduce Group B Streptococcus colonization in pregnant women: A randomized controlled trial. *Taiwanese journal of obstetrics & gynecology*, *55*(4), 515–518.

The purpose of the randomized double-blind clinical trial was to investigate the role that probiotics have in preventing Group B *streptococcus* (GBS) in pregnant people. Ming-Ho and his research team studied the effect of oral supplementation of *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 on 110 GBS positive pregnant people. The efficacy of the probiotic treatment was measured with a vaginal and rectal GBS colonization at the time of delivery. The study found significant data, with a *p* value of 0.007, suggesting that probiotics containing *L. rhamnosus* GR-1 and *L. reuteri* RC-14 reduce the conization of GBS in pregnant people.

This study seamlessly connects to our paper on the use of prophylactic probiotics to reduce the infection rates of GBS during pregnancy. While our PICOT aims to address all forms of probiotics, the data collected on two specific strains of *Lactobacillus* only adds to the base of knowledge we have on probiotics. The market for probiotics is vast. Thus, it is helpful to have data relating to specific probiotic strains to determine which

probiotics, if any, have effects on GBS. Furthermore, this study only included pregnant people who presented as GBS positive at 35-37 weeks' gestation and excluded people who used antibiotics during pregnancy, were diagnosed with acute illness during pregnancy, or were immunocompromised. While this limited the sample size to only 110 people from the initial 1200 people tested, it created a sample population with minimal confounding variables. Even then, not all confounding variables were be controlled for. The major limitation in the study was the uncontrolled variable of diet. Because people can ingest probiotics through diet, it is impossible to determine how diet played into the results of the study. Additionally, the study population was limited to the patient population of China Medical University Hospital in Taichung, Taiwan. This specific patient population means that the findings cannot be generalized. This further impacts the aspect of diet, as diet is largely based on family upbring, culture, and socioeconomic status. Despite its limitations, this clinical trial can be used to supplement other studies on prophylactic use of probiotics.

Sharpe, M., Shah, V., Freire-Lizama, T., Cates, E. C., McGrath, K., David, I., Cowan, S., Letkeman, J., & Stewart-Wilson, E. (2021). Effectiveness of oral intake of *Lactobacillus rhamnosus GR-1* and *Lactobacillus reuteri RC-14* on Group B *Streptococcus* colonization during pregnancy: a midwifery-led double-blind randomized controlled pilot trial. *The journal of maternal-fetal & neonatal medicine: the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians*, *34*(11), 1814–1821.

Using data from randomized double-blind controlled pilot trial (113 participants), researchers tested the hypothesis that treatment of pregnant persons with probiotic

supplements would reduce Group B *Streptococcus* colonization. The primary aim of the pilot study was to determine the feasibility of implementing a larger multisite appropriately powered study. The study found no statistically significant differences in the rates of colonization between pregnant people who did or did not receive probiotics. This study aligns perfectly with our paper's focus on testing whether probiotics impact GBS colonization rates in pregnant people, but unfortunately the study suffers from many shortcomings that make it less valuable. The researchers noted several weaknesses in the study: the study concluded before desired sample size of 200 was reached, it had no baseline GBS tests were administered, and the probiotic dosage may have been ineffective. We noted even more weaknesses: participants did self-swabbing, the probiotics were started between 23-25 weeks, when a longer treatment course may be required, and there was no evaluation of diet. Although the paper has strong merit being a double-blind randomized controlled trial, the limitations in the article were far too great for us to consider using it.

Appendix B

Evidence Table

Author last name, year of publication, and title	**Research Question/Research Objective**	**Type of Evidence (Qualitative, Quantitative, etc)**	**Sample (Population size, setting, etc.)**	**Design**	**Independent Variable OR Intervention**	**Dependent Variable/ Outcome**	**Significant Results**	**Limitations/Gaps**	**Strengths**	**Evidence level and quality**
Ho et al., 2016 Oral *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 to reduce Group B *Streptococcus* colonization in pregnant people: A randomized controlled trial.	The objective of this study was to examine the effect of oral supplementation of *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 on Group B streptococcus (GBS)-positive pregnant people with respect to becoming GBS negative.	Quantitative	1200 people were tested at the Obstetric Department of the China Medical University Hospital in Taichung, Taiwan. Pregnant people, singleton pregnancy, with positive GBS screening at 35-37 weeks of gestation. 219 were positive with GBS. Of those, 110 enrolled. Small sample size.	Experimental Double Blind randomized clinical trial.	Use of Oral Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 nightly.	Primary: Absence of vaginal and rectal GBS colonization at delivery in pregnant people who presented as GBS positive at 35-37 weeks' gestation after probiotics or placebo treatment. Secondary: Relationship between parity and newborn transfer units.	42.9% of the participants receiving probiotics achieved a negative GBS culture when compared to the 18.0% in the placebo group. Cha-Square analysis showed a p value of 0.007, which suggests a significant decrease in the negative GBS culture rate between the two groups.	Patients were only from one pt population and demographic. Findings cannot be generalized. There was no cost effectiveness analysis. They did not factor the pregnant people's diet in the data. Participants only took probiotics for an average of 20 days, which may not be long enough for the *Lactobacillus* to repopulate.	The double-blind randomized control. Specific probiotics were used in the study. Chi-square tests were used to confirm that there was no significant difference in the two groups in terms of: maternal age, education level, parity, gestational week of delivery, duration of drug taking,	AHRQ level of Evidence: Level 2: Randomized Control Trials AHRQ Grade of Research: Level A

									neonatal birth weight, newborn transfer units, and Apgar score.	
Hanson et al, 2022 Probiotic interventions to reduce antepartum Group B streptococcus colonization: A systematic review and meta- analysis.	Can antenatal probiotic decrease the probability of positive GBS results in pregnant persons? The in vitro studies looked at the use of probiotic interventions and mechanisms of actions. The in vivo studies looked at probiotics to reduce antenatal GBS colonization.	Quantitative Five studies contained in vitro studies of probiotic intervention to determine antagonist activity against GBS. Six clinical trials of probiotics to reduce antenatal GBS.	It varies. Research studies were limited to English language with no limit on publication date. Clinical trials were included if they reported 36-week GBS results. In vitro studies were included if probiotics were tested for antagonist activity against GBS.	Systematic review and meta-analysis Of the six clinical trials, there were three randomized controlled trials, one prospective study, one cohort open-label, and one quasi experiment.	Varies Each in vitro study used different probiotics including *L. crispatus, L. gasseri L. vaginalis, Lactobacillus salivarius, treptococcus salivarius, L. rhamnosus, L. acidophilis, L. crispatus, L. paracasei,* and *L. salivarius.* Each clinical trial used a different probiotic intervention	Varies Each in vitro studied the antagonist activity against strains of GBS. The strains varied by study. The dependent variables was a reduced antenatal GBS	Results in the in vitro studies showed that probiotics antagonize GBS by acidification, adherence, and immune modulation. Two of the studies found no statistical significance. Two studies found	Limited by the lack of in vitro and clinical trials of probiotics to reduce GBS colonization in pregnancy. Only one clinical had low risk of bias. Large variations in probiotic species. Over the counter probiotics vary vastly and specific strains studied in this trial may not be in those.	The review studied the efficacy of probiotics in vitro and in clinical trials. Significant data was presented in various backgrounds. Reviewers of the data reviewed the data separately.	AHRQ level of Evidence: Level 1: Systematic review/ Meta analysis AHRQ Grade of Research: Level B

					including UREX, *L rhamnosus* GR-1, *L salivarius* CECT 9145, iNatal®, and Florajen3®.	colonization at 36 weeks or at the time of delivery.	statistical significance for a decrease in GBS. One study found a 70% decrease in GBS but did not report statistical significance.			
Sharpe et al. 2019 Effectiveness of oral intake of *Lactobacillus rhamnosus GR-1* and *Lactobacillus reuteri RC-14* on Group B *Streptococcus* colonization during pregnancy: a midwifery-led double-blind randomized controlled pilot trial	The study was to evaluate whether probiotic supplements reduce GBS colonization	Quantitative	139 pregnant people, chosen from 19 midwifery practices in southern Ontario, Canada	Randomized controlled pilot trial	Probiotic w/2 dried bacterial strains (*L.rhamnosus GR-1* and *L. reuteri RC-14*) begun at 23-25 prenatal visit	The rates of GBS colonization	No statistically significant differences in the rates of GBS	Self-swab; 9 in probiotics group and 12 in placebo group received intrapartum antibiotic prophylaxis (IAP) during pregnancy and some received antibiotics during labor bec. of suspected infection (fever), unresolved maternal tachycardia, and/or foul/purulent amniotic fluid. Other variables not thought of health/diet of particiant (high carb/junk/processed food/sugar etc.	Few The theoretical concept	AHRQ level of Evidence: Level 2: randomized control trial AHRQ Grade of Research: Level B

								would allow for overgrowth of GBS or any other bacteria. No prior GBS swab before probiotic		