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Non-Antibiotic Alternatives for Reducing Antepartum Group B Streptococcus Colonization

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NON-ANTIBIOTIC ALTERNATIVES FOR REDUCING ANTEPARTUM GROUP B STREPTOCOCCAL COLONIZATION

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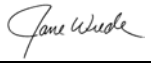
Director of Graduate Nursing Program Signature:	

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Acknowledgements

To LeAnn van den Bosch, CNM, for taking me off work at 40 weeks – and then when I complained about boredom, giving me a 'project'. After two years of graduate school, countless hours at my computer, several reams of paper, a Costco-size bottle of Excedrin Headache, and a full semester of Mac-trackpad-related wrist tendonitis…I will never again complain of boredom in your presence.

To my Dad, who was so surprised that I ever questioned my ability to do this. Everyone needs someone in their life who is convinced, beyond any doubt, of their ability to accomplish great things. 'Of course you're going to make it. You're my kid. You're a Toth. We are a bunch of eccentric misfits yes, but we are *intelligent* eccentric misfits!'

To my Mom, who expresses love in the form of comfort food and "I'm just going to quick do a little laundry while I'm here". Shep would like to thank you for making sure that he ate some meals with redeemable nutritional qualities while I've been in school!

To Dr. Kristin Sandau and Dr. Julie Ann Vingers, for doing the impossible – reigniting my love for research, which had been dormant for over a decade after a painful undergrad nursing research class! I look forward to following in your footsteps, offering my own contributions to the incredible fields of nursing and midwifery.

And to my longsuffering husband Will – behind every determined woman is her husband telling her the good news, that there is no cap on the student loan debt one individual can have. I promise I'm (almost) done!

La vita é questa niente é facile e nulla é impossibile.

Life is that nothing is easy, and nothing is impossible.

-Bishop Giacomo Donadei (1351-1431)

Abstract

Background: *Streptococcus agalactiae* (known as Group B strep, or GBS) is a bacterium that resides in the gastrointestinal tract and/or vaginal canal and is typically benign, but during pregnancy and birth it can potentially colonize the neonate, causing early-onset group B streptococcal disease (EOGBSD) of the neonate. The use of antibiotics has reduced the morbidity and mortality associated with neonatal infections, but it is not an entirely effective means of preventing the infections and the development of antibiotic resistance may render antibiotics ineffective for this purpose. Alternative methods of preventing GBS colonization are in use by consumers and out-of-hospital birth providers, but it is unclear whether or not these methods are supported in the literature.

Purpose: To evaluate the literature in support of alternative methods to reduce or prevent GBS colonization.

Results: Using germ theory as a theoretical framework, twenty-two articles were identified for review and appraised using the Johns Hopkins Research Evidence Appraisal Tool. The major findings of the reviewed literature were that chlorhexidine has not demonstrated consistent benefit in reducing GBS colonization, and both probiotics and garlic have in vitro effects against GBS, but they lack the testing necessary to support in vivo use.

Conclusion: There are many alternative methods in practice, but they all suffer from a lack of literature to guide their use.

Implications for Research and Practice: The findings of this review support the idea that alternative methods may be a viable alternative to antibiotics, but there is a critical need for research to definitively support the practice.

Keywords: group b streptococcus, GBS, GBS colonization, pregnancy, antibiotics, probiotics, garlic, chlorhexidine, neonatal sepsis, midwifery

List of Tables

Chapter I: Introduction

Need for Critical Review of a Nurse-Midwifery Problem

Streptococcus agalactiae, most commonly known as "Group B strep" (GBS), is a gram-positive coccus that can colonize in the vaginal canal. Unless abnormal overgrowth has taken place, GBS is a normal variant of vaginal flora and is harmless. During pregnancy however, it is a significant contributor to neonatal GBS infection, which is the most common cause of neonatal sepsis and meningitis, and the mortality rate with early-onset GBS disease (EOGBSD) is 2% to 30% (Siewert & Holida, 2010). In the absence of any other definitive and targeted approach, most birth professionals in the United States adhere to the universal approach of administering antibiotics to all women who have a positive GBS culture, which is typically obtained via vaginal and rectal swab at 36 to 37 weeks gestation. Vaginal colonization of GBS has been reported to occur in 10-30% of women (CDC, 2010) and those women are typically treated with antibiotics in labor to prevent EOGBSD in the neonate.

The use of antibiotics has become the gold standard of neonatal GBS disease prevention; however, the supporting evidence is weak. The three primary studies on which the practice was based are more than 20 years old (Boyer & Gotoff, 1986; Tuppurainen & Hallman, 1989; Matorras, García-Perea, Madero, & Usandizaga, 1991) included a total of only 500 women, and the studies have long been criticized for bias and poor methodology. Antibiotic prophylaxis is the accepted practice in the absence of other viable alternatives, but there is lack of evidence from well designed and conducted trials to recommend intrapartum antibiotic prophylaxis (IAP) to reduce neonatal EOGBSD (Ohlsson & Shah, 2014).

The risks of antibiotics for the woman include allergic reaction such as hives or pruritus, anaphylactic reaction, antibiotic drug resistance and perpetuation of drug-resistant bacteria and elimination of beneficial vaginal flora potentially causing opportunistic infections. For the neonate, bacterial flora in the gut can be protective against pathogens, and alternation of the flora by exposure to antibiotics is a potentially important risk factor in the development of allergic disease (McKeever, Lewis, Smith, & Hubbard, 2002). The combined use of ampicillin and gentamicin in early life can have significant effects on the evolution of the infant gut microbiota, the long-term health implications of which remain unknown (Fouhy, et al., 2012). For both mother and neonate, intrapartum antibiotic exposure is associated with higher rates of thrush within one month of delivery (Dinsmoor, Viloria, Lief, & Elder, 2005).

Some birth professionals, primarily midwives, use alternative methods to prevent or treat GBS colonization. With the goal of removing the need for antibiotics or minimizing the risk of transmission of GBS to the neonate, these methods have seen increased usage in recent years. As demonstrated in Table 1, there are a multitude of alternative methods that are in use. Many of these methods have not been tested in a research setting, so the goal of this synthesis is to determine what alternative practices, if any, are supported in the literature for primary prevention of GBS colonization.

Table 1: Alternative Methods to Prevent/Reduce GBS Colonization
Apple cider vinegar
Bentonite clay
Colloidal silver

Diatomaceous earth
Essential oils (*Melaleuca alternifolia, cinnamomum verum, syzygium aromaticum*)
Fermented foods (kefir, kombucha, sauerkraut, kimchi)
Garlic
Probiotics/yogurt
Water birth

Significance to Nurse-Midwifery

This topic is of high interest to midwives in practice, as neonatal sepsis related to GBS colonization has serious, and potentially fatal, outcomes in neonates. Approximately 10-30% of pregnant women are colonized with GBS in the vagina or rectum, and in the absence of any intervention, an estimated 1%-2% of infants born to colonized mothers develop early-onset GBS infections (CDC, 2010). In the United States mortality rates were reported to be between 4 and 6%, although it has been suggested that the rate of neonatal disease is considerably underestimated because the requirement for positive cultures from blood or cerebrospinal fluid underrepresents the true burden of disease (Johri, et al., 2006). Importantly, morbidity is high, as approximately 50% of neonates who survive GBS infection suffer complications, including neurological sequelae, cortical blindness, deafness, uncontrolled seizures, hydrocephalus, hearing loss, and speech and language delay (Johri, et al., 2006).

Some early onset infections can occur when the neonate is exposed to GBS during passage through the birth canal, but most early onset infections are probably caused by ascending movement of the organism from the maternal genital area through ruptured

membranes into the amniotic fluid, where the organism multiplies and ultimately colonizes the respiratory tract of the fetus (Johri, et al., 2011). However intact membranes do not preclude vertical transmission, as GBS can cross intact amniotic membranes (CDC, 2010).

The current approach of prophylactic antibiotics has been a valiant although only moderately effective effort to eliminate neonatal sepsis from GBS, and overuse and resistance to antibiotics is a public health concern. GBS has built resistance to antibiotics that were previously considered effective, and some strains of GBS have been found to be resistant to treatment by all currently used forms of antibiotics (Dabrowska-Szponar & Galinski, 2001). No new classes of antibiotics have been introduced since 2003, and despite the advances in antimicrobial and vaccine development, infectious diseases still remain as the third-leading cause of death in the United States (Conly & Johnston, 2005). The question of time left until there is no longer a viable antibiotic effective against GBS makes the prevention of GBS colonization particularly critical. Even more concerning is the treatment for women who are allergic to penicillin, as up to 29% of GBS strains have been shown to be resistant to non-penicillin antibiotics (Bland, et al., 2001).

Access to antibiotics is not universal, but in the United States access is a concern unique to midwives practicing outside of the hospital. Depending on the state legislation, certified professional or direct entry midwives may not be authorized to administer antibiotics in the home or birth center setting. This restriction forces women who are seeking to give birth in a home or birth center setting with a midwife to make a critical decision regarding GBS prophylaxis. Some women choose not to be tested, some choose

to transfer to a hospital for antibiotic administration if they are GBS positive, and others choose to prevent or treat GBS with alternative methods.

Many women who seek midwifery care, irrespective of birth setting, do so in part because of low rates of intervention, and the willingness of midwives to consider alternative approaches to typical interventions. Studies identified regarding the use of alternative solutions to antibiotics for GBS may support the practices of midwives who are unable to administer antibiotics in their practice, and for midwives whose clients prefer to avoid the use of antibiotics. Alternatives to antibiotics are currently in use amongst midwives, and further research may suggest that the reasoning behind some of these practices are not entirely unfounded and may find that the methods have merit.

Conceptual Model/Theoretical Framework

The theoretical model used in this review is germ theory, which is based on factors that alter the interactions and effects of microorganisms on human life. Germ theory was first introduced in the early 1800s as medicine developed, and as a theory has withstood the test of time. Germ theory is not a specific theory with a single author, but rather discoveries that culminate in the germ theory have had a long gestation period, and were a collective process (Snowden, 2010). Since its beginnings as a continued study by many scientists and physicians, most notably Louis Pasteur, Joseph Lister, and Robert Koch, it has become a foundational theory for virtually all aspects of human biology. At the time of Lister's medical practice, 'As many as 80% of all operations were followed by hospital gangrene, and almost one half of all patients died after a major operation' (Alexander, 1985, as cited in Jessney, 2012). After Pasteur, Lister and others showed the medical significance of bacteria in the 1860s, great progress was made in identifying new

kinds of bacteria and demonstrating their roles in a host of diseases, including diphtheria, tuberculosis, and cholera (Thagard, 1997). Each advancement was then built upon by subsequent research and scientists, starting with the identification of bacteria, recognition of specific bacteria as the cause of specific diseases, means of preventing infection, and methods for immunization. Application of germ theory to GBS in this review is the identification of methods that reduce the incidence of GBS colonization in the antepartum, defined as the time during labor and birth.

Statement of Purpose

The most common remedies that are in use by consumers and some out-of-hospital birth providers are garlic, chlorhexidine, and probiotics. The intent of this review is to determine if there is literature to support the use of these methods as a means of preventing GBS colonization.

Chapter II: Methods

Search Strategies Used to Identify Research Studies

The databases that were utilized were Embase, CINAHL, PubMED, and the Cochrane Database of Systematic Reviews. An initial search of PubMed was conducted using the keywords “group B strep” OR ‘streptococcus agalactiae” AND probiotics, which yielded 22 items. A search of CINAHL using the keywords “group B strep” OR ‘streptococcus agalactiae” AND probiotics, which yielded 47 items.

An Embase search was conducted with keywords “Streptococcus agalactiae” or “group B strep”, along with “prenatal”, “prenatal care”, “pregnancy”, “pregnant women”, “pregnan*” (to capture both pregnant and pregnancy), “antenatal”, “prevent” or “prophyl*”, “prevention”, “anti-infection agents”, “vaginal”, “antiseptic”, “complementary therapies”, “without antibiotics”, “no antibiotics”, “chlorhexidine”, “probiotic”, “probiotics”, “garlic”, “allium”, or “allicin”. This search yielded seven results.

A search of the Cochrane Database of Systematic Reviews with keywords “group b strep” and “prevention” yielded six results.

Criteria for Including or Excluding Research Studies

The inclusion criteria requires articles to have been written in English and published in the last 10 years. Two earlier published articles and one article written in Turkish were included after the initial inclusion and exclusion criteria applied due to their applicability to the research question. In addition, the four individual studies that made up the Cochrane Review were added, although they were older than the chosen criteria.

Articles were excluded if they focused on secondary prevention, defined as treating with antibiotics after GBS colonization testing has been performed and resulted in a positive culture. Articles regarding the creation, testing, and acceptance of a GBS vaccination or a rapid result GBS test were also excluded as outside of the scope of this project. Articles that focused on bacterial vaginosis were excluded unless the article had findings specific to GBS, as there can be multiple pathogens responsible for bacterial vaginosis. Also excluded were articles that focused on GBS in animal populations.

Number and Types of Studies Selected After application of the inclusion/exclusion criteria, 18 articles remained. With the four individual articles from the Cochrane Review, the total articles came to 22. The articles were categorized using the criteria from Johns Hopkins (Dearholt & Dang, 2012):

- Level I experimental studies (n=8)
- Level II, quasi-experimental studies (n=2)
- Level III, non-experimental studies (n=10)
- Level IV, clinical practice guidelines (n=0)
- Level V, non-research literature reviews and case studies (n=2)

Criteria for Evaluating Research Studies

Article quality was then determined using the Johns Hopkins Research Evidence Appraisal Tool (Dearholt & Dang, 2012). Level I, level II, and level III articles were evaluated for their consistency of results, sufficiency of sample size, whether or not controls were adequate, and the comprehensiveness of the literature reviews. Level IV articles were evaluated for the documentation of search strategy, consistency of results,

sufficiency of sample size, evaluation of included studies, strength of conclusions, and date of publication. Level V articles were evaluated for the clarity of expertise, definitive conclusions, and consistency and use of scientific evidence in recommendations. Strengths and weaknesses of the individual articles is discussed in Chapter III.

Chapter III: Literature and Analysis

Major Findings

Garlic. Garlic was frequently mentioned in midwifery periodicals for GBS prophylaxis, specifically the intravaginal use of garlic cloves, and garlic is a well-known and well-accepted natural remedy. But despite the general popularity of garlic, very little was found about the activity of garlic on GBS in the review of the literature.

Ankri and Mirelman (1999) reviewed the antibacterial activity of allicin, which is one of the active principles of freshly crushed garlic homogenates. Various garlic preparations have been shown to exhibit a wide spectrum of antibacterial activity against Gram-negative and Gram-positive bacteria (Ankri & Mirelman, 1999). Authors described the substrate alliin and the enzyme alliinase as a protective mechanism against microbial invaders from surrounding soil, and when the membrane that encloses the enzyme and substrate and compromised, the result is allicin which is antibacterial. However Ankri and Mirelman reported that GBS strains were found to be resistant to the action of allicin (1999). It was thought that the hydrophilic capsular layers of the Streptococcus bacteria prevent penetration of the allicin compound; however, the article did not articulate the basis for that conclusion.

Cohain (2009) reported using garlic with eight cases of confirmed symptomatic vaginal GBS of 6 months to 4 years during, not resolved by course(s) of oral antibiotics. This article references adjunctive local therapies including chlorhexidine and povidone-iodine, but garlic was chosen for its accessibility and ease of use. The women all successfully resolved the symptoms by using half a freshly cut clove of garlic inserted vaginally at night and removed in the morning, for 3-6 weeks followed by maintenance

doses of once every 2-4 days (Cohain, 2009). The subjects were not pregnant and already had active infections; however, it was the only article that specifically evaluated the use of an alternative method to treat GBS being used on human subjects.

Cutler, et al., (2009) evaluated the effectiveness of extracts of allicin in water or a novel gel formulation, specifically for prevention of GBS colonization. The solution was a 500mg/L aqueous solution, and a combination of the aqueous solution with a commercially available gel to make up the gel formulation. The two types of allicin formulations were used on seventy-six non-duplicate clinical isolates of GBS from vaginal swabs, and the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were determined using a microtiter plate, liquid culture system. There was a >6 log reduction in bacterial load in all cases compared with the growth control and there was no detectable growth (detection limit 100 cfu/mL) after 8 or 24 hours of treatment (Cutler, et al., 2009). While this article does support garlic as being a potentially effective remedy, it still lacks the in vivo testing needed for acceptance and there is no clear determination of how long the effects of garlic would last until re-colonization was possible. The most interesting aspect of this article is the assertion that the different types of preparations (such as cream vs. gel) can impact its efficacy.

All three articles offer support to garlic as a potential agent for reducing GBS colonization. However, it is clear that further research is needed to support and direct the use of garlic.

Chlorhexidine. Ohlsson, Shah, and Stade (2014) conducted a Cochrane review of the use of vaginal chlorhexidine during labor in an effort to prevent early-onset neonatal GBS infections. The four studies in the review are as follows.

Adriaanse, Kollée, Muytjens, Nijhuis, de Haan, and Eskes (1995) evaluated the effect of chlorhexidine gel placed intravaginally after active labor had started, and found a modest reduction in vertical transmission of GBS ($p = 0.069$). The timing of the intervention may be a factor to consider in future studies, as this study initiated the intervention after the woman was in active labor and it has been theorized that vertical transmission may occur before the onset of labor.

Burman and Christensen (1992) evaluated the effectiveness of vaginal flushing with chlorhexidine on admission rates to special-care neonatal units, and found a modest improvement. Compared with placebo, antepartum vaginal chlorhexidine disinfection reduced the overall admission rate to special-care neonatal units from 2.9% to 2.0% (RR 1.48, 95% CI 1.01-2.16; p =0.04). Additionally, the reduction in admissions after chlorhexidine prophylaxis vs. placebo was greater among infants born of GBS carrier mothers (5.4% to 2.8%; RR 1.95, 95% CI 0.94-4.03) than among those whose mothers were non-carriers (24% to 19%; RR 127, 95% CI 0.81-2.00) (Burman & Christensen, 1992).

Stray-Pedersen, Bergan, Hafstad, Normann, Grogaard, and Vangdal (1998) further reviewed the topic using vaginal douching with chlorhexidine as the intervention. They found that when comparing the two douched groups (chlorhexidine or saline), the infants of the chlorhexidine group had significantly less overall neonatal morbidity of infectious diseases (combined incidences of septicemia, respiratory problems and

superficial infections) than those of the saline group (p = <0.05, 95% confidence interval 0.00-0.06) (Stray-Pedersen, et al., 1998).

Hennquin, Tecco, and Vokaer (1995) wrote a letter to the editor of the *Acta Obstetrica et Gynecologica Scandinavica* that reviewed the results of a randomized controlled study that evaluated the use of gloves lubricated with chlorhexidine, and found no reduction in neonatal colonization with GBS.

Cutland, et al. (2009) studied the effect of intravaginal chlorhexidine washes during labor and full-body neonatal chlorhexidine washes on neonatal sepsis in South Africa. There were 289 cases of early-onset sepsis, and there was no significant difference in rates between the intervention group (34.6 per 1000 births) and control groups (36.5 per 1000 births).

Goldenberg, McClure, Saleem, Rouse, and Vermund (2006) did a literature review for all articles related to use of chlorhexidine in the context of pregnancy, vagina, infant, newborn, and neonatal. They further narrowed the review to include only chlorhexidine use vaginally during pregnancy or as a treatment of the newborn. The results were not significant improvements in overall maternal and neonatal outcomes; however, they identified that there was potential benefit in developing countries due to the low cost and ease of accessibility.

Probiotics. Rönnqvist, Ström, Forsgren-Rusk, and Håkansson (2005) identified two strains of Lactobacillus that held promise as a urogenital probiotic, using panty liners impregnated with freeze-dried Lactobacillus. Rönnqvist, Forsgren-Brusk, and Grahn Håkansson (2006) reported that higher amounts of lactobacilli in the genital tract was associated with a lower vaginal pH.

Açikgöz, Gamberzade, Göçer, and Ceylan (2005) reported inhibitory effects of strains of Lactobacilli on GBS, specifically *Lactobacillus rhamnosus*.

Ortiz, Ruiz, Pascual, and Barberis (2014) found that *Lactobacillus fermentum* and *Lactobacillus rhamnosus* may protect the vaginal epithelium through a series of barriers (self-aggregation, co-aggregation with potential pathogens, and adherence) and interference (receptor binding interference block) mechanisms. Similarly, Pradhan, Mohanty, and Mishra (2011) focused on specific strains of lactobacilli that had inhibitory action against pathogens, including GBS.

Ruiz, et al. (2012) describe a synergistic effect when two Lactobacillus species are used together, reporting the bacteriocin-like inhibitory substances (BLIS) from two Lactobacillus species being better than the BLIS of each one alone ($p = <0.05$) as GBS growth inhibitors. Zárate and Nader-Macias (2006) also evaluated the inhibitory action of lactobacilli on adhesion of GBS to vaginal epithelial cells, and found that *Streptococcus agalactiae* showed a high degree of inhibition by *L. acidophilus* CRL 1259 and *L. paracasei* CRL 1289. Reid, et al. (2003) found that oral use of lactobacillus strains could alter vaginal flora, supporting the idea that oral probiotic regimens could potentially be used to alter vaginal flora.

Ephraim, et al. (2012) evaluated the antagonistic effects of two probiotics on GBS. *Lactobacillus rhamnosus* HN001 is a specific strain of Lactobacillus, and Florajen 3 is a commercially available probiotic capsule containing *lactobacillus acidophilus*, *Bifidobacterium lactis*, and *Bifidobacterium longum*. They specifically evaluated the antagonistic effects of both probiotics against GBS in co-cultures, attachment of Florajen 3 and *L. rhamnosus* HN001 to cell monolayers, and inhibition of GBS adherence to Vero

cells and MDCK cells. This study also evaluated the affinity of probiotics to adhere to host cells which could exclude GBS through competition for attachment sites on host cells. The understanding of different strains having different effects on host cells is compelling and could help with identification of strains with the strongest effects against GBS.

Hanson, VandeVusse, Duster, Warrack, and Safdar (2014) found that 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. However, the two probiotic group participants who were positive for GBS at final culture had lower colony counts (2×10^2 CFU) on the quantitative cultures than the two control group participants (7×10^2 CFU and 2.07×10^7 CFU). In addition, the eight GBS negative averaged 90% adherence compared with two GBS positive women who averaged 68%, as well as a significant inverse relationship ($p = 0.02$) between yogurt ingestion and GBS colonization at 36 weeks gestation (Hanson, et al., 2014). While these findings are interesting, the sample size (10 in the probiotic group, 10 in the control group) was insufficient to demonstrate that probiotics can prevent GBS colonization although it does demonstrate diet as a potential confounding variable, and adherence to a probiotic regimen as a factor in potential efficacy of the regimen. This was also the only study to discuss the timing of initiation of probiotic therapy in pregnancy, as well as the amount of probiotic required to be ingested for efficacy.

The in vitro studies give credence to the idea that probiotics could potentially prevent GBS colonization. Lindsay, Brennan, and McAuliffe (2014) demonstrated that a prenatal probiotic capsule intervention had high acceptability and likelihood of

compliance amongst pregnant women. However, a randomized controlled trial with a larger sample size would be required before any definitive determination can be made regarding the efficacy of prenatal probiotic therapy against GBS. If similar results could be obtained in vivo, the use of these probiotics in preventing and treating GBS infection in pregnant women would be highly promising (Ephraim, et al., 2012).

Benzalkonium Chloride. Mosca, Russo, and Miragliotta (2005) evaluated the antimicrobial activity of benzalkonium chloride, which is used as an antiseptic. A total of 52 strains of GBS isolated from vaginal swabs of pregnant women were used, along with a solution of powdered benzalkonium chloride reconstituted with distilled water. After 24 hours of incubation at 37C, the MIC value was recorded as the lowest concentration of benzalkonium chloride that inhibited visible growth when compared with that in the control growth tube (Mosca, Russo, & Miragliotta, 2005). They investigated antibiotic susceptibility, and found that for all the strains tested benzalkonium chloride susceptibility was not related to antibiotic resistance. This was the only mention of benzalkonium chloride in the literature, but there is potential for further study, preferably a randomized controlled trial evaluating in vivo effects.

Strengths and Weaknesses of the Research Studies

Benzalkonium chloride and garlic may have potential efficacy in their antibacterial mechanisms, but there is currently so little research that their use is limited until larger studies can be done. Probiotics seem to have the most potential of any method in the literature, however they suffer from a lack of in vivo studies and there are many variables (e.g., required amount to survive the GI tract) that have not been addressed.

Chlorhexidine was the only method that had enough data to suggest that it was not a replacement for antibiotics, as literature showed only modest improvement in rates of neonatal EOGBSD.

Matrix of the Literature

<table>
<tr><td colspan="4">Source: Açikgöz, Z., Gamberzade, S., Göçer, S., & Ceylan, P. (2005). Inhibitor effect of vaginal lactobacilli on group B streptococci. Mikrobiyoloji Bulteni, 39(1), 17-23. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15900833
Location: Turkey</td></tr>
<tr><th>Purpose/Level/Quality</th><th>Design</th><th>Measurement</th><th>Findings/Outcomes</th></tr>
<tr><td>Purpose: To evaluate the effect of lactobacilli on GBS.

Level of Evidence: III

Quality of Evidence: A</td><td>Non-experimental study</td><td>51 lactobacilli from vaginal swabs and vaginal tablets used on five GBS (four clinical isolates and one standard strain) by sandwich plate technique and deferred antagonism well technique.</td><td>Ten clinical isolates (20%) and the drug-purified Lactobacilli had an inhibitory effect on GBS growth. Seven of the inhibitory clinical isolates were Lactobacillus rhamnosus. The inhibitory isolates had higher acid production than the non-inhibitory ones ($p = < 0.05$), and pH-adjustment destroyed their inhibitory effects entirely.</td></tr>
<tr><td colspan="4">Strengths: Identification of specific strains of Lactobacilli with inhibitory action, and identification of pH-adjustment as a factor effecting efficacy.</td></tr>
<tr><td colspan="4">Limitations: The study only measures in vitro effects. Further research is needed to determine if the effects have in vivo applicability.</td></tr>
<tr><td colspan="4">Implications: The inhibitory effects of Lactobacilli, specifically Lactobacillus rhamnosus, have potential as a safe and cost-effective method for preventing GBS colonization.</td></tr>
</table>

<table>
<tr><td colspan="4">Source: Adriaanse, A., Kollée, L., Muytjens, H., Nijhuis, J., de Haan, A., & Eskes, T. (1995). Randomized study of vaginal chlorhexidine disinfection during labor to prevent vertical transmission of group B streptococci. European Journal of Obstetrics & Gynecology and Reproductive Biology, 61(2). 135-141. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7556834
Location: The Netherlands</td></tr>
<tr><th>Purpose/Level/Quality</th><th>Design</th><th>Measurement</th><th>Findings/Outcomes</th></tr>
<tr><td>Purpose: Study the effect of chlorhexidine disinfection on the rate of GBS transmission to the neonate.

Level of Evidence: I

Quality of Evidence: B</td><td>Randomized controlled trial.</td><td>A sample of 1020 women were randomly assigned to three groups – the intervention group, the placebo group, and the control group who received no treatment. Chlorhexidine gel (containing chlorhexidine digluconate 0.3g) was applied once active labor was determined, and again 10 hours later if delivery had not occurred.</td><td>The vertical transmission rate of S. agalactiae was lower in the chlorhexidine group, but the difference did not reach significance (two-tailed Fisher’s exact text, p = 0.069).</td></tr>
<tr><td colspan="4">Strengths: Three groups – intervention, placebo, and control.</td></tr>
<tr><td colspan="4">Limitations: The intervention was not initiated until active labor was determined.</td></tr>
<tr><td colspan="4">Implications: It has a modest effect, but the results are not compelling enough to suggest this become standard practice.</td></tr>
</table>

<table>
<tr><td colspan="4">Source: Ankri, S., & Mirelman, D. (1999). Antimicrobial properties of allicin from garlic. Microbes and Infection / Institut Pasteur, 1(2), 125-129. doi:S1286-4579(99)80003-3
Location: Israel</td></tr>
<tr><th>Purpose/Level/Quality</th><th>Design</th><th>Measurement</th><th>Findings/Outcomes</th></tr>
<tr><td>Purpose: Review the literature to evaluate the antimicrobial properties of allicin (garlic) compounds.

Level of Evidence: V

Quality of Evidence: B</td><td>Literature review</td><td>NA</td><td>Allicin in its purest form has antibacterial, antifungal, and antiviral activity.</td></tr>
<tr><td colspan="4">Strengths: The article specifically referred to the action of allicin on GBS. There was detailed explanation of how allicin compounds antibacterial functions.</td></tr>
<tr><td colspan="4">Limitations: The article did not have any information as to how the review was conducted, and there were statements made with citing a source.</td></tr>
<tr><td colspan="4">Implications: GBS is a Gram-negative bacterium, and allicin has antibacterial activity against several strains of Gram-negative bacteria. While this article did state that GBS is resistant to allicin, it bears further sources and research before being considered definitive.</td></tr>
</table>

<table>
<tr><td colspan="4">Source: Burman, L., Christensen, P., Christensen, K., Fryklund, B., Helgesson, A., Svenningsen, N., & Tulles, K. (1992). Prevention of excess neonatal morbidity associated with group B streptococci by vaginal chlorhexidine disinfection during labour. The Swedish Chlorhexidine Study Group. Lancet, 340(8811), 65-69. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1352011
Location: Sweden</td></tr>
<tr><th>Purpose/Level/Quality</th><th>Design</th><th>Measurement</th><th>Findings/Outcomes</th></tr>
<tr><td>Purpose: To evaluate the effect of vaginal chlorhexidine flushes on rates of admission to special-care neonatal units.

Level of Evidence: I

Quality of Evidence: A</td><td>Randomized controlled trial.</td><td>The analysis included 4483 mothers and their full-term infants. Vaginal cultures were obtained from 4384 women on arrival in the delivery ward. 2238 women had vaginal flushings with chlorhexidine, and 2245 with placebo, at least once before delivery.</td><td>The reduction in admissions after chlorhexidine prophylaxis vs. placebo was greater among infants born of GBS carrier mothers (5.4% to 2.8%; RR 1.95, 95% CI 0.94-4.03) than among those whose mothers were non-carriers (24% to 19%; RR 127, 95% CI 0.81-2.00) (Burman & Christensen, 1992).</td></tr>
<tr><td colspan="4">Strengths: There was a pre-culture to determine GBS status, and evaluation of differences in admission rates between the GBS carriers, regardless of whether they were in the intervention or control groups.</td></tr>
<tr><td colspan="4">Limitations: Admission to the special-care nursery may or may not be for issues related to GBS colonization, and is subject to provider comfort level. No mention of whether or not mothers were previously cultured for GBS, and if any of the mothers were treated with antepartum antibiotics.</td></tr>
<tr><td colspan="4">Implications: Vaginal chlorhexidine offers a modest amount of benefit in preventing admissions to special-care neonatal units.</td></tr>
</table>

<table>
<tr><td colspan="4">Source: Cohain, J. (2009). Long-term symptomatic group B streptococcal vulvovaginitis: Eight cases resolved with freshly cut garlic. European Journal of Obstetrics, Gynecology, and Reproductive Biology, 146(1), 110-111. doi:10.1016/j.ejogrb.2009.05.028
Location: Israel</td></tr>
<tr><th>Purpose/Level/Quality</th><th>Design</th><th>Measurement</th><th>Findings/Outcomes</th></tr>
<tr><td>Purpose: To report the results of the use of intravaginal garlic in the setting of chronic GBS vulvovaginitis.

Level of Evidence: V

Quality of Evidence: B</td><td>Case reports</td><td>Eight patients with confirmed symptomatic vaginal GBS of 6 months to 4 years duration, not resolved by courses of oral antibiotics. The women used half a freshly cut clove of garlic inserted vaginally at night and removed in the morning for 3-6 weeks followed by maintenance doses of once every 2-4 days.</td><td>All eight women had resolution of their symptoms.</td></tr>
<tr><td colspan="4">Strengths: The only article that had in vivo use of garlic on vaginal GBS colonization.</td></tr>
<tr><td colspan="4">Limitations: The regimen was not detailed regarding the specifics of the regimen for treatment or maintenance. It was unclear if the patients were pre-cultured to establish that it was GBS.</td></tr>
<tr><td colspan="4">Implications: Sustained use of intravaginal garlic could be explored as a potential method of reducing GBS colonization.</td></tr>
</table>

<table>
<tr><td colspan="4">Source: Cutland, C. L., Madhi, S. A., Zell, E. R., Kuwanda, L., Laque, M., Groome, M., . . . PoPS Trial Team. (2009). Chlorhexidine maternal-vaginal and neonate body wipes in sepsis and vertical transmission of pathogenic bacteria in South Africa: A randomised, controlled trial. Lancet, 374(9705), 1909-1916. doi:10.1016/S0140-6736(09)61339-8
Location: South Africa</td></tr>
<tr><th>Purpose/Level/Quality</th><th>Design</th><th>Measurement</th><th>Findings/Outcomes</th></tr>
<tr><td>Purpose: To determine if chlorhexidine applied intravaginally in labor, or neonatal washes at birth reduce neonatal sepsis.

Level of Evidence: I

Quality of Evidence: A</td><td>Randomized controlled trial</td><td>8011 women (aged 12-51 years) were randomly assigned in a 1:1 ratio to chlorhexidine vaginal wipes or external genitalia water wipes during active labor, and their 8129 newborn babies were assigned to full body (intervention group) or foot (control group) washes with chlorhexidine at birth. In a subset of mothers (n=5144) maternal lower vaginal swabs and neonatal skin swabs were obtained after delivery to determine colonization with GBS.</td><td>Rates of neonatal sepsis did not differ between the groups (chlorhexidine 141 [3%] of 4072 vs control 148 [4%] of 4057; p = 0.6518).

289 cases of early-onset sepsis occurred, with no difference in rates in the chlorhexidine (34.6 per 1000 births) and control groups (36.5 per 1000 births).</td></tr>
<tr><td colspan="4">Strengths: Large, randomized sample. Done in developing nation.</td></tr>
<tr><td colspan="4">Limitations: Lack of screening for GBS colonization prior to labor, and the midwives knew who received the intervention and who did not. Some mothers received antibiotics per protocol, usually due to meconium-stained amniotic fluid.</td></tr>
<tr><td colspan="4">Implications: Because screening mothers for GBS colonization is not routine, its unclear whether or not chlorhexidine had an affect on existing GBS colonization. However the study does demonstrate that intravaginal and neonatal application of chlorhexidine does not affect neonatal sepsis rates.</td></tr>
</table>

<table>
<tr><td colspan="4">Source: Cutler, R. R., Odent, M., Hajj-Ahmad, H., Maharjan, S., Bennett, N. J., Josling, P. D., . . . Dall'Antonia, M. (2009). In vitro activity of an aqueous allicin extract and a novel allicin topical gel formulation against Lancefield group B streptococci. Journal of Antimicrobial Chemotherapy, 63(1), 151-154. doi:10.1093/jac/dkn457
Location: United Kingdom</td></tr>
<tr><th>Purpose/Level/Quality</th><th>Design</th><th>Measurement</th><th>Findings/Outcomes</th></tr>
<tr><td>Purpose: To investigate the in vitro activity of a novel allicin extract in aqueous and gel formulation against 76 clinical isolates of Lancefield GBS.

Level of Evidence: III

Quality of Evidence: A</td><td>Non-experimental study</td><td>Seventy-six non-duplicate clinical isolates of GBS, from vaginal swabs were streaked on blood agar plates and incubated overnight. A 5000mg/L solution of novel stabilized AEAllicin, and an allicin gel mixture was created by mixing the solution with a commercially available gel.
Growth in the presence of a range of concentrations of AEAllicin between 2500 and 2.5 mg/L and growth in Iso-Sensitest broth containing no antimicrobial agent (negative control) were assessed by spectroscopy.</td><td>By 8 hours there was a >6 log reduction in bacterial load in all cases compared with the growth control and there was no detectable growth (detection limit 100 cfu/mL) after 8 or 24 hours of treatment.

A purified allicin extract was active against all GBS strains tested, with MICs ranging between 35 and 95 mg/L.</td></tr>
<tr><td colspan="4">Strengths: This is the first study to demonstrate differences in types of preparation, and supports gel formulations as a viable method for using allicin extract.</td></tr>
<tr><td colspan="4">Limitations: The study was done in vitro and may not have the same applicability in vivo without further research being performed.</td></tr>
<tr><td colspan="4">Implications: Allicin extract is active against GBS, and different types of preparations (cream, gel) can impact its efficacy.</td></tr>
</table>

<table>
<tr><td colspan="4">Source: Ephraim E., Schultz R. D., Duster M., Warrack S., Spiegel C.A., & Safdar N. (2012). 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), 113-120. Retrieved from http://connection.ebscohost.com/c/articles/85948295/in-vitro-evaluation-antagonistic-effects-probiotics-lactobacillus-rhamnosus-hn001-florajen-3-against-group-b-streptococci
Location: United States</td></tr>
<tr><th>Purpose/Level/Quality</th><th>Design</th><th>Measurement</th><th>Findings/Outcomes</th></tr>
<tr><td>Purpose: To evaluate the effects of two probiotics (Lactobacillus rhamnosus HN001 and Florajen 3) against GBS.

Level of Evidence: III

Quality of Evidence: A</td><td>Non-experimental.</td><td>The probiotics (L. rhamnosus HN001 and Florajen 3) and all the GBS isolates were grown in MRS and Columbia broth, respectively, overnight and serially diluted in Columbia broth to achieve the required colony forming units per ml (CFU/ml). Three ml of each GBS isolate was co-cultured with 3ml of each probiotic, and co-cultures were grown in Columbia broth which should not favor the growth of one organism or another. The number of GBS at 0 hours, 24 hours and 48 hours was determined by plate dilution method on SBA plates.</td><td>When co-cultured, L. rhamnosus HN001 inhibited the growth of GBS 0191 and 0192 and decreased that of GBS 0193 significantly ($p = <0.0001$). Florajen 3 inhibited the growth of GBS 0191 and ATCC 12386 and decreased that of GBS 0192 by 5 logs. Both probiotics could attach well to MDCK and Vero cells and both protected Vero cells from GBS 0193 adhesion. Both probiotics decreased the number of adherent GBS 0192 significantly ($p = <0.05$).</td></tr>
<tr><td colspan="4">Strengths: Multiple features that make probiotic strains more effective were identified.</td></tr>
<tr><td colspan="4">Limitations: In vitro study may not be applicable to in vivo use.</td></tr>
<tr><td colspan="4">Implications: This study demonstrated that both L. rhamnosus HN001 and Florajen 3 have potential efficacy in reducing GBS colonization.</td></tr>
</table>

<table>
<tr><td colspan="4">Source: Goldenberg, R. L., McClure, E. M., Saleem, S., Rouse, D., & Vermund, S. (2006). Use of vaginally administered chlorhexidine during labor to improve pregnancy outcomes. Obstetrics and Gynecology, 107(5), 1139-1146. doi:107/5/1139
Location: United States</td></tr>
<tr><td>Purpose/Level/Quality</td><td>Design</td><td>Measurement</td><td>Findings/Outcomes</td></tr>
<tr><td>Purpose: To analyze the literature to determine the efficacy of vaginal and neonatal washes in reducing GBS infections.

Level of Evidence: III

Quality of Evidence: B</td><td>Systematic review</td><td>NA</td><td>While the studies from developed countries did not show a significant improvement in maternal and neonatal outcomes, there were improvements of those outcomes in developing countries.</td></tr>
<tr><td colspan="4">Strengths: The studies were split between developed and developing countries.</td></tr>
<tr><td colspan="4">Limitations: The two largest studies in developing countries were not randomized or blinded.</td></tr>
<tr><td colspan="4">Implications: Chlorhexidine may offer a low cost, low risk method of improving maternal and neonatal outcomes.</td></tr>
</table>

Source: Hanson, L., VandeVusse, L., Duster, M., Warrack, S., & Safdar, N. (2014). Feasibility of oral prenatal probiotics against maternal group B streptococcus vaginal and rectal colonization. *Journal of Obstetric, Gynecologic, & Neonatal Nursing, 43*(3), 294-304. doi:10.1111/1552-6909.12308 **Location:** United States			
Purpose/Level/Quality	**Design**	**Measurement**	**Findings/Outcomes**
Purpose: To evaluate the effects of probiotic use on GBS colonization in pregnant women. **Level of Evidence:** II **Quality of Evidence:** C	Quasi-experimental study.	A convenience sample of 20 healthy women at 28 weeks (+/- 2 weeks) gestation was recruited from a midwifery clinic in a large Midwestern city. Ten women received an oral probiotic to be taken once daily, and ten women served as the control group. All women completed a questionnaire about dietary intake, vaginal cleansing practices, and sexual activity. All participants had wet mount and vaginal and rectal cultures at 28, 32, and 36 weeks gestation (+/- 2 weeks).	No adverse effects were noted in the probiotic group. Two women in each group had positive GBS colonization at 36 weeks, however those women averaged 68% adherence to the probiotic regimen compared to the 8 who were negative (90%) based on returned pill counts. Of note, women who consumed yogurt were significantly more likely to be GBS negative ($p = 0.02$).
Strengths: Compliance with the regimen was evaluated and addressed.			
Limitations: Very small sample size. Researchers, midwives, and participants were aware of group assignment. The control group participants did not receive a placebo.			
Implications: This could potentially have clinical applicability but needs to be studied further, preferably with a randomized controlled trial.			

Source: Hennquin, Y., Tecco, L., & Vokaer, A. (1995). Use of chlorhexidine during labor: How effective against neonatal group B streptococci colonization? *Acta Obstetricia et Gynecologica Scandinavica, 74*(2), 168. doi:10.3109/00016349509008931 **Location:** Belgium			
Purpose/Level/Quality	**Design**	**Measurement**	**Findings/Outcomes**
Purpose: To evaluate the use of chlorhexidine lubricated gloves on the rates of colonization on the rates of neonatal GBS colonization. **Level of Evidence:** I **Quality of Evidence:** B	Randomized controlled trial.	59 pregnant women antenatally screened as GBS carriers were prospectively and randomly allocated in two groups at the onset of labor: vaginal examinations of the treated group with systematically performed with gloves lubricated with 5 ml chlorhexidine digluconate 1% cream; the control group was examined with uncoated gloves. Swabs for microbiological examination were sampled on different cutaneous areas of the newborn at delivery.	11 newborns out of 28 (39%) were colonized in the treated groups versus 13 out of 31 (42%) in the control group ($x^2 = 0.003$).
Strengths: The premise of lubrication rather than vaginal washings has increased comfort level for mother.			
Limitations: Small study group. Letter to the editor, this was not publication of the study.			
Implications: Chlorhexidine lubricant is not effective in reducing neonatal GBS colonization.			

<table>
<tr><td colspan="4">Source: Lindsay, K. L., Brennan, L., & McAuliffe, F. M. (2014). Acceptability of and compliance with a probiotic capsule intervention in pregnancy. International Journal of Gynaecology and Obstetrics: The Official Organ of the International Federation of Gynaecology and Obstetrics, 125(3), 279-280. doi:10.1016/j.ijgo.2014.01.004
Location: Ireland</td></tr>
<tr><th>Purpose/Level/Quality</th><th>Design</th><th>Measurement</th><th>Findings/Outcomes</th></tr>
<tr><td>Purpose: To evaluate the acceptability of and compliance with a probiotic regimen during pregnancy.

Level of Evidence: I

Quality of Evidence: B</td><td>Randomized controlled trial.</td><td>A total of 140 obese pregnant women completed a randomized controlled trial of a daily probiotic or placebo capsule for 4 weeks between 24 and 28 weeks of pregnancy. A questionnaire evaluated the willingness to consider taking a probiotic in a future pregnancy, ease of use, and level of convenience.</td><td>Completed questionnaires were returned by 121 women (57 probiotic, 64 placebo). Acceptability of and compliance with the intervention was high: 97% of women reported a willingness to take a probiotic in a future pregnancy, over 80% reported missing no more than 2 capsules. Only 2 participants did not complete the study. Non-responders to the questionnaire did not differ from responders in terms of capsule compliance (58% responders vs 63% non-responders missed 1 or more capsules; $p = 0.317$) or baseline characteristics (age, ethnicity, parity, education level).</td></tr>
<tr><td colspan="4">Strengths: The study being randomized and controlled is a strength, as well as the sample size.</td></tr>
<tr><td colspan="4">Limitations: The sample size may not be sufficient to make the results generalizable.</td></tr>
<tr><td colspan="4">Implications: If probiotic regimens are supported in the literature as beneficial, this study supports women being receptive to and compliant with a probiotic regimen.</td></tr>
</table>

Source: Mosca, A., Russo, F., & Miragliotta, G. (2006). In vitro antimicrobial activity of benzalkonium chloride against clinical isolates of streptococcus agalactiae. *Journal of Antimicrobial Chemotherapy, 57*(3), 566-568. doi:10.1093/jac/dki474 **Location:** Italy			
Purpose/Level/Quality	**Design**	**Measurement**	**Findings/Outcomes**
Purpose: To evaluate the inhibitory effect of benzalkonium chloride against GBS. **Level of Evidence:** III **Quality of Evidence:** B	Non-experimental study	The MIC or the MBC was determined by broth macrodilution. After 24 hours of incubation at 37°C, the MIC value was recorded as the lowest concentration of benzalkonium chloride that inhibited visible growth when compared with that in the control growth tube. After 24 hours of incubation at 37°C in a candle jar, the CFU were counted and MBC was defined as the lowest concentration of benzalkonium chloride resulting in the death of 99.9% or more of the initial inoculum.	When the capacity of benzalkonium chloride to interfere with GBS growth was evaluated, all the isolates tested were inhibited at MIC values ranging from 0.39 and 6.25 ml/L. The MIC_{90} (that inhibited 90% of the strains) was 3.12 mg/L. The MBC values ranged between 0.78 and 12.50 mg/L and were similar or slightly higher than the MIC values. Neither prolonged incubation (up to 48 hours) nor the use of different culture media interfered with the benzalkonium chloride antibacterial activity.
Strengths: Study was done on benzalkonium chloride and several antibiotics.			
Limitations: In vitro			
Implications: Benzalkonium chloride could be considered as a potential method for reducing GBS colonization			

Source: Ohlsson, A., Shah, V., & Stade, B. (2014). Vaginal chlorhexidine during labour to prevent early-onset neonatal group B streptococcal infection. Cochrane Database of Systematic Reviews, (12). Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/25504106 **Location:** Canada			
Purpose/Level/Quality	**Design**	**Measurement**	**Findings/Outcomes**
Purpose: Determine the effectiveness of chlorhexidine during labor in women who are colonized with GBS for preventing neonatal GBS infection. **Level of Evidence:** II **Quality of Evidence:** B	Systematic review with meta-analysis	Randomized and quasi-randomized trials comparing vaginal disinfection with chlorhexidine (vaginal wash or gel/cream) versus placebo, or no treatment were retrieved for review The authors evaluated for risk of bias, measurement of treatment effect, unit of analysis, missing data, and reporting biases.	There was no statistically significant difference in early-onset GBS disease
Strengths: Reviewed only randomized controlled trials.			
Limitations: Quality of the trials varied as did the risk of bias and quality of evidence.			
Implications: Does not support the wide use of chlorhexidine to prevent GBS transmission.			

<table>
<tr><td colspan="4">Source: Ortiz, L., Ruiz, F., Pascual, L., & Barberis, L. (2014). Effect of two probiotic strains of Lactobacillus on in vitro adherence of Listeria monocytogenes, Streptococcus agalactiae, and Staphylococcus aureus to vaginal epithelial cells. Current Microbiology, 68(6), 679-684. Retrieved from http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=emed12&AN=2014259859
Location: Argentina</td></tr>
<tr><th>Purpose/Level/Quality</th><th>Design</th><th>Measurement</th><th>Findings/Outcomes</th></tr>
<tr><td>Purpose: To evaluate the effect of two Lactobacilli strains and adherence of GBS on vaginal epithelial cells (VECs) by exclusion, competition, and displacement.

Level of Evidence: III

Quality of Evidence: A</td><td>Non-experimental study</td><td>Exclusion assay: VEC and lactobacilli were mixed in a 1:1 ratio and incubated with agitation at 37°C for 1 hour. Pathogens were added later, the resulting suspension was incubated.
Competition assay: VEC, lactobacilli, and pathogenic bacteria were incubated together with orbital shaking for 60 min.
Displacement assay: equal volumes of pathogenic bacterial suspensions and vaginal cells were mixed and incubated at 37°C with orbital shaking for 60 min. Then a suspension of lactobacilli was added to determine if lactobacilli displace adhered pathogens.</td><td>The lactobacilli showed a great capacity of adherence with a mean of 83.5 +/- 26.67 L. fermentum cells and 56.2 +/- 20.87 L. rhamnosus cells per VEC. In competition assays, the reduction of S. aureus and GBS adherence observed ranged between 91.2 and 94.3 (p = <0.05). L. fermentum showed the highest capacity of adherence (p = <0.05). In displacement assays, L. fermentum and L. rhamnosus were able to reduce the adherence of S. aureus, GBS, and L. monocytogenes in a significant level in this assay (p = <0.01).</td></tr>
<tr><td colspan="4">Strengths: The GBS strains were obtained from pregnant patients.</td></tr>
<tr><td colspan="4">Limitations: In vitro study that may not have the same applicability in vivo.</td></tr>
<tr><td colspan="4">Implications: These strains of lactobacillus have potential as a method to reduce colonization of GBS.</td></tr>
</table>

<table>
<tr><td colspan="4">Source: Pradhan, P., Mohanty, R., & Mishra, A. (2011). Selection of probiotic lactobacillus species to eradicate resistant urogenital pathogens in pregnant women. International Journal of Probiotics and Prebiotics, 6(1), 13-20. Retrieved from http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=emed11&AN=2013649332
Location: India</td></tr>
<tr><th>Purpose/Level/Quality</th><th>Design</th><th>Measurement</th><th>Findings/Outcomes</th></tr>
<tr><td>Purpose: To evaluate the antibacterial properties of Lactobacillus strains on common urogenital bacteria.

Level of Evidence: III

Quality of Evidence: B</td><td>Non-experimental study</td><td>Bacterial strains were cultivated in MRS broth using well diffusion assay, and 100 ul of Lactobacillus culture fluid were added to each well. The inoculated plates were incubated for 24 hours at 37°C and the diameter of the inhibition zone was measured.</td><td>The antibacterial properties of the Lactobacillus strains tested were very variable. Only L. plantarum showed inhibitory activity against GBS.</td></tr>
<tr><td colspan="4">Strengths: Several actions evaluated, including hydrogen peroxide activity and tolerability of acidic environments (i.e., the GI tract).</td></tr>
<tr><td colspan="4">Limitations: Focused on urogenital pathogens, specifically patients who had clinical symptoms of UTI and positive urine cultures.</td></tr>
<tr><td colspan="4">Implications: Lactobacilli are commonly found in the human vagina and stool, and may have efficacy against urogenital pathogens.</td></tr>
</table>

<table>
<tr><td colspan="4">Source: Reid, G., Charbonneau, D., Erb, J., Kochanowski, B., Beuerman, D., Poehner, R., & Bruce, A. W. (2003). Oral use of lactobacillus rhamnosus GR-1 and L. fermentum RC-14 significantly alters vaginal flora: Randomized, placebo-controlled trial in 64 healthy women. FEMS Immunology and Medical Microbiology, 35(2), 131-134. doi:S0928824402004650
Location: Canada</td></tr>
<tr><th>Purpose/Level/Quality</th><th>Design</th><th>Measurement</th><th>Findings/Outcomes</th></tr>
<tr><td>Purpose: To evaluate the effect or an oral regimen of two lactobacillus strains on vaginal flora.

Level of Evidence: I

Quality of Evidence: B</td><td>Randomized controlled trial.</td><td>64 women, ages 19-46, were randomly allotted to receive either a capsule contained two lactobacillus strains or a placebo. Two vaginal swabs were collected at days 0, 7, 28, 60, and 90, and cultured for total lactobacilli, yeast, and coliforms using standard diagnostic media and biochemical tests.</td><td>The culture findings showed that lactobacilli oral therapy led to a significant (log 10) increase in vaginal lactobacilli within 4 weeks (p = 0.01), plus a 0.8 log 10 decrease in yeasts (p = 0.01) and coliforms (p = 0.001) compared to the placebo.</td></tr>
<tr><td colspan="4">Strengths: Demonstration that lactobacillus have effect with taken orally.</td></tr>
<tr><td colspan="4">Limitations: Not specific to GBS.</td></tr>
<tr><td colspan="4">Implications: Oral regimens of lactobacillus strains can impact vaginal flora.</td></tr>
</table>

<table>
<tr><td colspan="4">Source: Rönnqvist, P., Ström, H., Forsgren-Brusk, U., & Håkansson, E. G. (2005). Selection and characterization of a lactobacillus plantarum strain promising as a urogenital probiotic. Microbial Ecology in Health & Disease, 17(2), 75-82. doi:10.1080/08910600510037992
Location: Sweden</td></tr>
<tr><th>Purpose/Level/Quality</th><th>Design</th><th>Measurement</th><th>Findings/Outcomes</th></tr>
<tr><td>Purpose: To identify strains of Lactobacillus that alter vaginal flora.

Level of Evidence: III

Quality of Evidence: B</td><td>Non-experimental study.</td><td>Lactobacillus strains (n = 511) were isolated from the vaginas of healthy fertile women from the northern part of Sweden.

Daily use of panty liners impregnated with freeze-dried lactobacilli, and adherence was measured as the number of lactobacilli transferred from the panty liners to the urogenital area.</td><td>Lactobacillus plantarum LB931 exerted a bactericidal effect on 93% of all ITS (n = 311), furthermore the growth of GBS was totally inhibited.

Lactobacillus plantarum LB931 could be isolated in the perineum in all girls as long as the panty liner was used.</td></tr>
<tr><td colspan="4">Strengths: Testing in age ranges that are not typically colonized with their own lactobacilli.</td></tr>
<tr><td colspan="4">Limitations: Not all strains may survive freeze-drying process.</td></tr>
<tr><td colspan="4">Implications: Lactobacilli has inhibitory action against urogenital bacteria.</td></tr>
</table>

<table>
<tr><td colspan="4">Source: Ronnqvist, P. D., Forsgren-Brusk, U. B., & Grahn-Hakansson, E. E. (2006). Lactobacilli in the female genital tract in relation to other genital microbes and vaginal pH. Acta Obstetricia Et Gynecologica Scandinavica, 85(6), 726-735. doi:743725790
Location: Sweden</td></tr>
<tr><th>Purpose/Level/Quality</th><th>Design</th><th>Measurement</th><th>Findings/Outcomes</th></tr>
<tr><td>Purpose: To evaluate the effect of lactobacilli on vaginal pH.

Level of Evidence: I

Quality of Evidence: A</td><td>Randomized controlled trial.</td><td>One hundred and ninety-one (191) subjects were divided into intervention and control groups. The LB931 group wore vapor-permeable panty liners impregnated with Lactobacillus plantarum LB931 24 hours a day for four consecutive menstrual cycles. Microbiological samples were taken and vaginal pH was registered the week preceding each menstruation during the study period.</td><td>Women with high numbers of lactobacilli were less prevalent with GBS than women with low numbers (p = 0.036). High numbers of lactobacilli may contribute to a low vaginal pH and seem to have a negative influence on GBS.</td></tr>
<tr><td colspan="4">Strengths: Alternative method of applying the Lactobacillus to the vagina.</td></tr>
<tr><td colspan="4">Limitations: Other factors, such as sexual activity, that can be associated with GBS was not considered in the analysis.</td></tr>
<tr><td colspan="4">Implications: Panty liners may be a viable option for application of Lactobacillus.</td></tr>
</table>

<table>
<tr><td colspan="4">Source: Ruiz, F. O., Gerbaldo, G., Garcia, M. J., Giordano, W., Pascual, L., & Barberis, I. L. (2012). Synergistic effect between two bacteriocin-like inhibitory substances produced by lactobacilli strains with inhibitory activity for streptococcus agalactiae. Current Microbiology, 64(4), 349-356. doi:10.1007/s00284-011-0077-0
Location: Argentina</td></tr>
<tr><th>Purpose/Level/Quality</th><th>Design</th><th>Measurement</th><th>Findings/Outcomes</th></tr>
<tr><td>Purpose: To evaluate the effect of two strains of lactobacillus on GBS.

Level of Evidence: III

Quality of Evidence: A</td><td>Non-experimental study.</td><td>A total of 57 S. agalactiae strains were isolated from 760 pregnant women at 35-37 weeks of gestation during 36 months. Inhibitory effects of the bacteriocin-like inhibitory substance (BLIS) was evaluated by well diffusion test on agar plates, on S. agalactiae as well as other pathogens.</td><td>The 52 strains showed different degree of susceptibility, but all of them were sensitive to L. fermentum L23 and L. rhamnosus L60. It was found that there is a synergistic effect between the strains of lactobacillus when used against a pathogen.</td></tr>
<tr><td colspan="4">Strengths: Sensitivity of pathogens to specific strain.</td></tr>
<tr><td colspan="4">Limitations: In vitro work has limited applicability to in vivo use at this time.</td></tr>
<tr><td colspan="4">Implications: The synergism aspect could support the use of multi-strain regimens, rather than just one strain.</td></tr>
</table>

Source: Stray-Pedersen, B., Bergan, T., Hafstad, A., Normann, E., Grøgaard, J., & Vangdal, M. (1999). Vaginal disinfection with chlorhexidine during childbirth. *International Journal of Antimicrobial Agents, 12*(3), 245-251. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10461843 **Location:** Norway			
Purpose/Level/Quality	**Design**	**Measurement**	**Findings/Outcomes**
Purpose: Determine whether chlorhexidine vaginal douching reduced mother-to-child transmission of vaginal microorganisms including GBS. **Level of Evidence:** I **Quality of Evidence:** B	Randomized controlled trial.	Vaginal cultures were obtained, and then vaginal douching with aqueous 0.2% chlorhexidine solution or sterile isotonic saline was used every six hours until delivery. Cultures were routinely obtained from the throat, nose, and ear immediately after birth. Infants born to mothers with vaginal GBS had additional eye and throat cultures obtained.	When comparing the two douched groups, the infants of the chlorhexidine group had significantly less overall neonatal morbidity of infectious diseases than those of the saline group (p = <0.05, 95% CI 0.00-0.06).
Strengths: Direct application of chlorhexidine.			
Limitations: Addressing GBS transmission retrospectively.			
Implications: Use of chlorhexidine could provide a reduction in neonatal morbidity.			

<table>
<tr><td colspan="4">Source: Zarate, G., & Nader-Macias, M. (2006). Influence of probiotic vaginal lactobacilli on in vitro adhesion of urogenital pathogens to vaginal epithelial cells. Letters in Applied Microbiology, 43(2), 174-180. doi:LAM1934
Location: Argentina</td></tr>
<tr><th>Purpose/Level/Quality</th><th>Design</th><th>Measurement</th><th>Findings/Outcomes</th></tr>
<tr><td>Purpose: To evaluate the ability of four vaginal lactobacillus strains to block the adherence of urogenital pathogens to vaginal epithelial cells.

Level of Evidence: III

Quality of Evidence: B</td><td>Non-experimental study.</td><td>The inhibitory effect of lactobacilli was measured by blockage by exclusion, competition, and displacement. Bacterial adhesion to VEC was quantified by microscopy (x1000) after Gram’s stain.</td><td>Only L. acidophilus CRL 1259 and L. paracasei CRL 1289 inhibited the attachment of GBS to VEC by exclusion and competition, respectively.</td></tr>
<tr><td colspan="4">Strengths: Identification of type of inhibition.</td></tr>
<tr><td colspan="4">Limitations: In vitro results may not have applicability for in vivo use.</td></tr>
<tr><td colspan="4">Implications: Lactobacillus strains have efficacy against urogenital pathogens.</td></tr>
</table>

Chapter IV: Discussion, Implications, and Conclusion

The research question posed was: *What are effective interventions of reducing GBS colonization and related intrapartum antibiotic administration?* Articles (n=22) on non-antibiotic methods that could potentially reduce GBS colonization were appraised using the Johns Hopkins Research Evidence Appraisal Tool (Dearholt & Dang, 2012). The findings were then synthesized to evaluate the strength of research about the alternative methods, evaluate trends and gaps in the literature, and identify further research opportunities. Germ theory was applied as the theoretical framework.

Trends

The idea that there should be non-antibiotic methods to prevent EOGBSD in the neonate has always been a matter of discussion. However, the methods being studied have shifted according to the research interests of the time. Chlorhexidine was studied primarily in the 1990's, but the Cochrane Review published in 2002 (updated in 2004 and 2014) discussed the lack of compelling results, seeming to signal the end of the focus on chlorhexidine as a method of interest. In the 2000's the increasing popularity and interest in probiotics brought forth a considerable amount of research, evaluating the potential of probiotics effect on pathogens with particular interest in vaginal flora, which could then potentially affect GBS. The articles in this review are dated as recently as 2014, so it remains to be seen if probiotics will evolve from hypothesis into effective intervention, or if they will simply fade away from lack of compelling results.

From a consumer perspective the trends are being driven by demands for alternatives to antibiotics, in light of the concern about antibiotic resistance and "superbugs", as well as

the idea that antibiotics may not truly be an effective method of prevention of neonatal EOGBSD. Anecdotal evidence suggests that the use of alternative methods, particularly preventative probiotics, or regimens using garlic or *Melaleuca alternifolia* (tea tree oil) to treat known GBS colonization, are increasing. The rates of women who choose not to be tested at all are increasing as well, as they cite lack of treatment availability (a birth provider not authorized to administer antibiotics), concern about long-term effects of antibiotic use, or concern that antibiotics are not effective. Further research on the human microbiome gives further credence to the idea that altering the flora of a human can have long-term effects on their health.

Gaps

Current studies of probiotics effect may lack generalizability to in vivo use, as there are many potential variables that its impossible to draw conclusions from research that is conducted entirely in vitro. The specifics that need to be addressed include which strains are the most beneficial, determination of the mount of probiotic intake required to survive GI tract and be found in vagina (Hanson, VandeVusse, Duster, Warrack, & Safdar, 2012) as well as whether or not probiotics need to be taken continually for best effect or if 'one-time dose' methods are effective.

Benzalkonium chloride and garlic are currently not supported in the research, each with so few studies that make it impossible to draw any reasonable conclusions. Considerable research about preparations, route of administration, and in vivo effect would be required before benzalkonium chloride or garlic could be considered reasonable alternatives.

The standard method of identifying GBS colonization is a culture carried out between 35 and 36 weeks, and the results are usually available within one week. However, Young, et al. (2011) found that the 35-36 week culture test only identified 69% of the women who actually had GBS during labor (as cited in Dekker, 2013).

Young, et al. (2011) found that 84% of those who tested positive at 35-36 weeks were still positive in labor, which suggests that 16% of that group were receiving unnecessary antibiotics. If this is broadened to the general population it would suggest that there is a percentage of women who are being treated with antibiotics but are GBS negative, but even more concerning are the women who were GBS negative at testing but are colonized with GBS at delivery and do not receive antibiotic prophylaxis. In women screened at 35-37 weeks, 91% of those who tested negative were still negative at labor (Young, et al., 2011) which suggests that 9% of women who became positive in that time were not receiving antibiotics.

Rapid tests that can provide results in labor are being developed and introduced to the market, however despite their use in Europe they have not become widely used in the United States. El Helali et al. (2012) followed a French hospital as it switched from prenatal testing to in-labor testing, and increased its rate of GBS identification, had fewer cases of early GBS infections in newborns, with the same financial cost. Its unclear why rapid tests are not being utilized in the United States, but they appear to have a potential to increase identification and lower the rates of GBS infections in infants. There is speculation that the time it takes to test and cost-effectiveness of this testing are contributing factors to its low utilization (Dekker, 2013).

Future Research

Use of probiotics in pregnant women has generally been regarded as safe, but there have been no studies published that specifically look at the effect of probiotic use on GBS colonization in pregnancy. Stanford University has a study currently ongoing, but the results of that study are not due to be published until November 2018 (ClinicalTrials.gov, 2016). Further research is needed on in vivo use of probiotics to reduce GBS colonization in pregnant women, as well as more strain-specific studies to determine what combination of strains has the most effect. Determining whether oral or vaginal probiotics are the most effective, and whether or not the regimen would be well-tolerated are important research considerations, as is the amount of probiotics that are required in order to survive the GI tract and alter vaginal flora.

Water birth had initially brought forth concerns about increased rates of GBS colonization. However, Jah (2014) specifically mentioned water birth as a method of reducing GBS colonization, citing the findings of Zanetti-Daellenbach, et al. (2006) that although the water had higher levels of GBS colonization, infants born in the water had decreased rates of GBS colonization, theorized as a "wash out effect". A later study by Zanetti-Daellenbach, et al. (2007) had a larger sample size and found that the outcomes of GBS colonization and EOGBSD of the neonate were comparable between the neonates born in the water versus those born out of the water. Further research would be warranted to determine if neonates born in the water to GBS positive mothers have lower rates of GBS colonization than infants born out of the water to GBS positive mothers.

Implications

While it would appear that antibiotics are the best solution at the current time, there is a paucity of research for any alternative methods that will be required soon if bacterial resistance continues its projected course. The fear of antibiotic resistance is well-founded, and it gives further urgency to the study of non-antibiotic methods, particularly methods that do not disrupt the existing microbiome.

Application and Integration of Theoretical Framework

No study of a microorganism's role in pathophysiology would be complete without the inclusion of germ theory. Once considered revolutionary, germ theory has become basic science that is part of the foundation of biomedicine. The challenge is that colonization with GBS is a normal part of vaginal flora, and rarely problematic in non-pregnant women. However benign it may be in the adult woman, it becomes a potentially life-threatening cause of infection if transmitted to the neonate. Unlike a surgical infection where new bacteria are introduced from an outside source, such as contaminated hands as a result of poor hand washing techniques, GBS is often pre-existing as a part of normal flora and only offers risk after reaching a certain threshold in specific situations. Concerns about the microbiome aside, the option of eliminating all vaginal flora with antibiotics will not remain an option forever in the era of antibiotic resistance. The ideal solution for preventing GBS colonization would be highly effective at eliminating GBS colonization in the vagina, minimally-invasive (i.e., not requiring intravenous access), low cost, be accessible without a prescription, readily available, easy to use or apply, and not disrupt the existing flora making up the host's microbiome.

Conclusion

The major finding of this review emphasizes that while non-antibiotic methods of reducing the colony count have limited support in the literature, it is almost without exception due to a lack of research, rather than the research not supporting the methods. The idea of garlic or probiotics as the answer to such a formidable foe as GBS seems prosaic, however it is not unlike the idea that hand washing can prevent death from infection. When Joseph Lister was practicing medicine, many refused to believe that tiny organisms could exist and, even when proven, most struggled with the concept that organisms existed in states of both health and disease (Jessney, 2012). It would be foolish to discount simpler methods for their simplicity, and the looming deadline of antibiotic resistance requires persistence and expediency. Researchers must aggressively focus on non-antibiotic methods of reducing neonatal EOGBSD to find reasonable alternatives, before once again babies are lost to a preventable disease.

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