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Effects of Probiotics on the Reduction in Incidence of Necrotizing Enterocolitis in Premature (< 37 Weeks Gestation) Neonates

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EFFECTS OF PROBIOTICS ON THE REDUCTION IN INCIDENCE OF NECTROTIZING ENTEROCOLITIS IN PREMATURE (<37 WEEKS GESTATION) NEONATES

by

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Abstract

Problem: Necrotizing Enerocolitis (NEC) is one of the leading causes of morbidity and mortality in neonatal intensive care units (NICU), affecting 7% to 14% of premature neonates weighing less than 1500g (Lin et al., 2008). Healthcare costs for the treatment of NEC account for roughly 20% of the 5 billion dollars spent on infants in the NICU annually (Gephart, McGrath, Effken & Halpern, 2012). Nutritional supplements, such as probiotics, may be used prophylactically to prevent NEC in this high-risk population.

Objective: A literature review was performed to examine which strains of probiotics show the most potential in reducing the incidence of necrotizing enterocolitis.

Method: A literature review was performed using CINAHL, Science Citation Index, Science Direct, Medline, Academic One file, PsychINFO, and PUBMED databases. Key words included enterocolitis, Necrotizing*/PC OR NEC* AND probiotics*. After applying exclusion criteria, 9 articles remained for this review.

Results: A variety of probiotic strains used to reduce the incidence of NEC were identified, along with inconsistent times of initiation, number of colony forming units and length of treatment. The most commonly studied probiotic strains include Lactobacillus species, Bifidobacterium species, and Saccharomyces species. After detailed analysis, it appears that a combination of Bifidobacterium species and Lactobacillus species reduce the incidence of NEC from an 8% (Fernández-Carrocera et. al, 2013) reduction up to 100% reduction in the incidence of NEC (Braga, Pontes da Silva, Cabral de Lira, & Lima, 2011). These two species, when combined, were more successful when compared to Saccharomyces species or Lactobacillus species alone.

Conclusion: Although there is positive support for the proactive use of probiotics for the reduction of the incidence of NEC in premature neonates, the inconsistencies between studies are a barrier for determination of a specific treatment recommendation. Although the combination of Bifidobacterium species and Lactobacillus species has been shown to have an impact on the reduction of NEC incidence, the research inconsistencies provide a barrier to generalizations for treatment. Additional research that focus on Bifidobactrium species in combination with Lactobacillus species is needed. Furthermore, the use of probiotics as a preventative treatment for NEC has not been thoroughly researched in extremely premature infant populations (<28 weeks gestation). Therefore, although the results are promising, further research is needed before this can be determined as a safe preventative method. The current questions remaining include: when prophylactic treatment should be initiated, how long prophylactic treatment should last, the number of colony forming units to be administered, and what is the long-term impact of probiotic administration on the normal gut flora, if any.

Dedication

To my family, including Samantha, you have always been my number one cheering squad. There have been so many moments in my life when I thought I could no longer hold it together. It was in those moments you strengthened me so that I could press on and I am eternally thankful that the Lord has blessed me with such an amazing family, “I love you bigger.”

To my friends, I have learned invaluable lessons from each and every one of you. I do not know how I could have survived this crazy, stressful journey without you as a support system. You guys have a knack for knowing when to buckle down to get the job done and when to simply laugh off the stress. There aren’t enough words to say how grateful I am for the love and friendship we share. Dream big dreams!

Finally, to all of the families of what I like to call “tiny humans”. The strength you have, as you watch your baby start its life in the NICU, is immeasurable. If this review can give hope to just one family, then it was worth it.

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I would like to thank the faculty and staff at the College of Nursing for inspiring students to go above and beyond. I am so proud to call myself a Knight Nurse.

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Introduction

Necrotizing Enerocolitis (NEC) is one of the leading causes of morbidity and mortality in neonatal intensive care units (NICU), effecting 7% to 14% of premature neonates weighing less than 1500g (Lin et al., 2008). A neonate is classified as premature when born before the 37th week of gestation. Of the neonates suffering from NEC, it is estimated that roughly 30% will not survive and although technological advances have improved care for increasingly premature neonates, the incidence of NEC has not decreased in over 20 years (Gephart, et al., 2012; Neu, Mshvildadze, &Mai, 2008). Treatment of NEC has a significant financial impact accounting for roughly 20% of the 5 billion per year cost for all NICU treatment costs in the United States (Gephart et al., 2012). The immediate treatment cost for a single neonate diagnosed with NEC ranges between $73,000 and $182,000 depending on surgical needs. In addition to financial hardship, neonates with NEC typically stay in the NICU 22 to 60 days longer than the average NICU patient (Gephart et al., 2012). This prolonged stay in the NICU also generates an emotional hardship on the family as well as disrupting early infant bonding.

Recent studies involving the use of probiotics have shown promising results in terms of reducing the incidence of NEC (Thompsom & Bizzarro, 2008). The World Health Organization defines probiotics as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (World Health Organization [WHO], 2001). Further studies need to be conducted to determine the most effective treatment plan including the optimal time of initiation, length of time treatment is implemented, as well as the combination of probiotics used for treatment.

Background

Necrotizing Enterocolitis

Necrotizing Enterocolitis (NEC) is a multifactorial gastrointestinal (GI) disease in which areas of a newborn's bowel become necrotic (the tissue dies). In 2011, the National Healthcare Safety Network (NHSN) updated the clinical and surgical criteria for the diagnosis of NEC. These updated criteria stated that for an infant to be diagnosed with NEC based on clinical and radiographic criteria, they must present with bilious aspirate, vomiting, abdominal distension, or occult blood in stool, plus one or more of the following radiographic signs: pneumatosis intestinalis, which is gas in the bowel wall, portal venous gas, or pneumoperitoneum (National Healthcare Safety Network [NHSN], 2011). Furthermore, the surgical criteria for NEC are that the infant must have more than two centimeters of necrotic bowel or surgical evidence of pneumatosis intestinalis, with or without intestinal perforation (NHSN, 2011).

Prematurity

NEC is almost exclusively seen in preterm neonates (Sari, et al., 2011). An infant born before the 37th week of gestation is considered premature. The presentation rate of necrotizing enterocolitis is indirectly proportionate to the age of a neonate (Gephart et al., 2012). That is, the earlier a neonate is born, the longer it will take for NEC to present. For example, the average onset of NEC in a neonate born at the 28th week of gestation is 40 days, whereas the average onset for a neonate born at 36 weeks is 5 days (Mayer, 2011).

Due to this near exclusive presentation, gut prematurity is believed to be a primary inciting factor of the disease. The gastrointestinal tract is developed from all three of the embryonic germ layers, the endoderm, mesoderm and ectoderm (Mayer, 2011). Development of

the fetal gut is broken down into four distinct phases: formation of the endoderm, morphogenesis and patterning of the gut tube, and organ morphogenesis and terminal differentiation (Mayer, 2011). While this maturation predominately takes place during the first trimester, the muscular maturity and hormonal maturity that control motility does not begin until the second trimester and continues until term (Braga et al., 2011). More significantly even, the "waves" of peristalsis needed for elimination are not seen in their entirety until a gestational age of 33 to 34 weeks (Braga et al, 2011). Therefore, gastric emptying is severely impaired for infants less than 32 weeks gestation. Due to the increased peristaltic waves seen at 33 weeks gestation, the majority of research investigating NEC excludes infants older than 32 weeks gestation (Braga et al., 2011). Despite this impaired gastric emptying, it is commonly suggested that enteral feeding commence as soon as possible once an infant is clinically stable (Ayede, 2011) This prevents a decrease in digestive enzymes as a result of mucosal and villous atrophy (Ayede, 2011).

The longitudinal and transverse muscles are responsible for the peristaltic action of the bowels (Mayer, 2011). Peristalsis is a mechanism used by the intestines to remove digested food and pathologic bacteria. Therefore, premature infants with immature intestinal smooth muscle consequently have decreased gut motility. This decreased motility may lead to a build-up in pathologic bacteria.

Bacterial Colonization

Gastrointestinal microbiota is a dynamic ecosystem with approximately 500 to 1000 differing species of bacteria that colonize the gastrointestinal tract of a healthy infant (Guaraldi, F. & Salvatori, G., 2012). This gut microbiota influences the growth and differentiation of gut epithelial cells and have a role immunologic and protective functions (Guaraldi, F. & Salvatori,

G., 2012) Molecular studies have shown that the guts of healthy breast-fed and formula-fed infants consist of 60% to 90% Bifidobactrium species with the most common strains of Bifidobactrium being *B. bifidium, B.longum,and B. breve* (Lin et al., 2008). Another primary physiologic bacterium are *Lactobacillus* species which have been shown to induce anti-inflammatory cytokines such as interleukin 10 (Lin et al., 2008).

Studies demonstrate that the gut microbiota of a term breast-fed infant and a term formula-fed infant have no significant differences (Guaraldi, F. & Salvatori, G., 2012). However, it has been reported that breast-fed newborns have a more uniform and stable population of bacteria and have two times the number of bacteria cells when compared to formula-fed newborns (Bezirtzoglou, Tsiotsias, & Welling, 2011). It is consequently appropriate to hypothesize that various strains of Bifidobacterium would have the greatest positive affect on reducing the incidence of NEC.

While a neonate is in utero, the intestine is sterile, surrounded by amniotic fluid (Guaraldi, F. & Salvatori, G., 2012). Bacterial colonization begins at birth and is influenced by several external factors such as mode of delivery, vaginal, or cesarean section, the environment, such as equipment, air and other people, and the ingestion of colostrum(the mother's initial milk) if the mother is breast feeding (Guaraldi, F. & Salvatori, G., 2012). During vaginal delivery, the newborn comes into direct contact with the maternal vaginal and intestinal flora via the ocular, nasal and oral cavities. This direct contact is absent in a cesarean section and gut bacteria is derived primarily from environmental factors (Guaraldi, F. & Salvatori, G., 2012). For this reason, vaginal delivery is associated with a greater biodiversity of healthy bacteria within the newborn gut (Godhia, M.L. & Patel, N., 2013). Colostrum is rich in antibodies that give passive

immunity to a newborn, growth factors that mature the gut epithelial cells, which prepare the newborn for human milk or formula, and antimicrobial peptides (Godhia, et al., 2013). Conversely, premature births are associated with higher rates of cesarean sections, hindered ability to breastfeed, due to delayed onset of lactation (Neu, J. & Rushing, J., 2011), and the newborn being surrounded with equipment in the NICU (Godhia, et al., 2013). These factors alter the introduction of bacteria to the newborn and as a result the preterm gut is predominately colonized by *Staphlococccus, Enterobacter, Enterococcus, and Clostridia* (Thompsom & Bizzarro, 2008). These pathologic bacteria then compete with the natural gut flora for nutrients, and if uncontrolled, an overabundance of pathologic bacteria can be a precursor to gastrointestinal disease such as NEC.

At birth, even in an infant born at term, the immune system has not fully matured. The adaptive immunity gains specificity into early childhood (Melville, J.M. & Moss, T., 2013). Immaturity of the immune system is even more pronounced in the preterm infant (Melville, J.M. & Moss, T., 2013). There is a reduced number of monocytes and neutrophils and a lower productions of cytokines which reduces the preterm infant's ability to recognize and kill pathogens when compared to the term newborn (Melville, J.M. & Moss, T., 2013). Due to this reduced ability to detect and neutralize bacteria and viruses, preterm infants are typically placed on broad-spectrum antibiotic therapy to reduce the bacterial load their immune system faces (Melville, J.M. & Moss, T., 2013). These antibiotics consequently further reduce the biodiversity of the preterm gut bacteria. Pathologic bacteria within the gut accumulate due to both decreased GI motility and these immunologic deficiencies.

Broad-spectrum antibiotics are also administered to mothers who go into premature labor or who have a cesarean section even prior to birth. This may also be a contributing factor that reduces the biodiversity of gut microbiota in preterm infants (Neu, J. & Rushing, J., 2011).

Oxygenation

A third inciting factor believed to contribute to NEC is hypoxic-ischemic injury. Any congenital or environmental factor that affects the oxygenation of the newborn, such as patent ductus arteriosus, respiratory distress, or in utero cocaine exposure, can lead to hypoperfusion. (Thompsom & Bizzarro, 2008). Decreased oxygenation and subsequent decreased organ perfusion is a common problem in premature infants. This can be attributed to the immature lung formation and lack of surfactant in the premature infant's lungs. Surfactant production begins at roughly 32 weeks gestation and reduces surface tension at the air-liquid junction of the alveolus subsequently allowing the alveoli to expand to capacity to perfuse the capillaries surrounding the alveoli (Nkadi, P., Merritt, T., & Pillers, D., 2009). To combat the lack of surfactant production, preterm infants are treated with antenatal corticosteroids and exogenous surfactant upon birth (Nkadi, P. et al., 2009). Without sufficient oxygen exchange between the lungs' alveoli and the blood there is hypo-oxygenation of organs throughout the body. During times of hypo-oxygenation, the body shunts blood to vital organs, such as the brain and heart, and away from "non-vital" organs, such as the intestines (Thompsom & Bizzarro, 2008). This subsequent decrease in oxygen to the gut causes a hypoxic-ischemic state. If the hypo-oxygenation is reversed, reperfusion can cause a proinflammatory cascade leading to damaged mucosal barrier by tumor necrosis factor α (TNF α) and platelet activating factor (Gephart et al., 2012). Inflammation, which reduces cell membrane integrity, is likely to cause hyperpermeability of the

intestinal wall, thus creating an opportunity for bacteria to permeate the gut lining. If bacteria continues past the gut lining, into the peritoneal cavity, sepsis and death can occur (Gephart, et al. 2012).

Diagnosis Necrotizing Enterocolitis

Diagnosis of NEC is oftentimes difficult due to its nonspecific clinical manifestations in the early stages. Temperature instability is one of the most common early signs of NEC while other signs may include apnea, low oxygen saturation, lethargy, and bradycardia (Thompsom & Bizzarro, 2008). As the disease progresses, there may be GI specific manifestations, such as a distended abdomen, feeding intolerance, and blood in the infant's stool (Thompsom & Bizzarro, 2008). Common diagnostic tests include an abdominal x-ray and serum analysis. According to Thompsom and Bizzarro (2008), radiographic signs of NEC may include dilated or fixed intestinal loops, air in the intestinal wall or free air in the abdomen. Serum analysis may show low platelet count and/or elevated white blood cells, although nonspecific, this is an indication of infection (Thompsom & Bizzarro, 2008). Based upon the combination of these diagnostic tests, the severity of NEC can be staged using the Bell's criteria. Stages range from IA: Suspected to IIIB: Advanced, severely ill, perforated bowel (Thompsom & Bizzarro, 2008).

Figure 1: Bell's Classification of NEC

Stage	Classification	Intestinal Signs	Radiologic Signs	Systemic signs
IA	Suspected NEC	Decreased gastric emptying, abdominal distention, emesis	Normal or intestinal dilation	Temperature instability, apnea, bradycardia, lethargy
IB	Suspected NEC	Bright red blood from rectum.	Same as IA	Same as IA
IIA	Proven NEC-mild	Same as IA plus absent bowel sounds, with or without abdominal tenderness	Intestinal dilation, ileus, and pneumatosis intestinalis.	Same IA
IIB	Proven NEC-moderate	Same as IIA with definite abdominal tenderness and with or without right lower quadrant mass	Same as IIA plus portal venous gas with or without ascites	Same as IA plus mild metabolic acidosis and thrombocytopenia
IIIA	Advanced NEC-bowel intact Requires surgery.	Same as IIB with generalized peritonitis, marked tenderness, and distention of abdomen	Same as IIB with definite ascites	Same as IIB with hypotension, bradycardia, apnea, respiratory and metabolic acidosis and neutropenia
IIIB	Advanced NEC-bowel perforation Requires Surgery.	Same as IIIA	Same as IIB with pneumoperitoneum	Same as IIIA

Adapted from Lee & Polin, 2003

Treatment of Necrotizing Enterocolitis

Treatment of NEC ranges from bowel rest for suspected NEC, the use of antibiotics along with bowel rest for proven but mild NEC, to surgery for advanced NEC. When NEC progresses into the "advanced" stages of NEC and requires surgery, it is termed "surgical NEC" and the long-term prognosis for the infant decreases. Long-term ramifications of surgical NEC include short bowel syndrome and neurodevelopment impairment (NDI) (Schulzle S.M., Desphande G.C., & Patole SK, 2007). Due to its costly and deadly nature, an effective preventative strategy needs to be further researched and implemented (Fanaroff, A. A. & Fanaroff J. M., 2013).

There are multiple proposed strategies for the prevention of NEC. These strategies include antenatal corticosteroids, which have been shown to mature the gut in a manner similar to the mechanism enhancing lung maturation; trophic feedings in which small volumes of enteral feedings are introduced in order to facilitate peristaltic action; oral antibacterials, in an effort to reduce the number of pathogenic bacteria; and prebiotics, used to selectively increase the population of commensal GI bacteria. And lastly, the use of probiotics, thought to be the most promising of therapies, and the focus of this literature review (Thompsom & Bizzarro, 2008).

The Pharmacodynamics of Probiotics

The knowledge that a healthy newborn delivered at full term has a gut primarily consisting of Bifidobactrium species (Lin et al., 2008), while the preterm newborn has a gut microbiota consisting primarily of staphlococci, enterobacter, enterococci, and clostridia bacteria (Thompsom & Bizzarro, 2008) led researchers to hypothesis that the use of probiotic supplementation would decrease the incidence of NEC. Probiotics are dietary supplements that introduce indigenous microbes to the human GI tract and confer a health benefit to the infant (Janvier, Malo & Barrington, 2014). It has been recently supported in research that probiotics "provide benefit to preterm neonates by enhancing the IgA mucosal response, improving the mucosal protective barrier, increasing the production of anti-inflammatory cytokines, decreasing intestinal wall permeability and competitively excluding pathogenic microbes" (Thompsom & Bizzarro, 2008, pp. 1234).

The indigenous microbes in a healthy human GI tract also aid in the digestion of protein and carbohydrates. Therefore, the administration of probiotics to preterm neonates could potentially result in a decrease in the rate of NEC, increased feeding tolerance, and a reduced rate

of sepsis. Of the studies investigating probiotics as a prevention strategy for NEC, the most commonly studied species have been those that include Bifidobacteria, Lactobacillus, and Saccharomyces (Thompsom & Bizzarro, 2008).

Problem

Plateau in Incidence

Although technology and treatment options have expanded which has increased the ability to care for and sustain increasingly premature infants, for example those born less than 28 weeks gestation, the incidence of NEC has plateaued rather than declined. The reason for this plateau is that the incidence of NEC is inversely proportional to gestational age. That is, there is a greater number of infants who develop NEC at 32 weeks gestation than there are at 36 weeks gestations (Gephart, et al. 2012). Due to this sustained occurrence rate recent research has shifted from treatment to prevention.

Mortality

NEC is a devastating disease that is responsible for 20%-25% percent of preterm and low birth weight deaths each year (Deshpande, G.C. Rao, S. Patole, S., & Bulsara, M., 2010).With this being a disease that ranks as the second highest cause of death among NICU patients (Deshpande et al., 2010), it is making its way to the forefront of neonatal research. However, there still remains little knowledge as to the intricate details and microbiota of the disease.

Cost of Hospitalization

NEC poses a significant financial burden, accounting for 20% of all neonatal treatment at nearly 5 billion dollars spent each year treating the disease (Gephart et al., 2012). It is estimated that an infant diagnosed with NEC, will stay on average an extra 22 days longer in the hospital than other infants of comparable gestational age (Gephart et al., 2012). Furthermore, the cost of treating one infant with NEC has been reported to be as high $216,666 per survivor (Deshpande,

et al., 2010). Research by Johnson and colleagues (2013) indicates that medically treated NEC, which is stages 1 and 2 that do not require surgery, increased hospital direct costs by $13,136 (p=0.034) and surgically treated NEC, which is Stage 3 or "advanced NEC", increased hospital direct costs by $22,328 (p=0.039) when compared with infants without NEC of similar gestational age, birth weight and demographics (Johnson, Patel, A., Jegier, Engstrom, & Meier, 2013). Directs costs include all chargeable items such as electrolyte panel, room charges and medications, during the infant's hospital stay (Johnson et al., 2013). These increased costs can be attributed to not only the increased length of hospital stay but also the additional care needs (Johnson et al., 2013). Given this significant financial burden the benefits of a prophylactic treatment aimed at reducing NEC, such as probiotics, is significant.

Lack of Research

There is a paucity of research and inconsistent methodologies amongst the current research concerning probiotics and their ability to reduce NEC. Specifically, there has yet to be a study design replicated to validate previously reported results. Without replicated studies that validate the benefits of a particular probiotic strain, dosage or administration protocol, health professionals are reluctant to implement the use of them in standard practice or recommend the use of probiotics to caregivers with any guarantee.

Cost of Probiotics

Probiotics are neither drug nor device and they are unregulated which makes then unique in terms of medical intervention (Taylor, R. S, 2014). Annie Javier and colleagues form the Sainte Justine University Health Center in Canada investigated the use of probiotics to reduce the incidence of NEC. They reported great success with nearly a 50% reduction in NEC incidence

(Janvier, 2014). Janvier et al., (2014) reports using a commercially available probiotic: FloraBABY. The researchers reported that this probiotic cost a mere 11 cents per day. In this particular study, the average length of treatment was 24 days. Therefore, the cost per infant was only $2.51(Janvier et al., 2014).

Purpose

The purpose of this review is to evaluate the current research findings regarding the potential for probiotics to prevent NEC in premature (<37 weeks gestation) neonates admitted to the neonatal intensive care unit (NICU). A literature review was performed to examine the current evidence. Probiotic strands will be identified that either decrease, increase or produce no effect on the reduction of NEC, and the findings will be discussed.

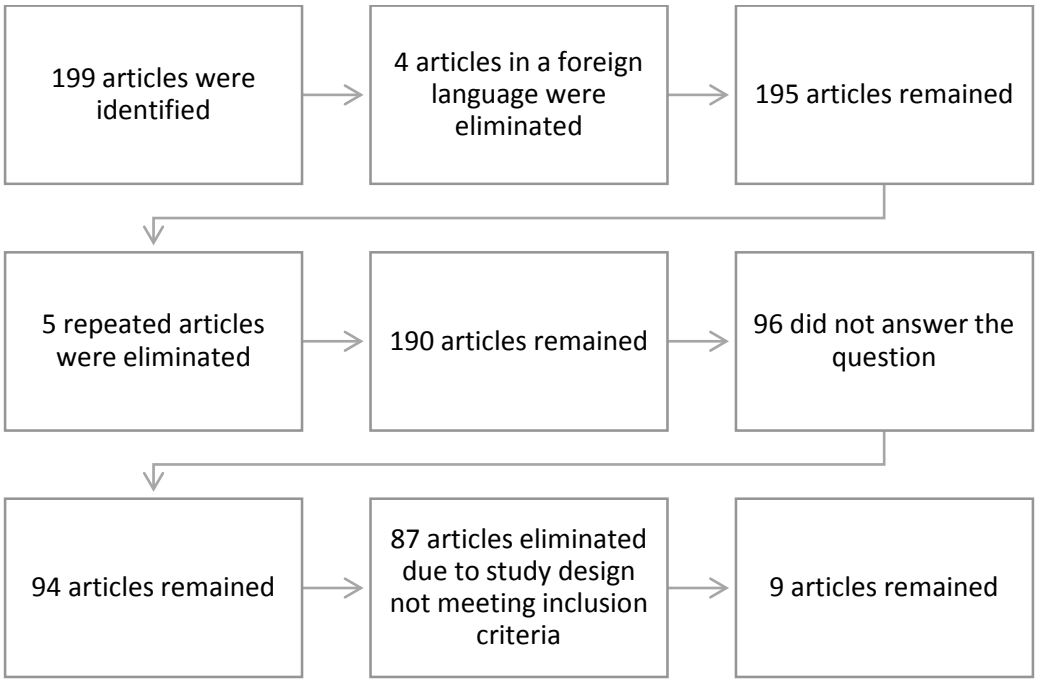
The secondary purpose of this research is to make recommendations for current practice and future practice based on the findings in this study. The final purpose of this study is to make recommendations for future research based on the findings in this study.

Current studies lack consistency in their choice and combinations of probiotics. These methodology differences have made it difficult to determine the most beneficial probiotic supplement to premature neonates. Therefore, the overall purpose of this research is to identify the probiotic strand or combination of probiotic strands that have the highest potential for decreasing the incidence of NEC in premature neonates.

Method

The focus of this comprehensive literature review will examine the effectiveness of probiotics in decreasing the incidence of NEC in premature neonates. Information will be obtained from CINAHL plus full text, Science Citation Index, Science Direct, Medline, Academic One file, PsychINFO, EBSCOhost databases, and PUBMED to determine research, which has been conducted, and the extent probiotics have shown to be effective. A scholarly search of these databases will be conducted using the key words enterocolitis, Necrotizing*/PC OR NEC* AND probiotics*. Literature criteria for this comprehensive review will consist only of randomized control trials (RCTs) and quasi-randomized trials, peer-reviewed articles, research articles, and articles written in the English Language no earlier than 2005. Population criteria for this literature review will consist of neonates that are premature (<37 weeks gestations). Neonates being fed breast milk, formula or a mixture of both will be eligible for inclusion in this review. Additionally, probiotic supplementation must have commenced prior to the corrected gestation of 40 weeks. This literature review will exclude data on neonates that have prior genetic anomalies, gastrointestinal malformations, and/or prior diagnosis of NEC based on Bell's criteria. After applying exclusion criteria, 9 articles remained for this review.

Figure 2: Method



Findings

The literature review returned over 12 strains of probiotics that have been studied to determine their effectiveness in preventing necrotizing enterocolitis. Staggering variances in methodologies between the studies created a problem when trying to identify a clinically significant approach to reduce the incidence of NEC. To better organize and discuss the numerous strains studied they have been divided into three categories that include Bifidobacterium species combined with Lactobacillus species, Lactobacillus species used alone, and Saccharomyces species. Within these three categories the research articles are compared based on: incidence of NEC, participant demographics, specific type and strength of probiotic and finally, the timing of the introduction, duration of administration and feeding regimes. Each section highlights evidence that shows how a particular species of probiotics may or may not prevent NEC.

Bifidobacterium Species combined with Lactobacillus Species

The literature review returned 3 research studies that met the inclusion criteria, which investigated the combined use of Bifidobacterium species and Lactobacillus species in terms of reducing the incidence of NEC.

Incidence of NEC

Both Lin et al., (2008) and Braga et al., (2011) found a statistically significant reduction in the incidence of NEC. Lin et al., (2008) reported that only 1.8% of the infants in the study group developed NEC, whereas 9.2% of infants in the control group developed NEC. With a p-value of 0.002, this was an incredibly significant decrease despite the potential center variations this study encountered. Likewise, Braga et al., (2011) reports similar results with 0% of

participants in the study group developing NEC versus 3.6% in the control group. With a p-value of 0.05 this is also statistically significant.

The only study included in this review, which investigated the combined effects of Bifidobacterium species with Lactobacillus species, that did not report a significant decrease in the incidence of NEC was a double-blind, randomized clinical trial undertaken by Fernandez-Carrocera et al., (2013). Although there was a decrease in NEC in the study group, 8% versus 16%, the p-value was 0.132 and therefore these results, while clinically significant are not statistically significant (Fernandez-Carrocera et al., 2013). It has been shown through molecular studies that in the healthy gut of a breastfed infant Bifidobactrium species can represent anywhere between 60% and 90% of the total fecal microbiota. Therefore, these results can possibly be attributed to the use of 4 Lactobacillus strains and only 1 Bifidobactrium stain. This specific combination does not mimic the microbiota of a healthy infant (Lin et al., 2008).

Population

The number of infants in the studies reviewed ranged from 150 to 434. Larger sample sizes carry more weight than small sample sizes, however small sample sizes are acceptable as when a power analysis is conducted to determine the minimal number of participants needed to produce valid results. A power analysis was done in all three studies to ensure the minimum participant number was met. However, in the Braga et al., (2011) trial, the research was discontinued after one year rather than continuing the originally planned 2-year period of time due to the trial producing clear benefits in the probiotic group. For this reason, Braga et al., (2011) did not met the minimum participant number of 282 in each group which weakens the

validity of their results. By comparison, both Lin et al., (2008) and Fernandez-Carrocera et al., (2013) met the minimum participant requirement.

Lin et al., (2008) was the only study of this entire literature review that categorizes its results based on birth weight. This helps to identify the true effects of probiotics on the extreme low birth weight infants (ELBW) which is any infant weighting less than 1000g at birth. Unfortunately, Lin et al., (2008) failed to stratify its participants based on birth weight during the randomization of its participants. This caused the study group to have a significantly lower average birth weight when compared to the control group (there were 33 infants weighting less than 750g in the study group and only 18 infants weighing less than 750g in the control group). This lower birth weight average in the study group puts the participants at a greater acuity and therefore increased risk of NEC than those infants in the control group. Despite this, there was still a significant reduction in the incidence of NEC.

Infants were excluded from all three trials if they had major congenital malformations such as congenital intestinal atresia or gastroschesis, previously diagnosed life-threatening chromosomal abnormalities, or severe asphyxia. Lin et al., (2008) included additional exclusion criteria such as exclusive formula feedings and infants who had been fasted for greater than 3 weeks. Clinical similarities in all three trials made it easier to accurately compare acuity of the participants.

Inclusion criteria of <1500g birth weight and <32 gestational age at birth was consistent between the three trials however, the average birth weight and gestational age varied. Gestational age ranged from 29.3 weeks (Braga et al., 2011) to 31.1 weeks (Fernandez-Carrocera et al., 2013). Lin et al., (2008) did not specify the average gestational age of the participants, they

simply stated that all infants were less than 32 weeks gestational age. Birth weight ranged from an average of 1,053g (Lin et al., 2008) to 1,173g (Braga et al., 2011).

Specific Type and Strength of Probiotic

There were various forms and doses of Bifidobactrium and Lactobacillus administered in the studies reviewed. Fernandez-Carrocera et al., (2013) even added one strain of streptococcus into the probiotic mix. The only two probiotic types seen in two studies were *L. acidophilus* (Fernandez-Carrocera et al., 2013; Lin et al., 2008) and *L. casei* (Fernandez-Carrocera et al., 2013; Braga et al., 2011). More over two of the three studies investigated the reduction of NEC using two strains of probiotic (Lin et al., 2008; Braga et al., 2011) while, Fernandez- Carrocera et al., 2013) employed the use of six various probiotics strains, four of which were Lactobacillus strains. The vast difference in type of probiotic studied makes it near impossible to identify which specific strain combination of Bifidobactrium and Lactobacillus create the greatest reduction in NEC incidence.

The dose presentation between the three studies also varies greatly. Although all three trials measure the probiotic by CFU's the dosing was random and ranged from 3.5 x10^7 (Braga et al., 2011) to 1x10^9 (Lin et al., 2008; Fernandez-Carrocera et al., 2013) and everywhere in between. The only consistency in dosing was in Lin et al., (2008) and Fernandez-Carrocera et al., (2013) where 1x10^9 CFU's of *L. acidophilus* are used. This inconsistency of dosage made it impossible to compare the doses of various probiotics.

Timing of the Introduction, Duration of Administration and Feeding Regimes

The introduction time, length of administration and feeding regimes were different for each study. Probiotic supplementation was started when the first enteral feeds were started

(Fernandez-Carrocer et al., 2013; Lin et al., 2008) or on the second day of life (Braga et al., 2011). Furthermore, the duration of administration ranged from 30 days (Braga et al., 2011) to until 6 weeks (Lin et al., 2008) to until discharge or death (Fernandez- Carrocera et al., 2013).

The feedings regimes were comparable between the three studies. Lin et al., 2008 and Braga et al., (2011) were both very detailed in the protocol for advancing feeds and neither study advanced feeds more than 20mL/kg per day. However, Braga et al., (2011) did not stop probiotic administration during times of feeding intolerance like Lin et al., (2008) and Fernandez-Carrocera et al., (2013). All three studies allowed the use of human milk and/or fortified preterm formula for feeding however, Braga et al., (2011) specifically fed the infants breast milk until the third week of life and then introduced fortified preterm formula to their diet. This inconsistent milk forms between these studies makes it difficult to analyze the effects of probiotics.

Lactobacillus Species

This literature review returned two articles that met the inclusion criteria, which focused on Lacrobacillus species as the single agent of investigation.

Incidence of NEC

Both Sari et al. (2011) and Oncel et al. (2014) found that the use of lactobacillus species produced no significant effect on the incidence of necrotizing enterocolitis stage 2 or greater. Oncel et al., (2014) reported the rate of NEC amongst the probiotic group participants to be 4%. This is only slightly reduced from the control group which had a NEC rate of 5%. With a p-value of 0.63, this reduction in NEC cannot be considered statistically or clinically significant. These results mirror the findings in the Sari et al., (2011). Sari et al., (2011) reported a NEC incidence rate of 5.8% in the study group and 9% in the control group with a p-value of 0.447. Like the

Oncel et al., (2014) study these are not statistically significant results. Sari et al, (2011) attributes the lack of change in incidence of NEC to the small population size and the interference of intestinal blood flow caused by umbilical venous catheters that were in place significantly longer in the study group than the control group.

Population

The researchers in both of the lactobacillus studies examined in this literature review excluded infants that were at an increased risk for NEC such as infants exposed to prenatal steroids, prolonged rupture of the amniotic membranes and asphyxia (Oncel et al., 2014; Sari et al., 2011). The demographic and clinical similarities between the participants of the two studies created an advantage for this review. Both research studies used event analysis with an α-error set at 0.05, the β-error set at 0.2 with an absolute reduction in incidence of NEC of 50%. Sari et al., (2011) determine that to verify the hypothesis the minimum number of participants needed in each arm of the study was 111 infants. There were 110 infants in the probiotic group and 111 infants in the control group therefore, the minimum number of participants was not achieved for the probiotic group which reduces the validity of the results presented in this study. Oncel et al., (2014) determined that the minimum number of participants in each arm needed to be 190. This minimum was achieved with 200 infants in both the study and control group thereby strengthening the validity of results in this study.

The participants in the Oncel et al., (2014) were slightly younger with an average gestational age of 28.0 weeks versus 29.6 gestational weeks in the Sari et al., (2011) participants. Likewise, the participants in the Oncel et al., (2014) study were also smaller than the Sari et al, (2011) participants with an average birth weight of 1,059.5g versus 1,254.5g, respectively. This

near two week and 200g difference between the participants of the two studies makes it difficult to comparatively analyze the results.

Specific Type and Strength of Probiotic

The specific strains of probiotic investigated in the Oncel et al., (2014) study and the Sari et al., (2011) study differed from one another. Oncel et al., (2014) supplemented feedings with *L. reuteri* and Sari et. al, (2011) supplemented feedings with *L. sporogens,* which was the first study to examine *L. sporogens.* Unfortunately, there is a deficit in research examining the effectiveness of these two strains of Lactobacillus in reducing NEC. For this reason, it is impossible to say which of these two strains carries the greatest benefit. However, it is thought that the main action of all Lactobacillus species is to aid and regulate the host- defense mechanisms of the intestines. It is possible then, that all lactobacillus species will produce similar results, in regards to decreasing NEC, as these two studies but further research is needed to confirm the validity of this hypothesis.

In terms of probiotic strength, the presentation of dose strength was the same which made it easy to decipher the equality of the strengths. Oncel et al., (2014) supplemented feedings with 100,000,000 CFU and Sari et al., (2011) supplemented feedings with 350,000,000 CFU. The supplementation in the Sari et al., (2011) study is significantly larger which makes it peculiar that there was no significant reduction in NEC incidence. This study design would need to be repeated in further research to determine the variables attributing to the insignificant NEC reduction.

Timing of the Introduction, Duration of Administration and Feeding Regimes

Introduction time, administration duration and feedings regimes were comparable in both lactobacillus studies. Probiotics were introduced with the first feed and when the participants were clinically stable (Oncel et al., 2014; Sari et al., 2011). However, details as to the exact day of first feed was not reported. For all participants in both studies, probiotics were administered until discharge or death. Furthermore, both studies started the feeding at a rate of 10-20mL/kg per day and restricted the advancement of feeds to no more than 20mL/kg per day. Also for participants in both study designs, feedings were stopped if there were two or more signs of feeding intolerance and held until the feeding intolerance resolved (Oncel et al., 2014; Sari et al., 2011).

The methodology differences between Oncel et al., (2014) and Sari et al., (2011) appeared in the mechanism of administration of probiotics. Oncel et al., (2014) administered the probiotic supplementation by placing 5 drops of *L. reuteri* in an oil based suspension on the back of the oropharynx of the infants while Sari et al., (2011) added the *L. sporogenes* to the milk feeds. There was no mention by Oncel et al., (2014) as to how this unique administration could have affected results nor has this study been repeated. For this reason, it is impossible to determine if the two administration methods cause variable results.

Saccharomyces Species

Two studies investigating the use of Saccharomyces species met the inclusion criteria for this literature review.

Incidence of NEC

Neither the study by Demirel, Erdeve, Celik & Dilmen (2013) nor the study by Serce, Benzer, Gursoy, & Ovali (2013) reported any decrease in NEC in the infants studied. Demirel et al. (2013) was the first randomized, controlled trial to investigate the use of *S. boulardii* as a preventative method to NEC. This study reported the rate of NEC in the study group as 4.4% which is just slightly lower than the rate of 5.1% in the control group however, this was not a statistically significant decrease with a 95% confidence interval and p-value of 1 (Demirel et al., 2013). Additionally, in both the study and the control group there were two cases of NEC stage 3 (severe NEC).

Population

The number of infants in the studies investigating Saccharomyces species were rather similar with 271 in the Demirel et al. (2013) study and 208 in the Serce et al. (2013) study. Through event rate analysis Serce et al. (2013) was able to determine that in order to verify their hypothesis they would need a minimum of 104 in the study group and 92 in the control group. With 104 in both the study and control group they met the minimum population size which strengthens the validity of the results presented in this study (Serce et al., 2013). Likewise, Demirel et al. (2013) determined through event analysis that the minimum population size for both the study and control arm was 111. This minimum population size was met with 135 in the study group and 136 in the control group therefore, the validity of the results reported is strengthened. Both Demirel et al. (2013) and Serce et al. (2013) used an α-error of 0.05 and a β-error set at 0.2 with an absolute reduction in the incidence of NEC of 50%.

In terms of average gestational age and weight of the participants, both the Demirel et al. (2013) and the Serce et al. (2013) studies were comparable with average gestational ages of 29.3 weeks and 28.7 weeks respectively, and average weights of participants at 1,147.5g and 1,144g, respectively. Furthermore, the demographics between the study participants were similar with both research groups excluding infants with clinical signs that put them at an increased risk for NEC.

Specific Type, Dose and Strength of Probiotic

Both Demirel et al. (2013) and Serce et al. (2013) investigated *Saccharomyces boulardii.* The difference between these two studies is in the dosage and strength of probiotic. Demirel et al. (2013) investigated S. *boulardii* at a strength of 250mg per day while Serce et al. (2013) investigated this probiotic strain at 50mg/kg/12 hour. Adjusting this to show comparisons this is 100mg/kg per day. The average weight in kg between both the study and control group was 1.14 kilograms (Serce et al., 2013). Therefore, the average dose of probiotic given to the infants in this study was roughly 114mg per day. Less than half the dosing given to the study participants in the study by Demirel et al. (2013).

The strength of *S. boulardii* used in these two studies was difficult to compare due to the differing presentation of the strengths. Demirel et al. (2013) reports using 5 billion colony forming units (CFU), while Serce et al. (2013) used a numerical value of 0.5 x 109 cell/kg/dose. This made it impossible to compare the strengths used in these two studies.

Timing of the Introduction, Duration of Administration and Feeding Regimes

The timing of probiotic introduction was slightly different for these two studies. Both studies started probiotic supplementation at the time feedings commenced. However, Demiral et

al. (2013) commenced feedings 48 hours after birth whereas Serce et al. (2013) commenced feedings at only 24 hours after birth. Although a 24-hour difference in the commencement of feedings does not appear to be clinically significant, in the life of a preterm infant, 24 hours is very clinically significant. Unfortunately, neither study comments as to why this particular initiation time was chosen and makes no mention about the participants being clinically stable at the time feedings commenced.

There were methodology similarities between Serce et al. (2013) and Demiral et al. (2013) in regards to the duration of administration and feeding regimes. Both studies continued probiotic supplementation until either discharge or death of the infant. Also, both studies began feeding each participant with 10-20mL/kg/day of either fortified formula or human breast milk. The variation between the studies appeared in the method of advancing feedings. Demiral et al. (2013) gave very scarce guidelines as to how the feedings were advanced, simply stating that the advancement of feeds did not exceed 20mL/kg/day. By comparison Serce et al. (2013) listed very systematic methods as to how the feedings were increased based upon the infant's weight: 10mL/kg/day for infants weighing <750g; 20mL/kg/day for infants weighing 750-1250g; and 30mL/kg/day for infants weighing 1250-1500g. Furthermore, Serce et al. (2013) added a fortifier to the human milk once the goal feeding of 100mL/kg/day was achieved. Demiral et al. (2013) makes no mention of additional fortifiers added to feedings.

Although the differences between these two studies are not extreme, the use of both breast milk and fortified formula amongst the participants makes evaluating the benefits of probiotics difficult.

Other Considerations

Rate of Sepsis

Although the study performed by Oncel et al., (2014) investigating the use of *L. reuteri* showed no significant reduction in the rate of NEC there was a statistically significant reduction in the rate of sepsis between the study group and the control group. With a p-value of 0.041, the rate of sepsis in the study group was only 6.5% whereas in the control group there was 12.5% of participants with proven sepsis (Oncel et al., 2014). Comparatively, the rate of culture-proven sepsis in the Sari et al., (2011) study was not statistically lower in the study group (26.4%) when compared with the control group (23.4%) with a p-value of 0.613. It is difficult to determine the cause of the drastically different results in sepsis occurrence due to the many inconsistencies between the two studies including but not limited to the specific strain of Lactobacillus investigated. More importantly however, in both studies, none of the positive blood cultures grew Lactobacillus bacteria. Therefore, the risk of sepsis as it relates to Lactobacillus supplementation appears extremely low.

Despite the Demiral et al. (2013) study showing *S. Boulardii* at a dose of 250mg/day ineffective in preventing NEC, their study did report a statistically significant reduction in the rate of clinical sepsis. With a rate of 34.8% in the study group and 47.8% in the control group with a 95% confidence interval. However, more importantly is that none of the positive blood cultures grew *S. Boulardii.* By comparison, Serce et al. (2013) reported no significant difference in the rate of sepsis between the probiotic study group and the control group.

Rate of Feeding Intolerance

Similar results were also reported in both the Lactobacillus studies. A statistically significant reduction was observed in feeding intolerance for both the Oncel et al., (2014) and the Sari et al., (2011) studies were reported: 28% vs. 39.5% with a p-value of 0.015 and 44.5% vs 63.1% with a p-value of 0.006, respectively.

Similarly, to the rate of sepsis, Demiral et al. (2013) reported that *S. Boulardii* at a rate of 250mg/day can provide a statistically significant decrease in feeding intolerance for premature infants. In the probiotic group, only 22.9% of the infants had at least one episode of feeding intolerance whereas 48.1% of the infants in the control group had at least one episode of feeding intolerance. By comparison, Serce et al. (2013) reported no significant difference in the rate of feeding intolerance or time to reach full enteral feedings between the study group and the control group.

Duration of Hospitalization

Amongst the studies investigating Lactobacillus species, a significant difference in length of hospital stay was reported from the Oncel et al., (2014) study with the probiotic group participants having an average stay of 38 days and the control group participants staying an average of 46 days in the hospital with a p-value of 0.022. The second study investigating Lactobacillus, Sari et al., (2011), did not have any mention of hospital stay amongst its participants.

The research studies investigating *S. Boulardii* by Serce et al. (2013) and Demiral et al. (2013) both reported that *S. boulardii* supplementation had no significant effect on the length of a neonate's hospital stay.

Summary

Table 1: Bifidobactrium combined with Lactobacillus Species

Author	Probiotic Used	Dose	Sample size	Length of treatment	Gestation at initiation of treatment	Increase or decrease of NEC	Incidence of NEC: Study vs. Control
Braga et.al., 2011	*B. breve* *L. casei*	3.5 · 107 to 3.5· 109 CFU;	231 babies <1500g (119 probiotics and 112 control)	Once daily for 30 days	2 days post birth	Decrease	0 in study group and 4 in control group. *P .05*
Fernández-Carrocera et. al, 2013	*B. breve* *B. bifidum* *B. infantis* *B. longum* *L. rhamnosus GG*	2 x 10^9 CFUs	294 participants in the study group; 317 participants in the control group	Once daily until 34 weeks gestation.	Starting with the first enteral feeding	Decrease	Probiotic: 5.4% Placebo: 9.8% *(P < .02)*
Janvier, A; Malo, J., & Barrington, K., 2014Javier,	*L. acidophilus* *L. rhamnosus* *L. casei* *L. plantarum* *B. infantis* *Streptococcus thermophillus*	1g per day diluted in 3ml of milk	150 babies (75 and 75) <1500g	Once daily. No other specifics noted.	Starting with the first enteral feeing	Decrease	6 (8%) versus 12 (16%) in the control group but the difference was not statistically significant. *(p=0.132)*
Lin et. al., 2008	*B. bifidum* *L. acidophilus*	10^9 CFU and 10^9 CFU respectively @125mg/kg/dose	434 babies <1500g <34 weeks	Twice daily for 6 weeks	Starting with the first enteral feeding	Decrease of NEC	≥stage 2 NEC/Death: *P.002*

Table 2: Lactobacillus used alone

Author	Probiotic Used	Dose	Sample Size	Length of Treatment	Gestation at initiation of treatment	Increase or Decrease of NEC	Incidence of NEC: Study vs Control
Oncel et. al., 2014	*L. reuteri*	100 million CFU/day (5 drops) lyophilized	400 babies with gestation ≤32 weeks and birth weight ≤1500g	Daily until discharge	Starting at first feeding	No effect	There was no statistically significant difference between groups in terms of frequency of NEC stage ≥2 (4% vs 5%; p=0.63) or overall NEC or mortality rates (10% vs 13.5%; p=0.27)
Sari et. al, 2011	*L. sporogenes*	350 000 000 CFU 1ml suspension was added to breast milk or formula	221 babies with gestation of <33 weeks or birth weight of <1500g (110 in the study group and 111 in the control group)	Daily Until discharge	Once they could enterally feed and then starting at first feeding	No effect	The incidence of NEC was not significantly lower in the probiotics group than in the control group (5.8 vs 9%, respectively; P 1⁄4 0.447).

Table 3: Saccharomyces used alone

Author	**Probiotic Used**	**Dose**	**Sample Size**	**Length of Treatment**	**Time of initiation of treatment**	**Increase or decrease of NEC**	**Incidence of NEC: Study vs Control**
Demirel et. al., 2013	*S. boulardii*	250 mg/ day of 5 billion CFU	217 babies with gestation ≤ 32 weeks and birth weight ≤1500g (135 in the study group and 136 in the control group)	Once daily until discharge	Once they could be enterally fed. Starting with first feed.	No significant effect	4.4% vs. 5.1%, 95% CI, !0.65–5.12; *p = 1.0)* However, it did improve feeding intolerance and reduced risk for sepsis
Serce et. al., 2013	*S. boulardii*	50 mg/kg every 12 h	208 babies with gestation ≤32 and birth weight ≤1500 g (104 in the study group and 104 in the control group)	Every 12 hours until discharge	Once they could be enterally fed. Starting with the first feed	No effect	The incidence of stage ≥2 NEC was 7 (6.7%) both in the control and study group, and it did not reach statistical significance (p = 1)

Discussion

Necrotizing enterocolitis remains one of the most devastating pathologic processes faced by premature infants with an unchanging incidence rate of 7%-14% and significant mortality of up to 30% (Deshpande et al., 2010). It is known that the pathogenesis of NEC involves a series of factors, with prematurity (less than 37 weeks gestation) consistently documented as one of the most influential (Braga et. al, 2011; Lin et. al, 2008). Aside from the high incidence and mortality rate associated with NEC, there is also an increased need for surgery to remove the areas of necrotic bowel and long-term ramifications, such as short bowel syndrome and its associated consequences of recurrent sepsis and total parenteral nutrition (TPN) dependence (Deshpande et al., 2010). It has been documented that surgically treated NEC, typically seen in Stage 3 or "advanced NEC" can prolong neonates' hospital stay up to 60 days and cause long-term neurodevelopmental impairment (NDI) (Deshpande et al., 2010; Schulzle S.M., Desphande G.C., & Patole SK, 2007). NDI encompasses cerebral palsy, bilateral blindness or bilateral deafness (Reese, C.M., Pierro, A., & Eaton, S., 2007). In the study by Schulzle et al. (2007) it was found that neonates with definite NEC (Bells stage 2 or greater) were significantly more at risk for long-term NDI when compared to infants who did not develop NEC with an odds ratio of 1.82. Factors causing NDI in premature infants are complex. Brain development continues into the second and third trimester. Therefore, infants born premature have a deficit in neuronal maturation which increases the risk for long-term NDI (Reese, C.M. et al., 2007). Proper nutrition is essential for the continued growth of the brain after birth. Nutrition becomes problematic for the infants with NEC considering NEC is associated with increased rates of feeding intolerance (Janvier, 2014). The issue of nutrition is furthered in the infant whose NEC is

treated surgically. When sections of gangrenous bowel are removed surgically due to NEC the infant has smaller gastrointestinal surface area to absorb necessary nutrients (Reese, C.M. et al., 2007). This is known as short bowel syndrome.

The microbiota within the gastrointestinal system must remain balanced between healthy gut bacteria, such as Bifidobactrium and Lactobacillus, and pathologic bacteria, such as staphlococcci, enterobacter, enterococci, and clostridia. If this balance is disturbed, for example by an over proliferation of pathologic bacteria, disease such as NEC can occur (Lin et al., 2008).

It has been hypothesized that preterm infants fed probiotic supplements containing bacteria found within the healthy newborn gut would have a decreased incidence of NEC (Braga et al., 2011; Fernandez-Carrocera et al., 2013; Janvier, 2014; Lin et al., 2008) . There is strong evidence supporting the benefits of prophylactic use of probiotics but more research is necessary to support the practice of routine probiotic supplementation. Among the literature reviewed there was a lack of consistency in study design (probiotic strain, dosage, feeding protocol, and duration) and this has led to debate about the effectiveness of probiotics in preventing NEC as some studies (Demirel et. al., 2013; Serce et. al., 2013) show no reduction in NEC, while others report astounding reduction (Braga et. al., 2011; Javier et al., 2014; Lin et. al., 2008). Inconsistency in feeding protocol, human milk versus formula, may be attributed to reports, such as by Guaraldi, F. & Salvatori, G. (2012), stating that the type of milk fed does not significantly alter the gut ecosystem, and therefore causing researchers to believe the type of milk does not alter results.

The strains investigated in this literature review included Bifidobactrium strains combined with lactobacillus strains, lactobacillus strains used alone and Saccharomyces strains

used alone. These three categories of probiotic species were investigated because they were the most frequently studied among current research (Deshpande et al., 2010). Despite the narrowed categories and the exclusionary criteria used in this review, a total of 12 different strains are represented between the studies. While there is some overlap in probiotic strains, little similarities in the duration of administration/administration parameter, time of initiation, or combination of strains was found. It is important to note that the mechanism of action of probiotics is strain specific (Serce et al., 2013). With no two studies matching in probiotic strains investigated a significant limitation was placed on the ability to compare studies in this review.

Bifidobactrium combined with Lactobacillus

It is well known among health professionals versed in the intestinal health of infants that the healthy newborn's intestines are colonized primarily by species of Bifidobactrium bacterial species. In fact, molecular studies show that 60%-90% of gut colonization in a healthy newborn is by Bifidobactrium species specifically, *Bifidobactrium bifidum, Bifidobactrium longum* and *Bifidobactrium breve* (Lin et al., 2008). The knowledge of this provides a clear rationale as to why the studies using a combination of Bifidobactrium and Lactobacillus strains (Braga et al., 2011; Fernandez-Carrocera et al., 2013; Janvier, 2014; Lin et al., 2008) are reporting a reduction in NEC among their study participants. All but one (Fernandez-Carrocera et al., 2013) of the research studies using the combination of Bifidobactrium and Lactobacillus found that there was a statistically significant decline in the incidence of NEC when these species of probiotics were added to feedings. More specifically, *B. breve* appears to be the most frequently studied Bifidobacrium strain studied due to its high affinity with the immature intestine and its large presence in the gut of a healthy newborn (Deshpande et al., 2010).

Among the studies investigating Bifidobactrium and Lactobacillus, (Braga et al., 2011; Fernandez-Carrocera et al., 2013; Janvier, 2014; Lin et al., 2008) three of the four studies reported a statistically significant reduction in NEC. This suggests that the use of probiotics, specifically Bifidobactrium and Lactobacillus may prevent the occurrence of NEC stage 2 and greater. The American Academy of Pediatrics (Section on Breastfeeding, 2012) recommends exclusively feeding preterm infants human breast milk, citing that one of the benefits is the reduced incidence of NEC. While human milk contains oligosaccharides that are beneficial to the neonatal gastrointestinal system, and has even shown to reduce NEC (Lin et al., 2008), feeding human milk alone cannot completely eradicate the incidence of NEC (Deshpande et al., 2010). This is attributed to interleukin 10 deficiency in preterm newborns. As previously mentioned, Bifidobactrium species and Lactobacillus species have both been shown to induce interleukin 10 production (Lin et al., 2008). Therefore, probiotics and human milk may work synergistically to reduce the incidence of NEC (Lin et al, 2008). This hypothesis would need to be studied further due to a scarcity of research investigating the use of probiotics in conjunction with exclusively human milk fed preterm infants.

Despite the promising results with Bifidobactrium and Lactobacillus, the number of studies that have evaluated the association of these two probiotics remains extremely low (Braga et al., 2011) and not a single study design has been repeated. As a result, health professionals are hesitant to implement probiotic supplementation into standard practice (Braga et al., 2011).

Lactobacillus Species Used Alone and Saccharromyces Species Used Alone

As previously stated, this literature review supports prior meta-analyses in showing that when only one species of probiotic is used alone, there is no reduction in the incidence of NEC (Deshpande et al., 2010). The studies that investigated Lactobacillus species or Sacharromyces showed no significant reduction to NEC. It should be pointed out that Saccharromyces is actually a yeast rather than a bacterium. Still considered a probiotic, yeast such as Saccharromyces species have been shown to antagonize both other yeasts and bacteria in a similar fashion as bacterial probiotics (Serce et al., 2013). Despite animal studies showing the potential for Saccharromyces species to inhibit pro-inflammatory mediator release, there was no reduction of NEC seen in this review (Serce et al., 2013). These results may be attributed to a differing immune response by the gut mucosal cells due to the substantial difference in cell wall structure between yeast and bacteria (Serce et al., 2013). This however, is just a hypothesis and further studies investigating the use of yeast would need to be conducted. These two probiotic species cannot be completely dismissed however. Both Lactobacillus and Saccharomyces studies showed that there was a significant reduction in the rate of feeding intolerance (Demirel et al., 2013; Oncel et al., 2014; Sari et al., 2013). For improved overall prognosis of this high-risk population, it is essential to establish optimal enteral nutrition as early as possible in the postnatal life (Deshpande et al., 2010). For this reason, supplementing feedings with these two species of probiotics would not be futile if its purpose was to enhance enteral nutrition via improved gastric emptying, and gut barrier function (Deshpande et al., 2010).

Results in terms of sepsis reduction and overall hospital stay were sporadic between the Lactobacillus and Saccharomyces studies and therefore it is inconclusive whether these species

can produce a significant reduction in these two events. The variation in results could be attributed to the heterogeneity in study designs (Deshpande et al., 2010). The inconsistent reduction in sepsis incidence seen in this review may also be attributed to the multiple sources of entry for various pathogens such as endotracheal tubes, central venous catheters, lipid infusions and TPN (Deshpande et al., 2010). Therefore, probiotics may have the ability to reduce the presence of coagulase-negative Staphylococcus (CONS), which is primarily found in the gut, but it it less likely that probiotics could overcome the burden of pathogens from these other entry points (Deshpande et al., 2010). It is reassuring however, that there were zero sepsis related deaths and zero probiotic related sepsis occurrences in this literature review (Braga et al., 2011; Demireal et al., 2013; Fernandez-Carrocera et al., 2013; Janvier, 2014; Lin et al., 2008; Oncel et al., 2014; Sari et al., 2011; Serce et al., 2013)

Conclusion

Necrotizing Enterocolitis is a severe life threatening gastrointestinal disease that often leads to death for premature neonates. In order to reduce the mortality rate associated with this disease it is vital to determine an effective preventative strategy . There is currently insufficient knowledge regarding the precise etiology, disease process, or the long-term consequences of NEC to prescribe a definitive preventative method. For this reason, research into the preventative use of probiotics, among other interventions such as, mothers own milk, increased oxygenation, prebiotics and steroids is ongoing. Furthermore, the use of probiotics as a preventative treatment for NEC has not been thoroughly researched in the extremely premature infant population (<28 weeks gestation). Therefore, although the results are promising further research is needed before the use of probiotics can be determined a safe prophylactic treatment for the generalized premature population.

The consensus between all studies investigating the use of probiotics is that it is an attempt to mimic the natural bowel colonization and microbiota of a healthy infant born full term (Deshpande et al., 2010). Although the use of probiotics appears promising, it should be highlighted that all of the studies included in this literature review reported NEC when it was confirmed at stage 2 or greater according to Bell's Criteria. This omits the early stages of NEC, or "suspected NEC". There is little to no research related to probiotics and their ability to prevent NEC development in these early stages. While NEC stage 2 and greater is associated with the greatest risk for mortality, NEC stage 1 still has the ability to cause morbid conditions with life-long health consequences such as a distended abdomen (Braga et. al, 2011). It is possible that if the confirmed outcome was expanded from "Confirmed NEC stage 2 or greater" to simply

"NEC, including suspected NEC", the effects of probiotics in reducing NEC incidence would be less significant.

Considering the results vary among the species specific research designs it is impossible to determine if there is a precise strain of probiotic more suitable to prevent NEC in the preterm infant. Despite the variable results and large variety of probiotic strains, this literature review further supports prior meta-analysis such as Deshpande et. al. (2010) that conclude that the administration of only one probiotic strain (eg. only one Lactobacillus strain or one Saccharomyces strain) is ineffective in reducing the incidence of NEC. By comparison, studies investigating the use of two or more strains of probiotics used in conjunction saw a significantly reduced incidence in the disease (Braga et al., 2011; Lin et al., 2008)

However, in conclusion, the great majority of research studies show a promising correlation between the combined use of Bifidobactrium species with Lactobacillus species and a decreased incidence in NEC for neonates born premature (Desphande et al., 2010).

Recommendations for Current Practice

Due to the financial costs and deadly nature of NEC in premature neonates it is recommended that health care professionals discuss the potential benefits of probiotics with caregivers. As seen in this literature review, the risk for sepsis as a result of probiotics is extremely rare. There is also the added benefit of increased nutrition due to the reduced rate of feeding intolerance seen in infants given probiotics. Therefore, probiotics used as a prophylactic treatment for NEC comes with little risk other than cost of the probiotic itself which, in comparison to the cost of NEC treatment, is minimal (Janvier et al., 2014). To ensure that this discussion occurs routinely it may be beneficial to provide an informational handout to parents or caregivers of premature neonates. This informational handout could educate parents on what NEC is and why their preterm newborn is at an increased risk of developing NEC. This would include how their preterm infant's gut microbiota differs from that of a term newborn. The informational handout would also need to include the purpose of probiotics, which is to introduce "good" bacteria that mimics the bacteria seen in term newborns, and how the supplementation of probiotics might prevent the occurrence of NEC. It would have to be stressed however, that research is yet to be definitive on the most beneficial strain, dosage or supplementation method. That there is simply a strong correlation between probiotic supplementation and a reduction in NEC incidence. Therefore, health care professionals could not guarantee the prevention of NEC while discussing this preventative approach.

In addition to suggesting the use of probiotics there are other preventative measures that that can incorporated in the NICU setting. This includes the continued use of antenatal

corticosteroids and exogenous surfactant to aid in lung develop which subsequently increases oxygenation of organs including the gastrointestinal tract.

Finally, it would be beneficial that, when possible, strict feeding protocols of human milk feeds only be implemented in current practice. This implementation can be accomplished by supporting mothers who choose to breast feed if their infant is able. This also includes supporting the mother during the time when a baby cannot adequately breast feed, by supporting the use of the breast pump and milk storage. Human breast milk differs from formula milk in the nutrient composition but more importantly human milk contains growth factors, not found in formula, that aid in the maturation of a newborn's gut epithelial cells and subsequent peristalsis and immunologic defenses necessary to maintaining a healthy gut ecosystem (Guaraldi F. & Salvatori G., 2012).

Recommendations for Future Practice

As research continues to be conducted pointing towards the benefits of Bifidobacterium species and Lactobacillus species for NEC prevention, it is recommended that health care professionals begin using these probiotic strains as supplementation routinely on all neonates born before the 37th week of gestation or born weighing less than 1500g. It may be interesting to note that since Japan has introduced the routine use of probiotics to prophylactically treat NEC, their NEC incidence rate has decreased to approximately 1% (Taylor, R. S., 2014).

Probiotic supplemented feedings would slightly increase the cost of routine care. However, the objective is to reduce the long term treatment costs associated with NEC and subsequently decrease the length of hospitalization for NICU patients. If incidence of NEC is reduced, the length of hospitalizations, cost of treatment for NEC and overall health of the neonate can improve. Current research shows that supplementing feedings with these probiotic strains produce a negligible risk for sepsis, and therefore has a beneficial risk-benefit balance (Taylor, R.S. 2014). The reality of the situation is that the gastrointestinal tracts of preterm infants are going to become colonized. Therefore, it becomes a matter of what type of bacteria that will colonize the GI tracts. The risk of an extremely rare case of sepsis occurring associated with the administration of probiotics, which is easily resolved via antibiotics (Janvier et al., 2014), should be weighed against the significantly larger risk accrued when an infant's GI tract is colonized by pathologic bacteria (Janvier et al., 2014).

Additionally, there is a strong correlation with improved feeding tolerance and the use of probiotic supplemented feedings (Braga et al., 2011; Demiral et al., 2013; Oncel et al., 2014; Sari

et al., 2011) along with a reduced hospital stay (Oncel et al., 2014). Therefore, the benefits outweigh the cost.

Recommendations for Future Research

This review has identified several probiotic strains researched to reduce the incidence of NEC. It has also identified that the current research has vast inconsistences in methodology. Even among research studies that look at similar probiotic strands, the other independent variables such as number of colony forming units, time of initiation, and length of treatment vary so significantly that there is not a single repeated study design. This hinders the growth of knowledge on how to prevent NEC in NICU patients.

Therefore, it is important that going forward in the research of NEC prevention that study designs shown to be successful, such as the study by Lin et. al in 2008, are repeated to validate the effectiveness of these researcher's methodology and validate the results. Only once variances between independent variables are reduced will HCPs have a basis for how to move forward in practice and prevention of this deadly disease process.

Due to the long-term consequences of NEC, such as short bowel syndrome, NDI, and frequent sepsis, research investigating the long-term ramifications of NEC, specifically in these areas, would greatly improve the overall knowledge of the pathogenesis of NEC. Additionally, as the ability to care for preterm infants increases it would be wise to look into the effects of prophylactic probiotic supplementation on extremely preterm infants (less than 28 weeks old).

Further research should also evaluate the effectiveness of incorporating additional prevention strategies such as improving oxygenation and tissue perfusion, strict feeding protocols that establish the use of human breast milk only, and slow advancements of enteral feeds.

Limitations

As previously mentioned, the relationship between the incidence of NEC and prematurity is inversely related (Gephart et al., 2012). The typical neonate in a level III NICU is of high acuity, often times extremely premature at less than 32 weeks gestation, presenting with comorbidities, and significant genetic anomalies (Levels of Neonatal Care, 2004). The participants in this literature review were all less than 32 weeks gestation however, the exclusionary criteria was such that infants with genetic anomalies or gastrointestinal comorbidities were excluded. Therefore, it can be concluded that this literature review contains studies that did not always look at infants with the greatest risk for NEC. This is a limitation because it is not applicable to the extremely premature infant with confounding morbidities and therefore not generalizable to all NICU patients (Rohan & Wainwright, 2014).

Another limitation to this literature review was that the scarcity of research made it so that no two studies were identical in probiotic strain, dosage or method of administration. It is important to note that the mechanism of action of probiotics is strain specific (Serce et al., 2013). Without repetition of probiotic strains investigated it is near impossible to determine with any certainty a single strain or combination of strains that is most effective in preventing NEC. Therefore, no definitive conclusion could be drawn as to which probiotic strain, dosage or method was most beneficial.

References

Ayede, A. I. (2011). Achieving optimal feeds for preterm babies, recommendations and realities in practice: Nigerian perspective. *Annals of Ibadan Postgraduate Medicine, 9*(1), 1–7.

Bezirtzoglou, E., Tsiotsias, A., & Weeling, G. (2011). Microbiota profile in feces of breast- and formula-fednewborns by using fluorescence in situ hybridization (FISH). *Anaerobe, 17*(6), 478-82. doi: 0.1016/j.anaerobe.2011.03.009

Braga, T.D., Pontes da Silva, G.A., Cabral de Lira, P.I., & Lima, M.C. (2011). Efficacy of Bifidobactrium breve and Lactobacillus casei oral supplementation on necrotizing enterocolitis in very-low-birth-weight preterm infants: a double-blind, randomized, controlled trial. *American Journal of Clinical Nutrition, 2011*(93), 81-86.

Demirel, G., Erdeve, O., Celik, I., & Dilmen, U. (2013). Saccharomyces boulardii for prevention of necrotizing enterocolitis in preterm infants: a randomized, controlled study. *Acta paediactrica, 102*(12), e560-e565. doi: 10.1111/apa.12416

Deshpande, G., Rao, S. Patole, S. & Bulsara, M. (2010). Updated meta-analysis of probiotics for preventing necrotizing enterocolitis in preterm neonates. *Pediatrics, 125*(5), 921-930. doi: 0.1542/peds.2009-1301

Fanaroff, A. A., & Fanaroff, J. M. (2013). *Klaus & Fanaroff's care of the high-risk neonate* – 6th ed. Philadelphia, PA: Saunders, an imprint of Elsevier Inc.

Fernandez-Carrocera L.A., Solis-Herrera, A., & Cabanillas-Ayon, M., et al. (2013). Double blind, randomized clnical assay to evaluate the efficacy of probiotics in preterm newborns weighing less than 1500g in the prevention of necrotizing enterocolitis. *Arch Dis child Fetal NeonatalEd, 2013*(98), F5-F9. doi: 10.1136/archdischild-2011-300435

Gephart, S. M., McGarth, J. M., Effken, J. A., & Halpern, M. D. (2012). Necrotizing enterocolitis risk: State of the science. *Advances in Neonatal Care, 12*(2), 77-89. doi:10.1097/ANC.0b013e31824cee94

Godhia, M & Patel, N. (2013). Colostrum-its composition, benefits as a nutraceutical: a review. *Food and Nutrition Journal, 1*(1), 37-47. Retrieved from http://www.foodandnutritionjournal.org/pdf/vol1no1/1_1_4_p37_47_Colostrum_MEENA.pdf

Guaraldi, F., & Salvatori, G. (2012). Effect of Breast and Formula Feeding on Gut Microbiota Shaping in Newborns. *Frontiers in Cellular and Infection Microbiology*, *2*, 94. http://doi.org/10.3389/fcimb.2012.00094`

Janvier, A., Malo, J., & Barrington, K. (2013). Cohort study of probiotics in a North American neonatal intensive care unit. *The Journal of Pediatrics, 164*(5), 980-985. doi: 10.1016/j.jpeds.2013.11.025

Johnson, T. J., Patel, A. L., Jegier, B., Engstrom, J. L., & Meier, P. (2013). The Cost of Morbidities in Very Low Birth Weight Infants. *The Journal of Pediatrics*, *162*(2), 243 49.e1. http://doi.org/10.1016/j.jpeds.2012.07.013

Lee, J.S., & Polin, R.A. (2003). Treatment and prevention of necrotizing enterocolitis. *Seminarsin Neonatology, 8*(6), 449-459. doi: 10.1016/S1084-2756(03)00123-4

Levels of Neonatal Care. (2004). *Pediatrics, 114*(5), 1341-1347. doi: 10.1542/peds.2004 1697

Lin, H., Hsu, C., Chen, H., Chung, M., Hsu, J., Lien, R., & ... Su, B. (2008). Oral probiotics prevent necrotizing enterocolitis in very low birth weight preterm infants: A multicenter, randomized, controlled trial. *Pediatrics*, *122*(4), 693-700.

Mayer, A.N. (2011). Chapter 381. Normal Structure and Function of the Gastrointestinal Tract. In Rudolph C.D., Rudolph A.M., Lister G.E., First L.R., Gershon A.A. (Eds), *Rudolph's Pediatrics, 22e.* Retrieved March 24, 2016 from http://accesspediatrics.mhmedical.com.ezproxy.net.ucf.edu/content.aspx?bookid=455&Sectionid=40310685.

Melville, J. M., & Moss, T. J. M. (2013). The immune consequences of preterm birth. *Frontiers in Neuroscience*, *7*, 79. http://doi.org/10.3389/fnins.2013.00079

National Healthcare Safety Network. (2011, December). Important surveillance guidance to incorporate beginning January 1, 2012. *NHSN e-News, 6*(4), 3. Retrieved from http://www.cdc.gov/nhsn/PDFs/Newsletters/NHSN_NL_Dec_2011.pdf

Neu, J., Mshvildadze, M., & Mai, V. (2008). A roadmap for understanding and preventing necrotizing enterocolitis. *Current Gastroenterol Report, 10*(5), 450-457.

Neu, J., & Rushing, J. (2011). Cesarean versus Vaginal Delivery: Long term infant outcomes and the Hygiene Hypothesis. *Clinics in Perinatology*, *38*(2), 321–331. http://doi.org/10.1016/j.clp.2011.03.008

Nkadi, P. O., Merritt, T. A., & Pillers, D.-A. M. (2009). An Overview of Pulmonary Surfactant in the Neonate: Genetics, Metabolism, and the Role of Surfactant in Health and Disease. *Molecular Genetics and Metabolism*, *97*(2), 95–101. http://doi.org/10.1016/j.ymgme.2009.01.015

Oncel, M., Sari, F., Arayici, S., et al. (2014). Lactobacillus reuteri for the prevention of necrotising enterocolitis in very low birthweight infants: a randomized controlled trial. *Arch Dis Child Fetal Neonatal ED, 99*(2), F110-115. doi: 10.1136/archdischild-2013

304745

Reese, C. M., Pierro, A., & Eaton, S. (2007). Neurodevelopmental outcomes of neonates with medically and surgically treated necrotizing enterocolitis. *Archives of Disease in Childhood. Fetal and Neonatal Edition, 92*(3), F193–F198. doi:10.1136/adc.2006.099929

Rohan, T., & Wainwright, L. (2014). Does administering probiotic treatment (J) to infants under 1500 g, decrease the incidence of necrotizing enterocolitis? A systematic literature review. *Journal of Neonatal Nursing, 20*(1), 37-42. doi:10.1016/j.jnn.2013.04.007

Sari, F., Dizdar, E., Oguz, S., Uras, N., & Dilmen, U. (2011). Oral probiotics: lactobacillus sporogenes for prevention of necrotizing enterocolitis in very low-birth weight infants: a randomized, controlled trial. *European Journal of Clinical Nutrition, 65*, 434-439. doi: 10.1038/ejcn.2010.278

Schulzke, S., Desphande, G., & Patole, S. (2007). Neurodevelopmental outcome of very low birth weight infants with necrotizing enterocolitis: a systemic review of observational studies. *Archives of Pediatrics and Adolescent Medicine Journal, 161*(6), 583-590.

Section on Breastfeeding. Breastfeeding and the use of human milk. Pediatrics 2012;129:e827–e841

Serce, O., Benzer, D., Gursoy, T., Karatekin, G., & Ovali, F. (2013). Efficacy of saccharomyces boulardii on necrotizing enterocolitis or sepsis in very low birth weight infants: a randomized controlled trial. *Early Human Development, 89*(2013), 1033-1036. doi: 10.1016/j.earlhumdev.2013.08.013

Taylor, R. S. (2014). Probiotics to prevent necrotizing enterocolitis: Too cheap and easy? *Pediatrics & Child Health, 19*(7), 351–352.

Thompson, A., & Bizzarro, M. (2008). Necrotizing enterocolitis in newborns: Pathogenesis, prevention and management. *Drugs*, *68*(9), 1227-1238.

World Health Organization. (2001). *Evaluation of health and nutritional properties of powder milk and live lactic acid bacteria.* Retrieved from ftp://ftp.fao.org/es/esn/food/probio_report_en.pdf