Flora, Flora, Flora… Microflora!: Evaluating the Use of Probiotics in Preterm Infants for Necrotizing Enterocolitis

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Learning Objectives:

1. Discuss the prevalence, pathophysiology, and treatment options for necrotizing enterocolitis in preterm infants.
2. Discuss the history of probiotic supplementation used in the healthcare setting.
3. Identify common strains of probiotics and understand what safety risks are concerning for the use of probiotics in preterm infants.
4. Evaluate the literature examining the use of probiotics for prevention of necrotizing enterocolitis in preterm patients, and formulate an evidence-based recommendation for their use.
I. INTRODUCTION TO NECROTIZING ENTEROCOLITIS (NEC)

A. Definition: A life-threatening gastrointestinal tract disease that commonly affects premature neonates.

B. Epidemiology
   i. The incidence of NEC in infants born at <29 weeks is approximately 7%\(^1\)
   ii. About one-third to one-half of affected neonates require surgical intervention\(^2\)

C. The leading cause of mortality and morbidity in premature, very low birth weight (VLBW) infants\(^3\)
   i. Average mortality from NEC is 20-30% and as high as 50% in infants requiring surgical intervention\(^2\)
   ii. NEC results in:
      a. Long-term complications:
         1. Neurodevelopment impairment
         2. Short-gut syndrome
         3. Intestinal Stricture
      b. Increased risk of death prior to discharge
      c. Longer duration of hospitalization
      d. Cost burden

D. Pathophysiology\(^5\)\(^-\)\(^10\)
   i. Bacterial colonization
      a. Preterm infants have reduced microbial diversity
         1. Increased numbers of Enterobacteriaceae (i.e. Klebsiella pneumonia, Escherichia coli, and Clostridium difficile)
         2. Delayed colonization of Lactobacillus and Bifidobacterium species
            i. Lactobacillus and Bifidobacterium are typical microflora of breast-fed infants

Figure 1: Rate of mortality by birth weight category\(^4\)
ii. VLBW infants do not receive enteral feedings immediately following birth and later may be formula fed rather than breast fed

b. Other factors leading to delayed or resistant intestinal bacteria colonization
   1. NICU environment
   2. Use of prophylactic broad spectrum antibiotics

ii. Ischemia to the intestine
   a. Exaggerated inflammation and tissue injury leads to intestinal necrosis

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**Figure 2:** Factors that predispose preterm infants to developing necrotizing enterocolitis

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E. Risk Factors

i. Prematurity (especially <32 weeks)
   a. Immature motility, digestion, absorption, immune defenses, and barrier function
   b. Genetic Variation
      1. Increased expression of bacterial receptor toll-like receptor 4 (TLR4) which predisposes the gut to a hyper-active state in response to gram-negative colonizing microorganisms.
      2. Activation of TLR4 can lead to enterocyte apoptosis, impaired mucosal healing and enhanced pro-inflammatory cytokine release.
      3. Higher serum levels of cytokines and chemokines have been observed in preterm infants with NEC.

ii. VLBW <1500 grams

iii. Bacterial colonization of the gut

iv. Formula feeding

v. Congenital malformations
   a. Gastroscisis
   b. Chromosomal abnormalities
   c. Congenital heart disease
F. Common Pathogens
   i. Bacterial Pathogens
      a. *Escherichia coli*
      b. *Klebsiella pneumoniae*
      c. *Proteus mirabilis*
      d. *Enterobacter cloacae*
      e. *Clostridium perfringens*
      f. *Pseudomonas aeruginosa*
   ii. Fungal Pathogens
      a. *Candida*

G. Clinical Presentation
   i. There is an inverse relationship between gestational age and the onset of NEC
      a. Severely premature (~27 weeks) ⇒ 4 weeks after birth
      b. Neonates born closer to term (~37 weeks) ⇒ within the 1st two weeks of life
   ii. Signs/Symptoms:
      a. Abdominal distension
      b. Feeding intolerance
         1. Gastric residuals
         2. Vomiting
      c. Bloody stools
      d. Lethargy
      e. Signs of sepsis including:
         1. Bradycardia
         2. Hypotension
         3. Temperature instability
         4. Apnea
   iii. Abdominal radiography
      a. Pneumatosis intestinalis
      b. Portal venous gas
      c. Pneumoperitoneum

*Figure 3:* Anteroposterior abdominal radiograph of a 27 day-old male with portal venous gas (top arrow) and pneumatosi (bottom arrow)
iv. Laboratory
   a. Neutropenia
   b. Thrombocytopenia
   c. Elevated C-reactive protein (CRP)
   d. Electrolyte imbalances
   e. Acidosis

H. Diagnosis

Table 1: Modified Bell Staging Criteria\textsuperscript{12}

<table>
<thead>
<tr>
<th>Stage</th>
<th>Classification</th>
<th>Systemic Signs</th>
<th>Intestinal Signs</th>
<th>Radiologic Signs</th>
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</thead>
<tbody>
<tr>
<td>IA</td>
<td>Suspected NEC</td>
<td>Apnea, bradycardia, lethargy, temperature instability</td>
<td>Gastric residuals, mild abdominal distension, emesis, positive guaiac fecal occult blood test (gFOBT)</td>
<td>Normal or intestinal dilation, mild ileus</td>
</tr>
<tr>
<td>IB</td>
<td>Suspected NEC</td>
<td>Same as IA</td>
<td>Bright red blood from rectum</td>
<td>Same as IA</td>
</tr>
<tr>
<td>IIA</td>
<td>Proven NEC: Mildly Ill</td>
<td>Same as IA</td>
<td>Same as above + absent bowel sounds, +/- abdominal tenderness</td>
<td>Intestinal dilation, ileus, pneumatosis intestinalis</td>
</tr>
<tr>
<td>IIB</td>
<td>Proven NEC: Moderately Ill</td>
<td>Same as IA + mild metabolic acidosis, mild thrombocytopenia</td>
<td>Same as above + abdominal tenderness, +/- abdominal cellulitis, right lower quadrant mass</td>
<td>Same as IIA + portal venous gas, +/- ascites</td>
</tr>
<tr>
<td>IIIA</td>
<td>Advanced NEC: Severely Ill – Bowel intact</td>
<td>Same as IIB + hypotension, combined respiratory and metabolic acidosis, disseminated intravascular coagulation, neutropenia</td>
<td>Same as above + signs of generalized perforititis, marked tenderness and distension of abdomen</td>
<td>Same as IIB + definite ascites</td>
</tr>
<tr>
<td>IIIB</td>
<td>Advanced NEC: Severely Ill – Bowel perforated</td>
<td>Same as IIIA</td>
<td>Same as above</td>
<td>Same as IIB + pneumoperitoneum</td>
</tr>
</tbody>
</table>

I. Treatment\textsuperscript{2,13}

i. Initiation of bowel rest
   a. Enteral feedings are stopped and patient is labeled “nothing by mouth” (NPO)
   b. Total parenteral nutrition is initiated
   c. Gastric decompression

ii. Broad spectrum antibiotics\textsuperscript{12}
   a. Required: gram positive, gram negative, and anaerobic coverage
   b. Additional: antifungal coverage
   c. More information on options and dosing for empiric antibiotics is located in the Appendix (Table 7 and 8)

iii. Fluid resuscitation and inotropic support

iv. Surgical intervention (if bowel perforation is present)
   a. Exploratory laparotomy
   b. Percutaneous drainage
   c. Bowel resection
J. Prevention
   i. Probiotics
      a. The use of probiotics has been studied in preterm, VLBW infants to decrease the risk of NEC in this population.
      b. A Cochrane review was conducted in 2014 that included 24 randomized and quasi-randomized control trials
         1. Participants included neonates <37 weeks of gestation and/or a birth weight <2500 grams
         2. The study found that probiotic supplementation significantly reduced the incidence of severe NEC (RR 0.43, 95% CI 0.33 to 0.56).
         3. Mortality was also significantly reduced (RR 0.65, 95% CI 0.52 to 0.81) when a combination probiotic is used.
      c. Although the evidence supports the use of probiotics, there is no consensus on the best strain, duration of therapy, or timing of initiation.

II. BACKGROUND ON PROBIOTICS

A. Definition: A dietary supplement that contain living organisms that are found in normal flora and has the potential to provide a health benefit to the host.

B. History of probiotics

Figure 4: A timeline highlighting the introduction of probiotics as a dietary supplement

i. Elie Metchnikoff theorized that the longevity of the Bulgarian peasant population was contributed to the consumption of yogurt containing lactic acid producing bacteria which he called “Bulgarian bacillus.”
ii. Ferdinand Vergin conceived the term “probiotic” in 1954 which comes from a Greek term that means “for life.”
iii. The World Health Organization (WHO) defined probiotics as “live organisms that when administered in adequate amounts, confer a health benefit to the host.”
iv. Following this report, the Food and Agriculture Organization (FAO) and WHO issued the “Guidelines for the Evaluation of Probiotics in Food.”
   In October 2013, an expert panel by the International Scientific Association for Probiotics and Prebiotics (ISAPP) met in London to discuss the appropriate use of the term “probiotic,” and published their conclusions in June 2014.
      a. Any claim beyond “contains probiotics” must provide substantial evidence
C. **Types of Probiotic Strains**

   i. Most common organisms used in probiotic:
      a. *Lactobacillus* species
         1. Gram-positive, non-spore forming, catalase-negative, facultative anaerobe
      b. *Bifidobacterium* species
         1. Gram-positive, non-spore forming, catalase-negative, anaerobe
      c. *Streptococcus* species
         1. Gram-positive, facultative anaerobe
      d. *Saccharomyces boulardii*
         1. Yeast

   ii. **Mechanism of action**
      a. The organisms used for probiotics ferment carbohydrates and produce lactic acid.
      b. Production of lactic acid lowers the intestinal pH and helps to suppress the growth of pathogenic bacteria.

**Table 2**: List of commonly used probiotics and their active ingredients

<table>
<thead>
<tr>
<th><strong>Product</strong></th>
<th><strong>Active Organism(s)</strong></th>
<th><strong>Dosing (based off of box)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Align®</strong></td>
<td><em>Bifidobacterium infantis</em></td>
<td>1 capsule (1 X10⁹ CFU)*</td>
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<tr>
<td></td>
<td><em>Bacillus coagulans, Lactospore</em></td>
<td>1 gummy (5 x 10⁸ CFU)**</td>
</tr>
<tr>
<td><strong>Florababy®</strong></td>
<td><em>Bifidobacterium breve,</em> <em>Bifidobacterium bifidum,</em> <em>Bifidobacterium infantis,</em> <em>Bifidobacterium longum,</em> <em>Lactobacillus rhamnosus</em></td>
<td>1 scoop = 1 gram (4 billion CFU)**</td>
</tr>
<tr>
<td><strong>Culturelle®</strong></td>
<td><em>Lactobacillus rhamnosus GG</em> (LGG)</td>
<td>1 tablet (5 billion CFU)**</td>
</tr>
<tr>
<td><strong>Florastor®</strong></td>
<td><em>Saccharomyces boulardii</em></td>
<td>1 powder stick (5 billion CFU)***</td>
</tr>
<tr>
<td><strong>Primadophilus®</strong></td>
<td><em>Lactobacillus plantarum,</em> <em>Lactobacillus acidophilus,</em> <em>Bifidobacterium bifidum,</em> <em>Bifidobacterium lactis</em></td>
<td>1 chewable tablet (3 billion CFU)**</td>
</tr>
</tbody>
</table>
D. Other Trending Indications for Probiotic Use in the Pediatric Population\textsuperscript{15}

i. Acute infectious diarrhea
   a. Strain dependent: *LGG* the most effective
   b. Evidence does not report routine use for prevention

ii. Antibiotic associated diarrhea
   a. Evidence for pretention but not treatment
   b. RCT have used: *LGG*, *Bifidobacterium lactis*, *Streptococcus thermophilus*, *Saccharomyces boulardii*

iii. Atopic diseases
   a. Children with atopic disease have been shown to have more *Clostridium* and fewer *Bifidobacterium* organisms.
   b. Hypothesis for use in at risk infants

iv. Chronic inflammatory bowel disease
   a. Routinely used as adjunctive therapy, but not recommended for long-term use in children.

E. Regulation of Probiotic Manufacturing\textsuperscript{15}

i. In the United States, products marketed as dietary supplements (i.e. probiotics) do not require premarket review and approval by the US Food and Drug Administration (FDA).

ii. Probiotics or prebiotics marketed specifically for the treatment or prevention of a disease are classified as biological products and require FDA review and approval.

iii. Infant formulas must be made in compliance with good manufacturing practices under the Infant Formula Act of 1980.
   a. Probiotics and prebiotics added to commercial infant formulas are classified as generally regarded as safe (GRAS).

F. Risks of probiotic use in neonates\textsuperscript{20}

i. Increased risk of translocation of intestinal microbes into lymphatic/systemic circulation leading to sepsis
   a. At risk: immunocompromised patients such as ill preterm neonates and/or children with intravenous catheters or indwelling medical devices

ii. Possible contamination of commercially available products
   a. In 2015, three lots of Solgar's ABC Dolophilus Powder were found to be contaminated with *Rizopus oryzae* resulting in an infant death from mucormycosis after administration of the probiotic product in the NICU.\textsuperscript{24}

iii. Variation between lots of a given product

iv. Cross-contamination in the NICU
   a. Contaminated surfaces or by hands of the caregiver and/or healthcare worker

G. Reasoning for Probiotic Use in neonates

i. Probiotics can help to establish normal nonpathogenic flora in the preterm infant’s gut\textsuperscript{25}
III. LITERATURE REVIEW

A. Clinical Question: Is the use of daily probiotic supplementation in preterm infants safe and beneficial to prevent the incidence of necrotizing enterocolitis?

B. Studies in preterm infants to be reviewed:
   i. Jacobs SE, et al. 2013
   iii. Robertson, et al. 2019
   iv. Additional studies summarized in the Appendix (Table 9)

Table 3: Jacobs SE, et al. Probiotic effects on late-onset sepsis in very preterm infants: a randomized controlled trial. 2013

<table>
<thead>
<tr>
<th>Study Overview</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Objective</strong></td>
</tr>
<tr>
<td><strong>Study Design</strong></td>
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<table>
<thead>
<tr>
<th>Selection and Enrollment</th>
<th><strong>Inclusion:</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• Infants born &lt;32 weeks gestation</td>
</tr>
<tr>
<td></td>
<td>• Birth weight &lt;1500 grams</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Exclusion:</strong></th>
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</thead>
<tbody>
<tr>
<td>• Major cogenital abnormality</td>
</tr>
<tr>
<td>• Chromosomal abnormality</td>
</tr>
<tr>
<td>• Mother taking nondietary probiotic supplements</td>
</tr>
<tr>
<td>• If death was considered likely within 72 hours of birth</td>
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<table>
<thead>
<tr>
<th><strong>Intervention</strong></th>
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<tbody>
<tr>
<td>Probiotic combination (Bifidobacterium infantis, Bifidobacterium lactis, and Streptococcus thermophilus – containing 1 x10^8 total organisms per 1.5g) vs. placebo</td>
</tr>
<tr>
<td>• Administered only if infant was receiving at least 1ml Q4H of milk, and withheld when not feeding</td>
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<tr>
<td>• 3mL of milk = 1.5g (2mL) of powder mixed into breast milk/formula</td>
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<tr>
<th><strong>Outcomes</strong></th>
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<tbody>
<tr>
<td><strong>Primary:</strong></td>
</tr>
<tr>
<td>• Incidence of at least 1 episode of definite late-onset sepsis before 40 weeks postmenstrual age or discharge</td>
</tr>
</tbody>
</table>

| **Secondary:** |
| • Incidence of NEC (stage 2+) |
| • Mortality |
| • Definite sepsis with a probiotic species |
| • Duration of primary hospitalization |

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<tr>
<th><strong>Statistical Analysis</strong></th>
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<tbody>
<tr>
<td>• Intention-to-treat</td>
</tr>
<tr>
<td>• Subgroup analyses within gestation age and birth weight stratification</td>
</tr>
<tr>
<td>• Differences assessed using Chi square analysis</td>
</tr>
<tr>
<td>• 1100 infants were required to have at least 80% power to detect a 7% difference with a 0.05 2-sided significance level</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Baseline Characteristics</strong></th>
<th><strong>Characteristic</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Probiotic Group</strong> N= 548</td>
<td>Gestational age &lt;28 weeks 40%</td>
</tr>
<tr>
<td></td>
<td>Birth Weight &lt;1000 g 42.9%</td>
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<tr>
<td></td>
<td>Male 49.6%</td>
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<tr>
<td></td>
<td>5 min Apgar score, median 8</td>
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<td></td>
<td>Multiple birth 35.9%</td>
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<tr>
<td></td>
<td>Antenatal steroid 81.6%</td>
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<td></td>
<td>Maternal antibiotics 47.8%</td>
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<td></td>
<td>Maternal infection 8.6%</td>
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<tr>
<td><strong>Control Group</strong> N = 551</td>
<td>Gestational age &lt;28 weeks 42.8%</td>
</tr>
<tr>
<td></td>
<td>Birth Weight &lt;1000 g 43.4%</td>
</tr>
<tr>
<td></td>
<td>Male 54.4%</td>
</tr>
<tr>
<td></td>
<td>5 min Apgar score, median 8</td>
</tr>
<tr>
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<td>Multiple birth 35%</td>
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<tr>
<td></td>
<td>Antenatal steroid 90.7%</td>
</tr>
<tr>
<td></td>
<td>Maternal antibiotics 49%</td>
</tr>
<tr>
<td></td>
<td>Maternal infection 8.7%</td>
</tr>
<tr>
<td>Age at enrollment, d</td>
<td>2</td>
</tr>
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<td>---------------------</td>
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<tr>
<td>Age at commenced study power, d</td>
<td>5</td>
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</tbody>
</table>

### Results

#### Primary Outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Probiotic Group N= 548</th>
<th>Control Group N= 551</th>
<th>RR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definite late-onset sepsis</td>
<td>72 (13.1)</td>
<td>89 (16.2)</td>
<td>0.81 (0.61 to 1.08)</td>
<td>0.16</td>
</tr>
</tbody>
</table>

#### Secondary Outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Probiotic Group N= 548</th>
<th>Control Group N= 551</th>
<th>RR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEC (stage 2 or more)</td>
<td>11 (2.0)</td>
<td>24 (4.4)</td>
<td>0.46 (0.23 to 0.93)</td>
<td>0.03</td>
</tr>
<tr>
<td>Mortality</td>
<td>27 (4.9)</td>
<td>28 (5.1)</td>
<td>0.97 (0.58 to 1.62)</td>
<td>0.91</td>
</tr>
<tr>
<td>Sepsis with a probiotic species</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Length of primary hospital admission, d, median</td>
<td>71</td>
<td>74</td>
<td></td>
<td>0.09</td>
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### Discussion and Conclusions

**Author’s Conclusions**
- Probiotics did not significantly effect definite late-onset sepsis or all-cause mortality.
- The rate of NEC (stage 2 or more) was significant reduced by 50%.

**Strengths and Limitations**
- **Strengths:**
  - The study included a large population size with reduced bias due to double-blinded, randomized placebo.
  - The target population were preterm infants with low birth weights who are the most at risk for incidence of NEC.
- **Limitations:**
  - The primary outcome was to evaluate the effect of probiotics on late-onset sepsis and the rate of NEC was a secondary outcome
  - The baseline rate of NEC was low, and therefore the number needed to treat was 333 infants.
  - The trial took place in Australia and New Zealand, and may not be applicable to the demographics in the United States.

**Reader’s Conclusions**
- The use of the 3 strain combination probiotic significantly reduced the incidence of stage 2 or greater NEC in preterm infants with low birth weights. There was no incidence of sepsis caused by a probiotic bacterial species which provides evidence of decreased risk of bacterial translocation. Further studies are needed to assess the incidence of NEC as a primary outcome.

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<td><strong>Intervention</strong></td>
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<td><strong>Outcomes</strong></td>
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<td><strong>Statistical Analysis</strong></td>
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**Results**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Probiotic Group N= 654</th>
<th>Control Group N= 661</th>
<th>aORR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEC (stage 2 or more)</td>
<td>61 (9%)</td>
<td>66 (10%)</td>
<td>0.93 (0.68 to 1.27)</td>
</tr>
<tr>
<td>Sepsis</td>
<td>73 (11%)</td>
<td>77 (12%)</td>
<td>0.97 (0.73 to 1.29)</td>
</tr>
<tr>
<td>Mortality</td>
<td>54 (8%)</td>
<td>56 (9%)</td>
<td>0.93 (0.67 to 1.30)</td>
</tr>
</tbody>
</table>
### Secondary Outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Probiotic Group N= 654</th>
<th>Control Group N= 661</th>
<th>aORR (99% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite of primary outcomes</td>
<td>143 (22%)</td>
<td>147 (22%)</td>
<td>0.99 (0.79 to 1.25)</td>
</tr>
<tr>
<td>Positive blood culture (skin commensal)</td>
<td>141 (22%)</td>
<td>161 (24%)</td>
<td>0.88 (0.69 to 1.13)</td>
</tr>
<tr>
<td>B breve isolated from a sterile site</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Stool cultures containing B breve (2 weeks postnatal)</td>
<td>436 (74%)</td>
<td>122 (21%)</td>
<td>3.51 (2.83 to 4.34)</td>
</tr>
<tr>
<td>Stool cultures containing B breve (36 weeks postmenstrual)</td>
<td>438 (84%)</td>
<td>253 (49%)</td>
<td>1.69 (1.50 to 1.91)</td>
</tr>
</tbody>
</table>

### Discussion and Conclusions

**Author’s Conclusions**

There was no evidence of benefit for the use of *B breve* in the reducing the incidence of NEC or mortality for preterm infants.

**Strengths and Limitations**

**Strengths:**
- The study included a large population size with reduced bias due to double-blinded, randomized placebo.
- There was sufficient statistical power to provide evidence.
- The study focused on a single strain of probiotic and looked at a specific duration.

**Limitations:**
- There was no restriction on birth weight.
- The trial took place in England and may not be applicable to the demographics in the United States.
- High colonization rate of the placebo group masked any benefit of the probiotic intervention.

**Reader’s Conclusions**

The incidence of NEC when using a single strain probiotic, *B breve*, did not significantly differ from the placebo group.

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**Table 5: Robertson, C. Incidence of necrotising enterocolitis before and after introducing routine prophylactic Lactobacillus and Bifidobacterium probiotics. 2019**

### Study Overview

**Objective**

To compare the rates of NEC, late-onset sepsis, and mortality pre and post implementation of routine daily probiotics administered to high-risk neonates.

**Study Design**

- Single Center, retrospective cohort study
- N= 982 at a level 3 NICU at the Norfolk and Norwich University Hospital in the UK
- 10-year study period
  - January 1, 2008 to December 31, 2012 = pre-probiotic group
  - January 1, 2013 to December 31, 2017 = post-probiotic group

**Selection and Enrollment**

**Inclusion:**
- Gestational age <32 weeks
- Gestational age 32 to 36 weeks and birth weight of <1500 grams
- Babies transferred into the NICU within 72 hours of birth that met criteria.

**Exclusion:**
- Transferred babies with abdominal concern present at referral.
**Intervention**

Daily probiotic:
- Jan 2013 to April 2016: Infioran dual species probiotic (*Lactobacillus acidophilus* and *Bifidobacterium bifidum*) ½ capsule twice daily
- After April 2016: Labinic Drops triple species probiotic (*Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and *Bifidobacterium infantis*) four drops daily

**Outcomes**

Primary:
- Incidence of NEC (Stage 2+)
- Incidence of late-onset sepsis

Secondary:
- Mortality

**Statistical Analysis**

Time-to-event
- Controlling for confounding variables
- Sub-hazard ratios (sub-HR)

**Baseline Characteristics**

- Baseline characteristics well matched except “NSAID treatment for PDA”
  - Indomethacin 8% vs 0%
  - Ibuprofen 2% vs 5%
- Median for birth weight matched (1100 grams)

**Results**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Pre Probiotic N= 469</th>
<th>Post Probiotic N= 513</th>
<th>Sub-HR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of NEC</td>
<td>35 (7.5%)</td>
<td>16 (3.1%)</td>
<td>0.44 (0.23 to 0.85)</td>
<td>0.014</td>
</tr>
<tr>
<td>Late-onset sepsis</td>
<td>106 (22.6%)</td>
<td>59 (11.5%)</td>
<td>0.74 (0.49 to 1.12)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mortality</td>
<td>67 (14.3%)</td>
<td>47 (9.2%)</td>
<td></td>
<td>0.155</td>
</tr>
</tbody>
</table>

**Safety Outcomes**

No reports of sepsis due to *Lactobacillus* or *Bifidobacterium*

**Discussion and Conclusions**

The introduction of routine probiotics in neonates with risk factors for NEC resulted in rates of NEC and late-onset sepsis significantly decreasing. However, there was no significant difference in mortality between the two groups.
Strengths and Limitations

Strengths:
- The target population were preterm infants with low birth weights who are the most at risk for incidence of NEC.

Limitations:
- It is a retrospective and observational study.
- The trial took place in the UK, and may not be applicable to the demographics in the United States.

Reader’s Conclusions

The incidence of NEC in preterm infants at high risk for NEC was significantly reduced and shows promising safety results of no sepsis resulting from *Lactobacillus* or *Bifidobacterium*.

Table 6: Summary of Evidence

<table>
<thead>
<tr>
<th>Study</th>
<th>Efficacy Results</th>
<th>Safety Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jacobs SE, et al. 2013.</td>
<td>A combination probiotic consisting of <em>Bifidobacterium</em> and <em>Lactobacillus</em> species significantly reduced the incidence of NEC (Stage 2+) compared to placebo in preterm, low birth weight infants (p=0.03). However, incidence of NEC was not the primary outcome of the study.</td>
<td>No reports of sepsis caused by a probiotic bacterial species.</td>
</tr>
<tr>
<td>Costeloe K, et al. 2016.</td>
<td>A single strain probiotic (BBG-001) did not provide benefit to reduce the incidence of NEC in preterm infants.</td>
<td>No reports of <em>B breve</em> growing from normally sterile sites. Increased colonization of <em>B breve</em> found in stools.</td>
</tr>
<tr>
<td>Robertson C, et al. 2019.</td>
<td>The introduction of a daily combination probiotic consisting of <em>Bifidobacterium</em> and <em>Lactobacillus</em> species reduced the incidence of NEC (Stage 2+) in preterm, low birth weight infants.</td>
<td>No reports of sepsis resulting in positive blood cultures with <em>Lactobacillus</em> or <em>Bifidobacterium</em> species as the causative organism.</td>
</tr>
</tbody>
</table>

D. Conclusions

i. Population for daily probiotic use
   a. Infants at risk of developing NEC
      1. Infants born at <32 weeks gestation admitted into the NICU and/or
      2. Infants weighing <1500 g
   b. Exclusion:
      1. Major congenital abnormality
      2. Poor prognosis following birth
      3. Severely immunocompromised

ii. Type: Combination probiotic consisting of *Bifidobacterium* and *Lactobacillus* species

iii. Duration: Within 48 hours after birth and continued until 36 weeks old or discharge if sooner

E. Future Directions

i. More randomized control trials are needed in order to assess for a specific strain and amount of probiotic, timing of initiation, and duration of therapy.

ii. More studies on if monitoring is necessary including stool samples and/or samples from sterile site for probiotic species.
### IV. APPENDICES

#### Appendix A

| Table 7: Common Antibiotic Regimens used for Empiric Coverage in NEC<sup>18</sup> |
|---------------------------------|--------------------------------------------------|
| **Option 1:**                   | Ampicillin + gentamicin + metronidazole          |
| **Option 2:**                   | Ampicillin + cefotaxime + metronidazole          |
| **Option 3:**                   | Meropenem                                        |
| **Option 4:**                   | Vancomycin (substituted for ampicillin if MRSA suspected) |

**Duration:** 7-14 days

#### Appendix B

| Table 8: Antibiotic Dosing in Neonates<sup>28</sup> |
|---------------------------------|--------------------------------------------------|

**Abbreviation Chart:**
- GA = Gestational Age
- PNA = Postnatal Age
- BW = Body Weight

**Antibiotic** | **Dosing** |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ampicillin</strong></td>
<td>GA &lt;34 weeks</td>
</tr>
<tr>
<td></td>
<td>- PNA ≤7 days: 50 mg/kg/dose Q12H</td>
</tr>
<tr>
<td></td>
<td>- PNA 8 to 28 days: 75 mg/kg/dose Q12H</td>
</tr>
<tr>
<td></td>
<td>GA &gt;34 weeks</td>
</tr>
<tr>
<td></td>
<td>- PNA &lt;28 days: 50 mg/kg/dose Q8H</td>
</tr>
<tr>
<td><strong>Gentamicin</strong></td>
<td>GA &lt;30 weeks</td>
</tr>
<tr>
<td></td>
<td>- PA ≤14 days: 5 mg/kg/dose Q48H</td>
</tr>
<tr>
<td></td>
<td>- PA ≥15 days: 5 mg/kg/dose Q36H</td>
</tr>
<tr>
<td></td>
<td>GA 30 to 34 weeks</td>
</tr>
<tr>
<td></td>
<td>- PA ≤14 days: 5 mg/kg/dose Q36H</td>
</tr>
<tr>
<td></td>
<td>- PA 15 to 60 days: 5 mg/kg/dose Q24 to 36H</td>
</tr>
<tr>
<td></td>
<td>GA &gt;35 weeks</td>
</tr>
<tr>
<td></td>
<td>- PA ≤7 days: 4 mg/kg/dose Q24H</td>
</tr>
<tr>
<td></td>
<td>- PA 8 to 60 days: 5 mg/kg/dose Q24H</td>
</tr>
<tr>
<td><strong>Metronidazole</strong></td>
<td>BW ≤2 kg</td>
</tr>
<tr>
<td></td>
<td>- PNA ≤28 days: 7.5 mg/kg/dose Q12H</td>
</tr>
<tr>
<td></td>
<td>- PNA 29 to 60 days: 10 mg/kg/dose Q8H</td>
</tr>
<tr>
<td></td>
<td>BW &gt;2kg</td>
</tr>
<tr>
<td></td>
<td>- PNA ≤7 days: 7.5 mg/kg/dose Q8H</td>
</tr>
<tr>
<td></td>
<td>- PNA 8 to 60 days: 10 mg/kg/dose Q8H</td>
</tr>
<tr>
<td><strong>Cefotaxime</strong></td>
<td>GA &lt;32 weeks</td>
</tr>
<tr>
<td></td>
<td>- PNA ≤14 days: 50 mg/kg/dose Q12H</td>
</tr>
<tr>
<td></td>
<td>- PNA 14 to 28 days: 50 mg/kg/dose Q8H</td>
</tr>
<tr>
<td></td>
<td>GA ≥32 weeks</td>
</tr>
<tr>
<td></td>
<td>- PNA ≤7 days: 50 mg/kg/dose Q12H</td>
</tr>
<tr>
<td></td>
<td>- PNA 8 to 28 days: 50 mg/kg/dose Q8H</td>
</tr>
<tr>
<td><strong>Meropenem</strong></td>
<td>GA &lt;32 weeks</td>
</tr>
</tbody>
</table>
## Appendix C

### Table 9: Summarization of Supplementary Studies on Probiotic Use in NEC²⁹,³⁰

<table>
<thead>
<tr>
<th>Study</th>
<th>Method</th>
<th>Population</th>
<th>Primary Outcomes</th>
<th>Author’s Conclusion</th>
</tr>
</thead>
</table>
| Lin, 2005, et al. | RCT | VLBW infants | Incidence of death or NEC (≥ stage 2) | • Incidence of NEC was significantly lower in the study group. (1.1% vs 5.3%)  
• Number needed to treat (NEC) = 27  
• Higher incidence of severe NEC in the control group (6 cases vs. 0 cases)  
• No incidence of positive blood cultures growing the probiotic species. |
| Singh, 2018, et al. | Retrospective cohort study | Infants with gestational age <29 weeks | Rates of NEC, late-onset sepsis, and mortality. | • Prophylactic probiotics were associated with a reduction of NEC and mortality in preterm infants.  
  o Incidence of NEC, aOR 0.64, 95% CI 0.41 to 0.996  
  o Mortality, aOR 0.41, 95% CI 0.26 to 0.63  
• There was no significant difference between groups for rates of LOS> |

**RCT = Randomized Control Trial, LOS = late-onset sepsis**

Vancomycin

| BW <1.2 kg | PNA <28 days: 15 mg/kg/dose Q18-24H |
| BW 1.2 to 2 kg | PNA <7 days: 10 to 15 mg/kg/dose Q12-18H  
PNA ≥7 days: 10 to 15 mg/kg/dose Q8-12H |
| BW >2 kg | PNA <7 days: 10 to 15 mg/kg/dose Q8-12H  
PNA ≥7 days: 10 to 15 mg/kg/dose Q6-8H |

*PNA <14 days: 20 mg/kg/dose Q12H  
PNA ≥14: 20 mg/kg/dose Q8H  
GA ≥32 weeks |

<table>
<thead>
<tr>
<th>Study</th>
<th>Method</th>
<th>Population</th>
<th>Primary Outcomes</th>
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  o Mortality, aOR 0.41, 95% CI 0.26 to 0.63  
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**RCT = Randomized Control Trial, LOS = late-onset sepsis**

Vancomycin

| BW <1.2 kg | PNA <28 days: 15 mg/kg/dose Q18-24H |
| BW 1.2 to 2 kg | PNA <7 days: 10 to 15 mg/kg/dose Q12-18H  
PNA ≥7 days: 10 to 15 mg/kg/dose Q8-12H |
| BW >2 kg | PNA <7 days: 10 to 15 mg/kg/dose Q8-12H  
PNA ≥7 days: 10 to 15 mg/kg/dose Q6-8H |

*PNA <14 days: 20 mg/kg/dose Q12H  
PNA ≥14: 20 mg/kg/dose Q8H  
GA ≥32 weeks |

Vancomycin

| BW <1.2 kg | PNA <28 days: 15 mg/kg/dose Q18-24H |
| BW 1.2 to 2 kg | PNA <7 days: 10 to 15 mg/kg/dose Q12-18H  
PNA ≥7 days: 10 to 15 mg/kg/dose Q8-12H |
| BW >2 kg | PNA <7 days: 10 to 15 mg/kg/dose Q8-12H  
PNA ≥7 days: 10 to 15 mg/kg/dose Q6-8H |

*PNA <14 days: 20 mg/kg/dose Q12H  
PNA ≥14: 20 mg/kg/dose Q8H  
GA ≥32 weeks |


