Probiotics in the NICU

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Conflicts of Interest

I receive speaker honoraria from Prolacta Bioscience.
To review recent efficacy and safety data on probiotic use for very low birth-weight (VLBW) infants.
• Normal/premature flora
• Probiotic review
• Probiotics and feeding tolerance
• Lactoferrin study
• Cochrane 2014 review
• Limitations of probiotics in the US
• Future considerations/directions
• Over 1000 different species identified
• GI Flora functions
  – Digestion
  – Nutrient absorption
  – Gut associated lymphoid tissue (GALT)
  – Neuroimmune interaction
• Not sterile at birth
• Quickly colonized after delivery

Indrio 2011; Gritz 2015; Guarner 2003; Morowitz 2010
Gut Flora of Premature Infants

- Delivery mode
- Environment
- Intestinal immaturity
- Delay in enteral feeding initiation
- Frequent broad-spectrum antibiotic use
- Infection control procedures
- Pasteurization of breast milk
  - Banked breast milk
  - Used in Dani 2002 study (Italy)

- Decreased diversity
- Increased pathogenicity

Gritz 2015; Morowitz 2010; Schwiertz 2003
<table>
<thead>
<tr>
<th>Breast Milk</th>
<th>Formula</th>
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<tbody>
<tr>
<td>• <em>Bifidobacterium</em> predominant</td>
<td>• More diverse species, less</td>
</tr>
<tr>
<td>• Some <em>Lactobacillus</em></td>
<td><em>Bifidobacterium</em></td>
</tr>
<tr>
<td>• Human milk oligosaccharides (HMOs)</td>
<td>• <em>Enterobacteria</em></td>
</tr>
<tr>
<td></td>
<td>• <em>Clostridium</em></td>
</tr>
<tr>
<td></td>
<td>• <em>Bacteroides</em></td>
</tr>
</tbody>
</table>
Probiotics

• Bacteria or yeast added to the GI tract as a supplement to provide potential benefit to the host
• Usually live bacteria
• ~1 billion colony forming units (CFUs) per strain per dose
• May be given alone or in conjunction with other probiotics

Deshpande 2011
GI Function Enhanced by Probiotics

- **Metabolic**
  - Fermentation of carbohydrates (colon)
  - Vitamin production and metabolism
- **Trophic**
  - Epithelial cell production and differentiation
  - Immune system development and homeostasis
  - Modification of host response to microbial products
  - Competitive inhibition of pathogens
- **Protective**
  - Protection against pathogens
  - Prevention of bacterial translocation

Guarner 2003

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Probiotics in Exclusive Human Milk Diet

- Lin 2005
  - 367 infants <1500g
  - *L. acidophilus, B. infantis*
  - Decreased mortality, NEC, sepsis

- Manzoni 2006
  - 80 infants <1500g
  - *Lactobacillus GG*
  - Increased colonization with LGG
  - No change in sepsis or NEC
Lactoferrin

- Iron-carrying compound abundant in breast milk (20% of protein content)
- Bacteriocidal/bacteriostatic and antiviral to most pathogenic organisms
- Maintains probiotic flora such as *Bifidus* and *Lactobacillus*
- Resistant to most proteolytic enzymes
- Anti-inflammatory
- Immune system development
- Facilitates adaptation of infant intestine

Duffy 2000; Lonnerdal 2014; Siqueiros-Cendon 2014; Sherman 2014
Potential Risks of Probiotics

- Crossover colonization
- Systemic infections
- Deleterious metabolic activities
- Excessive immune system stimulation
- Antibiotic resistance
  - Some probiotics contain antibiotic-resistant genes
  - European guidelines prohibit antibiotic resistance in probiotics

Doran 2015; Kitajima 1997; Sharma 2014
• 25 RCTs → 19 included in analysis; n=5895
• <37 weeks or <2500g BW
• Primary aim: Time to full enteral feeding
  – Subgroup analysis
    • Bifidobacterium v. non Bifidobacterium strains
    • Single v multiple strain usage
    • Early (<72h) or late (>72h) initiation
• Secondary aims
  – Time to regain BW
  – Number of feeding intolerance episodes
  – Length of stay
• Significant decreased time to full enteral feeding
• Significant improvement noted for:
  – *Bifidobacterium* and non-*Bifidobacterium* strains
  – Single or multiple strains used
  – Early and late initiation
• Significantly decreased LOS, feeding intolerance
• Improved weight gain and growth velocity
• Double blind, placebo-controlled RCT
• 11 Italian NICUs
• 472 VLBW infants
• 3 groups: Bovine lactoferrin (BLF) ± Lactobacillus rhamnosus GG (LGG) vs. placebo for 30 days
• Primary aim: late onset sepsis
• Outcome: Significantly less bacterial and fungal sepsis in both BLF (5.9%) and BLF +LGG (4.6%) groups as compared to placebo (17.3%)
• Primary endpoints:
  – Efficacy and safety of prophylactic enteral probiotic administration vs placebo or no treatment
  – Prevention of severe (stage II or greater) NEC, sepsis or both in preterm infants

• Secondary endpoints: subgroup analysis
  – VLBW <1500g; ELBW <1000g
  – According to species, time of initiation, duration of probiotic administration
• Significant decrease in **severe NEC** (stage II-III)
  – Lactobacillus or mixture of probiotics only
  – RR 0.43
• Significant decrease in **mortality**
  – Mixture of probiotics only
  – RR 0.65; NEC-related mortality 0.39

• Significant decrease in length of stay by 3-4 days
• Decreasing trend, but no significant change in sepsis
• Lactobacillus ± bifidus for ≥ 7 days shown to be effective in reduction of NEC or mortality
• Duration: at least 6 weeks or until discharge
• Inadequate data for BW <1000g
Cochrane 2014
BW <1000g

- 2 studies included only ELBW infants
- No significant reduction in NEC, sepsis, or mortality
- Inadequate data to recommend probiotics for this group
Limitations

- Highly variable regimens
- Baseline NEC rate in control groups
- Timing, dose and formulation of probiotics
- Feeding regimens
- Type of milk given
Organizational Recommendations

• ESPGHAN 2010: Inadequate evidence
• ASPEN 2012: Insufficient data
• AAP 2010: Insufficient data
• Denmark guidelines for infants <30w GA

Agostoni 2010; Fallon 2012; Thomas 2010
Questions

- Adequacy of evidence
- Dose
- Patient safety
- Safe supply
## Comparison of Cochrane Analyses

<table>
<thead>
<tr>
<th>Topic</th>
<th>Year</th>
<th># of studies</th>
<th># of infants</th>
<th>Mortality RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probiotics for prevention of necrotizing enterocolitis in preterm infants</td>
<td>2014</td>
<td>17</td>
<td>5112</td>
<td>0.65</td>
</tr>
<tr>
<td>Animal-derived surfactant treatment of RDS</td>
<td>2009</td>
<td>13</td>
<td>1611</td>
<td>0.68</td>
</tr>
<tr>
<td>Cooling for newborns with HIE</td>
<td>2007</td>
<td>11</td>
<td>1468</td>
<td>0.75</td>
</tr>
<tr>
<td>Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth</td>
<td>2006</td>
<td>18</td>
<td>3956</td>
<td>0.77</td>
</tr>
</tbody>
</table>

AlFaleh 2014; Dalziel 2006; Jacobs 2008; Seger 2009; Janvier 2014; Deshpande 2011
Probiotics - Pooling Interventions

• “Effects are strain-specific and cannot be extrapolated to other strains of the same species” Guarner, 2003
  – Pooling interventions of the same class is common practice
  – Antibiotics, β-blockers, surfactants, immunoglobulins, hydrolyzed formulas, etc.
Dosing

- Appropriate dose needed to survive the GI tract and populate with appropriate levels in the intestine
- Minimum effective dose unknown
- EU guidelines $1 \times 10^9$ CFU/dose
- Deshpande et al. recommended $3 \times 10^9$ CFU (with half starting dose)
- Single v. multi strain

Deshpande, 2011; Indrio, 2011
**Patient Safety**

- **PROPATRIA trial**
- RCT probiotic prophylaxis for severe acute pancreatitis in adults
  - 24 died in probiotic group (16%), 9 in control group (6%)
    - 11 of the 24 had severe bowel ischemia
    - 4 had bowel necrosis
- Probiotics cannot be considered harmless
• *Lactobacillus* sepsis has been reported in infants, children, and adults
• Recent case series of 3 patients developing bacteremia or NEC from *B. longum*
• Separate case series of 2 patients resulting surgical NEC from *B. longum*
• 12-44% of non-treated patients in the unit may become colonized with probiotics (Kitajima 2012)

Bertelli 2015; Kitajima 1997; Gritz 2015; Zbinden 2015
# FDA Oversight

<table>
<thead>
<tr>
<th>Intention</th>
<th>Dietary supplement</th>
<th>Biotherapeutic product</th>
</tr>
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<tbody>
<tr>
<td>Product taken by mouth to supplement the diet</td>
<td>Intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease</td>
<td></td>
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</table>

**Premarket**
- Notify FDA of use
- Demonstrate safety if new ingredient since 1994
- Requires Investigational New Drug (IND) application
- Conduct studies proving safety and efficacy before marketing
- Demonstrate safety, purity, potency
- Inspection, receive license

**Postmarket**
- List ingredients on label*
- Follow Good Manufacturing Practices*
- Report adverse events*
- Ongoing inspections of Good Manufacturing Practices
- Reporting of adverse events

*Manufacturer responsible for self-reporting. [http://www.fda.gov/Food/DietarySupplements](http://www.fda.gov/Food/DietarySupplements); Vaillancourt 2005
• US 2005 - 5 samples analyzed
  – 2 of the 5 claimed to have *B. bifidum* but DNA was not found
  – *Lactobacillus* species not listed on the label was found in 2 samples
• Italy 2011 - 24 samples examined
  – 58% compliant
  – 42% did not contain labeled amount
  – 17% no identification of at least one strain
  – 2 had no identifiable DNA from any probiotics listed
• US 2014 - 5 mass-market US probiotics were analyzed
  – Species and colony counts appropriate to label
ABC Dophilus

- Used in ProPrems trial
- On November 22, 2014, FDA found *Rhizopus oryzae* in unopened containers of ABC Dophilus (Mold contaminant)
- Preterm infant died of necrotic bowel with heavy fungal burden of *Rhizopus oryzae*.
- 12/9/14 FDA issued a letter to healthcare providers regarding the case
  - Suggested IND application for use
• Microbiology department to assess each container of probiotics for:
  – Taxonomy
  – Exclusion of contaminants
  – Colony counts in the reconstituted product
• Contaminant identification available
• Bifidobacterium media not commercially available
• Strain isolation and identification very problematic
• DNA sequencing most reliable, expensive

Deshpande 2011; Masco 2004; Toscano 2011
Concerns with Probiotics

• Several species involved in studies, all of which have diverse effects
• No optimal dose, dosing strategy
• Gut colonization is important, but no confirmation that active colonization is necessary for disease prevention
• Safety of available products cannot easily be confirmed
• Long-term effects unknown

Indrio et al, 2011
Summary and Future Considerations

• Probiotics are efficacious for preterm neonates
  – Reduced mortality
  – Reduced NEC
  – Reduced LOS
• There is no safe supply currently available in the US
• Standard hospital labs are not equipped to isolate and verify some typical strains of probiotics, only contaminants
• Lactoferrin may be a promising sterile synbiotic alternative, further study required