Routine probiotics for preterm neonates for prevention of Necrotizing Enterocolitis (NEC)

Dr Girish Deshpande, FRACP
Senior Neonatologist, Department of Neonatology
Nepean Hospital Sydney
Senior Lecturer, University of Sydney
Disclosures

- Presentation involves comments or discussion of unapproved or off-label, experimental or investigational use of **probiotics**

<table>
<thead>
<tr>
<th>Affiliation / Financial Interest</th>
<th>Organization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speaker support</td>
<td>International scientific association of Probiotics and Prebiotics (ISAPP) – 2012 and 2014</td>
</tr>
</tbody>
</table>
Distance: 10,394 miles, > diameter of earth 7926 miles
• Concise review of clinical evidence supporting routine probiotics
  • RCTs - Updated meta-analysis of RCTs, long term follow-up
• Evidence based guidelines to use probiotics and pathway to introduce probiotics
• Routine use of probiotics observational studies and the meta-analysis
• Impact of intervention
Necrotising enterocolitis (NEC)

• Commonest GI emergency of unknown cause
• Incidence
  • 5-6% in <32 weeks & VLBW
  • 9-10% in <28 weeks
• Mortality
  • (20-40%), higher in <1000 grams
• Morbidity – cerebral palsy
• Expensive
  • $100,000 to $250,000/NEC case
  • USA – $1 billion/year

Bisquera JA, Pediatrics 2002
Neu NEJM 2011
Pathophysiology of NEC

Prematurity

Immaturity of intestinal:
- Motility and digestion
- Circulatory regulation
- Barrier function
- Immune defense

Genetic predisposition?

NEC

Abnormal bacterial colonization

Feeding

Lin P et al, Semin Perinatol 2008
• 29 premature neonates <30 weeks

Claude EC 2001
Probiotics for prevention of NEC

Possible mechanisms

• Appropriate bacterial colonization
• Improve gut barrier function
  • Tight junction protein, Mucous secretion
• Reduced TLR-4 mediated inflammation
• Regulation of NFκB signalling
• Improved gut motility
• Paneth cells-antimicrobial peptides

Patole AJOG 2014
<table>
<thead>
<tr>
<th>Study</th>
<th>Probiotic agent/s</th>
<th>Dose (CFU)</th>
<th>Duration</th>
<th>Type of Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kitajima 1997</td>
<td>B Breve</td>
<td>0.5 X 10⁹</td>
<td>28 days</td>
<td>BM or FM</td>
</tr>
<tr>
<td>Dani 2002</td>
<td>LB- GG</td>
<td>6 X 10⁹</td>
<td>till discharge</td>
<td>BM or DM or FM</td>
</tr>
<tr>
<td>Costalos 2003</td>
<td>SB</td>
<td>10⁹ /kg BD</td>
<td>30 days</td>
<td>FM</td>
</tr>
<tr>
<td>Bin Nun 2005</td>
<td>BI, BB, ST</td>
<td>1.05 X 10⁹</td>
<td>36 weeks corrected</td>
<td>BM or FM</td>
</tr>
<tr>
<td>Lin 2005</td>
<td>LB-A, BI</td>
<td>2 X 10⁷</td>
<td>till discharge</td>
<td>BM or DM</td>
</tr>
<tr>
<td>Manzoni 2006</td>
<td>LB-Casei</td>
<td>6 X 10⁹</td>
<td>till discharge</td>
<td>BM or DM</td>
</tr>
<tr>
<td>Mohan 2006</td>
<td>BB-Lactis</td>
<td>1.6 X 10⁹</td>
<td>day 1 to day 3</td>
<td>FM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.8 X 10⁹</td>
<td>day 4 to day 21</td>
<td></td>
</tr>
<tr>
<td>Samanta 2008</td>
<td>LA, BI,BB,BL</td>
<td>2.5 X 10⁹</td>
<td>till discharge</td>
<td>BM or FM</td>
</tr>
<tr>
<td>Lin 2008</td>
<td>BB, LA</td>
<td>2 X 10⁹</td>
<td>for 6 weeks</td>
<td>BM or FM</td>
</tr>
</tbody>
</table>
Systematic reviews of probiotics for prevention of NEC
Probiotics for prevention of necrotising enterocolitis in preterm neonates with very low birthweight: a systematic review of randomised controlled trials

Girish Deshpande, Shripada Rao, Sanjay Patole


7 trials, n=1393

<table>
<thead>
<tr>
<th>Outcome</th>
<th>RR</th>
<th>95% CI</th>
<th>p-values</th>
<th>NNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definite NEC</td>
<td>0.36</td>
<td>0.20-0.65</td>
<td>p&lt;0.00001</td>
<td>25</td>
</tr>
<tr>
<td>Mortality</td>
<td>0.47</td>
<td>0.30-0.73</td>
<td>p&lt;0.00001</td>
<td>20</td>
</tr>
<tr>
<td>Sepsis</td>
<td>0.94</td>
<td>0.74-1.20</td>
<td>p=0.80</td>
<td></td>
</tr>
</tbody>
</table>
Conclusive meta-analysis

Updated Meta-analysis of Probiotics for Preventing Necrotizing Enterocolitis in Preterm Neonates
Girish Deshpande, Shripada Rao, Sanjay Patole and Max Bulsara
Pediatrics published online Apr 19, 2010;
DOI: 10.1542/peds.2009-1301
Methods

- Standard Cochrane Methodology for systematic review was followed

Inclusion Criteria:

- RCT involving preterm neonates (gestation <34 weeks and birth weight <1500 grams) reporting on ≥ stage II NEC (Modified Bell staging)
- Oral probiotic/s commenced within the first 10 days of life and continued for at least 7 days
Results (11 trials n=2176)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>RR</th>
<th>95% CI</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definite NEC</td>
<td>0.35</td>
<td>0.23-0.55</td>
<td>p&lt;0.00001</td>
</tr>
<tr>
<td>Mortality</td>
<td>0.42</td>
<td>0.29-0.62</td>
<td>p&lt;0.00001</td>
</tr>
<tr>
<td>Sepsis</td>
<td>0.98</td>
<td>0.81-1.10</td>
<td>p=0.80</td>
</tr>
</tbody>
</table>

Trial sequential analysis

**A**: Alpha 0.05 and power 80%

**B**: Alpha 0.01 and power 80%

FIGURE 6
Trial sequential analysis.
Conclusion (Pediatrics 2010)

- It is unlikely that ongoing RCTs will change the results of meta-analysis.
- Probiotics should be offered routinely provided safe and effective product is available.
- A RCT of 2000 neonates and a baseline incidence of 8% would have to show a doubling of the incidence of NEC to overturn the benefits shown in the trials completed to date. Such a reversal of effects has never been demonstrated in clinical medicine.

Barrington KJ, Arch Dis Child Educ Pract Ed 2011
Proprems RCT  *(Jacobs et al, Pediatrics 2013)*

- Preterm neonates <32 wks and <1500g (n=1099)
- ABC acidophilus (BI, ST, BL) \(1 \times 10^9\) CFU

Results:
- Primary outcome: No difference in LOS
- Secondary outcomes
  - NEC (probiotics 4.4% vs. placebo 2.0%, \(p=0.03\))
  - No differences in mortality
- Breast feeding: 96.9%
Pips/Cochrane 2014

PiPS - Probiotics in preterm babies trial (UK):

- **N=1300**, Gestation <31 weeks, < 48 hours old, *B breve BBG*
- Primary outcomes: NEC, LOS, Death (Power: 90%, 40% RRD)
- BBG-01
- Completed 2013, results awaited

Cochrane updated meta-analysis 2014 *(Al-Faleh 2014)*

- 24 RCT n=5529
- Similar results (reduction in NEC, mortality)
- Supports change in practice
Deshpande 2014

- 27 RCTs n=6000 (part A/B)
- Part A: nutritional benefits of probiotics

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Probiotics</th>
<th>No Probiotics</th>
<th>Mean Difference IV, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Total</td>
</tr>
<tr>
<td>Bin Nun 2005</td>
<td>14.6</td>
<td>8.7</td>
<td>72</td>
</tr>
<tr>
<td>Braga 2010</td>
<td>15.2</td>
<td>5.2</td>
<td>119</td>
</tr>
<tr>
<td>Demirel and Erdeve 2013</td>
<td>11.7</td>
<td>4.74</td>
<td>135</td>
</tr>
<tr>
<td>Fernandez-Carrocera 2013</td>
<td>23</td>
<td>16.3</td>
<td>75</td>
</tr>
<tr>
<td>Havranek 2013</td>
<td>23.9</td>
<td>8.3</td>
<td>15</td>
</tr>
<tr>
<td>Hikaru 2010</td>
<td>13.9</td>
<td>6.89</td>
<td>108</td>
</tr>
<tr>
<td>Jacobs 2013</td>
<td>12.25</td>
<td>2.02</td>
<td>548</td>
</tr>
<tr>
<td>Kikajima 1997</td>
<td>17</td>
<td>8.4</td>
<td>45</td>
</tr>
<tr>
<td>Lin 2008</td>
<td>29.8</td>
<td>19.7</td>
<td>217</td>
</tr>
<tr>
<td>Manzoni 2006</td>
<td>15</td>
<td>8</td>
<td>39</td>
</tr>
<tr>
<td>Mihatsch 2010</td>
<td>17.9</td>
<td>6.8</td>
<td>91</td>
</tr>
<tr>
<td>Oncel 2013</td>
<td>9.1</td>
<td>3.2</td>
<td>200</td>
</tr>
<tr>
<td>Patole 2014</td>
<td>16.29</td>
<td>11.8</td>
<td>77</td>
</tr>
<tr>
<td>Rouge 2009</td>
<td>16</td>
<td>23.3</td>
<td>45</td>
</tr>
<tr>
<td>Roy 2014</td>
<td>11.22</td>
<td>5.04</td>
<td>56</td>
</tr>
<tr>
<td>Samanta 2008</td>
<td>13.76</td>
<td>2.28</td>
<td>91</td>
</tr>
<tr>
<td>Sari 2011</td>
<td>17.3</td>
<td>8.7</td>
<td>110</td>
</tr>
<tr>
<td>Serce 2013</td>
<td>11.9</td>
<td>7</td>
<td>104</td>
</tr>
<tr>
<td>Totsu 2014</td>
<td>11</td>
<td>3.6</td>
<td>119</td>
</tr>
<tr>
<td><strong>Subtotal (95% CI)</strong></td>
<td><strong>2266</strong></td>
<td><strong>2261</strong></td>
<td><strong>100.0%</strong></td>
</tr>
</tbody>
</table>

Heterogeneity: Tau² = 5.12; Chi² = 253.22, df = 18 (P < 0.00001); I² = 93%
Test for overall effect: Z = 2.48 (P = 0.01)

<table>
<thead>
<tr>
<th>Total (95% CI)</th>
<th><strong>2266</strong></th>
<th><strong>2261</strong></th>
<th><strong>100.0%</strong></th>
<th><strong>-1.54 [-2.75, -0.32]</strong></th>
</tr>
</thead>
</table>

Heterogeneity: Tau² = 5.12; Chi² = 253.22, df = 18 (P < 0.00001); I² = 93%
Test for overall effect: Z = 2.48 (P = 0.01)
Test for subgroup differences: Not applicable
Barriers preventing clinicians to use of probiotics routinely

- Regulatory approval: food vs. drug?
- Probiotic product availability
- Suitability of product in high risk preterm neonates
- Quality control and safety
- Dose, dilution and protocol
- Long term data
Safety: Long term outcomes

- Prospective follow up of preterm neonates (<32 weeks) in the RCTs of oral probiotics) for prevention of NEC
- Neurological and sensory performance BDSD-II at 24 months corrected age: No significant differences.


Improved neurological outcomes: RCT

- 249 preterm (<37 weeks) VLBW neonates randomised to: L. reuteri (n=83), L. rhamnosus (n=83) and No probiotic (n=83)
- Probiotic treated neonates had a significantly lower incidence of abnormal neurological outcome at 12 months

  Romeo et al, 2010
Routine probiotics for preterm neonates: experience in a tertiary Australian neonatal intensive care unit

Deshpande G, Shingde V, Downe L, Brandenburg U, Kumar K, Leroi M, Xiao J

Department of Neonatal Paediatrics, Nepean Hospital Sydney, University of Sydney, Department of Microbiology, Nepean Hospital Sydney, Department of Pharmacy Nepean Hospital Sydney
Which Probiotic?
Probiotics quality !!

- Tested 14 products by T-RFLP assays
- Only 1/14 products contained the exact species stated on the label
- 8/14 products contained all specified species plus additional unspecified/bonus organisms
- 5/14 products had at least one species missing

Marcobal et al. JPGN 2008
Probiotic sepsis in neonates

- Kunz et al. Two cases of lactobacillus *bacteremia* during probiotic treatment of short gut syndrome. *JPGN* 2004
- Land et al. Lactobacillus *sepsis* associated with probiotic therapy. *Pediatrics* 2005
Access to Unapproved Therapeutic Goods

- Authorised Prescribers
Access to unregistered drugs

- Special Access Scheme (SAS Category A or Category B)
- Clinical trials (CTN or CTX)
- Authorised prescriber pathway
Selecting the optimal product

- Single strain Lactobacillus

- Single strain Bifidobacterium
Combinations (Lacto and Bifido)

- 7 trials N=1558
- NEC: RR: 0.30, 95% CI 0.17-0.512, \( p<0.0001 \)
Stepwise approach at Nepean

1. Develop evidence based guidelines to use routine probiotics
2. Identifying a suitable probiotic product
3. Approval of selected probiotic product (overseas) from local area drug and therapeutics committee.
4. Endorsement from local Ethics Committee and prepare parent information sheet
5. Authorised Prescriber application to TGA, Australia
6. Approval from authorities regarding importing probiotic product from overseas
7. Independent quality assessment of the product
8. Develop the practical protocol for routine use
9. Perform prospective audit after introducing routine probiotics for prevention of NEC and monitor safety
Evidence-based guidelines for use of probiotics in preterm neonates

Girish C Deshpande\textsuperscript{1,2}, Shripada C Rao\textsuperscript{3,4,5}, Anthony D Keil\textsuperscript{3,6} and Sanjay K Patole\textsuperscript{3,5*}

\textit{Deshpande et al, BMC Medicine 2011:92}
Table 3 Specific recommendations for major clinical decisions

<table>
<thead>
<tr>
<th>Specific recommendations</th>
<th>LOE[^a][reference]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selection of strains</td>
<td>Combination containing <em>Lactobacillus</em> and at least one <em>Bifidobacterium</em> species is preferable. <em>Lactobacillus GG</em> alone may not be effective.</td>
</tr>
<tr>
<td>Dose</td>
<td>$3 \times 10^9$ organisms per day, preferably in a single dose</td>
</tr>
<tr>
<td>When to start?</td>
<td>When the neonate is ready for enteral feeds, preferably within first 7 days of life</td>
</tr>
<tr>
<td>How long to continue?</td>
<td>At least until 35 weeks corrected age, or discharge</td>
</tr>
<tr>
<td>Supplementation during acute illness</td>
<td>Stopping the supplementation during an acute illness such as sepsis, NEC[^b] or perinatal asphyxia may be safe.</td>
</tr>
</tbody>
</table>

[^a]: Level of evidence.
[^b]: Necrotising enterocolitis.
Nepean Sydney protocol

Preterm neonates <32W and <1500g

Infloran

- L. Acidophilus, B. Bifidum, 250mg capsule (2 X 10⁹ CFU)
- Laboratorio Farmaceutico SIT and Desmapharma, Italy, Switzerland

Dose/Duration

- 1 capsule dissolved in 2ml of milk (2 X 10⁹ CFU)
- 1ml daily till baby is on 2ml 2hrly feeds, Later 2ml once daily up to 34 weeks corrected age

Cost

- $20 to $50/baby

Use

- Australia/NZ, Singapore, Malaysia, Netherlands, Germany etc.
Demographic Data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Probiotics cohort n=146 (Jan 2012-Jan 2013)</th>
<th>Controls n=144 (Jan 2010-Dec 2011)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA (weeks)</td>
<td>28.2 (24 - 32)</td>
<td>28.2 , (23.4 – 32)</td>
</tr>
<tr>
<td>Bwt</td>
<td>1.110 (290.2) (400-1790)</td>
<td>1088.2 (260.5), (565-1500)</td>
</tr>
<tr>
<td>Sex (Male/Female)</td>
<td>33/47</td>
<td>61/81</td>
</tr>
<tr>
<td>ANS (complete course)</td>
<td>86 (58%)</td>
<td>84 (59.%)</td>
</tr>
<tr>
<td>C. Section</td>
<td>59 (73.5%)</td>
<td>90 (63.3%)</td>
</tr>
<tr>
<td>PDA requiring treatment</td>
<td>21 (27.9%)</td>
<td>48 (33.7%)</td>
</tr>
<tr>
<td>Breast milk</td>
<td>94%</td>
<td>94.3%</td>
</tr>
</tbody>
</table>

Two protocol violation- GA<32 weeks and BW1790g
Preterm – meningitis commenced at 4 weeks of life
Key outcomes

<table>
<thead>
<tr>
<th>Variable</th>
<th>Probiotics n=146 (Jan 2012-Jan 2014)</th>
<th>Controls n=142 (Jan 2010-Dec 2011)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEC (≥Stage II)</td>
<td>1 (0.06%)</td>
<td>8 (5.5%)</td>
<td>0.01</td>
</tr>
<tr>
<td>NEC (≥Stage II) BW&lt;1000g</td>
<td>0/61</td>
<td>6/65 (9.2%)</td>
<td>0.02</td>
</tr>
<tr>
<td>LOS - Proven</td>
<td>16 (10.8%)</td>
<td>34 (23.6%)</td>
<td>0.004</td>
</tr>
<tr>
<td>Death (&gt;7days)</td>
<td>4</td>
<td>5</td>
<td>0.49</td>
</tr>
<tr>
<td>TFF 150ml/kg*</td>
<td>13.7(6.3) (n=129)</td>
<td>16.43(8.62) (n=124)</td>
<td>-2.73 days (-4.88 to -0.58), p=0.02</td>
</tr>
</tbody>
</table>

Deshpande et al, Psanz 2013, submitted for publication
Probiotics for prevention of NEC in preterm neonates - systematic review of observational review (Routine probiotic use)

Deshpande G, Balegar K, Rao S and Patole S
Nepean Hospital, Sydney Medical School, Nepean, University of Sydney
Centre for newborn research and education, School of Paediatrics and child health, University of Western Australia
KEM Hospital for Women and
Princess Margaret Hospital for Children, Perth, WA
Methods

- The guidelines for reporting of the Meta-analysis of Observational studies in Epidemiology Group were followed
- Standard search strategy of Cochrane review group
- Inclusion criteria: observational studies with retrospective cohort
  - Preterm neonates <32 weeks and <1500g
  - NEC> stage II by modified by Bell stage
  - Routine probiotics
<table>
<thead>
<tr>
<th>Study</th>
<th>Location</th>
<th>Probiotic cohort</th>
<th>Probiotic product</th>
<th>Historical controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bonsante 2012</td>
<td>France</td>
<td>GA 24-31 weeks Year 2008-2011</td>
<td>Lcr Restituo 1 X 10⁸ CFU BD till 36 weeks</td>
<td>GA 24-31 weeks Year 2003-2008</td>
</tr>
<tr>
<td>Deshpande 2014</td>
<td>Australia</td>
<td>GA &lt;32 w and &lt;1500g Year 2011-2012</td>
<td>Infloran (LA, BI) 2 X 10⁹ till 34 weeks</td>
<td>GA &lt;32w and &lt;1500 Year 2009-2010</td>
</tr>
<tr>
<td>D Li 2013</td>
<td>USA</td>
<td>BW &lt;1500g Year 2008-2011</td>
<td>ST, BBB, BI till 36 weeks</td>
<td>BW &lt;1500 Year 2003-2007</td>
</tr>
<tr>
<td>Hunter 2012</td>
<td>USA</td>
<td>BW &lt;1000g Year 2009-2011</td>
<td>L Reut (1 X 10⁸ CFU) till discharge</td>
<td>BW &lt;1000g Year 2004-2009</td>
</tr>
<tr>
<td>Satoh 2007</td>
<td>Japan</td>
<td>BW &lt;1500g Year 1999-2003</td>
<td>BB (1 X 10⁹ CFU) till discharge</td>
<td>BW &lt;1500g Year 1994-1998</td>
</tr>
<tr>
<td>Barrington 2013</td>
<td>Canada</td>
<td>GA&lt;32W Year 2011-2012</td>
<td>Flora-baby 2 X 10⁹ till 34 weeks</td>
<td>GA&lt;32W Year 2010-2011</td>
</tr>
</tbody>
</table>

Deshpande et al 2013 (under review), PSANZ 2013
Stage $\geq$ II NEC

- **OR**: 0.31, 95% CI (0.15, 0.63) $p=0.001$
### All cause mortality

**OR:** 0.64, 95% CI (0.47, 0.89)  
*p*=0.007
Late onset sepsis

- **OR**: 0.67, 95% CI (0.51, 0.88) $p=0.004$
Probiotics for preterm neonates – current evidence for change of practice

- 27 RCTs (NEC, sepsis, feeding), N=6000
- 2 RCTs (Candida colonisation), N= 329
- 7 Reports on routine use, N= 4614
- Grand total: 11000
- No adverse events reported including probiotics induced sepsis
- Routine use: Finland, Italy, Japan

Recently completed RCTs with no safety concerns:
Pips (n=1300), PANTS (n=160) Total=1490

"Probiotics should be used routinely in preterm neonates"
Impact on health care

Possible *short term* cost savings (assuming a modest 50% reduction in NEC)

- Nepean Hospital, Sydney 1.1 million dollars/year
- 15 to 20 million dollars /year in Australia
- In billions if probiotics are offered routinely in preterm neonates worldwide
- Cost of probiotics $30 to $50 per baby

Just not about the money: difficult to put any cost for the emotional trauma of parents with a child who has a cerebral palsy after suffering from severe NEC
Improved Neonatal Outcomes With Probiotics

**Evidence is a spectrum.** The US Food and Drug Administration (FDA) Guidance for Industry\(^1\) states that “[w]ith regard to quantity, it has been FDA’s position that Congress generally intended to require at least two adequate and well-controlled studies, each convincing on its own, to establish effectiveness.” However, in practice, care is often changed if standard of care is limited otic products in the trials. As readers are aware, Cochrane reviews are very conservative with the wording of their conclusions and data interpretation; however, Alfaleh et al\(^6\) state that their “updated review of available evidence supports a change in practice.” It is difficult to find such a definitive conclusion statement in other contemporaneous Cochrane reviews.

Shane A, Deshpande G, Merenstein D.

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Role of ISAPP

- Consensus statement based on the current evidence
- Bridge the gap – regulatory issues etc.
The Nepean NICU team
NNICUPS Association Nepean
First baby in Australia to receive routine probiotics
Born at 26 weeks and birth weight 620 grams