Probiotics for Preterm Infants - The Western Australian Experience

Sanjay PATOLE
Australia
Probiotics for preterm infants - the Western Australian experience

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University of Western Australia, Perth
Australian & New Zealand Neonatal Network

- **ANZNN**: A collaborative network that monitors the care of high risk newborn infants to provide quality assurance.
- **28 Level III NICUs**: Australia: 22 + NZ: 6

**Criteria**: Gestation < 32 weeks *or* BW <1500 g *or* needed ventilation for at least 4 hours *or* died before 4 hours while receiving mechanical ventilation *or* major surgery *or* received therapeutic hypothermia
Australia New Zealand Neonatal Network

Royal Darwin
Townsville
Children’s at Westmead
Liverpool Nepean
Royal Hospital for Women
Royal North Shore
Sydney Children’s Westmead
RPA Women and Babies
Mater Mothers’ Royal Brisbane & Women’s
John Hunter Children’s
Canberra
King Edward Memorial and Princess Margaret
Women’s & Children’s Flinders Medical Centre
Mercy Women’s Monash Medical Centre
Royal Children’s Royal Women’s
Royal Hobart
Auckland City Waikato
Middlemore Wellington Women’s
Christchurch Women’s
Dunedin
Necrotising enterocolitis: ANZNN data

Year 2015

- Total 3,449 infants <32 weeks, including 2,856 <1,500 g
- Denominator: All infants born at the gestation concerned

Incidence of NEC ≥ Stage II: Year 2006-2015

- < 28 weeks: 6.6% to 10% (Average: 7.7%)
- 28-31 weeks: 1 to 2%
- ≥ 32 weeks: <1%

ANZNN Annual Report 2015
Probiotics for preterm infants in Australia

(1) Authorised Prescriber Pathway (TGA, Australia):
- Approval of the probiotic product from hospital’s drug and therapeutics committee
- Endorsement from hospital ethics committee
- TGA approval for the applicant to become an authorised prescriber for the specific probiotic product for the specific site for a specific period.
- Informed consent required from parents/guardian

(2) Special Access Scheme (TGA, Australia):
- Allows urgent import of any life saving drug for ‘a’ patient
Probiotics for preterm infants in Australia

- Significant reduction in ≥ Stage II NEC after introducing RPS for preterm neonates (0%, n=84 vs. 4.8%, n=144) in Nepean NICU, Sydney, Australia.
- Infloran 250 mg capsule (Laboratorio Farmaceutico Italy, Desma Pharma SIT, Switzerland) *L. acidophilus NCDO 1748; B. bifidum NCDO 1453*: 2 billion CFU/day start with the first feed and continue until 34 weeks’ CGA.
- Currently, 16/20 Australian tertiary NICUs offer RPS using a similar protocol.

Probiotics for preterm infants: Western Australia

- *B. breve* M16-V, Morinaga Milk Industries, Japan
- One gram sachets; stored at 18-20 degrees; shelf life 2 years
- 3 billion CFU once daily till 37 weeks’ CGA for infants <34 weeks
- Infants <28 weeks: Start with 1.5 billion CFU/day till milk feeding of 50 ml/kg/day, increase thereafter to 3 billion CFU/day
- Start ASAP after admission, preferably within 24-48 hours
- Stop probiotic during suspected/proven sepsis and/or NEC.
- On site microbiology back up.
Routine use of *B. breve* M-16 V: Perth data

- Data from 24 months 'before' vs. 24 months 'after' implementing routine suppl. with *B. breve* M-16V for preterm infants <34 weeks.
- **1755 infants (835 vs. 920)** with comparable gestation and BW.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>&lt;34 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEC ≥ Stage II</td>
<td>3% vs. 1%; aOR: 0.43 (0.21-0.87)</td>
</tr>
<tr>
<td>NEC ≥ Stage II or Mortality</td>
<td>9% vs. 5%; aOR: 0.53 (0.32-0.88)</td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>7% vs. 4%; aOR: 0.58 (0.31-1.06)</td>
</tr>
<tr>
<td>Late onset sepsis</td>
<td>14% vs. 9%; aOR: 0.57 (0.42-0.78)</td>
</tr>
<tr>
<td>Safety</td>
<td>No cases of probiotic sepsis</td>
</tr>
</tbody>
</table>
Benefits in extremely preterm infants

- Clinically important benefits in infants <28 weeks (n=250 vs. 220) but many did not reach statistical significance.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>&lt;28 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEC ≥ Stage II</td>
<td>6% vs. 3%; aOR: 0.51 (0.20-1.27)</td>
</tr>
<tr>
<td>NEC ≥ Stage II or Mortality</td>
<td>21% vs. 14%; aOR: 0.59 (0.29-1.18)</td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>17% vs. 11%; aOR: 0.63 (0.28-1.41)</td>
</tr>
<tr>
<td>Late onset sepsis</td>
<td>32% vs. 29%; aOR: 0.53 (0.35-0.82)</td>
</tr>
<tr>
<td>Safety</td>
<td>No cases of probiotic sepsis</td>
</tr>
</tbody>
</table>

Patole et al. PLoS One 2016 March
Strain-specific systematic review of *B. breve* M-16V

- 5 RCTs (n=482): Patole (n=159), Hikaru (n=208), *Li* (n=30), *Fuji* (n=19), *Wang* (n=66), *Ishizeki* (n=44; 3-strain product)

- *Patole et al* studied fecal bifidobacteria response after *B. breve* suppl. and was the only trial with low risk of bias (ROB).
- Other 4 RCTs including *Hikaru* had high/unclear ROB in many domains.
- Data from RCTs was inadequate to derive firm conclusions

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Studies</th>
<th>RR(95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEC ≥ Stage II</td>
<td>Patole</td>
<td>0.33 (0.01-7.95)</td>
<td>0.49</td>
</tr>
<tr>
<td>Late onset sepsis</td>
<td>Patole, Hikaru, Fuji</td>
<td>0.71(0.31-1.62)</td>
<td>0.42</td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>Patole, Hikaru</td>
<td>Low event rate</td>
<td>NA</td>
</tr>
<tr>
<td>Age at full feeds</td>
<td>Patole, Hikaru</td>
<td>-2.05 (-9.29- 5.18)</td>
<td>0.58</td>
</tr>
</tbody>
</table>

*Athalye-Jape et al. JPEN 2017 Aug*
Meta-analysis: Routine use of *B. breve* M-16V

- **4 observational studies (n=2496)** in preterm infants
- Significant benefits on routine use of *B. breve* M-16V

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Studies</th>
<th>OR (95% CI)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEC ≥ Stage II</td>
<td>2 (1 case)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Late onset sepsis</td>
<td>3 (n=2452)</td>
<td>0.56 (0.45-0.71)</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>2 (n=2319)</td>
<td>0.61 (0.44-0.84)</td>
<td>P = 0.002</td>
</tr>
<tr>
<td>Age at full feeds</td>
<td>2 (n=361)</td>
<td>-2.42 (-2.55, -2.3)</td>
<td>P &lt; 0.00001</td>
</tr>
<tr>
<td>Adverse effects</td>
<td>Nil</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

- **GRADE analysis**: Overall quality of evidence was very low.

*Athalye-Jape et al JPEN 2017 Aug*
Probiotics for preterm infants: New Zealand

- Retrospective cohort; Infants <32 weeks or <1500g
- LGG and 100mg lactoferrin daily (2011-2015 vs. 2004-2008)
- NEC reduced from 3% to 1%; RR: 0.29 (CI 0.1-0.9) NNT: 50.
- The cost of preventing one case of NEC: NZ $2800
- Late onset culture positive sepsis: No significant difference.
- LGG sepsis in a 23 week infant with abdominal pathology; one infant developed NEC after stopping prophylaxis.

Probiotics for preterm infants: New Zealand

- **Objective:** To study if RPS was associated with earlier removal of peripherally inserted central catheters (PICC) in extremely preterm (EP) infants born \( \leq 28 \) weeks' gestation.

- **Design:** Retrospective, cohort study; 2 years before vs. 2 years after introducing RPS with Infloran in a large tertiary NICU.

- **Primary outcome:** Age at removal of PICC in infants whose first PICC lines were inserted before day 14 and remained in-situ for at least 4 days was compared.

*Rajput et al. Turk J Pediatr 2017*
Results

- Mean (95% CI) age at PICC removal: 25.9 (22.6-29.2) vs. 23.1 (20.9-25.2) days.
- The result was independent of birth weight, gender, type of PICC line and age at insertion but related significantly to gestation at birth (p < 0.001).
- No difference in the incidence and microbiologic profile of PICC infections (120 vs 130 PICC).

Conclusion: PICC were removed 2.8 days earlier in infants receiving probiotics (p=0.070), a potentially beneficial outcome.

Rajput et al. Turk J Pediatr 2017
Probiotics for preterm infants - Global scenario

- Tertiary level NICUs in many countries are providing probiotic prophylaxis as a standard treatment for preterm VLBW infants
- Australia, Austria, Canada, Columbia, Denmark, Finland, France, Germany, Italy, Japan, Netherlands, New Zealand, Singapore, Spain, Switzerland, Taiwan
- Some units in UK, and USA (Dang, Hunter, Li, Kane)
- India: Many units are providing routine probiotic prophylaxis but no published data
Probiotics for preterm infants in UK

- Multicentre trial (PiPs) of *B. breve* BBG-001*
- 1310 infants, Median gestation 28 weeks, BW 1010g
- No benefit in NEC, late onset sepsis, all cause mortality
- Current scenario: No published data but probably <25% of big NICUs in UK offer probiotics for preterm VLBW infants.

“…PiPS dominates UK thinking; basically I am not convinced the ‘big data’ have enough detail yet.”

*Costeloe et al. Lancet 2016 Dec*
Probiotics for preterm infants in USA

- 6.7% of VLBW infants in the US were exposed to probiotics in 2014: Vermont Oxford Network (VON)

A phone survey of all NICUs in VON (May and September 2015)

- Response rate: 70.3% (500/711)
- 14.0% (70/500) of NICUs were using probiotics
- Routine suppl. to all VLBW infants in 8.8% (44/500) units
- Suppl. for selected VLBW infants in 5.2% (26/500) of units for feeding intolerance and antibiotic use.

Viswanathan et al. J Perinatol 2016 Dec
Probiotics for preterm infants in USA

- 16 commercial probiotics products were identified.
- Most commonly used products: Culturelle (27.1%), Biogaia (14.3%), Gerber Soothe (14.3%), and Florababy (8.6%).
- Literature search: Evidence for only 4/16 products (Culturelle, Align, Biogaia and ABC Dophilus) used.
- Only ABC Dophilus was reported to be protective against NEC, but was used sparingly (2.9% of NICUs).

**Conclusion:** No evidence for safety or efficacy of 90% of probiotics currently used in US NICUs; caution is warranted.

*Viswanathan et al. J Perinatol 2016 Dec*
Probiotics for preterm infants in USA

- The probiotics use in VLBW infants within the US is increasing, but is still limited.

- There was no evidence for safety or efficacy of 90% of the probiotics currently used in US NICUs; and therefore, caution is warranted.

Viswanathan et al. J Perinatol 2016 Dec
Probiotics for preterm infants in USA

- Incidence of NEC ‘before vs. after’ implementing routine supplementation with LGG was 10.2% vs. 16.8%

  Ken et al J Pediatr 2018 April

- “However, none of these trials were conducted in the USA.”

- “Additional clinical trials are needed to evaluate both safety and efficacy of the most commonly used probiotic agents for prevention of NEC in VLBW infants in the United States.”

  Heikamp J Pediatr April 2018
Low and middle income countries (LMIC)

- 23 (n=4783) RCTs from 4 continents and 10 LMICs included in the systematic review and meta-analysis.
- Significantly reduced risk of NEC, LOS, and all-cause mortality.

- **NEC ≥ Stage II**: RR: 0.46; (95% CI: 0.34 to 0.61), *p*<0.00001
- **LOS**: RR: 0.80; (95% CI: 0.71 to 0.91), *p*=0.0009
- **All-cause mortality**: RR 0.73 (95% CI: 0.59 to 0.90), *p*=0.003
- Results significant after excluding studies with high risk of bias.

*Deshpande et al. BMJ Open 2017 Dec*
Probiotics for preterm infants - controversies

The "Golden age" of probiotics in preterm infants

Dermyshi et al. Neonatology 2017 Feb

Why haven't the documented benefits of probiotics on preterm babies led to their wider acceptance and use?

Probiotics for preterm infants - controversies

1: Results of the PIPs trial from UK showed ‘no benefits’
2: Probiotic effects are ‘strain-specific’, so data from different strains should NOT be pooled in a meta-analysis.
3: No evidence to support probiotic use in ELBW infants
4: Most of the trials were conducted outside the USA
5: Probiotic sepsis and product contamination is a serious issue.
6: Clinically proven ‘drug’ quality products are not available
7: We don’t know how probiotics work
8: Probiotics are not needed in breast milk fed infants
9: Probiotics are not needed when the baseline risk of NEC is low
10: We don’t know the long-term safety of probiotics
The future of probiotics for preterm infants

- Well designed cluster RCTs and ‘Observational’ studies
- Head to head comparison of different probiotic strains and their combinations, research on synbiotics, lactoferrin, post-biotics
- Alternatives to live strains (e.g. Heat inactivated/killed strains)
- Need data from units providing probiotic supplementation as a standard practice for preterm VLBW infants

Whether we offer probiotics for research and/or routine use, we need a ‘good/drug quality’ product!!
Thanks for listening!
Strain selection - a pragmatic approach


- **L. reuteri DSM 19938**: Athalye-Jape et al. JPEN 2016 Aug


- **L. rhamnosus GG**:

- **B. breve BBG-001**: Kitajima et al (???) Costeloe et al)
Network meta-analysis ESPHAGN 2018

- Conventional meta-analyses don’t help in determining the most effective probiotic strain.
- A network meta-analysis using Bayesian hierarchical random effects models was used to identify strains with greatest efficacy.
- 51 RCTs involving 11,231 preterm infants were included.
- Most strains or their combinations were studied only in one or a few RCTs.

Network meta-analysis ESPHAGN 2018 Jan

- Only 3/25 studied probiotic combinations showed significant reduction in mortality, 7 reduced NEC, 2 reduced LOS, and 3 reduced TFEF.
- No clear overlap of strains which were effective on multiple outcome domains.

**Conclusion:** Further large and adequately powered RCTs using strains with the greatest apparent efficacy will be needed in order to more precisely define optimal treatment strategies.

Selecting a probiotic – a checklist for clinicians

- **P**: Evidence for being a ‘Probiotic’ strain?
- **R**: Resistance to antibiotics? Regulatory approval?
- **O**: Effective Dose? *(Duration?)*, Evidence? (RCT, Non-RCT)
- **B**: Bovine vs. Human derived?
- **I**: Indigenous vs. Imported?
- **O**: Osmotic load?
- **T**: Taxonomy? *(Strain details)*
- **I**: Indwelling tubes, catheters, lines? (ICU set up; Asepsis)
- **C**: CFU? Contents? Contamination?
- **S**: Storage, Stability, Shelf life, Safety?
Our research so far
Antimicrobial proteins and peptides (AMPP) and probiotics

- APP provide critical first line defence in neonates due to broad-spectrum antimicrobial and antifungal activity.
- APP are likely involved in regulating the local microbiome and immune homeostasis, a mechanism by which probiotics may reduce the risk of NEC and LOS.

- It is not clear if probiotics directly alter local or systemic APP expression in preterm infants, enhancing gut integrity and reducing colonisation by pathogens, and gut-associated sepsis.
- Limited studies have assessed APP expression in preterm infants and majority have focussed on faecal levels of APP.
**B. breve M-16V and AMPP in preterm infants**

- Levels of secreted *and inducible* APP studied in a prospective cohort of preterm (<30 weeks) infants with (n=13) or without (n=62) *B. breve M-16V* (*3 × 10⁹* cfu) during first month of life.

- **Plasma** (D1, 14, 28) and **stool** (before probiotic, D21) samples

- **APP:** Bactericidal/Permeability Inducing (BPI) protein, Human beta defensins (**HBD1, 2**), Lactoferrin (**Lf**), Human cathelicidin **LL-37**, Secretory phospholipase A2 (**SPLA2**)

- **Induced APP:** Whole blood cultured with *live S. epidermidis* or with agonists of TLR2, 6, 4.

Stool, plasma & stimulated APP levels changed significantly during 1st month. *B. breve* didn’t affect APP maturation except for transient rise in inducible BPI.

**Figure 1** APP levels (pg/mL) in stool of very preterm infants who did or did not receive probiotics. Data show levels of (A) HbD1, (B) HbD2, (C) LL-37, (D) lactoferrin(LF), (E) sPLA2 and (F) BPI in available stool samples from individual infants with (closed circles, n = 9) and without (open circles, n = 10) probiotic supplementation. Median is shown as bar.

*Strunk et al. Acta Pediatr 2017*
Human milk oligosaccharides (HMO) and probiotics

- HMO are abundant in human milk, but infants don’t produce glycosidases to digest HMO.
- HMO help in shaping gut microbiota as only few commensals produce transport molecules and glycosidases to ingest and digest HMO as food.
- Undigested HMO in stool and urine: Potential marker of dysbiosis.

- Only *B. longum subsp. infantis* can consume all HMO. *B. bifidum* digests some HMO and transports their select parts into cytoplasm for consumption.

- The capacity of *B. breve* strains to use HMO as an energy source varies.
- Need data on HMO and *B. breve M-16 V* as probiotic in very preterm infants.
HMO digestion by *B. breve* M-16 V in preterm infants

- 29 preterm infants, Gestation: 28 (23-32) weeks
- Suppl. with *B. breve* (1.5x10^9 followed by 3x10^9 cfu/day).
- Faeces, urine, milk samples: At start of probiotic and 3 weeks later.
- Fecal 16S rRNA analysis: Next-generation sequencing.

- **Infants** with <6% bifidobacteria in 2nd stool sample: *Non-responders*
- **Mothers** with a relative α(1,2) fucosylated abundance >6% in breast milk: *Phenotypic ‘secretors’*

*Underwood et al. JPGN 2017 Oct.*
Results

- Marked differences in microbial composition over time (A)
- Significant increase in Enterobacteriaceae over time
- Non-responders had significantly higher percentages of Enterobacteriaceae (P=0.001) and Clostridiaceae (P=0.02) in 2nd sample than responders (B).
- Maternal secretor status, delivery type, and antibiotic exposure did not differ between responder and non-responders (C).

As a selective consumer of HMOs. *B. breve* M-16V consumed lacto-N-tetraose (LNT), and 3’FL (3’-Fucosyllactose) but not 2’FL. Like other *B. breve* strains, it was able to utilize large but not small sialylated HMOs.

*Underwood et al. JPGN 2017 Oct.*
Results
Evidence ‘for’ probiotics in preterm infants
Systematic review of randomised trials of probiotics

- 42 RCTs (Probiotics: 5304 vs. Control: 5216), <37 weeks/<2.5 kg

<table>
<thead>
<tr>
<th>Outcome</th>
<th>RR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEC ≥ Stage II</td>
<td>0.53 (0.42-0.66); p &lt; 0.00001</td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>0.79 (0.68-0.93); p &lt; 0.003</td>
</tr>
<tr>
<td>Late onset sepsis</td>
<td>0.88 (0.77- 1.0); p &lt; 0.05</td>
</tr>
<tr>
<td>Time to full feeding</td>
<td>1.2 (-2.2, -0.1)</td>
</tr>
<tr>
<td>Safety</td>
<td>No probiotic sepsis reported</td>
</tr>
</tbody>
</table>

Sawh et al. PeerJ 2016
Systematic review of non-randomised studies

14 observational studies with 13,779 preterm infants

- Probiotics: 7,878 vs. Controls: 5,961
- NEC ≥ Stage II: OR: 0.51 (0.37-0.70)
- All-cause mortality: OR: 0.71 (0.62-0.81)
- Late onset sepsis: OR: 0.81 (0.69, 0.96)
- No significant benefits for these outcomes in ELBW infants

*Dermish et al. Neonatology 2017*
Routine use of probiotics: German network

- Retrospective analysis of NEO-KISS data (German surveillance system for LOS in VLBW infants)
- All units that implemented prophylactic probiotics (2004-2014) with ≥ 2 strains
- Interrupted time series analyses: 36 months before vs. 36 months after implementing probiotics
- 10,890 VLBW infants (including 4,683 ELBW) from 44 units
- Effects more pronounced in preterm ELBW infants

PIPS trial UK

- Multi-centre double blind randomised placebo controlled trial
- *B. breve BBG-001* (2.1 to $5.3 \times 10^8$ cfu daily)
- Gestation <31 weeks
- Randomised before 48 hrs.

**Primary outcomes:** NEC $\geq$ Bell Stage II, LOS, Death.
- ITT analysis adjusted for sex, gestation and randomisation within 24 hours and allowing for clustering of multiples.

*Costeloe et al. Lancet 2016 Feb*
PIPS results

- 1310 infants, Median gestation 28 weeks, BW 1010g
- Age starting intervention 44 hours, Colonisation: 85 vs 37%
- No probiotic related adverse events
- No benefits in ANY of the outcomes of interest

Conclusions

- *B. breve BBG-001* did not have any advantage
- Highlight need to assess the efficacy of different strains
- Challenges the validity of combining trials using different probiotic interventions in meta-analyses
Primary outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>B. breve (n=650) vs Placebo (n=660)</th>
<th>aRR(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEC ≥ Stage II</td>
<td>61 (9.4%) vs. 66 (10.0%)</td>
<td>0.93 (0.68 to 1.27)</td>
</tr>
<tr>
<td>Late onset sepsis</td>
<td>73 (11.2%) vs. 77 (11.7%)</td>
<td>0.97 (0.73 to 1.29)</td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>54 (8.3%) vs. 56 (8.5%)</td>
<td>0.93 (0.67 to 1.30)</td>
</tr>
</tbody>
</table>

Possible reasons for the inconclusive results:

- Ineffective strain
- Low dose
- Cross contamination
- Random variation

Note: Significant benefits for all outcomes in ‘colonised’ infants
Effect of different probiotic species on NEC

- Data from systematic review of randomised trials (n=42)
- NEC ≥ Stage II was significantly reduced by probiotics

- Lactobacillus species (8 trials): RR: 0.61 (0.40–0.95)
- Bifidobacterium species (6 trials): RR: 0.37 (0.14–0.97)
- Multispecies (i.e. ≥ 2: 18 trials): RR: 0.41 (0.29–0.56)

Sawh et al. PeerJ 2016
Gut dysbiosis precedes NEC in preterm infants

- Systematic review of studies comparing gut microbiome in preterm infants who developed NEC vs. controls.
- Culture-independent molecular techniques: 16S rRNA sequence data, $\alpha$ and $\beta$-diversity indices, microbial profiles.
- **Microbial dysbiosis preceding NEC**: Increased relative abundances of Proteobacteria and decreased relative abundances of Firmicutes and Bacteroidetes in preterm infants.
- Microbiome optimization may prevent NEC.

  *Pammi et al. Microbiome. 2017 Mar*
Gut dysbiosis precedes NEC in preterm infants

Pammi et al. Microbiome. 2017 March
Outcomes after *B. breve* vs. placebo supplementation

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Probiotic N=74</th>
<th>Placebo N=66</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary outcome</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>• Fecal <em>B. breve</em> (cfu/g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sample 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count (log10) Median (IQR)</td>
<td>BD (6.7–9.1)</td>
<td>BD (BD-8.0)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Colonised</td>
<td>29 (39%)#</td>
<td>2 (3%)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td><strong>Sample 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count (log10) Median (IQR)</td>
<td>8.6 (8.9–9.4)</td>
<td>BD (7.6–9.5)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Colonised</td>
<td>67 (91%)</td>
<td>25 (38%)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Difference (S2-S1) (log10)</td>
<td>3.1 (4.3–4.9)</td>
<td>0 (3.2–5.0)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td><strong>Secondary outcomes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• NEC ≥ Stage II</td>
<td>0</td>
<td>1</td>
<td>P=0.497</td>
</tr>
<tr>
<td>• Late onset sepsis</td>
<td>60 (78%)</td>
<td>64 (84%)</td>
<td>P= 0.465</td>
</tr>
<tr>
<td>• All-cause mortality</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

Patole et al. PLoS one 2014 Mar
Are probiotic effects strain-specific?

- Although data demonstrate strain-specific immunologic effects, a consistently decreased risk of NEC in trials using variable probiotic regimens suggests strain nonspecific protection.
  
  *Ganguly K, Walker WA, J Clin Gastroenterol 2011*

- There is broad consensus that strain specificity is important in probiotic research. However, clinical data to sustain this statement are limited.
  
  *Vandenplas Y, Veereman-Wauters G. JPGN 2012*
Are probiotic effects strain-specific?

- Numerous meta-analyses conclude, with some cautions, that “probiotics” (as a class) are beneficial. This suggests that many probiotic strains do share the same effects.

- Perhaps it is time to be open to the idea that there may be a spectrum of probiotic functions, some of which involve capabilities unique to only one or a few strains but others which are more general to larger groups of microbes.

*Saunders ME. California Dairy Research Foundation, 2013 Mar1*
Long term effects of probiotics

- Romeo et al. Role of probiotics in the prevention of the enteric colonization by Candida in preterm newborns: incidence of late-onset sepsis and neurological outcome. *J Perinatol* 2011
- Jacobs et al: ProPrems trial follow up. *JPCH Abstract*

*Keunen et al, and Partty et al, Ped Res 2015*
How probiotics work?

**Direct antimicrobial effects:**
- ‘Crowd out’ pathogens, Compete for elements (e.g. iron)
- Act as ‘decoy binding sites’
- Produce antibacterial products (e.g. bacteriocins) that inhibit other bacteria, hydrogen peroxide and organic acids.

**Enhance gut mucosal barrier:** IgA, Zonal proteins, Goblet cells

**Effects on mucosal immunity:** Nonspecific humoral immune responses, production of protective cytokines and induction of regulatory T cells, which have an anti-inflammatory effect.

*Robinson JL. Can J Infect Dis Med Microbiol 2015*
Evidence from experimental studies

- 158 premature Sprague-Dawley rats were enrolled.

- Probiotic strains *B. bifidum*, *B. longum*, *L. acidophilus*, *L. plantarum*, and *B. breve* were fed as a single strain or mixture with 2 or 3 strains for a total of 9 study groups

- Control groups received no probiotic supplement.

- *L. plantarum* alone (*P = 0.0026*) and *B. bifidum* with *B. longum* (*P = 0.0017*) were more effective in reducing NEC vs controls.

*Wu et al. JPGN 2013 Jul.*
Evidence from experimental studies

- All study groups except *B. breve* and *B. bifidum* with *B. breve* definitely prevented death compared with controls.
- *B. bifidum* and *B. longum* together had significantly lower mortality than the control group (*P* < 0.0001).
- Fecal E. coli and Klebsiella counts were decreased significantly in *B. bifidum*, *B. longum*, and *L. plantarum* group compared with other study and control groups after 36 hours.

*Wu et al. JPGN 2013 Jul.*
Probiotics for preventing NEC and mortality: An updated meta-analysis: 25 trials (n=7345)

**Multistrain probiotics:** Markedly reduced NEC [OR: 0.36; 95% CI: 0.24-0.53; P < .00001] and mortality [OR: 0.58; 95% CI: 0.43-0.79; p=.0006]

**Single strain probiotics:** Lactobacillus species had a borderline effect in reducing NEC (OR: 0.60; 95% CI: 0.36-1.0; p=.05), but not mortality.

- Bifidobacterium species (6 RCTs) and S. boulardii (3 RCTs) did not show reduction in NEC or mortality.

*Chang et al. PLoS One. 2017 Feb*
**B. bifidum in preterm infants - a cluster RCT**

- A double-blind cluster RCT conducted in 19 hospitals
- **10 hospitals**: *B. bifidum OLB6378* (1.25 x 10⁹ viable cells) started within 48 h of birth) and **9 hospitals**: Placebo
- **Primary outcome**: Postnatal day at which feeding exceeded 100 mL/kg/day. **Secondary outcomes** included morbidity and growth before discharge.
- **283 VLBW infants** (*Bifido: 153 vs. Placebo: 130*)

**B. bifidum OLB6378- a cluster RCT**

- Enteral feeding was established within 21 days after birth in 233 infants of which 119 received *B. bifidum* and 114 received placebo until reaching 2000 g.
- Enteral feeding was established significantly earlier in Bifido vs. Placebo group (11.0 ± 3.6 vs. 12.1 ± 3.8 days; P < 0.05).
- Growth during NICU stay was not different between groups.
- LOS was significantly lower in Bifido vs. Placebo group: 6/153 (3.9%) vs. 13/130 (10%); P < 0.05
- No differences in other adverse outcomes including mortality.

**B. animalis subsp. lactis in preterm VLBW infants- a systematic review (4 RCTs, n= 324)**

- *B. lactis* CNCM 1-3446 had no effect on NEC ≥ Stage II (RR: 0.53, 95% CI: 0.16-1.83), LOS (RR: 0.6, 95% CI: 0.07-5.2), and antibiotic use (RR: 0.67, 95% CI: 0.28-1.62).
- *B. lactis* suppl. has the potential to increase fecal bifidobacteria and reduce Enterobacteriaceae and Clostridium spp. counts.
- *B. lactis* can reduce stool pH and fecal calprotectin levels, increase fecal IgA and SCFA concentrations, and decrease intestinal permeability.

*Szajewska et al. JPGN 2010 Aug*
Term infants breast fed by secretor vs. non-secretor mothers

- Term infants breast-fed by ‘secretor’ mothers have higher numbers of fecal bifidobacteria than infants of ‘non-secretor’ mothers.

- *B. infantis* is the dominant Bifidobacterium in the feces of infants of ‘secretor’ mothers.

- *B. breve* was found in the feces of infants of both ‘secretor’ and ‘non-secretor’ mothers.

Table 1: Incidence of NEC, mortality and sepsis before probiotics

<table>
<thead>
<tr>
<th>Outcome</th>
<th>VLBW</th>
<th>ELBW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necrotising enterocolitis</td>
<td>2.5</td>
<td>4.6</td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>6.1</td>
<td>11.9</td>
</tr>
<tr>
<td>Late onset sepsis</td>
<td>15</td>
<td>24.2</td>
</tr>
</tbody>
</table>

Table 2: NEC, mortality and sepsis reduced after routine probiotics

<table>
<thead>
<tr>
<th>Outcome</th>
<th>VLBW</th>
<th>ELBW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necrotising enterocolitis</td>
<td>0.48 (0.39–0.62)</td>
<td>0.48 (0.36–0.64)</td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>0.60 (0.44–0.83)</td>
<td>0.59 (0.41–0.84)</td>
</tr>
<tr>
<td>Late onset sepsis</td>
<td>0.89 (0.81–0.98)</td>
<td>0.83 (0.71–0.94)</td>
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</table>