



Probiotics for prevention of necrotizing enterocolitis—where do we stand in 2017?

Joan L. Robinson

Department of Pediatrics, University of Alberta and Stollery Children's Hospital, Edmonton, Canada

Correspondence to: Joan L. Robinson. Department of Pediatrics, University of Alberta and Stollery Children's Hospital, 3-558 ECHA, 11405-87 Ave, Edmonton, AB T6G 1C9, Canada. Email: jr3@ualberta.ca.

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Necrotizing enterocolitis (NEC) affects primarily preterm infants and can lead to the devastating outcomes of intestinal failure secondary to resection of extensive necrotic bowel or death. A disordered intestinal microbiome is thought to be a risk factor for NEC. Probiotics are live microorganisms administered as food supplements or as pharmaceuticals in an attempt to improve human health. If the intestine of a preterm infant could be deliberately colonized with non-pathogenic organisms administered as a probiotic, the risk of NEC may decrease (1).

A 2014 review by the Cochrane Collaboration concluded that probiotics are useful for prevention of NEC when studied in infants with either gestational age (GA) <37 weeks or birth weight <2,500 grams or both (1). There was great heterogeneity in the 24 included randomized controlled trials (RCTs) in this review in terms of maximum GA or birth weight at enrollment, dose and type of probiotics administered (*Lactobacillus* species in 8, *Bifidobacterium* species in 5, *Saccharomyces boulardii* in 2, and a mixture of probiotics in 9 trials), the timing of initial administration of the probiotic (varying from the first day of life to the first week that the infant tolerated enteral feeds), and the total duration of probiotics (varying from 2 weeks to the entire hospital stay). Eleven of the 24 trials had a low risk of bias. Twenty trials reported the primary outcome of definite or advanced NEC in 5,229 infants, demonstrating a relative risk (RR) of 0.43 [95% confidence interval (CI) 0.33–0.56] in the probiotic group with 16 of the 20 trials showing a statistically significant decrease or a trend towards a reduction in NEC. I calculated the number needed to treat to prevent one case (NNTT) and it is 30.5.

The relative risk of all-cause mortality prior to hospital discharge was 0.65 (95% CI: 0.52–0.81) in the 17 studies that reported this outcome. There was no evidence for prevention of nosocomial sepsis, the third primary outcome analyzed. Safety concerns were not identified in any of the trials.

The increasing number of positive trials led authors from Canada (2) and the United States (3) to plead that “settled science” shows that routine use of this simple, safe and relatively inexpensive intervention should be adopted in all infants at high risk of NEC with a moratorium on further studies with a placebo group. However, there is still limited data in extremely low birth weight (ELBW) infants (defined as those with a birth weight less than 1000 grams). Only two of the 24 studies in the Cochrane review enrolled primarily ELBW infants. In these two trials of 1,200 ELBW infants, there was only a trend towards a decrease in the incidence of necrotizing enterocolitis [risk ratio 0.76 (95% CI: 0.37–1.58)] (4,5).

Moving ahead then to 2016 when the largest study to date was published with Costeloe *et al.* enrolling 1,315 infants less than 31 weeks GA (6). *Bifidobacterium breve* GGB-001 or placebo was started as soon as practical after birth. This appears to be a high quality study with a low risk of bias and a neutral funding source. Minor criticisms of the study are that the term “very preterm infants” in the title is ambiguous, blinding of the laboratory staff is not mentioned, and the method by which three clinicians reached consensus on the diagnosis of NEC is not explained. Outcomes were almost identical in the treatment and placebo groups for the incidence of NEC, all-cause

mortality before discharge and nosocomial sepsis. There was an apparent lack of efficacy for prevention of NEC irrespective of GA or birth weight.

The Costeloe study is the first RCT to report colonization rates with the probiotic strain in treatment and control groups over time. Colonization was documented by culture in 74% of study infants and 21% of controls at 2 weeks of age and in 84% of study infants and 49% of controls at 36 weeks postmenopausal age. Colonized control infants were detected at all 24 trial sites. There is unfortunately no colonization data collected from a site not involved in the study to determine whether infants who are not directly or indirectly exposed to *Bifidobacterium breve* in probiotics ever become colonized. However, assuming that such colonization is rare, the data would suggest that nosocomial spread of the probiotic strain is common, resulting in contamination of the control group and dilution of any treatment effect. Analysis of outcomes by *Bifidobacterium breve* colonization status at 2 weeks of age showed a trend towards a decrease in NEC (RR =0.68; 96% CI: 0.43–1.09) and a less impressive trend towards a decrease in all-cause mortality before discharge (RR =0.68; 95% CI =0.35–1.29) and in nosocomial sepsis (RR =0.88; 95% CI: 0.59–1.31), keeping in mind that the sample size was inadequate for these outcomes.

Colonization appeared to be more difficult to achieve at a lower GA [which may explain why results were less encouraging in ELBW infants in previous studies (4,5)] and in infants still on antibiotics after the fifth day of life. A prolonged course of antibiotics in the neonatal period no doubt alters the intestinal microbiome to a degree that is not readily overcome by probiotics. Even when colonization is achieved, it is not always sustained as 54 (63%) of infants in the study group with stools tested at both time points were colonized at 2 weeks but not at 6 weeks of age (7).

The incidence of NEC is postulated to be lower in infants fed human milk versus those fed formula, thought to be related to the presence of specific human milk components (8). It is not known whether donor breast milk is equivalent to mother's own breast milk or whether exclusive use of breast milk is necessary to attain this benefit (9). The Costeloe probiotic trial reported very similar types of feeds in cases and controls with about half of infants receiving only human milk for the first 14 days of life (6). However, use of formula was not analyzed as a confounding risk factor for NEC, possibly because the potential increased risk of NEC from formula was thought to be minimal at the time that the trial was designed in

2005 (7). Perhaps unexpectedly, colonization rates with the probiotic strain were lower with exclusive use of breast milk. The authors postulate that this is because formula can contain oligosaccharides that promote the growth of *Bifidobacterium* (6,7).

Probiotics are presumed to have to contain live organisms to confer maximal benefit. There is no practical means to determine whether organisms were always viable at the time of administration in any of the NEC studies. The Costeloe study report that the average number of viable organisms per dose in unused sachets fell from 9.2 to 8.2 log₁₀ CFU during the trial (6), with the latter still being presumably an adequate dose. However, they do not specify how many sachets were tested or whether any tested sachets contained no viable organisms. Procedures for storage of probiotics would presumably differ if they were used routinely in preterm infants rather than as a study drug which might alter the number of viable organisms in an unpredictable direction.

Addition of the results of the large Costeloe study to the Cochrane review changes the relative risk for prevention of NEC by probiotics to 0.58 (95% CI: 0.47–0.71). A 2017 meta-analysis that included the Costeloe study and several small RCTs published since the Cochrane review and that also considered observational studies yielded a very similar result to this revised Cochrane relative risk (10). Another 2017 meta-analysis suggested that probiotics with multiple strains were much more effective than single strains for prevention of NEC (11), which may explain the unexpected negative result from the Costeloe trial. Another possibility is that *Bifidobacterium* is not the ideal organism for prevention of NEC. In the Cochrane review, the incidence of NEC was 4/211 in infants given *Bifidobacterium* alone and 8/198 controls in four studies (with only one using *Bifidobacterium breve*) (1) but this is clearly an inadequate sample size to warrant comparison.

Beyond efficacy, other residual concerns about routine use of probiotics in infants at high risk for NEC include the following:

- (I) There is the potential for harm if almost all infants in a neonatal intensive care unit (NICU) are colonized with the same bacteria or fungus which then acquires genetic material that allows it to become more pathogenic or more antimicrobial-resistant;
- (II) There is a lack of certainty about the optimal probiotic product, regimen and duration of administration. Perhaps efficacy of a given probiotic

is not uniform in all geographic areas given the marked variation in the intestinal microbiome worldwide;

- (III) Although there have not been invasive infections with probiotic strains detected in any of the RCTs of probiotics in preterm infants, there are a small number of case reports with genomic typing confirmed that organisms in probiotics can cause invasive infections in this population (12);
- (IV) In most countries, probiotics are regulated as food additives rather than as drugs so there are lower standards for product manufacturing and uniformity. There is a case report of a fatal fungal infection in a preterm infant potentially linked to a probiotic (13).

I for one have changed my mind about the issue (14) and now think that there is a need for further placebo-controlled trials. The authors of the Costeloe trial call for cluster randomized trials in the future to overcome the apparent contamination of controls that occurred in their study (6,7). It seems counter-intuitive to call for a cluster randomized trial given that there are multiple RCTs and that the incidence of NEC seems to vary markedly over time in a single NICU, but the authors may be correct that it is the optimal trial design to overcome the contamination issue. There is also a place in the literature for further “before-and-after” studies from NICUs that have introduced routine use of probiotics in high risk infants (15), hopefully with no publication bias towards trials with positive results.

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