Probiotics for Preterm Neonates: What Will It Take to Change Clinical Practice?

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Key Words

Neonates · Practice · Probiotics · Routine

Abstract

Necrotizing enterocolitis (NEC) is a major cause of mortality (25%) and morbidity including recurrent sepsis, dependence on parenteral nutrition, need for surgery, and survival with short bowel syndrome in preterm very low birth weight infants. Mortality (45–100%) and morbidity including the risk of long-term neurodevelopmental impairment are higher in extremely preterm infants needing surgery for NEC. Systematic reviews of randomized controlled trials (RCT) indicate that probiotics significantly reduce the risk of NEC (RR 0.39; 95% CI 0.29–0.52; $p < 0.00001$) and all-cause mortality (RR 0.52; 95% CI 0.40–0.69; $p < 0.00001$) while facilitating enteral feeds in preterm infants. At present, data from 25 RCT (~5,000 neonates) and reports on routine use (~3,000 neonates) indicates that significant adverse effects of probiotics are rare. Despite the robust evidence, there is still reluctance in incorporating routine probiotic prophylaxis in clinical practice. If the goal is to have zero tolerance for NEC, then probiotic prophylaxis must be adopted as soon as possible. Current gaps in knowledge can be addressed by continued research while providing routine probiotic supplementation. We believe that the concept of evidence-based practice of medi-

cine has been stretched too far in this case. Trial sequential analysis has already shown that the evidence for probiotic supplementation was conclusive after 10 trials. Results of the ongoing trials are unlikely to change the conclusions of the systematic reviews significantly. Currently we are at trial number 25; how many more trials do we need? What will it take to change clinical practice?

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Necrotizing enterocolitis (NEC; $\geq$ stage II) is one of the major causes of mortality and morbidity in preterm infants. NEC affects about 6–7% of very low birth weight (VLBW; <1,500 g) infants, with mortality ~25% and morbidity that includes recurrent sepsis, complications of prolonged parenteral nutrition, the need for surgery, and survival with short bowel syndrome [1]. Mortality (45–100%), and importantly the risk of long-term neurodevelopmental impairment (NDI), is higher in extremely low birth weight (ELBW) infants needing surgery for the illness [1]. The economic burden of NEC is significant considering the prolonged hospital stay due to multiple complications. The USA alone is estimated to spend ~1 billion dollars per year for NEC, not accounting for the cost of caring for its survivors with NDI [1].

Table 1. Prophylactic interventions reducing the risk of NEC and death in preterm neonates

Intervention	First author (year)	Sample size, n	Study type	Effect size and significance
<i>NEC</i>				
Probiotics	Wang [2] (2012)	3,816	Systematic review of RCT	RR 0.33; 95% CI 0.24–0.46; $p < 0.00001$
Antenatal glucocorticoids	Roberts [28] (2010)	4,269	Systematic review of RCT	RR 0.46; 95% CI 0.29–0.74; $p = 0.0012$
Breast milk	McGuire [29] (2003)	343	Systematic review of RCT	RR 0.25; 95% CI 0.06–0.98; $p = 0.015$
Standardized feeding protocols	Patole [30] (2005)	453	Systematic review of observational studies	RR 0.13; 95% CI 0.03–0.5; $p = 0.03$
Restricted fluid intake	Bell [31] (2008)	526	Systematic review of RCT	RR 0.43; 95% CI 0.21–0.87; $p = 0.019$
Bovine lactoferrin + LGG (neonates < 1 kg)	Pammi [32] (2011)	472	Systematic review of RCT	RR 0.34; 95% CI 0.17–0.70; $p = 0.002$
<i>All-cause mortality</i>				
Probiotics	Wang [2] (2012)	3,816	Systematic review of RCT	RR 0.56; 95% CI 0.43–0.73; $p < 0.0001$
Antenatal glucocorticoids	Roberts [28] (2010)	4,269	Systematic review of RCT	RR 0.69; 95% CI 0.58–0.81; $p < 0.00001$
Vitamin A	Darlow [33] (2011)	1,391	Systematic review of RCT	RR 0.93; 95% CI 0.88–0.99; $p = 0.027$ (death/BPD)

RCT = Randomised controlled trial; RR = relative risk; CI = confidence interval; LGG = *Lactobacillus* GG.

Probiotics are live microbial supplements that colonize the gut while providing benefits to the host. Their benefits include an improved gut barrier, enhanced mucosal IgA responses, and increased production of anti-inflammatory cytokines. The rationale for probiotic prophylaxis for NEC relates to the role of bacterial colonization of the gut in the pathogenesis of the illness. Similar to earlier reviews, results of a recent systematic review and meta-analysis of 20 randomized controlled trials (RCT; total sample size = 3,816) confirm that probiotic supplementation significantly reduces the risk of NEC (RR 0.33; 95% CI 0.24–0.46; $p < 0.00001$) and all-cause mortality (RR 0.56; 95% CI 0.43–0.73; $p < 0.0001$) without any significant adverse effects, including probiotic sepsis, in preterm VLBW infants [2]. Experts agree that probiotics reduce the risk of NEC and death, and the evidence for mortality reduction is as conclusive as that for other well-established interventions such as antenatal corticosteroids, surfactant, and cooling [3–5]. Others passionately remind us that ‘we will never conquer NEC until we strive to significantly reduce its incidence; otherwise we will not be able to look parents in the eyes and say that their preterm infant has avoided all the major risks of NDI’ [6]. Despite the seriousness of the underlying issue, there is still a lot of reluctance in adopting probiotic prophylaxis for reducing the risk of NEC and death in preterm infants [7].

Probiotics have the best effect size (RR 0.33; 95% CI 0.24–0.46; $p < 0.00001$) compared with other strategies

for preventing NEC (table 1) [2]. We reviewed the Cochrane systematic reviews database for primary prophylactic strategies ($n = 49$) for preterm infants and selected those that have been incorporated in clinical practice. Sixteen of these 25 strategies have been reported as beneficial in improving the primary outcome, with only 4 reducing mortality. Compared with other strategies that reduce mortality, probiotics have the largest effect size (RR 0.56; 95% CI 0.43–0.73; $p < 0.0001$) (table 1) [2].

Primary prophylaxis involves exposing a large population of healthy subjects to an intervention for preventing disease in only a few. It is therefore crucial to prove that the intervention is very safe. Demonstrating the safety of probiotics in preterm infants is more critical considering their immunologic immaturity and decreased intestinal barrier integrity. The potential adverse effects of probiotics include probiotic sepsis, antibiotic resistance, and altered long-term immune responses. A review [8] of the level of evidence for probiotics in preterm infants concluded that so far no side effects have been reported and long-term outcomes are reassuring. Probiotic sepsis, even if it occurs, is easy to treat. Experts argue that infection with lactobacilli is preferable over potential pathogens like *Klebsiella*, *Enterobacter*, or yeast [9]. The availability of effective killed/inactivated probiotic strains or probiotic components may overcome the issue of probiotic sepsis [10].

Data from routine use rather than RCT is important for optimal assessment of the adverse effects of any interven-

Table 2. Characteristics of probiotic trials in preterm neonates

No.	First author (year)	Sample size, n	Birth weight/ gestation	Probiotic strain(s)	Dose and duration	Type of milk	Primary outcome
1	Rheuman [34] (1986)	30	<2 kg	LB-A	5×10 ¹⁰ probiotic formula or organisms started with 72 h until discharge	FM	Death, stool colonization, hospital infection
2	Kitajima [35] (1997)	91	<1,500 g	BB	0.5×10 ⁹ organisms once daily from first feed for 28 days	MM or FM	Gut colonization by BB
3	Dani [36] (2002)	585	<33 weeks or <1,500 g	LB-GG (Dicloflor)	6×10 ⁹ CFU once daily from the first feed until discharge	MM or DM or FM	UTI, sepsis, NEC
4	Costalos [37] (2003)	87	28–32 weeks	SB	10 ⁹ /kg twice daily from the first feed for 30 days	FM	Gut function and stool colonization; tolerance by preterm infants
5	Bin-Nun [38] (2005)	145	<1,500 g	BI, ST, BBB	BI: 0.35×10 ⁹ CFU, ST: 0.35×10 ⁹ CFU, and BBB: 0.35×10 ⁹ CFU once daily from the first feed to 36 weeks corrected age	MM or FM	NEC
6	Lin [39] (2005)	367	<1,500 g	LB-A, BI	LB-A: 1004356, and BI: 1015697 organisms twice daily from day 7 until discharge	MM or DM	NEC or death
7	Manzoni [40] (2006)	80	<1,500 g	LB-C (Dicloflor)	6×10 ⁹ CFU once daily from the 3rd day of life to 6 weeks or discharge from the NICU	MM or DM	Gut colonization by <i>Candida</i> species
8	Mohan [41] (2006)	38	<37 weeks ^a	BB-L	1.6×10 ⁹ CFU once daily from day 1 to day 3 4.8×10 ⁹ CFU once daily from day 4 to day 21	FM	Gut colonization by BB-L and enteric pathogens
9	Stratiki [42] (2007)	69	27–37 weeks ^a	BB-L	Preterm formula 1×10 ⁷ CFU/g started within 48 h to 30 days	FM	Intestinal permeability
10	Lin [43] (2008)	434	<34 weeks and <1,500 g	BBB, LB-A	2×10 ⁹ CFU daily for 6 weeks	MM or FM	NEC or death
11	Samanta [44] (2009)	186	<34 weeks and <1,500 g	BBB, BB-L, BI, LB-A	2.5×10 ⁹ CFU daily until discharge	MM or FM	NEC, time to full feed, sepsis, death, and hospital stay
12	Rouge [18] (2009)	94	<32 weeks and <1,500 g	BB-LG, LB-GG	1×10 ⁸ CFU daily until discharge	MM or DM or FM	Enteral feed intake at day 14
13	Mihatsch [8] (2010)	180	<30 weeks and <1,500 g	BB-L	6×10 ⁹ CFU daily for 6 weeks	MM or FM	Sepsis
14	Braga [45] (2010)	231	750–1,499 g	BB, LB-C	3.5×10 ⁷ –3.5×10 ⁹ CFU daily from the 2nd day to day 30	MM or DM or FM	NEC
15	Hikaru [46] (2011)	208	<32 weeks and <1,500 g	BB-M16V	1×10 ⁹ CFU daily until 37 weeks or a weight of 2,300 g	MM or FM	Sepsis
16	Sari [47] (2011)	221	<33 weeks and <1,500 g	LB-S	3.5×10 ⁸ CFU daily until discharge	MM or FM	NEC 2–3 or death
17	Al-Hosni [48] (2011)	101	<1,000 g	LB-GG, BI	LB-GG: 0.5×10 ⁹ , and BI: 0.5×10 ⁹ daily with the first feed until discharge	Unclear	Weight gain
18	Rojas [49] (2012)	360	<2,000 g ^a	LB-R	LB-R: 1×10 ⁸ CFU from the date of consent to discharge	Unclear	Death or nosocomial infection

Table 2 (continued)

No.	First author (year)	Sample size, n	Birth weight/ gestation	Probiotic strain(s)	Dose and duration	Type of milk	Primary outcome
19	Fernandez-Carrocera [50] (2013)	150	<1,500 g	BB-S, LB-A	(LB-A: 1.0×10^9 CFU, LA-R: 4.4×10^8 CFU, LB-C: 1.0×10^9 CFU, LB-P: 1.76×10^8 CFU, BI: 2.76×10^7 CFU, ST: 6.6×10^5 CFU)/g and dose 1 g/day	MM or FM	NEC
20	Sadowska-Krawczyńska [51] (2012)	55	<32 weeks and <1,500 g	LB-Rm	LB-Rm: 1×10^{12} CFU twice daily till discharge	Unclear	NEC
21	Ke [52] (2008)	598	<33 weeks and/or <1,500 g	Unclear ^b	Unclear ^b	Unclear ^b	Unclear ^b
22	Huang [53] (2009)						
23	Di [54] (2010)						
24	Ren [55] (2009)						
25	Garland [56] (2011)	1,099	<32 weeks and <1,500 g	BI, ST, BBB	BI: 0.35×10^9 CFU, ST: 0.35×10^9 CFU, and BBB: 0.35×10^9 CFU once daily from the first feed to discharge	MM or FM	Sepsis

^aData for <34 weeks and <1,500 g was obtained by contacting the authors. ^bAll 4 trials are in a Chinese database. Their details are not accessible to us but have been assessed and included in the meta-analysis by Wang et al. [3]. BB = Bifidobacterium breve; LB-GG = Lactobacillus GG; SB = Saccharomyces boulardii; BI = Bifidobacterium infantis; ST = Streptococcus thermophilus; BBB = Bifidobacterium bifidus; LB-A = Lactobacillus acidophilus; LB-C = Lactobacillus casei; LB-R = Lactobacillus reuteri; BB-LG = Bifidobacterium longum; LB-S = Lactobacillus sporogenes; LB-P = Lactobacillus plantarum; LB-Rm = Lactobacillus rhamnosus; CFU = colony-forming units; MM = mother's milk; DM = donor milk; FM = formula milk; NICU = neonatal intensive care unit.

tion. Bonsante et al. [11] reported on 1,130 preterm infants (gestation: 24–31 weeks) given routine probiotic prophylaxis. The risk of NEC, death, late-onset sepsis, and rectal bleeding was reduced and full feeds were reached earlier without any adverse effects. Hunter et al. [12] assessed the impact of routine *Lactobacillus reuteri* supplementation on NEC in ELBW infants ($\leq 1,000$ g). The incidence of NEC was significantly lower in probiotic-treated [2/79 (2.5%)] versus untreated infants from a historical cohort [35/232 (15.1%)]. Probiotic supplementation prevented 1 NEC case for every 8 infants given the prophylaxis [12]. No probiotic-related adverse events occurred. The reports by Luoto et al. [13] (n = 644) and Manzoni et al. [14] (n = 811) also indicate the safety of routine probiotic supplementation in preterm VLBW infants practiced for 6–12 years [13, 14]. Probiotic prophylaxis has not been associated with long-term NDI in preterm infants [15, 16]. Romeo et al. [17] reported that probiotic-treated infants had a significantly lower incidence of NDI at 12 months. There is little evidence that antibiotic resistance is a significant problem with lactobacilli and bifidobacteria [18]. These strains have a high degree of genetic stability, but the risk justifies continual assessment [19]. The previously reported risk of sensitization in infants exposed to probiotics has not been apparent in the long term [20].

Adopting an effective prophylaxis in a low-incidence setting may be difficult if adverse effects are a concern because many who are not at risk would unnecessarily be exposed to the its potential adverse effects, even if they are rare. However, systematic reviews show the safety and efficacy of probiotics even when the incidence of NEC is low (<6%) [2].

Considering that severe adverse effects are rare, a reasonably sized clinical study would be insufficient to detect a two-fold rise in the rate of an adverse effect with a 1% incidence. Current evidence from 25 RCT (~5,000 infants) and the reports on routine use (~3,000) indicate that significant adverse effects of probiotics are rare (table 2).

If the goal is to have 'zero tolerance' [6] for NEC, then probiotic prophylaxis must be adopted as the most effective strategy to reduce the risk of this disastrous illness associated with a huge health burden, and immense suffering [1]. This globally available intervention that significantly reduces death and NEC at less than a dollar per day should not be denied to preterm infants if we are to look their parents in the eyes without any hesitation to say that we have done everything for their baby [6].

We have provided evidence-based guidelines for the practical aspects of probiotic supplementation (strain se-

lection, dose, duration, surveillance, etc.), and for dealing with nonclinical but important issues (regulatory requirements, product format, etc.) [21] and addressed all concerns raised by researchers, including inadequate data on extremely preterm/ELBW infants, reproducibility in different set-ups, and the role of breast milk [22]. Others have pointed out the ethics and politics of probiotics for preterm infants [7]. Our experience in introducing routine probiotic supplementation in Australia confirms that, just as in other nations including the USA [23], access to clinically effective and safe probiotic products is not difficult [11–14, 21]. Defining probiotics as ‘foods for specialized health use’ or ‘biotherapeutic agents’ may overcome the regulatory difficulties while not compromising on quality [21, 24]. Cooperation between all stakeholders is needed to increase the availability of high-quality probiotics provided there is a will to do so [21].

Assessment of the most effective strains (singularly or in combination), dose, duration, and mechanisms of benefits of probiotics does not need placebo-controlled trials. Current evidence from RCT and observational studies indicates the safety and efficacy of probiotics in extremely preterm neonates who are at the highest risk of NEC, death and feed intolerance. Investigators have voiced a concern that ‘the microbiota of a preterm infant without a stable-background gut flora may be significantly skewed by the intentional colonization with billions of organisms in a narrow group of probiotics’ [25]. We believe that introducing billions of probiotic microorgan-

isms, if at all, will skew the unstable and less diverse flora towards being more stable and diverse and potentially less pathogenic. RCT comparing single versus multi-strain/species probiotics can address the concern about introducing a narrow group of probiotics. Adopting the purist’s view of waiting until the fine details of the various mechanisms of the benefits of probiotics are understood is unrealistic and perhaps unjustified in view of the available evidence.

We believe that the concept of evidence-based practice of medicine has been stretched too far in the case of probiotics for preterm neonates. Many level III neonatal units in Japan, Italy, Finland, and Colombia have been using probiotics routinely for over a decade and have not reported any significant adverse effects [21]. Trial sequential analysis has already shown that the evidence for probiotic supplementation was conclusive after 10 trials [26]. Experts had commented that the results of the ongoing (PiPS, UK: ISRCTN No. 05511098; ProPrem, Australia: ACTRN012607000144415) trials are unlikely to change the conclusions of the systematic reviews significantly [3, 4]. The preliminary results of the ProPrem trial support the current evidence that probiotics do not reduce the risk of late onset sepsis (primary outcome) but can reduce the risk of NEC (secondary outcome, not powered for) even in the presence of a low baseline incidence (4.4%) of the illness [27]. Currently we are at trial number 25; how many more trials do we need to change clinical practice? At what potential cost?

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