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Review Article

Probiotic for preventing necrotising enterocolitis in preterm neonates- The past, present, and the future

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Abstract. Although survival of very preterm neonates has improved in the surfactant era necrotizing enterocolitis (NEC) continues to be major cause of mortality and morbidity. Despite decades of research the pathogenesis of NEC is poorly understood. Historically, apart from antenatal glucocorticoids and postnatal preference to early feeding with human milk, neonatologists have had not many options to minimize the risk of NEC in preterm neonates. Probiotics supplementation has given a fresh outlook for prevention of NEC in preterm neonates. Evidence from many recent randomized controlled trials have shown that probiotics can significantly reduce the risk of NEC, all cause mortality and time to full feeds in preterm VLBW neonates. Some important issues need to be addressed (e.g. optimal strains, dose, and duration, combination of probiotic organisms) before this therapy becomes a safe routine in high-risk preterm neonates. This brief review covers the current evidence, and future for probiotic supplementation in preterm neonates.

Key words: Neonates, necrotizing enterocolitis, preterm, probiotics, systematic review

NEC is the commonest neonatal gastrointestinal emergency. It is mainly associated with prematurity with full term neonates accounting for only 5-25% of all cases (1). The incidence of this potentially fatal illness is reported to be 5-10% in VLBW neonates (2). Extremely low birth weight (ELBW: birth weight < 1000 grams) neonates with gestation < 28 weeks are the most susceptible (3). The overall mortality (20-40%) and morbidity (recurrent sepsis, prolonged dependence on parenteral nutrition, intestinal strictures, short bowel syndrome etc) continue to be high especially in survivors of surgical NEC (4).
Long-term neurodevelopmental impairment (NDI) in preterm VLBW survivors of surgical NEC has also been recently recognized as a significant issue.

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The socioeconomic burden of NEC is significant. Based on the length of stay, the yearly additional hospital charges for NEC are estimated to be $6.5 million or $216 666 per survivor (5). Despite decades of research, the pathogenesis of NEC continues to be poorly understood. Prematurity is however universally accepted as the single most important risk factor for the illness. Currently an interplay of various risk factors (e.g. hypoxia, gut colonization with pathogens, formula feeding, sepsis, and intestinal ischemia-reperfusion injury) against the background of an immature and vulnerable gut is thought to contribute to the inflammatory cascade that precipitates NEC (6). Given the poorly understood pathogenesis of the illness primary prevention of NEC is a very difficult task. Except for antenatal glucocorticoids and postnatal preference to early feeding with human milk, not many options are currently available to minimize the risk of NEC in preterm neonates. Human milk has been reported to reduce the incidence of NEC by up to sevenfold compared with formula milk, probably due to its anti-inflammatory components (e.g., cytokines, growth factors), lysozyme, IgG, as well as pre- and probiotics that modulate intestinal microflora composition to the advantage of the host (7, 8–11). Following the benefits reported in experimental studies (12-14),

a significant reduction in the incidence of NEC following antenatal glucocorticoids was reported in subsequent clinical trials (15,16). It is however important to note that NEC continues to be reported in preterm neonates who have been fed exclusively with fresh human breast milk and received antenatal glucocorticoids. Currently there is no specific cure for NEC, and its management continues to be mainly supportive in the form of bowel rest, provision of broad spectrum antibiotics, and careful surveillance for early detection of complications, and need for surgical interventions including insertion of a peritoneal drain. A recent animal experiment involving preterm rat pups with NEC, has shown that pentoxifylline significantly reduced the frequency as well as the severity of the illness (17). Data from well designed randomised controlled trials (RCTs) is needed to confirm its role as a secondary prophylaxis in definite medical NEC.

Almost a century ago (1908) Russian scientist and Nobel prize Laureate Elie Metchinkoff proposed the idea of non-pathogenic bacteria for healthy lifestyle on observing the association between prolonged lifespan and dairy products in the diet of Bulgarian people. What he thought was contained in the diet is what we now know as probiotics. The term probiotic was first introduced in 1965 and the definition has since undergone many variations (18). In 2001, the Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) defined probiotics as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (19).

The human gastrointestinal tract harbours a complex eco-system of microbes. Approximately 10^{14} microbes exist in the intestine, consisting of over 500 species (20). Microbial flora exists in symbiotic relationship with the host. A neonate is born with a sterile gut that is usually colonised within 12 to 24 hrs. Intestinal microbes from the mother are usually the first source of bacteria. Colonisation by anaerobes usually commences in next 2 or 3 days. *Lactobacilli* and *Bifidobacteria* from breast milk colonise the gut subsequently. It is important to note that many other factors including mode of birth, surrounding environment and dietary factors also influence initial colonisation in a neonate (21). There are at least four different strains of *Lactobacilli* and *Bifidobacteria* isolated form human breast milk, which colonise newborn gut (22,23).

The microbial flora of gut of preterm neonates in neonatal intensive care units differs from that of normal term neonates. Very low birth weight (VLBW) preterm neonates usually acquire microbial flora mainly from intensive care environment rather than from their own mother. Few studies have demonstrated that there is delay in appearance of *Bifidobacteria* species in VLBW neonates until third week of life, even in those receiving only breast milk (24). Stools of breastfed neonates have predominance of *Bifidobacterium* and *Lactobacillus* species, which compete with pathogens such as *Bacteroides, Clostridia* and *Enterobacteriaceae* species (25). Unfortunately VLBW preterm neonates are at risk of gut colonisation with pathogens which can alter the permeability of intestines and promotes inflammatory cascade which facilitates NEC (26-27).

Probiotics may prevent NEC and sepsis by promoting colonisation with beneficial organisms (eg *Bifidobacteria* species), preventing colonisation by pathogens (eg *Enterobacteria, Clostridia,* and *Staphylococcal* species), improving the maturity and function of gut mucosal barrier in dealing with the flow of food and microbial antigens across it, and by modulating the immune system to the advantage of the host (19,28). Recent animal studies have also reported effects of probiotics on platelet activating factor (PAF), tool-like receptor (TLR)-4 and nuclear factor-kB in prevention of NEC (29,30). The human TLRs are a family of pattern recognition receptors that transduce signals from specific ligand components of pathogenic organisms (31). Various pathogens including gram negative bacteria could activate TLR-4 receptor which ultimately leads to nuclear factor-kB translocation from cytoplasm to nucleus expressing multiple pro-inflammatory cytokines (32). These factors may contribute to NEC and probiotics could also prevent NEC by reducing TLR4 signalling and increasing TLR9 (anti-inflammatory TLR response) (33).

Hoyas et al (1999) were the first to report significant reduction in the incidence of NEC following probiotic supplementation in a cohort of preterm neonates (34). *Lactobacillus Acidophilus* and *Bifidobacterium Infantis* were given to 1237 preterm neonates and the results were compared with 1282 historical controls from the previous year. Significant reduction in NEC (85 vs 34) was noted in probiotics group. Since then many trials have assessed the benefits of probiotics in reducing NEC, sepsis, and allergy in neonates and infants. Prevention of NEC and sepsis however has been the main focus of probiotic trials in preterm neonates.

Table 1. Trials included in the analysis [38]

Study	Birth weight/GA	Probiotic agent/s	Dose and duration	Jadad's Quality score[39]	Number of subjects	Primary Outcome
Kitajima 1997[40]	<1500g	*BB*	0.5 X 10^9 organisms once daily from first feed for 28 days.	3	88	Gut colonisation by BB
Dani 2002[41]	< 33w or <1500g	*LB- GG (Dicloflor)*	6 X 10^9 CFU once daily from first feed till discharge	4	585	UTI, Sepsis, NEC
Costalos 2003[42]	28 to 32 w	*SB*	10^9 /kg twice daily from first feed for 30 days	5	87	Gut function and stool colonisation
Bin Nun 2005[43]	< 1500g	*BI, ST,BBB*	BI- 0.35 X 10^9 CFU, ST- 0.35 X 10^9 CFU and BBB- 0.35 X 10^9 CFU once daily from first feed to 36 weeks corrected age	4	145	NEC
Lin 2005[44]	< 1500g	*LB-A, BI*	LB-A: 1004356 and BI*:* 1015697 organisms twice daily from day 7 till discharge	4	434	NEC
Manzoni 2006[45]	< 1500g	*LB-C (Dicloflor)*	6 X 10^9 CFU once daily from 3rd day of life to 6 weeks or discharge from NICU	4	80	Gut colonisation by *Candia* species
Mohan 2006[46]	< 37w*	*BB-L*	1.6 X 10^9 CFU once daily from day 1 to day 3 4.8 X 10^9 CFU once daily from day 4 to day 21	4	38	Gut colonisation by BB-L and enteric pathogens
Stratki 2007 [47]	27 to 37 weeks	*BB-L*	Preterm formula 1 X 10^7 CFU/g started within 48 hours to 30 days			Intestinal permeability
Lin 2008[48]	<34w and <1500g	*BBB, LB-A*	2 X 10^9 CFU daily for 6 weeks	5	434	NEC or Death
Samanta 2009[49]	<34w and <1500g	*BBB,BB-L, BI, LB-A*	2.5 X 10^9 CFU daily till discharge	3	186	NEC, time to full feed, sepsis, death and hospital stay
Rouge 2009[50]	<32w and <1500g	*BB-LG, LB GG*	1 X 10^8 CFU daily till discharge			Enteral feed intake at day 14

*Data for < 34 weeks and <1500grams obtained by contacting the authors.
BB: *Bifidobacterium breve,* LB GG*: LactobacillusGG,* SB: *Saccharomyces boulardii,* BI: *Bifidobacteria infantis,* ST: *Streptococcus thermophilus,* BBB: *Bifidobacterium bifidus,* LB-A:*Lactobacillus acidophillus,* LB-C: Lactobacillus casei; BB-L: *Bifidobacterium lactis,* CFU: *Colony forming units*

Deshpande et al were the first to systematically review the RCTs evaluating efficacy and safety of any probiotic supplementation (started within first 10 days, duration: ≥7 days) in preventing ≥stage II NEC in preterm VLBW (gestation<33 weeks, birth weight<1500 grams) neonates (35). A total of 7 out of 12 retrieved RCTs (N=1393)

Table 2. Comparison of results of the old (2007) [35] versus new (2010) [38] meta-analysis

	2007 review [35]				2010 review [38]			
Outcome	N	RR	95% CI	P-value	N	RR	95% CI	P-value
NEC	1393	0.36	0.20, 0.65	<0.0008	2176	0.35	0.23, 0.55	<0.00001
All cause mortality	1268	0.47	0.30, 0.73	<0.0007	1888	0.42	0.29, 0.62	<0.00001
Sepsis*	1355	0.94	0·74, 1·20	0.68	2138	0.98	0·81, 1·18	0.80

N: number of subjects, RR: Relative risk, CI: confidence interval, *****blood culture positive sepsis

were eligible for inclusion in the analysis. Meta-analysis using a fixed effects model (7 trials, N=1393) estimated a lower risk of ≥ Stage II NEC [RR: 0·36(95% CI: 0·20, 0·65)] in the probiotic group. The numbers needed to treat (NNT) with probiotics to prevent one case of NEC was 25 (95% CI: 17, 50). The risk of blood culture positive sepsis (6 trials, N=1355) did not differ significantly between groups [RR: 0·94 (95% CI: 0·74, 1·20)]. The risk of death (5 trials, N=1268) was reduced significantly in the probiotic vs control group [RR: 0·47(95% CI: 0·30, 0·73)] NNT to prevent one death by treatment with probiotics was 20 [95% CI: 12, 50]. However there was no significant difference in mortality due to NEC or sepsis. Additionally the time to full enteral feeds (3 trials, N= 316) was significantly shorter in the probiotic group [WMD=-2·74 days (95% CI: -4·98, -0·51]. Overall the results indicated that probiotics may significantly reduce the risk of all cause mortality and ≥ Stage II NEC in preterm neonates <33 weeks' gestation while significantly shortening the time to full enteral feeds. These significant results were subsequently confirmed by two more systematic reviews indicating the tremendous potential of probiotic supplementation in saving preterm neonates from death and disease (36,37).

Deshpande et al (2010), (38) have recently reported their updated meta analysis of RCTs (11 Trials, N= 2176) of probiotics in preterm neonates (Table 1). The results have confirmed the previously reported benefits of probiotics while further increasing their precision and reducing the role chance alone (Table 2). The risk of blood culture positive sepsis (10 trials, N=2138) however did not differ significantly between groups [RR: 0·98 (95% CI: 0·81, 1·18)]. Trial sequential analysis results were conclusive of at least 30% reduction in the incidence of NEC (38).

Expert bodies such as the Cochrane Neonatal Review Group have concluded that except for those under 1000 grams (due to lack of specific data in this high-risk population), a change in practice was supported by the data (37). However two recent studies have documented safety of probiotics for those under 1000 grams (48,51). Individual experts have also commented that based on current data, those wishing to offer probiotic supplementation as a routine therapy in preterm neonates can not be faltered (52). Many important issues need to be addressed before accepting probiotics as a routine therapy in preterm neonates.

The optimal strains, dose, and duration of probiotic organisms is not clear. It is also not clear whether a combination of probiotics is more effective than a single probiotic. Individual organisms are known to have variable rates of colonization in different populations (40,53-54). Maturity of the host is also an important factor in colonization by probiotic organisms. For example, colonization rates have been reported to be much lower (25% vs 50%) in VLBW neonates compared with those weighing from 1500 to 1999 grams at birth (40). Whether colonization with a particular probiotic will result in benefits over only a specific period of postnatal life is not known.

The current data do not provide details on the frequency of neonates fed exclusively or predominantly with breast milk, and also on the local policies about the frequency, type, and duration of antibiotic exposure for sepsis. Whether the reported benefits of probiotics will significantly differ with variations in the level of exposure to breast milk and antibiotic therapy is not known. The degree of benefits may also vary depending on the baseline mortality and morbidities including NEC in a given population. The tolerance of a given probiotic supplement with regards to pH, osmolality, presence of dairy products such as lactose, preservative, and coloring agents etc is an important practical issue, especially for ELBW neonates.

Safety of probiotic supplements is an important issue in preterm neonates. It is reassuring to know that sepsis by probiotic organisms has not been reported in any of the trials included in the systematic reviews (38). However caution is necessary before adopting probiotics for prophylaxis in immunocompromised hosts such as preterm neonates given that the possibility of probiotic sepsis can never be ruled out, especially if probiotic supplementation becomes a routine (55,56). Other practical issues include difficulties in accessing a suitable, stable, and safe product with documented probiotic properties in this high-risk population, that is approved by the regulatory agencies and in assuring regular quality assurance and supply by the industry (57,58).

The future of probiotic supplementation in preterm neonates depends on the interpretation of the current evidence by the scientific community. Considering the significant benefits in critical areas (death, significant disease, and enteral nutrition), the certainty and precision of the results and the negligible role of chance alone, some may accept that the current evidence is adequate enough to justify introducing probiotics as a standard treatment in preterm neonates. Others however may still want more data specifically on ELBW neonates before introducing probiotics as a routine therapy for all preterm neonates.

Design, conduct, and timely completion of large definitive RCTs in this specific population will however be difficult given the significant resources required in enrolling a large number of such neonates across many centres. The obligation of clearly conveying the reported benefits of probiotics in preterm neonates to the parents as part of the informed consent process may also make recruitment difficult.

The solution may lie in tightly controlled prospective observational studies after introducing probiotics as a routine therapy for all preterm neonates in the nursery. For those who believe that the current data are adequate to justify routine use of probiotics, the future research in this area will involve head on trials of different products to evaluate safety and efficacy of one product over the other, and assessment of the role of prebiotics and synbiotics.

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