*Review article*

# Probiotics in neonatology: can it be adopted as a standard of care?

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## ABSTRACT

Probiotics are micro-organisms that confer health benefits to the host. There is a growing body of evidence documenting the immune-modulatory ability of probiotic bacteria. The need for probiotic formulations has been appreciated for the health benefits in "topping up your good bacteria" or indeed in an attempt to normalise the dysbiotic microbiota associated with immunopathology. This review will focus on the use of probiotics in clinical practice, particularly related to neonatal practice, including explanations of what they are and how they work. Evidence for the health benefits of consuming probiotic bacteria are examined in several clinical conditions. Lastly we have tried to solve the debated question that "should probiotics be used as standard of care in neonatal practice?"

### Key Points

1. Probiotics are enterally administered live "good' micro-organisms that colonise the gastrointestinal tract to modulate the functions of the innate microbial community and immune system.
2. This may result in significant health benefits; for example enteral probiotic supplementation significantly reduces both severe necrotising enterocolitis and all-cause mortality in preterm infants.
3. There is a debate on routine enteral probiotic supplementation for all preterm infants. Others advise caution pending results from large clinical trials designed to address issues regarding safety and efficacy in the smallest, most vulnerable newborn population.
4. Probiotics may also reduce atopic eczema in high-risk infants when administered to the mother during pregnancy and to the infant post-natally and improve feeding tolerance in neonates.

## INTRODUCTION

Probiotics are used extensively in therapeutic preparations, added to foods and have a major influence on gastrointestinal flora. They are defined as live micro-organisms, which when administered in adequate amounts, confer health benefits to the host.[1] A probiotic micro-organism selected for use in humans should ideally be: a micro-organism of human origin, non-pathogenic, resistant to destruction by technical processing and gastrointestinal tract (GIT) secretions, able to colonise the GIT, capable of producing antimicrobial substances, modulating immune responses and influencing human metabolic activities.[2] It should be unlikely to develop resistance to commonly used antibiotics.[3] The ability of supplemental probiotics to colonise the human GIT has been demonstrated in clinical studies.[4–6] It is hypothesised that probiotics may alter the microbial community in the digestive tract to mimic the 'healthy' GIT microbiome through the same mechanisms of action as the host's original microflora.[7]

At birth, an infant's gastrointestinal tract is sterile. Colonization of the gastrointestinal tract starts immediately after birth with the initiation of enteral feedings and is well established within the first few days of life.[8] In breastfed infants, ***Bifidobacterium*** and ***Lactobacillus*** predominate, with other enteric organisms being present less frequently.[9-13] This is not the case for formula fed infants. In these infants, coliforms, ***Enterococci,*** and ***Bacteroides*** predominately colonize the intestinal tract. Preterm infants are particularly susceptible to abnormal colonization. A combination of antibiotic use, delayed initiation of enteral feedings, and exposure to the unusual microorganisms that populate the nursery intensive care unit (NICU) may lead to

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abnormal patterns of colonization. Feeding oral probiotic bacteria may be an effective way to change this pattern of colonization.

Probiotics are marketed in several countries and widely used by pediatric health care providers. Although probiotics can be helpful for specific disorders, they have been broadly prescribed for disorders without clear evidence to support their use. We have reviewed the evidence of its use in various pediatric disorders.

## MECHANISM OF BENEFIT

The American Academy of Pediatrics Committee on Nutrition defined probiotics as 'microbes that generate small molecular metabolic by-products that exert beneficial regulatory influence on host biological functions and may function as immuno-modulators'.[14-16]

Probiotics have varied mechanisms through which it confers benefit. The mechanisms of benefits include modification of the gut flora, competitive adherence to the mucus and the epithelium, strengthening of the gut epithelial barrier, and modulation of the immune system (Table I).[17]

Probiotics have been reported to be beneficial in a wide spectrum of conditions ranging from antibiotic-associated diarrhea (AAD), irritable bowel syndrome (IBS), and travellers diarrhoea to dental caries, ulcers due to *Helicobacter pylori*, hepatic encephalopathy, and neonatal necrotizing enterocolitis (NEC).

**Table I**

**Postulated mechanism of action of probiotics [17]**

| Mechanism | Benefit |
|---|---|
| a. Reduction of mucosal permeability<br>b. Strengthening of intestinal tight junctions | Maintains mucosal barrier integrity |
| a. Modulation of microflora growth and adherence<br>b. Reduction of intraluminal pH<br>c. Competitive exclusion of pathogenic bacteria from binding sites | Regulation of appropriate bacterial colonisation |
| a. Enhanced mucosal IgA response<br>b. Production of short-chain fatty acids | Activation of general intestinal immune defense |
| a. Increased T cell production of cytokines<br>b. Increased production of anti-inflammatory cytokines | Modulation of intestinal inflammation |

## THE HUMAN INTESTINAL MICROBIOME

The mature human GIT microbial community encompasses several hundred species of micro-organisms, which perform vital functions, including digestion of nutrients, regulation of fat storage, metabolism of endogenous and exogenous compounds and immune-regulation plus limiting colonisation with potentially pathogenic microbes.[18-21] Term infants acquire their intestinal microbial community from the birth canal and close parental contact.[13,22,23] Failure to establish a normally functioning IM is associated with disease locally (inflammatory bowel disease) and distally (allergic disease).[24,25] Preterm infants may develop a dysfunctional gut microbiome as they preferentially acquire colonising bacteria from the intensive care environment.[26] The process may be adversely affected by mode of delivery, administration of antibiotics and prolonged hospitalisation.[27-30] This leads to delayed colonisation with healthy commensals (e.g. *Bifidobacterium* and *Lactobacillus* species (spp) and increased colonisation with pathogenic organisms.

As postulated in Table I, there is evidence that probiotics and their by-products exert these effects both locally within the GIT and remotely in tissues, such as the lung and brain. For example, animal models show that probiotics have immuno-modulatory effects on mediators implicated in injury to the nervous system (e.g. brain-derived neurotrophic factor, interleukin-10, interferon-γ (IFN-γ), etc.). In these studies, the probiotic-induced immuno-modulation improved neurological function, including memory, stress response behaviours and peripheral nervous system reflexes.[31]

Probiotic effects on immune system responses, with associated improved clinical outcomes, have been reported in studies of pregnant women and their infants. These include reduced breast milk tumour necrosis factor-α and inflammatory cells from mothers who received *Lactobacillus casei*, with decreased gastrointestinal disturbances in the infants.[32] In an Australian study, women who received pre- and post-natal probiotics had increased levels of breast milk immunoglobulin A (IgA) and their infants had increased blood levels of IFN-γ.[33] Therefore, there is evidence that probiotics administered pre-and/or postnatally can influence the developing

immune system. The effects of probiotics may be enhanced by the addition of synergistic substances, such as oligosaccharides (prebiotics) or lactoferrin (an iron-binding protein).[34]

## PROBIOTICS AND PRETERM INFANTS

Recent meta-analyses[35,36] and systematic reviews[37,38] demonstrate that probiotic supplementation in preterm infants reduces the incidence of significant NEC and all-cause mortality, with a relative risk of 0.35 (95% confidence interval (CI) 0.24, 0.52) for NEC and 0.40 (95% CI 0.27, 0.60) for all-cause mortality in the Cochrane database review.[39-41] (Table II) NEC-affected infants have reduced microbial diversity in their GIT compared with controls, which may limit the ability of their micro-biome to perform its important immunoprotective functions. Supplemental probiotic microbes may assist the innate microflora with these functions. Other reported positive effects of probiotics in preterm infants include: improved GIT motility and feeding tolerance, reduction in days of hospitalization, improved weight gain, intestinal colonisation with probiotic organisms and favourably altered immune responses in the probiotic versus placebo group.[42-44] In 2011, Manzoni *et al*, who previously reported decreased fungal colonisation in preterm infants treated with *Lactobacillus rhamnosus* GG(LGG), concluded that routine use of LGG to be 'microbiologically safe and clinically well tolerated'.[45]

*Bifidobacterium* and *Lactobacillus* spp. are the most studied probiotic organisms in neonates to date, and one systematic review suggested that the best clinical outcomes are achieved with combinations of probiotic organisms, rather than a single strain.

Current evidence on the effects of probiotic supplementation on late-onset sepsis, weight gain or neuro-developmental outcome in preterm infants is not supportive for routine use. Results from ongoing large clinical trials, including ProPrems[46] in Australia and New Zealand, and PiPs (Probiotics in Preterm Infants Study) in the United Kingdom, are awaited to determine further benefits of probiotic supplementation in this vulnerable population (Table III). Evidence-based guidelines for the use of probiotics in preterm neonates have been recently published.[47]

## PROBIOTICS AND TERM INFANTS

Studies of probiotic effects in term infants have focused on allergic diseases, which result from dysfunction of immune regulation.[48] Children with abnormal perinatal intestinal colonisation are at increased risk of developing allergic diseases, and probiotics can reduce this risk by regulating immune mediators, such as IgA.[49,50] Some studies have demonstrated that administration of probiotics to pregnant women and/or their infants prevented allergic diseases, particularly infants considered at high risk of developing these conditions.[51]

The Cochrane review[52] reported an inconsistent effect of probiotics in atopic eczema and concluded that 'There is insufficient evidence to recommend the addition of probiotics to infant feeds for prevention of allergic disease or food hypersensitivity'. Furthermore, there is debate regarding the best timing of pro-biotic administration to prevent or reduce allergic diseases. Supplementation appears to be most beneficial if commenced antenatally and continued postnatally.

## EVIDENCE OF PROBIOTICS USE IN NEONATOLOGY

Evidence from a recent updated systematic review[35-38] and conclusive meta-analysis of randomized controlled trials (RCTs) indicates the significant benefits of probiotic supplementation in preventing all cause mortality and definite (Stage II) NEC with no significant adverse effects in preterm neonates. [All cause mortality: risk ratio 0.42, 95% confidence interval (CI) 0.29 – 0.62, $p<0.00001$; definite NEC: risk ratio 0.35, 95% CI 0.23 – 0.55, $p<0.00001$][35] Given the precision of these results, an almost insignificant role of the play of chance alone, and the magnitude of the benefits, experts have recommended that a change in practice favouring routine probiotic supplementation is justified if a safe and effective probiotic product is available.

Results of observational studies involving routine use of probiotic supplementation in preterm including extremely low birth weight (ELBW) neonates, and long-term follow-up support such recommendations. Evaluating the benefits and risks of live vs dead probiotics is an exciting area of research Evidence indicates that the action of probiotics could be a dual one. Live probiotics influence the gut flora and the immune response, whereas the dead cell components exert an anti-inflammatory response. Variable amounts of dead cells may thus explain the variation in response to live probiotics. Products with dead probiotics may have several advantages, including safety and a long shelf-life.

**Table II**
**Evidence: Probiotics in Preterm Neonates**

| | NEC 2/3 | NEC <1500 | Mortality | Sepsis | Others |
|---|---|---|---|---|---|
| **Cochrane** [35]<br>· 16 trials<br>· 2842 babies<br>· Updated till Nov 2010<br>· Inclusions: < 37 wk and/ or <2500 gm<br>· Any pro biotic/ variable dose x 7 days vs placebo or no treatment | 0.35 (0.24-0.52) [65 % reduction] NNT=25<br><br>High quality studies 0.25 (0.23-0.49) [75 % reduction] | 0.34 (0.23- 0.50) [66% reduction] | 0.40 (0.27-0.600 [60 % reduction] NNT =25<br><br>NEC reported mortality 0.31(0.10-0.94) [69 % reduction] | 0.90 (0.76-1.07) | a. TPN days 0.80 ( -0.30-1.90)<br><br>b. Hospitalisation days -6.80 ( -7.08 to -5.90)<br><br>c. Weight gain 0.28 ( -0.93,1.49)<br><br>d. Time to full feeds -4.2 (-4.8,-3.7)<br><br>e. Long term outcomes 1.02 (0.35-6.94) |
| **Walter** [53]<br>· 15 RCTs included<br>· 2764 neonates<br>· Up to date Nov 2010<br>· GA <37 wk or BW <1500 gm | Did subgroup analysis for different strains and found significant difference in NEC for 5 studies and not rest<br>No difference in mortality and sepsis<br>Comments by Walter<br>• Non-inclusion of unpublished studies (b) Heterogeneity in studies (c)A considerable variety of probiotics has been studied in preterm infants in RCTs (d)Publication bias (e) Probiotic therapy alone or probiotics in combination with bovine lactoferrin (f) blinding or not blinding (g) definition of the primary outcome a priority or during the ongoing trial (h) appropriate sample size calculation or not | | | | |
| **Wang et al** [54]<br>Subgroup analysis of *Bifidiobacteria, Lactobacilli* , and *Bifidiobacteria + Lactobacilli* | 0.33 (0.24-0.46) (20 studies) | 0.56 (0.43-0.73) | 0.90 (0.71-1.15) | | |

Data is represented as RR (95 % CI) ; RR -relative risk, CI - confidence interval, NNT -number needed to treat, TPN- total parentral nutrition, GA- gestational age, BW- birth weight

## DISCUSSION

Evidence of the beneficial effects of probiotics on human health is accumulating. There is support for routine introduction to prevent certain diseases in specific populations. For example, the Cochrane Neonatal Review Group states that current evidence for the use of probiotics to prevent NEC and all-cause mortality in preterm infants supports a change in practice[35] (i.e. from non-routine to routine administration). Other authors and experts also advocate that probiotics should be a part of regular clinical practice, stating that 'current probiotics could prevent tens of thousands of deaths annually, and that 'further placebo-controlled trials are not warranted'. So, if there is emphatically favourable evidence for the routine use of probiotics in neonatology, why has practice not changed?

Other concerns have been raised regarding the generalisability of the results of published RCTs and reviews. Very few of the most vulnerable extremely low birth weight (below 1000 g) and extremely low gestational age (below 28 weeks' gestation) infants, who are most at risk of morbidity and adverse out-comes, have been included in the RCTs or reviews, such that results from meta-analyses cannot be extrapolated to these infants.

**Table III**
**Future studies (results awaited)**

| Study | Primary outcome | Inclusion criteria | Sample size | Time of completion |
|---|---|---|---|---|
| Garland SM Proprem trial (Australia, Newzeland) [44] | LONS | <1500 g and < 32 wk | 1100 | March 2012 |
| Costeloe PIP trial (UK) [55] | Sepsis, NEC, death | <31wk | 1300 | 2013 |

**LONS:** late onset neonatal sepsis; **NEC:** necrotizing enterocolitis; **ProPrem:** Probiotics for premies; **PIP**: Probiotics in preterms

On the other hand, Deshpande *et al*[36,48] described the use of probiotics as very favorable and commented that "The significant effect, size, precision, consistency, extremely low p values almost ruling out the role of chance alone, low risk of publication bias, no statistical heterogeneity, critical areas of benefit, all indicate that withholding probiotics from high-risk neonates is now almost unethical. Further he described that "It will be very difficult to justify the need for additional placebo-controlled trials".[48]

It is clear that there is great reason to be hopeful regarding the effect of probiotics. However, meta-analyses and multiple small trials have led us astray before and should not be over interpreted. The barrier to probiotics use is not in whether the neonatal community is convinced by the available data and believes that these drugs might be effective but in the need to have a more careful evaluation that addresses the optimum type of probiotics, the dosage, and multiple other factors such as their effect in ELBW and breastfed infants.

## CONCLUSION

The evidence from clinical trials of probiotics in neonates is promising, particularly the use of probiotics to prevent NEC in preterm infants and atopic eczema in high-risk term infants. However, the current guidelines from the European Society for Pediatric Gastroenterology, Hepatology and Nutrition and the American Academy of Pediatrics state that there is insufficient evidence at this time to recommend routine administration to all neonates.[56,57]

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