Probiotics decrease the incidence of NEC and death – so why aren't we using them?

A recent meta-analysis suggested that oral probiotics decreased the incidence of necrotising enterocolitis (NEC) and its associated mortality; however, there is much debate about the interpretation of this evidence. No UK units currently offer prophylactic probiotics as a routine, and there are practical difficulties in obtaining a suitable product. This article reviews how probiotics might work, the risks and benefits associated with their use, and the potential disadvantages of doing nothing while we await further research.

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Key points

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1. The microbial environment of the preterm gut differs vastly from those born at term and is a key factor in the development of NEC.
2. Probiotics are live bacteria (or fungi) that confer a health benefit on the host.
3. Controlled trials and meta-analyses suggest that oral probiotics decrease the incidence of NEC and death.
4. Probiotics appear generally safe but questions remain about efficacy.
5. There may already be sufficient data to suggest that this strategy should be adopted using careful monitoring without further delay.

Probiotics – what are they?

Probiotics are bacteria that confer a health benefit to their host[1,2]. 'Healthy bacteria' are present in all mammals and are not simply restricted to colonisation of the gastrointestinal tract. However, the gut is probably the most important location within the body, and certainly the site that is most amenable to therapeutic manipulation. Humans have co-evolved over millions of years with a multitude of different bacteria and other microbes. Our guts play host to more than a 1000 different bacterial species[3]. In total there are about ten times as many bacterial cells as human cells in our body[4] – humans might therefore be considered a 'super-organism'.

Many of these gut bacteria cannot be cultured because they live in locations difficult for sampling access such as the small intestine, and many do not grow using standard culture techniques and media[5]. Some are difficult to clearly identify, but sophisticated molecular techniques are now able to detect their DNA[6]. Not all of these bacteria would be considered 'probiotic' as such, but because they are involved in important metabolic processes, one could argue that every bacterium that normally resides in the intestines of healthy humans is important for health.

The majority of probiotic species that are consumed in food (most commonly dairy products), or as supplements, belong to the Lactobacillus (see **FIGURE 1**) or Bifidobacterium genera. But, there are other bacteria (eg certain Streptococci) and fungi (eg Saccharomyces) that are also considered probiotic[7]. There are hundreds

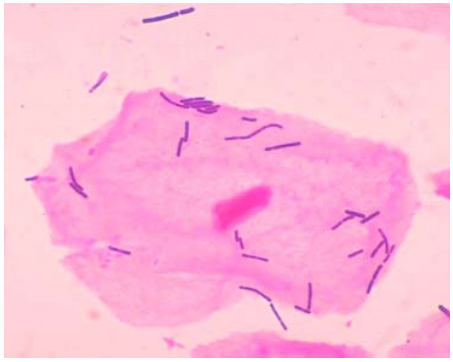

FIGURE 1 Lactobacilli (reprinted from Wikipedia from an original PD-US Government–HHS-CDC).

of different species of Lactobacillus or Bifidobacterium, not all exert the same beneficial effects, and there are population differences in colonisation patterns that probably reflect the local environment and food exposures. Probiotics might work best in combination with other bacteria or probiotic species. There are no probiotic food supplements then, that will duplicate 'normal' colonisation patterns.

Microbial colonisation of newborn babies' gastrointestinal tract

During fetal life the gut is essentially sterile but is rapidly colonised as the amniotic membranes rupture and delivery progresses. Normal vaginal delivery at term exposes the newborn baby to bacteria that live within the maternal genital and gastrointestinal tracts resulting in acquisition of bacteria that are presumed to be living healthily within the mother. These maternally derived bacteria can be cultured from the newborn baby's gut within the first week[6]. During this time, breast fed infants develop a predominance of Lactobacillus and Bifidobacterium

species[8]. The pattern in those delivered by caesarean section, who receive antibiotics or are formula fed is quite different. Even brief exposure to antibiotics (eg 48 hours) results in changes that are detectable for several weeks. Given the essentiality of gut colonisation for health, and the persistence of early ecosystems it is possible that any of these interventions will result in changes which may be sub-optimal for health[9].

Probiotics or prebiotics – what's the difference?

Prebiotics are non-absorbable substances derived from the diet that promote the existence of probiotic species. There is a wide range, but most prebiotics are non-absorbable carbohydrates such as oligosaccharides, and are an important constituent of soluble fibre. Galactose and fructose oligosaccharides (GOS and FOS) are now added to certain foods and milk formula and are fermented within the gut. A recent trial suggested they may have a small effect on enteral tolerance in preterm infants[10].

Shorter molecules tend to undergo fermentation more quickly than larger ones. This means that FOS for example, may preferentially affect composition and activity of bacteria in the first part of the colon, whereas inulin (a longer molecule commonly added to foods as it can taste sweet) may exert a greater effect in the descending colon. One reason breast milk is so beneficial is because it contains naturally occurring oligosaccharides that promote the predominance of probiotic species in the colon[11]. While these can (and are) added to formula milk, the optimal quantity and combination is not known. In addition to prebiotics, breast milk has now been shown to contain bacteria (including probiotic species) that grow within milk ductules in the mammary gland, and are expressed along with the milk[12]. This may be an additional mechanism through which breast milk exerts an advantage.

How might probiotics exert beneficial effects?

Probiotics affect health through a bewildering array of mechanisms, many of which are probably not yet known, and involve cellular, immunological, and nutritional processes. They produce substances ('bacteriocidins') that directly inhibit the growth of more pathogenic species, but also compete for available nutrients limiting that available for pathogens. Integrity of the gut wall is essential to prevent bacterial translocation, and this is improved by probiotics exerting effects on 'tight junctions' – the point at which two gut epithelial cells are joined together. Probiotics have also been shown to affect aspects of the immune system helping to improve the production of immunoglobulin, cytokines (chemical substances involved in regulating the immune process) and phagocytosis, and there is increasing evidence of 'cross-talk' between gut cells and probiotic bacteria. Bacteria are involved in several nutritional processes including recycling of the nitrogen in urea to make new amino acids.

Probiotics and preterm infants

It is easy to appreciate how the gut of a premature baby might differ from that of a baby born at term given the clear evidence of the effect of the interventions that preterm babies are exposed to: antibiotics, caesarean section, sterile environment, immature immune systems, delayed or absent enteral feeding, H_2 antagonists, lack of breast milk and use of formula, and exposure to pathogens residing in SCBU environments (eg pseudomonas) etc. Preterm infants become colonised with a smaller range of organisms than term babies, and tend to lack the predominance of lactobacilli or bifidobacteria.

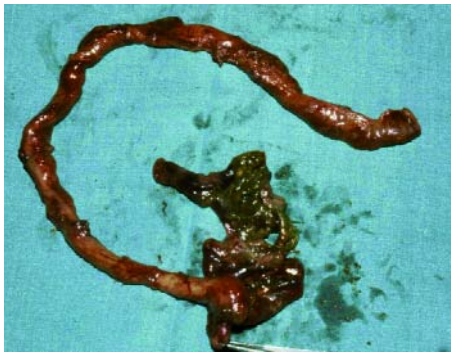

FIGURE 2 NEC specimen. Figure courtesy of Mr Bruce Jaffray.

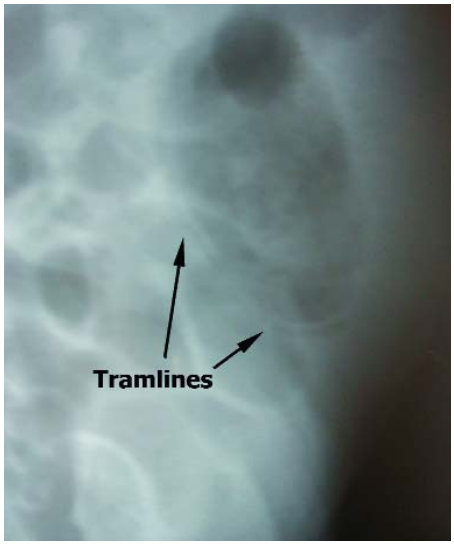

FIGURE 3 NEC tramlines. Figure courtesy of Mr Bruce Jaffray.

Necrotising enterocolitis (NEC) is a devastating disease with a high mortality rate that predominantly affects preterm infants and is one of the commonest reasons (along with sepsis) for death after the first few days[13] (**FIGURE 2**). Tramlines on X-ray represent gas in the bowel wall ('pneumatosis intestinalis') indicative of NEC (**FIGURE 3**). Initially thought of as having an essential element of ischaemia, the promoting factor is now thought more commonly to be an inflammatory process affecting the gut wall. Given the evidence that an optimal pattern of bacterial colonisation is essential for normal immunological and nutritional functioning, it is not difficult to understand why even subtle perturbations might result in an imbalance and initiate a process that eventually progresses to NEC. Studies demonstrating an increase in NEC with interventions such as prolonged use of cephalosporins or H_2 antagonists would appear to support this[14,15]. Prophylactic administration of probiotics either as single species or in combination appears to result in decreased rates of NEC and might therefore be an important therapeutic option for large numbers of babies born prematurely.

Are probiotics safe?

There are both short- and long-term safety considerations. Robust evidence of long-term safety in preterm infants does not exist, but that which is available suggests no evidence of harm. There are equivocal effects on later allergy with one or two studies showing slightly increased rates of allergic disease in children who received probiotics, but few data relate to preterm infants. Medium-term follow-up studies have shown no evidence for an adverse effect on growth or neuro-development in infancy[16].

There are several case reports of invasive sepsis caused by administered probiotic species, with lactobacilli being more commonly reported than bifidobacteria[17]. These have mainly occurred in patients with immuno-compromise (which includes preterm infants), but these cases have been successfully treated with antibiotics. Probiotic organisms generally

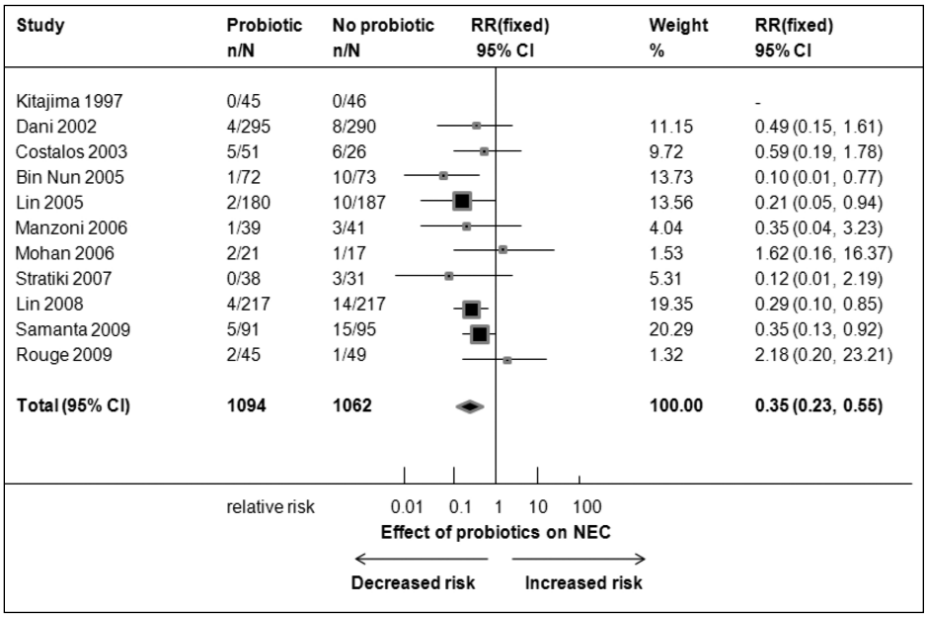

FIGURE 4 Meta-analysis of RCTs of probiotic administration to reduce incidence of NEC (Deshpande et al, 2010).

Scenario	Estimated baseline risk of NEC	Estimated risk reduction due to probiotic	Power	Number of babies needed in study
1	10%	50%	80%	700
2	10%	30%	80%	2100
3	10%	25%	80%	3100
4	7.5%	30%	80%	2300
5	5%	30%	80%	4500

TABLE 1 Number of babies needing enrolment in potential probiotic trial in five different hypothetical scenarios.

require anaerobic culture so might not be as easily detected as more common pathogens. Nevertheless, there were no reports of invasive sepsis in over 1000 preterm infants exposed to probiotics in the 11 randomised controlled trials (RCTs) that are included in the recent meta-analysis. It is possible that there are important safety issues that are not currently apparent, but in general terms probiotics appear relatively safe and any theoretical concern of harm needs to be counter-balanced by existing evidence of efficacy.

It is also important to note that even large RCTs (n >1000) will not reliably confirm safety. As with pharmaceutical products, safety (or lack of it) is often only confirmed in phase IV (post-marketing) surveillance studies. The potential for cross-contamination with other babies on the unit is important and needs careful consideration. In some of the pilot studies, non-treated babies became colonised with species administered to other babies within the same SCBU[18]. However, there is no suggestion that this is necessarily 'harmful' as colonisation usually ceases after exposure whether it is deliberate or accidental. It might even be regarded as beneficial if the numbers of pathological bacteria within a unit are thus reduced. There is no evidence that currently available commercial probiotic species transmit antibiotic resistance to more pathogenic bacteria, but this remains an important issue. This concern will not be answered by standard RCT trial designs.

Meta-analysis of probiotics for preventing NEC in preterm infants

The question "do prophylactic probiotics decrease the incidence of NEC" can only be answered by large RCTs combined with meta-analysis. Case-control and cohort comparison studies are important and provide additional data, but cannot on their own provide evidence of efficacy. In total there have been 11 RCTs considered suitable for inclusion in the most recent meta-analysis[7,19-29]. **FIGURE 4** is reproduced from the meta-analysis of Deshpande et al. The black square represents the result of each study – the relative risk (RR) of getting NEC. The length of the line tells you the confidence interval, ie you are 95% confident that the true result will be somewhere on that line.

For example, Manzoni 2006[24] shows a relative risk of 0.35, ie you are only about a third as likely to get NEC if you received probiotics. But the confidence intervals range from a potentially massive reduction with a tiny RR of 0.04 (only 4% as likely to get NEC) through to a RR of 3.23 equivalent to a potential increased risk of NEC of more than three-fold if you received probiotics[24]. The black diamond is the 'average' of all the studies having accounted for the size and effect of the study – the % weight. Large studies and those with large effects contribute more to the overall effect than small ones. The overall RR suggests probiotics decrease definite NEC by more than 50%. Note that the study of Samanta (2009) contributes 20% of the 'weight' of the overall result, although it enrolled less than 10% of all the babies[28].

Problems with the trials – number of infants studied

There are several potential methodological problems with all of the studies and there is insufficient space here to address all of them. Although some of the trials recruited several hundred infants, most have insufficient power to detect a change where NEC (Bell's stage ≥2[30]) was the primary outcome. This is because the background rate of NEC in most units is relatively low. Although many quote an incidence of NEC of 10%, this would be the typical rate of 'definite' NEC (ie NEC with unequivocal signs, requiring surgery or causing death) only in those born <28 weeks. Background rates of definite NEC of >13% in those born <1500g (as seen in the Bin Nun trial[22]) are much higher than in most UK units. **TABLE 1** gives an estimate of how large trials might need to be based on differing incidence and estimated treatment effects.

In **TABLE 1** the estimated baseline risk of NEC and the risk reduction vary between five hypothetical scenarios. Power tells the research team what the chance is of missing a genuine positive effect of the intervention (a false negative result). A power of 80% means that every one in five times the trial took place you might expect to miss a genuine effect of the intervention. Most funding bodies would not fund an expensive trial if there was a higher than 1:5 chance of missing a genuine effect. If

power is only 50%, every other time you did the trial you would miss the genuine effect. This would then be pretty pointless.

Risk reduction in the current 11 trials 'averages' over 50%, but many feel this might be over-estimated: there are very few interventions in any branch of medicine as effective as that, so 30% decrease might be a more reasonable assumption. Comparing scenarios 1 and 2 shows that an apparently small change in effect (changing from 50% to 30% reduction) increases the number of infants needed to study by a factor of three, and this increases by more than four times if we estimate just a 25% reduction. Even a 25% reduction in NEC would be important, but to show that a new RCT would have to recruit more babies than all the babies in the previously published 11 studies together.

Scenario 4 shows the numbers needed if the background rate of NEC was only 7.5% – this is perhaps a more realistic rate of definite NEC if slightly more mature babies were studied in the UK, eg <30 weeks. If babies <32 weeks were studied the rate of 'serious' NEC might only be 5%. Even if probiotics decreased that risk by 30% (ie decreased it to a rate of 3.5%) the study would need to recruit 4500 infants. No single neonatal RCT of any intervention has ever been conducted with that number of enrolled infants (although this has been achieved in adult trials examining myocardial infarction.) Many would argue that we simply have not studied enough babies yet to know whether probiotics are a good thing.

Problems with the trials – populations studied, initiation and duration of treatment

Some of the trials did not recruit the sickest babies who might either have developed NEC before approached for enrolment, or might have been unwell with other pathologies. This means trials may have included infants who were relatively well. Some (but not all) studies exclude those on breast milk where the beneficial effect of probiotic might be less. In addition the time at which the probiotic was administered and the duration of exposure was different for each of the studies. Although there are >2000 infants in the meta-analysis many would point out that the conclusions are based on just 100 informative cases who actually got NEC, although others would argue that the babies who did not get NEC were of more interest.

Problems with the trials – choice of probiotic product and long-term effects

Almost every trial studied a different probiotic or probiotic combination, and in the only two trials that used the same species a different dose was used[25,26]. There have been no 'head to head' trials so not only are we uncertain of which product we should use, but we also do not know the optimal dose that balances risk and benefit. However, head to head trials would likely require several thousand infants and for that reason might never be performed. Some would argue that it is inappropriate to group all these studies together as they are all using a different treatment (species), while some would argue that the consistency of effect is a strength in justifying probiotics as a 'class' of drug or treatment[31]. Long-term follow-up studies will take several more years – should we withhold what appears to be a promising method of preventing NEC or death for another five or ten years while we wait for 'further evidence'?

What should clinicians conclude? Are more research trials needed before we can start?

This is a matter of fierce debate[31-36]. There is no doubt more research is needed and there are many unanswered questions. Without clear data it might be unwise to introduce a new therapy. If probiotics 'improve' gut flora, why do they appear to have no effects on rates of sepsis? Despite this, we feel the current data suggest that probiotics as a class are not only likely to be effective, but also appear reasonably safe. Not only do they appear to decrease the risk of NEC, other analyses suggest they also decrease the overall risk of dying. This would at a minimum, suggest that significant unmeasured harm is not occurring. Despite the methodological flaws, the evidence base for probiotics in preterm infants is stronger than for many other interventions we use in neonatal medicine. Further trials might be justified as long as parents are fully informed about the existing evidence in a balanced fashion. Alternative methodologies could be employed for further research studies. Patient preference trials allow parents to opt for probiotic or no probiotic, but also allow those who are uncertain to consent to randomisation if they wish. Patient preference trial designs can affect key outcomes, but are a valid statistical design especially in this case where there is complete separation between the preference (parent) and biological effect (NEC in the baby).

What should we tell parents?

Despite the difficulties, challenges and uncertainties, we now feel parents could be provided with this information in a balanced fashion. While some may feel there is still insufficient evidence to recommend routine use of probiotics as a 'standard of care', the current evidence on efficacy and the lack of any clear evidence of harm is persuasive. Although long-term outcomes are uncertain ('you don't know what you don't know'), it seems highly unlikely that any long-term adverse effects (eg allergy, or developmental effects) will be either common enough, or severe enough to outweigh the reduction in mortality. We do not yet know whether probiotics will colonise non-treated infants in the same SCBU, but even if they did, it seems unlikely this will be harmful. RCTs will not be able to answer questions concerning risks of transmission of antibiotic resistance – this will require detailed studies involving microbial-genetics. From a practical neonatal aspect the key question appears not to be safety, but efficacy.

What probiotics are available for use in the UK?

In the UK the use of medicines is regulated by the MHRA (Medicines and Healthcare products Regulatory Agency) but probiotics are currently considered to be a food supplement – much like breast milk fortifier (BMF). This means they are not regulated by the MHRA but would come under the control of the Food Standards Agency (FSA). Manufacturers cannot make a medicinal or therapeutic claim for a food product: it is possible to say that BMFs improve growth, but it would not be legal to claim that they might prevent infections even if, in fact, they did have a beneficial effect on the immune system by improving a baby's nutritional status. Probiotics cannot be prescribed as a drug, and are not licensed or recommended by the BNF, or any professional body for this indication. Probiotics similar to those used in the existing trials can be obtained in the UK, and given to preterm infants if the clinician determines that they might be beneficial as a food supplement (just as the case is for BMF).

Is it possible our view is wrong and we are 'jumping the gun'? It is possible that ongoing large trials will produce data that are at variance with the current studies and that the conclusions of the meta-analyses may change. Probiotics may not be as effective as they appear to be at present, or might be shown to be associated with, as yet, unrecognised harm. As with any area of practice, clinicians must retain responsibility for audit and regular review of the available evidence and be prepared to change their recommendations in the light of those findings. Most importantly, clinicians should ask themselves "…do parents have a right to this information, and a right to be involved in decision making?" Would parents of babies who now develop NEC feel aggrieved if we chose not to share the information with them.

We are aware of units in the UK which are currently exploring introducing probiotics. In the south of England, a large RCT of probiotics is underway – the PiPS study (see www.npeu.ox.ac.uk/pips for more information). Staff working within hospitals and networks linked to these centres are strongly encouraged to contact the National Perinatal Epidemiology Unit (NPEU) and consider participation if they remain uncertain as to whether probiotic administration is appropriate. We do not consider it inappropriate to conduct further placebo controlled RCTs, but equally do not feel that further RCTs are *essential* before probiotics can be introduced. As with any new intervention, information sharing, parental assent, and rigorous audit are essential.

The most important intervention available for babies is mother's own breast milk. Neonatal staff must strive to increase initiation and duration rates for expressed breast milk (EBM). Unfortunately, NEC still occurs in those only ever exposed to EBM. Colonisation of the preterm infant gut will always be 'abnormal' to some extent. But some bacteria have to live there and we already operate numerous practices that adversely manipulate the microbiome: sterile environments, parental skin contact, frozen EBM, formula milk, BMF, alcohol gel, antibiotics etc. Despite the clear uncertainties surrounding the use of probiotics in preterm infants, are we right to withhold this information and intervention from parents?

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