Clinical effects of probiotics: scientific evidence from a paediatric perspective

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Abstract

Probiotics are live micro-organisms that when given in adequate amounts can cause health benefits. The safety and efficacy of probiotics in the prevention and treatment of various clinical conditions have been evaluated in randomised controlled clinical trials, systematic reviews and meta-analyses. Generally, their safety has been documented. As a supplement to standard rehydration therapy, probiotics have been demonstrated to shorten the duration of diarrhoea resulting from acute viral gastroenteritis and in preventing antibiotic-associated diarrhoea in healthy children. Preliminary evidence suggests that probiotics might prevent necrotising enterocolitis in very-low-birth-weight infants, but further studies are needed before definite conclusions can be drawn. Probiotics have also been assessed in the treatment and prevention of allergic disease but the results, although promising, need further confirmation. Targeting a paediatric population, probiotics have been evaluated in the treatment of irritable bowel syndrome, ulcerative colitis, *Helicobacter pylori* gastritis and infantile colic, but at this stage, there is no evidence to support their routine use for these indications. There is a great need for studies aiming at disentangling the mechanisms by which probiotics mediate their clinical effects and for comparative studies between various probiotic bacteria. We still need to know which probiotic(s) to use and for which indications. A clearer message on dosages, optimal timing and duration of administration is needed. For this purpose, more carefully designed and sufficiently powered, randomised controlled trials with predefined outcomes are needed.

Under normal conditions, the gut microbiota and the host thrive in symbiosis, i.e. in close mutuality. The healthy fetal intestine has been considered 'sterile' (or with few bacteria present) and an intense colonisation process starts during delivery. This is a highly dynamic process recently suggested to begin already in utero, and it takes years to develop an adult-type gut microbiota with mostly harmless bacteria – the commensal microbiota. In adults, the numbers of bacteria in the gastrointestinal tract are approximately 10^{14}, thus outnumbering the number of cells in our body by a factor 10. Over the past decades, our modern way of living has contributed to a shift in gut microbial colonisation patterns and composition. Not only intestinal but also extra-intestinal disorders have been proposed to be linked to gut microbial aberrancies[1,2]. In turn, this has led to intense interest in manipulation of the gut microbiota by non-pathogenic micro-organisms such as probiotics in the treatment and prevention of various clinical conditions. Here, we review the current level of evidence (LoE) for probiotics in paediatric clinical practice.

The concept of probiotics

The use of fermented milk goes back several hundred years to pre-biblical times. Nevertheless, it was not until a century ago that the study of health-promoting effects of consumption of soured milk was initiated by the pioneering work of the Nobel Prize laureate Ilya Metchnikoff. He proposed that soured milk could antagonise harmful bacteria in the lower gut and that regular ingestion of soured milk have an impact upon the longevity of Bulgarians. During the same time period, Henri Tissier demonstrated that bifidobacteria were predominant in the gut microbiota of breast-fed infants. He then proposed that administration of these bifidobacteria could restore gut microbial balance and resolve diarrhoeal disease. Thus, the concept of probiotics was born. However, despite these early findings, clinical research in this area has only been carried out in the last decades.

Definition and proposed mechanisms of probiotics

The meaning of probiotics is 'for life'. 'Probiotics' is a broad term, analogous to the term 'antibiotics', for different strains and species of micro-organisms with a diverse variety of specific clinical, immunological and metabolic effects. The WHO and United Nations Food and Agriculture Organization have defined probiotics as 'live microorganisms, which when administered in adequate amounts confer a health benefit on the host'[3]. To date, the most commonly used probiotics are various strains of lactobacilli and bifidobacteria, but other micro-organisms, such as the yeast *Saccharomyces boulardii*, have also been used as probiotics.

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Probiotics share the universal feature that they are non-pathogenic micro-organisms. Conversely, there is significant diversity in confirmed modes of action between single probiotic strains. Probiotics can have direct effects on the chyme and microbiota, and/or effects related to changes in the microbial ecosystem[4]. Moreover, they may exert effects on enterocytes and immune-competent cells in the gut mucosa[5]. However, it must be stressed that most of the information on their effects stems from *in vitro* studies and animal models.

Experience from clinical trials on probiotics

Infectious diarrhoea

Several meta-analyses on the effect of probiotics for treatment of infectious diarrhoea, when used as a supplement to treatment with oral rehydration solutions, have concluded that probiotics reduce the duration of the diarrhoeal period with about 1 d (24 h). In a recent Cochrane review based on sixty-three studies, of which fifty-six recruited infants and young children, and with a total of more than 8000 participants, the duration of diarrhoea was reduced by 27·46 (95 % CI 15·9, 33·6) h[6]. The authors concluded that *Lactobacillus rhamnosus* GG (LGG) was the most effective probiotic based on available studies at the time of the analysis, and dose-dependent for doses exceeding 10^{10} colony-forming units. They further concluded that there was a large heterogeneity between studies including the definition of acute diarrhoea, end of illness, risk of bias and a wide range of different settings, of micro-organisms tested, dosages and participant characteristics.

In a recent expert opinion on the efficacy of *S. boulardii* for treatment of infectious diarrhoea, Dinleyici *et al.*[7] found similar results. This expert opinion was based on a systematic review and meta-analysis of eleven randomised placebo-controlled trials, including a little more than 1300 children. *S. boulardii* shortened the duration of acute diarrhoea with about 24 h (95 % CI −1·40, −0·58) and that of hospitalisation by approximately 20 h (pooled weighted mean difference −0·84 d; 95 % CI −1·14, −0·54). The latter observation was based on a subgroup of 449 children. The results of these two meta-analyses support the joint statement expressed by the European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) and the European Society for Paediatric Infectious Diseases that only probiotic strains with proven clinical efficacy and in appropriate dosages are recommended as an adjunct to rehydration therapy for the management of children with acute gastroenteritis[8]. They further concluded that based on meta-analyses of properly designed randomised controlled trials (RCT) of appropriate size, there is a strong LoE for the efficacy for treatment with LGG and *S. boulardii*[8]. Besides these two probiotics, the World Gastroenterology Organization included *Lactobacillus reuteri* ATCC 55730 and *Lactobacillus casei* DN-114 001 in their evidence-based recommendation and pointed out that timing of administration (the sooner the better) is of much importance[9]. In conclusion, a shorter duration of acute infectious diarrhoea, preferably when caused by viral infection and when added as an adjunct to conventional rehydration therapy, is the most convincing effect shown for probiotics.

Szajewska *et al.*[10] undertook the interesting approach to systematically review whether probiotics would reduce healthcare-associated diarrhoea and found three RCT that involved 1092 children (admitted to the hospital for any reason). Compared with placebo, LGG administration for the duration of hospital stay was associated with significantly lower rates of diarrhoea (two RCT encompassing 823 children; relative risk (RR) 0·37, 95 % CI 0·23, 0·59), and symptomatic rotavirus gastroenteritis (three RCT; *n* 1043; RR 0·49, 95 % CI 0·28, 0·86). The numbers needed to treat were 7 (95 % CI 8, 21) and 35, respectively. There were, however, no significant differences between the intervention and placebo groups in the incidence of asymptomatic rotavirus infection, the duration of hospitalisation or the duration of diarrhoea. However, the analyses were based on few studies and relatively few children. On the other hand, only one probiotic strain was included in the analysis, which is a strength, but, on the other hand, no conclusion can be drawn on the efficacy of other probiotics.

Antibiotic-associated diarrhoea

Besides infectious diarrhoea, antibiotic-associated diarrhoea is probably the most common indication used for probiotics. A recent systematic review addressed that topic for children. Randomised, parallel, controlled trials in children (2 weeks to 17 years of age) receiving antibiotics and placebo actively or as prophylactic, or no treatment were included and the incidence of diarrhoea secondary to antibiotic use was evaluated. In total, sixteen studies met inclusion criteria. The incidence in the probiotic group was 9 % compared with 18 % in the control group (3392 participants; RR 0·81, 95 % CI 0·63, 1·04). Including only studies using a high dose of probiotics (defined as $>5 \times 10^9$ colony-forming units/d), the prevalence of diarrhoea was 8 and 22 %, respectively (1474 participants; RR 0·40, 95 % CI 0·29, 0·55, and numbers needed to treat 7, 95 % CI 6, 10). The main conclusion was that LGG and *S. boulardii* at high dose (5×10^9–4×10^{10} colony-forming units/d) may prevent the onset of antibiotic-associated diarrhoea. However, a GRADE analysis (a consensus on rating quality of evidence and strength of recommendations) indicated overall low quality of the evidence, particularly due to high loss to follow-up and sparse data (few events). The authors suggested that the result obtained needs to be verified in a large, well-designed randomised study. No conclusions about the safety and efficacy of other probiotics could be drawn with respect to paediatric antibiotic-associated diarrhoea[11].

Upper respiratory tract infections

In the most recent Cochrane review on the efficacy of probiotics in preventing upper respiratory tract infections (URTI) including ten placebo-controlled randomised trials (3451 participants including both children and adults), Hao *et al.*[12] concluded that probiotics were superior to placebo when

measuring the number of participants experiencing at least one episode of acute URTI (six studies; RR 0·58, 95 % CI 0·36, 0·92). For at least three episodes, the corresponding values were RR 0·53 (95 % CI 0·36, 0·80). There was also a reduced rate of antibiotic prescription in the probiotic group (based on only three studies), but no difference in duration of the URTI episodes[12]. Overall, the authors concluded that the analysis indicated that probiotics may be more beneficial than placebo for preventing acute URTI, but that evidence is weak. It is of note that RCT in children show no consensus, but then again, few studies targeting a paediatric population were included in the meta-analysis. Hence, there is not sufficient evidence for a general recommendation to use probiotics for the prevention of URTI in this population.

Necrotising enterocolitis

Necrotising enterocolitis (NEC) is a serious disease that affects the bowel of premature infants in the first few weeks of life. Although the cause of NEC is not entirely known, type of feeding and bacterial growth play a role, and there is a clear variation between countries and neonatal units. A recent meta-analysis based on sixteen studies (2842 infants with birth weight <1500 g) concluded that included trials were heterogeneous with respect to birth weight, gestational age, baseline risk of NEC in control groups and probiotic formulation. Moreover, data did not allow to extrapolate the efficacy and safety regarding extremely low-birth-weight infants (birth weight <1000 g). With these precautions, the authors concluded from their analyses that probiotics compared with placebo reduced the incidence of severe NEC (Bell's stage II or more; RR 0·35, 95 % CI 0·24, 0·52) and mortality (RR 0·40, 95 % CI 0·27, 0·60), but not the risk of nosocomial sepsis (RR 0·99, 95 % CI 0·76, 1·07). Although the results are promising, the authors stated that more studies are needed to assess the efficacy in extremely low-birth-weight infants as well as the most effective dose and formulation to be used[13].

In their recent systematic review of the LoE of published RCT for routine use of probiotics for reduction of mortality and prevention of NEC and sepsis in preterm infants, Mihatsch et al.[14] used a slightly different approach. Their conclusions were based on fifteen trials of which methodological assessment revealed considerable heterogeneity. Overall, they concluded that there is no conclusive evidence (LoE 1a, i.e. meta-analyses of well-designed RCT, meaning LoE 1b studies) on which to base a general recommendation for routine use of probiotics in preterm infants, or in special subgroups such as very-low-birth-weight preterm infants to reduce mortality, incidence of NEC or sepsis. They found that only two probiotic strains, LGG and *Bifidobacterium lactis*, had been used in more than one trial. When analysing these two probiotics separately (*B. lactis*, three studies; LGG, two studies), no significant reduction of the incidence of severe NEC, mortality or culture-proven sepsis was found for either of them. The authors, however, acknowledge that in specific situations, such as local high incidence of NEC or mortality, clinicians may feel justified to consider off-label use of specific probiotics with some documented efficacy and safety (LoE 2b,

i.e. lesser-quality RCT). Individual risk–benefit considerations would then be required. However, based on the limited evidence from current available trials, no optimum strain, dosing or protocol could be defined. Finally, the authors calculated that given a NEC incidence (Bell's stage >2) of 5 % in very-low-birth-weight infants, a sufficiently powered double-blinded RCT to detect a NEC reduction of 50 % would require 714 infants per group, which is much larger than any study conducted so far. Importantly, the authors point out that data generated with one strain do not necessarily apply to another strain, i.e. safety and efficacy need to be proven for each strain separately. Nor is it known if single strain or multiple strain products is the most efficacious[14].

Allergic disease

Mounting evidence indicates that early environmental exposures may influence susceptibility to development of immune-mediated disease such as allergic disease[15]. One of the most undeniable hypotheses proposed to explain the epidemic rise in allergic disease has been the observation of aberrancies in early gut microbial composition and patterns in infants that subsequently develop allergic disease[16,17].

Observational studies have proposed a link between declining microbial exposure and allergic disease[18]. This is further supported by animal models showing that early exposure to pathogenic[19] or non-pathogenic microbial products[20] can either prevent or adjust allergic responses. The gut microbiota mediates specific immune-protective effects through intricate pathways within (and potentially even beyond) the gut-associated lymphoid tissue. These effects include local IgA production and induction of tolerogenic dendritic cells and regulatory T-cell populations, with production of immune-modulatory cytokines such as IL-10 and transforming growth factor β (reviewed in Prescott & Björkstén[21]). These mechanisms are proposed to collectively regulate local inflammation, improve gut barrier mechanisms and, consequently, decrease the risk of inappropriate systemic immune responses. Taken together, this provided a basis for intervention studies designed to shape aberrant gut microbiota or to affect postnatal colonisation. Consequently, the effects of probiotics have been assessed in both allergy treatment and prevention studies.

Probiotics in treatment of allergic disease. There are now several RCT that have examined the role of probiotics in allergic disease, most studies including infants and young children with eczema with or without associated food allergy (reviewed in Tang et al.[22] and Prescott & West[23]). In a meta-analysis by Tang et al.[22] including seven trials, there was no significant reduction in eczema symptoms with probiotic treatment compared with placebo (mean difference 0·90 points on a 20-point visual analogue scale; 95 % CI 1·04, 2·84). Further, there was no significant decrease in investigator-rated eczema severity by probiotic treatment (588 participants). Subgroup analysis by eczema severity or evidence of sensitisation could not reveal a population with different treatment outcomes, and there was no evidence of

a difference in eczema symptoms between treatment groups. The authors observed significant heterogeneity between studies, which might be explained by the use of different probiotic strains. They further stated that a lack of effect based on pooled data from different probiotics does not exclude the possibility that specific strains or a combination of strains could be effective.

Currently, probiotics for treatment of respiratory allergic disease are scarcer (summarised in Tang *et al.*[22] and Prescott & West[23]) and basically more studies are needed before any definite conclusions can be drawn.

Probiotics in prevention of allergic disease. A number of randomised trials have evaluated the effects of probiotics in allergy prevention (reviewed in West & Prescott[24]). At least fourteen published trials have assessed the effects of probiotics in primary prevention of allergic disease. At present, just about half of the reported studies have shown a reduction in eczema, while the remaining studies have not (summarised in Tang *et al.*[22], West & Prescott[24] and Pelucchi *et al.*[25]). The majority of studies have shown no decrease in food allergy, allergic disease in general or allergic sensitisation. However, the design of the studies varies in most aspects, which hinders direct comparison[21,24]. Most studies have targeted infants at a high risk of allergic disease and some studies have combined prenatal and postnatal probiotic administration. In the meta-analysis by Tang *et al.*[22], the authors concluded that studies combining pre- and postnatal administration were more likely to have a benefit[22]. Then again, the only randomised trial to explicitly examine the prenatal effects of probiotics with no postnatal administration found no benefit[26]. Only one study[27] has specifically examined the effects of probiotic supplementation with a *Lactobacillus paracasei* strain during weaning from breast-feeding (from 4 to 13 months) and observed a reduction in eczema. These studies suggest that prenatal administration is not the only determinant for a clinical benefit. This view is supported by the most recent meta-analysis including fourteen studies and about 3000 infants[25]. This meta-analysis demonstrated that probiotics decreased the incidence of eczema (RR 0·79, 95 % CI 0·71, 0·88). The corresponding RR of IgE-associated eczema was 0·80 (95 % CI 0·66, 0·96). No substantial difference emerged across strata (study period, type of study subject, dose and duration of intervention). The authors concluded that there is evidence in support of a moderate benefit of probiotics in the prevention of eczema and IgE-associated eczema in infancy, and that the favourable effect was comparable regardless of the time of probiotic use (pregnancy or early life) or the subject(s) receiving probiotics (mother, child or both).

Very few published studies[28,29] have evaluated the long-term effects on respiratory allergic disease, but those that have, observed no benefit of probiotics.

Taken together, meta-analyses[22,25] suggest a role for probiotics in the prevention of eczema, but the effect appears to be modest. However, long-term follow-ups of these study cohorts have not yet been sufficiently assessed. There is less promise for probiotics in treatment of eczema, and it has been discussed that when gut colonisation and the allergic phenotype are established, the beneficial effect of probiotics is reduced compared with at younger ages when there is more plasticity[24]. With the exception of eczema prevention, there is no consistent evidence that probiotics are effective in the treatment or prevention of any allergic condition. More definitive clinical studies are needed before any general recommendations can be given. Thus, with the available evidence, probiotics are not recommended in the treatment or prevention of any allergic condition. Several ongoing clinical trials are anticipated to provide a clearer message.

Recommendations on use of probiotics in infants and children

Recently, both the ESPGHAN Committee on Nutrition and the American Academy of Pediatrics Committee on Nutrition reviewed the evidence for use of probiotics in infants and children. ESPGHAN[30] concluded that currently evaluated probiotic- and/or prebiotic-supplemented formulae to healthy infants do not raise safety concerns with regard to growth and adverse effects, but also that the safety and clinical effects of one product should not be extrapolated to other products. It remains to define the optimal doses and intake durations, as well as to generate more information about the long-term safety of probiotics. The committee further concluded that there are insufficient data to recommend the routine use of probiotic- and/or prebiotic-supplemented formulae, but that supplementation of formula with probiotics and/or prebiotics is an important research area. The committee called for well-designed and carefully conducted RCT, with relevant inclusion/exclusion criteria and adequate sample sizes, and for validated clinical outcome measures to assess the effects of probiotic and/or prebiotic supplementation of formulae.

American Academy of Pediatrics concluded in their review[31] that there is some evidence that probiotics prevent NEC in very-low-birth-weight infants (1000–1500 g), but that more studies are needed. They further stated that results of RCT on treatment of childhood *Helicobacter pylori* gastritis, irritable bowel syndrome, ulcerous colitis and infantile colic, as well as in preventing childhood allergic disease, although encouraging, are preliminary and require further confirmation. Moreover, they stated that probiotics have not been proven to be beneficial in treating or preventing human cancers or in treating children with Crohn's disease. This committee also noted that there are safety concerns with the use of probiotics in infants and children who are immune-compromised, chronically debilitated or seriously ill with indwelling medical devices. Finally, American Academy of Pediatrics also stressed that the current lack of evidence of efficacy does not mean that future clinical research will not establish significant health benefits for probiotics[31].

Conclusions

In conclusion, although some published studies have shown a benefit of probiotics specifically in the treatment of acute infectious viral diarrhoea, in the prevention of eczema and possibly in antibiotic-associated diarrhoea, there are still many gaps in our understanding and therefore many unanswered questions. More research is needed to provide a clearer message on the

mechanisms behind the potential effects of probiotics and the benefits and limitations of their use. More uniform criteria would help to make results of studies conducted with different strains and in different populations more comparable[24,32]. However, the effects of probiotics are probably influenced by various host and environmental factors (24). Maternal microbiota, general microbial burden, mode of delivery, feeding practices and weaning diet, antibiotics, and other immune-modulatory influences may have secondary effects on colonisation[21,24]. These factors may explain some of the discrepancy seen in subpopulations and apparently similar studies. Further, differences in the probiotic supplement such as strain, dose, viability, timing, duration, adherence and method of administration may result in differences in clinical effect. Although there are some promises, besides supplement to oral rehydration solutions for treatment of acute infectious diarrhoea, the current evidence is insufficient to recommend probiotics for general treatment or prevention of other diseases. With a number of ongoing clinical trials, it seems likely that there will be more distinct answers for some proposed indications in the near future.

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