

## Probiotics in Paediatric Practice

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This review summarizes the clinical indications for the use of probiotics in paediatric clinical practice, based on the available scientific evidence. The recent panel of the International Scientific Association of Probiotics and Prebiotics recommended in 2014 that the term “probiotic” should only be used for products that deliver live microorganisms with a suitable viable count of well-defined strains with a reasonable expectation of delivering benefits for the wellbeing of the host. In this review we searched the relevant guidelines on the use of probiotics in children, recommendations and position papers covering the paediatric clinical indications and summarize the high – quality evidence. Some specific probiotic strains are effective in preventing antibiotic-associated diarrhoea, nosocomial diarrhoea and upper respiratory tract infections, in the treatment of acute gastroenteritis, infantile colic in breastfed infants, and mild ulcerative colitis, but have disappointing results in remission of Crohn’s disease and prevention of food allergies. **Conclusion** – The capacity of probiotics to prevent, improve illnesses and relieve symptoms varies, as well as their mechanisms, effects and safety, are strain specific. Therefore, every strain is disease specific and not all probiotics should be considered equal. Special caution is advised in immunocompromised and severely ill patients in the Intensive Care Unit.

## Introduction

The new term – microbiota – replaced the previous one – “gut flora” – based on the fact that microbiota plays a key role in the intestinal ecosystem. Microbiome implies all the microbes present with their genes and interactions with each other. It is estimated that the human microbiome comprises about 100 million cells, which is 10 times more than the number of human cells (1, 2). Most of this microbiota controls numerous physiological and pathological processes. Any disruption to the microbial equilibrium may be associated with a serious gastrointestinal disease or a disease outside the gastrointestinal tract. The focus of medical research has become prima-

ry prevention of gastrointestinal diseases, improving the composition of the microbiome and maintaining ‘gut health’. Therefore, the approach has changed and the management of common pathological conditions involves deliberate probiotic-based modulation of gut microbiota composition (3). The first beneficial effect of bacteria was described by the Ukrainian scientist Eli Metchnikoff, a Nobel laureate in medicine in 1908, who is today known as the ‘father’ of modern probiotics. He was the first to draw the attention of the medical public to the longevity of Bulgarian peasants due to their extensive and regular use of fermented dairy products which contain lactic acid bacteria (*Lactobacillales*), and

he called them ‘*the Bulgarian bacillus*’. The first consensus on definitions in the field of probiotics between the Food and Agriculture Organization of the United Nations and the World Health Organization was adopted in 2001. An expert panel of the International Scientific Association for Probiotics and Prebiotics defined probiotics as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” and this was reinforced as relevant. To date, the mechanisms of probiotics have not yet been entirely deciphered, but the scope of their use has been significantly extended over the past few years, driven by the global advancement in the understanding of the role of microbiota in both health and disease (4, 5).

This review summarizes the clinical indications of the use of probiotics in pediatric clinical practice, based on the available scientific evidence.

### Probiotic Use in the Prevention of Infections in Day Care Centers

Infectious diseases are the most common cause of morbidity in children, where respiratory and gastrointestinal infections (GI) account for the majority of them. Two major

settings where children acquire respiratory and GI infections are hospitals and day care centers. Children who attend daycare centers usually have 2-3 times more infections than children who stay at home. Respiratory tract and GI infections are some of the most common health care problems for pediatricians who have to separate children who are at higher risk, and try to offer preventive measures (including good hand hygiene, the absence of ill children from daycare and vaccination for influenza and rotavirus). Very often all these measures are ineffective, opening room for possible new modalities, such as probiotics (6, 7). Several randomized controlled trials (RCTs) have investigated probiotic use in the prevention of common infections in children who attend daycare centers. Most of them found that probiotics were able to diminish upper respiratory tract infections, but without any clear explanation about the strains to use or the duration of use (7). On the basis of well-designed RCTs in children, *Lactobacillus rhamnosus GG* (LGG) was examined in 3 studies (8-10) involving 1,375 children receiving doses from  $10^8$  to  $10^9$  colony forming units (CFU)/day, and all of them reported a positive effect on the incidence of respiratory tract infections (Table 1). The other strain in-

Table 1. Probiotics in the Prevention of Respiratory and Gastrointestinal Infections in Children Attending Day Care Centers

Author(s)	Probiotic strain	N (Age)	Probiotic dose (CFU)*	Effect on respiratory infection (RCTs)†	Effect on GI infection
Hatakka et al. (2001)	<i>Lactobacillus rhamnosus GG</i>	571 (1-6 y)	$1-2 \times 10^8$ CFU/day	Lower number of URTI‡ and Lower number of PA§	Not significant
Kumpu et al. (2012)	<i>Lactobacillus rhamnosus GG</i>	523 (2-6 y)	$6.7 \times 10^5$ to $1.9 \times 10^6$ CFU/mL	Lower risk of respiratory infection	Not assessed
Hojsak et al. (2010)	<i>Lactobacillus rhamnosus GG</i>	281 (1-7 y)	$10^9$ CFU	Lower number of URTI‡	Not significant
Weizman et al. (2005)	<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> (Bb12) or <i>Lactobacillus reuteri</i> 55730	210 (4-10 mo)	min $10^7$ CFU	No significant difference in the incidence of URTI‡	Not assessed
Merenstein et al. (2010)	<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> (Bb12)	182 (13 y)	$10^{10}$ CFU	Not significant	Not significant
Merenstein et al. (2011)	<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> (Bb12)¶	172 (2-4 y)	$(10^{10})$ CFU	Not significant	Not significant

\*Colony Forming Units; †Randomized Controlled Trials; ‡Upper Respiratory Tract Infections; §Prescribed Antibiotics; ¶Yoghurt Containing Streptococcus Thermophilus and Lactobacillus Bulgaricus.

investigated in four RCTs (11-14) was *B. animalis subsp. lactis* BB-12, and in contrast to LGG, all the results were negative. In conclusion, LGG should be considered for the prevention of upper respiratory tract infections in children attending day care centers. At present, there is no evidence to recommend the use of probiotics for the prevention of gastrointestinal infections in children in day care centers (7, 15).

### **Probiotic Use in the Prevention of Nosocomial Infections**

Nosocomial infections develop during a hospital stay and they are not present upon admission. Infections that occur more than 48 hours after admission are usually considered nosocomial. The incidence of nosocomial infections on pediatric wards is still high even in developed countries and most of them are GI and respiratory tract infections. Nosocomial infections have several negative impacts on the treatment outcome: they prolong hospitalization and significantly increase hospital costs. Standard preventive measures, including hand hygiene, separation of sick children and a reduction in the number of hospitalized patients, cannot successfully prevent them, hence the new approach – the use of probiotics (7, 15). On the basis of the evidence currently available, the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Working Group (WG) for Probiotics and Prebiotics, recommends using LGG in the prevention of nosocomial diarrhea in children, at least  $10^9$  CFU/day, for the duration of the hospital stay, (quality of evidence: moderate; recommendation: strong) (16). This WG performed a systematic review of the role of different probiotic strains in the prevention of nosocomial diarrhea. Eight RCTs were included and 3 of them investigated LGG. The use of LGG decreased the risk of nosocomial diarrhea from

13.9% to 5.2% (2 RCTs, N=1823; Risk ratio (RR), 0.35; 95% Confidence interval (CI), 0.19 to 0.65) (16). *Lactobacillus reuteri* DSM 17938 was investigated in two studies (the same probiotic strain but different doses,  $10^8$  CFU/day (17) and  $10^9$  CFU/day (18), but had negative results (RR, 1.11; 95% CI, 0.68 to 1.81) (16).

There is not enough evidence to recommend the use of probiotics for the prevention of nosocomial respiratory tract infection. The authors also noted that children who stayed longer in hospital and who were younger had more chance of acquiring upper respiratory tract infections (6).

### **Probiotic Use in the Prevention of Antibiotic-Associated Diarrhea**

Antibiotic-associated diarrhea (AAD) is a common complication of antibiotic therapy, defined as diarrhea that occurs in relation to antibiotic treatment with the exclusion of other etiologies (19). It is more commonly caused by antibiotics that target anaerobic bacteria (e.g. clindamycin, penicillin, amoxicillin/clavulanic acid etc.) which cause significant disruption of the enteric microbiome (7). AAD may present as mild diarrhea, but it can also present as fulminant pseudomembranous colitis caused by *Clostridium difficile* (20). Therapies that can prevent AAD are limited, mainly by reduction of antibiotic use, the type of antibiotic prescribed and the use of probiotics. ESPGHAN WG for Probiotics and Prebiotics performed a systematic review with meta-analysis, aiming to provide evidence-based guidelines for every specific probiotic strain in the prevention of AAD (21). This systematic review found only two probiotic strains with enough evidence (efficacy proven in more than 2 well-designed RCTs). LGG was investigated in 5 RCTs (N=445) and its administration in children reduced the risk of AAD from 23% to 9.6% while

only one trial evaluated the effect of LGG in the prevention of *Clostridium difficile*-associated diarrhea in children, and found no effect (22). Similarly, *Saccharomyces boulardii* (*S. boulardii*) reduced the risk of AAD based on 6 RCTs (N=1,653) from 20.9% to 8.8% (RR, 0.43; 95% CI, 0.30 to 0.60) (21). The administration of *S. boulardii* also reduced the risk of *Clostridium difficile*-associated diarrhea in children (2 RCTs, N=579); RR, 0.25; 95% CI, 0.08 to 0.73) (21).

In summary, the recent review identified only two strains as effective in prevention of AAD. These are LGG (quality of evidence:

moderate; recommendation: strong) and *S. boulardii* (quality of evidence: moderate; recommendation: strong). For prevention of *Clostridium difficile*-associated diarrhea, only *S. boulardii* showed efficacy (quality of evidence: low; recommendation: conditional) (19) (Table 2). There is always a question for the clinicians about when to administer probiotics so they are not killed by antibiotics and there is no scientific evidence for this. However, some probiotic strains (such as *S. boulardii*) are resistant to antibiotics used for bacterial infections (23).

Table 2. Probiotics with a Positive Recommendation in the Prevention of Antibiotic-Associated Diarrhea in Children

Probiotics for preventing AAD* in children			
Probiotic	Quality of evidence	Strength of recommendation	Dose (CFU) <sup>†</sup>
<i>Lactobacillus rhamnosus</i> GG	Moderate	Strong	1–2 × 10 <sup>10</sup> CFU/day for the duration of ABT <sup>‡</sup>
<i>Saccharomyces boulardii</i>	Moderate	Strong	250–500 mg (1 × 10 <sup>10</sup> ) for the duration of ABT <sup>‡</sup>
Probiotics for preventing <i>Clostridium difficile</i> - associated diarrhea in children			
<i>Saccharomyces boulardii</i>	Low	Conditional	500 mg (1 × 10 <sup>10</sup> )

\*Antibiotic-Associated Diarrhea; <sup>†</sup>Colony Forming Units; <sup>‡</sup>Antibiotics Therapy.

## Probiotic Use in the Treatment of Acute Diarrhea

Acute gastroenteritis (AGE) is a very common disease in children. The incidence of AGE is still high, even in Western industrialized countries and it still represents one of the major causes of death. ESPGHAN has defined AGE as a decrease in the consistency of stools – loose or liquid and/or an increase in the frequency of evacuations, at least three in 24 hours, with or without fever or vomiting (15, 24). The major cause of AGE in children is still rotavirus, which is decreasing in countries with a high rate of rotavirus vaccination, followed by norovirus. The treatment strategy aims to treat and prevent dehydration, shorten the duration of diarrhea and to prevent prolonged diarrhea. This treatment can be provided orally in the

majority of children by using oral rehydration solutions (ORS), the first-line therapy. Other well-defined treatment modalities include probiotics (7, 15). Administration of probiotics may be considered if there is solid proof of efficacy. To maximize efficacy, active treatment should be administered early in the course of the disease. Administration of any product should not replace oral rehydration therapy and should be always used as an addition to ORS treatment. Since investigation of active therapies is rapidly evolving, the choice of best treatment should be always made according to the recommendations of evidence-based guidelines and in compliance with well-done RCTs. Recently, ESPGHAN WG for Probiotics and Prebiotics performed a systematic review and provided guidelines on the use of different probiotic strains for the treatment of AGE (21). Based on the

available, well designed RCTs, ESPGHAN WG recommended probiotic strains that had proved to be effective in at least two RCTs. These probiotics were LGG and *S. boulardii*. LGG was investigated in 11 RCTs (N=2,072) and this meta-analysis found that the use of LGG reduced the duration of diarrhea, with a mean of 27 hours (95% CI, -41 to -13) (25). Later on, a systematic review identified 15 RCTs (N=2,963). This review also confirmed the superiority of LGG in significantly decreasing the duration of diarrhea compared to a placebo [Mean difference (MD) -1.05 days; 95% CI, -1.7 to -0.4; based on 11 RCTs]. A dose  $\geq 10^{10}$  CFU was more effective than  $<10^{10}$  CFU (26).

Another strain with a well proven effect is *S. boulardii*. A Cochrane review found 6 RCTs (N=606) and reported a reduced risk of diarrhea lasting  $\geq 4$  days (RR 0.37; 95% CI, 0.2 to 0.65) if *S. boulardii* was used (25). A more recent systematic review analyzing 11 RCTs (N=1,306) showed that *S. boulardii* significantly reduced diarrhea duration (MD, -0.99 days; 95% CI, -1.4 to -0.6) (27).

None of the studies evaluated the influence on stool volume.

The third strain, *Lactobacillus reuteri* ATCC 55730 showed a moderate clinical effect in treating acute gastroenteritis in children, but because this strain was found to carry a transferable resistance trait for antibiotic resistance, it was replaced by a new strain, *Lactobacillus reuteri* DSM 17938 (28). The new strain, *Lactobacillus reuteri* DSM 17938, was investigated by 3 RCTs. Two of them (N=196) were analyzed in a systematic review and showed a significant reduction in diarrhea duration (MD, -32 hours; 95% CI, -41 to -24) (29).

Generally, after reviewing these results, ESPGHAN WG for Probiotics and Prebiotics recommended the use of LGG and *S. boulardii*, with a strong recommendation and of *Lactobacillus reuteri* DSM 17938 with a weak recommendation, as adjuncts to rehydration therapy (Table 3). For clinicians, it is of great importance to know that probiotics have been proven efficacious mostly in watery, usually viral diarrhea (21).

Table 3. Probiotics Recommended in the Treatment of Acute Gastroenteritis in Children

Probiotic	Quality of evidence	Recommendation	Dose (CFU)*
<i>Lactobacillus rhamnosus</i> GG	Low	Strong	$10^{10}$ CFU/day (typically 5–7 days)
<i>Saccharomyces boulardii</i>	Low	Strong	250–750 mg/day (typically 5–7 days)
<i>Lactobacillus reuteri</i> DSM 17938	Very low	Weak	$10^8$ to $4 \cdot 10^8$ (typically 5–7 days)

\*Colony Forming Units.

### Probiotics and Helicobacter Pylori Eradication Therapy

The role of supplementary probiotic therapy to reduce adverse effects, improve adherence, and increase the efficacy of *Helicobacter pylori* eradication regimens remains controversial. Several meta-analyses suggest that probiotics in *Helicobacter pylori* therapy in children reduce the incidence of adverse effects of the therapy. This effect depends on the probiotic strain. One meta-analysis found that

*S. boulardii* significantly decreased some therapy-related side effects, and significantly increased the eradication rate, but it was still below the desired level of success (30). Another meta-analysis investigated the efficacy of eradication regimens supplemented with a *lactobacillus*-containing probiotic. They concluded that the *Lactobacillus*-containing probiotic was effective for eradication, while the side effects caused by eradication treatment may not decrease (31).

In summary, there is currently insufficient evidence to support the concept that a probiotic or combination of probiotics may be helpful as an adjuvant therapy along with antibiotics in *Helicobacter pylori* eradication (32, 33).

### **Probiotics in the Prevention and Treatment of Allergic Diseases**

In contrast to the World Allergy Organization, which recommends the use of probiotics in pregnant women and breastfeeding women with high risk of allergy, and in infants with atopic predisposition, other guidelines do not recommend the use of probiotics in the prevention of atopic disease (15). The recommendation for probiotic use to prevent allergic diseases must be supported by evidence that special strains or mixtures of specific probiotic strains (administered during pregnancy, breastfeeding, or directly through supplements) reduce the risk of subsequent allergic manifestations. Although there have been many systematic reviews and meta-analyses in this field, their results were sometimes controversial. The best example of diverse reactions to the use of the identical probiotic strain LGG in pregnant women and their infants in two different populations (from Finland and Germany), but with similar study design, presents controversial results. While the Finnish study showed a significant reduction in the risk of atopic eczema in the probiotic group versus the placebo (34), the German group proved the opposite (35, 36). One recent meta-analysis included 17 RTCs, with 4755 children and found that the use of probiotics decreased the risk of atopic dermatitis, but there were no significant differences in preventing asthma, wheezing or rhinoconjunctivitis. In line with the currently available evidence, probiotics cannot be recommended for the prevention of atopic diseases (15, 36).

### **Probiotics in the Prevention of Necrotizing Enterocolitis in Preterm Infants**

Necrotizing enterocolitis (NEC) is a disease in preterm infants strongly associated with gestational age. Its pathogenesis is not completely understood as its occurrence may be the result of a variety of different etiologies and early detection is difficult. Delayed enteral feeding, frequent use of antibiotic therapy, and altered acquisition of normal digestive microflora are believed to be the primary contributing factors for increased risk of NEC in preterm infants. There is much evidence showing that, in addition to the effect of human milk, feeding probiotics may be important in preventing NEC and reducing mortality (37, 38). The ESPGHAN WG for Probiotics and Prebiotics and the Committee on Nutrition performed a network meta-analysis (NMA) and established that there were some significantly effective strains or combinations of strains that reduced NEC grade 2 or 3. The positive results of probiotic administration in the prevention of NEC relate to preterm infants who weigh more than 1800 gr at birth. The high-risk group (extremely low-birth-weight infants who weighed less than 1800 gr at birth) could not be used to estimate the efficacy and safety of probiotic supplementation reliably. However, there is not enough evidence to recommend specific bacterial strains, doses or combinations of probiotics in the prevention of NEC (37, 38).

### **Probiotics in Treatment of Inflammatory Bowel Disease**

The term “inflammatory bowel disease” (IBD) describes disorders that involve chronic inflammation of digestive tract. The Pediatric IBD Porto group of ESPGHAN published the “PIBD-Classes” criteria that standard-

ized the differentiation of pediatric IBD into 5 categories: typical ulcerative colitis (UC), atypical ulcerative colitis, IBD-unclassified (IBDU), Crohn's colitis and Crohn's disease (CD) (39). Recent advances suggest that IBD may have a multifactorial etiology, where complex interactions between environmental factors, epigenetics, genetics, and the host immune system lead to abnormal immune responses and chronic inflammation (40).

Symptoms may range from mild to severe, and periods of active illness are followed by periods of remission. The recent guidelines for managing children with UC, CD and IBD – unclassified, providing a modern management strategy, were developed to assist practitioners at all levels of health care, while recognizing that each patient is unique. Probiotics have been investigated for induction and maintenance of remission in UC. There were one pediatric and 3 adult trials that found *E coli Nissle 1917* to be as successful as mesalamine in maintaining remission (41, 42). The dosage used in all these studies, including the pediatric one, was 200 mg/day (100 mg contain 25 x 10<sup>9</sup> viable *E coli* bacteria), administered as capsules. A recent systematic review and meta-analysis selected six trials, with 719 patients (390 in the study group and 329 in the control group), where *E coli Nissle 1917* induced remission in 61.6% of cases, while in the control group (mesalazine) remission was achieved in 69.5% of cases, with a MD of 7.9% (43). Another small, randomized, placebo-controlled trial involving 29 children treated with 5-ASA reported that the combination of VSL#3, in conjunction with concomitant steroid induction and mesalamine, was superior to a placebo in inducing and maintaining 1-year remission (44). A small open-label study in 18 children with mild-moderate UC, investigated the efficacy of VSL#3 added to standard treatment with a 56% remission rate (45). All studies on VSL#3 in IBD patients were performed

with the original formulation containing 8 bacterial strains (*Lactobacillus paracasei* DSM 24733, *Lactobacillus plantarum* DSM 24730, *Lactobacillus acidophilus* DSM 24735, *Lactobacillus delbrueckii subspecies bulgaricus* DSM 24734, *Bifidobacterium longum* DSM 24736, *Bifidobacterium infantis* DSM 24737, *Bifidobacterium breve* DSM 24732, and *Streptococcus thermophilus* DSM 24731). It is worth noticing that different manufacturers changing the manufacturing processes also changed the clinical efficacy and safety, as shown in the data report published (41).

One randomized pediatric trial assessed 40 children (median age 7.2 years, range 6-18) with active distal UC and showed that rectal infusion of *Lactobacillus reuteri* was effective in improving mucosal inflammation and changing the mucosal expression levels of some cytokines involved in the mechanisms of IBD. Evaluation of cytokines demonstrated that IL-10 significantly increased ( $p < 0.01$ ) whereas IL-1 $\beta$ , TNF $\alpha$  and IL-8 significantly decreased ( $p < 0.01$ ) in the *Lactobacillus reuteri* group alone (46). The dose of probiotic in the enema solution was 10<sup>10</sup> CFU of *Lactobacillus reuteri* ATCC 55730.

The European Crohn's and Colitis Organization (ECCO) and ESPGHAN guidelines recommended (98% agreement) the use of VSL#3 and *E coli Nissle 1917* in UC (Table 4). No dosing recommendation for *E coli Nissle 1917* is available for young children. In contrast to these results, other RCTs where probiotics were used in pediatric patients with CD did not show benefits. Therefore, the use of probiotics in CD could not be recommended. According the Consensus Guidelines of European Crohn's and Colitis Organization (ECCO) and ESPGHAN on the medical management of pediatric CD probiotics are not recommended for maintenance of remission [Evidence level (EL) 3] 96% agreement (47).

Table 4. Dosage of Probiotics Recommended in the Treatment of Children with UC\* and IBD† – Unclassified

Age (year)	Weight (kg)	Daily/dose (bacteria/day)
VSL#3 (Mielle, et al.)		
4–6	17–23	1 sachet (450 billion)
7–9	24–33	2 sachets (900 billion)
11–14	34–53	3 sachets (1350 billion)
15–17	54–66	4 sachets (1800 billion)
<i>E coli</i> Nissle 1917		
Adults and adolescents only	-	200 mg/day <sup>§</sup>

\*Ulcerative Colitis; †Inflammatory Bowel Disease; §No Dosing Recommendation Is Available for Young Children.

### Probiotics in the Treatment of Functional Gastrointestinal Disorders

Functional gastrointestinal disorders (FGIDs) in children are common in the entire age group and affect the quality of life of both patients and their families. The latest consensus from the Rome Foundation suggest that these disorders are “the product of interactions of psychosocial factors and altered gut physiology via the brain–gut axis” Functional abdominal pain (FAP) in children represents a group of functional gastrointestinal disorders that yet have no clear etiology. Accordingly, there is no causal treatment (48, 49). Since one of the findings for a cause is altered intestinal microbiota, probiotics were proposed as one of the treatment modalities. Before suggesting the probiotic-based treatment of FAP in children, the efficacy of specific probiotic species and strains or their combinations must be proven through clinical studies, which should include strain specific analysis (15). However, the 2017 meta-analysis did not perform strain-specific analysis, although it showed that probiotics, in general, significantly reduced the frequency of abdominal pain compared to the placebo (standardized MD 0.55; 95% CI –0.98 to –0.12). Therefore, clinically relevant recommendations cannot be provided (50). Some of the latest summary evidence from 3 systematic reviews of treatment effectiveness showed the lack of evidence of efficacy

for any drug suggested including probiotics (49). There is some evidence that probiotics could decrease pain intensity in children with FAP but only two strains (LGG and *Lactobacillus reuteri* DSM 17 938) were proven to be effective in more than two RCTs (15). It was, however, difficult to interpret the results because they included different study protocols, durations of interventions, primary outcomes and type of pain. In conclusion, there is insufficient evidence for the use of probiotics in FAP. Only LGG seems to reduce the frequency and intensity of abdominal pain, but only in children with irritable bowel syndrome (51).

Infantile colic is a common FGID in infants aged 1 to 5 months, involving long periods of inconsolable crying, with prevalence rates varying from 3%-28%. According to the Rome IV criteria, infantile colic may be defined in an infant who is “less than 5 months of age when their symptoms start and stop, they present with recurrent and prolonged periods of crying, fussing or irritability that occur without an obvious cause that cannot be prevented or resolved by caregivers, and in whom there is no evidence of failure to thrive, fever or illness” (52). The etiology of this functional disorder in infants is still undefined, but intestinal dysbiosis has been a possible underlying condition, suggesting that probiotics could be useful in prevention. In accordance, an individual



participant data meta-analysis (IPDMA) included 4 double-blind RCTs involving 345 infants with colic, 174 receiving a probiotic and 171 a placebo. The intervention groups in all 4 studies received the same probiotic, *Lactobacillus reuteri* DSM17398, in the same dose ( $0.2 \times 10^8$  CFU/per drop, 5 drops orally per day), with all control groups receiving the same placebo (maltodextrin in oil suspension). All 4 studies included had the outcome measurement of infant crying and/or fussing duration. The probiotic group averaged less crying and/or fussing time than the placebo group at all time points (day 21 adjusted MD in change from baseline (minutes)  $-25.4$ , 95% CI:  $-47.3$  to  $-3.5$ ). Intervention effects were better in breastfed infants but were not significant in formula-fed infants. The conclusion was that *Lactobacillus reuteri* DSM 17938 was effective in treating breastfed infants with colic, but not formula-fed infants (53). A recent systematic review and meta-analysis included altogether seven randomized controlled trials, with 471 participants. Compared with the placebo, the administration of *Lactobacillus reuteri* DSM 17938 (daily dose of  $10^8$  CFU) was associated with treatment success (RR 1.67, 95% CI: 1.10–2.81, number needed to treat: 5, 95% CI: 4–8) and reduced crying times at the end of the intervention (MD  $-49$  min, 95% CI:  $-66$  to  $-33$ ). Notably, this effect was mainly seen in exclusively breastfed infants. One more recent RCT using *Lactobacillus reuteri* DSM 17938 in treating infantile colic in breastfed infants has been published (54) and included 60 colic infants. The results showed that *Lactobacillus reuteri* significantly decreased the daily crying time over the 30-day intervention period. In conclusion, *Lactobacillus reuteri* DSM 17938 is effective and can be recommended for breastfed infants with colic. The dose of *Lactobacillus reuteri* DSM17938 should be at least  $10^8$  CFU/day and provided for 21 to 30 days. There

were insufficient data to draw conclusions for formula-fed infants with colic, and also no evidence for other strains of probiotics or probiotic mixtures.

Constipation is one of the most common problems in children, with a prevalence ranging from 7 to 30%. It is usually treated with defecation training and laxatives. Most studies did not demonstrate any significant effect of probiotics on defecation frequency, fecal incontinence, or painful or difficult defecation. There was insufficient evidence to recommend probiotics in the treatment of children with functional constipation (55). In addition to limited evidence which did not support the use of probiotics in the treatment of functional constipation in children (56) one study compared *Lactobacillus casei rhamnosus* LCR35 to a placebo in 94 children with constipation. Treatment success (at least 3 spontaneous stools per week without fecal soiling) was comparable between the groups. Although stool frequency was significantly lower in the probiotic group, *Lactobacillus casei rhamnosus* LCR35 as the sole treatment was not more effective than the placebo in the management of functional constipation in children <5 years (57). In the most recent trial, the effect of *Lactobacillus reuteri* DSM 17938 and macrogol versus macrogol and a matching placebo was studied in 129 constipated children for 8 weeks. Stool frequency increased in almost all the patients and in a comparable amount in both groups. Moreover, there were no significant differences between the groups in the number of patients with hard stools, painful defecation, large stools, fecal soiling or abdominal pain. *Lactobacillus reuteri* DSM 17938 supplementation as an additional therapy to macrogol did not have any beneficial effect on the treatment of functional constipation in children aged 3–7 years (58).

In conclusion, there is insufficient evidence to recommend probiotics in the treat-

ment of children with functional constipation (51).

### The Safety of Probiotics

Probiotics have been useful in treating a wide range of childhood diseases, and there is strong evidence for their efficacy. Given the increasingly widespread use of them, a thorough understanding of the risks and benefits of probiotics is imperative. In 2011, the US Agency for Healthcare Research and Quality published a report on the safety of probiotics, based on a systematic review of 622 RCTs (59). There were four main conclusions to this report. The first referred to the Generally Recognized as Safe Status (GRAS) that the evidence properly addressing the safety of probiotics was limited, but the majority of strains that were studied should be generally regarded as safe. Secondly, the report stated that the adverse effects were more frequent in patients with compromised health. Another key finding was that there was no conclusive evidence that using a mixture of different probiotic strains had more adverse events than using one probiotic strain. The final finding was that the long-term effects of use of probiotic strains were unknown (59). In general, there were several theoretical concerns regarding the safety of probiotics: the occurrence of diseases, such as bacteremia or endocarditis, toxic or metabolic effects on the gastrointestinal tract, immune stimulation in susceptible populations, and the transfer of antibiotic resistance in the gastrointestinal flora (60). Of the gut microbiota, the probiotics most often used are strains of *Bifidobacterium*, *Lactobacillus* and *Saccharomyces*. In children, sepsis with *Lactobacillus* strains has been reported in association with prematurity, short-gut syndrome, cardiac surgery, immunosuppression and cerebral palsy (15). Minor risk factors are the presence of a central venous catheter, impaired intestinal barrier, short-gut syndrome, administration

of probiotics by jejunostomy, concomitant administration of broad-spectrum antibiotics (probiotic resistance), high mucosal adhesion or the known pathogenicity of probiotic strains and cardiac valvular disease (61). Also, a recent systematic review documented that probiotic products such as *S. boulardii*, have been shown to increase the risk of complications in specific patient groups, such as immunocompromised subjects (62). The transfer of antimicrobial resistance has been demonstrated for *Lactobacillus reuteri* ATCC 55730, which had a transferable resistance trait for tetracycline and lincomycin and therefore was replaced by a new strain, *Lactobacillus reuteri* DSM 17938 (28). In conclusion, practice points about the safety of probiotic use suggest that probiotics in children seem to be safe in general, but should be used with special caution in some conditions such as prematurity, immunocompromised patients, critically ill patients, those with central venous catheter, cardiac valvular disease and short-gut syndrome. Some probiotic strains are not recommended for use in children, such as *Enterococcus faecium* SF68, due to the possible transfer of vancomycin-resistance genes. In children with a *Clostridium difficile* infection, *S. boulardii* has been proven efficacious, but special caution is required in critically ill patients.

Increased awareness and knowledge of the potential benefits of probiotics have resulted in raised doubts about their quality. That is why the ESPGHAN Working Group for Probiotics and Prebiotics performed a literature search and provided recommendations (63). Based on their review of the literature, the authors recommended: precise identification of the microorganisms to strain level; products prescribed for specific clinical indications and situations to be subjected to rigorous clinical trials; systematic quality controls by the respective authorities to confirm the viability and strain-level identification of

the active ingredients; adverse events, potentially related to probiotic products should be reported, and a register of those events should be maintained by health authorities. Control mechanisms by the respective regulatory agencies (ESPGHAN, WHO) are required to ensure that patients receive commercial probiotic products that meet the expected quality (63).

### Conclusion

To summarize, based on the recent high-quality evidence, positive recommendations are suggested for the use of probiotics in pediatric practice, with strictly defined strains, for the prevention of upper respiratory tract infections in children attending day care centers, nosocomial diarrhoea and prevention of antibiotic-associated diarrhoea. There are also positive recommendations about managing children with UC, acute gastroenteritis, and infantile colic in breastfed babies. Probiotics are not recommended for the prevention of gastrointestinal infections in day care centers, of nosocomial respiratory tract infection, or atopic diseases in the prevention of infantile colic. There is not enough evidence to recommend specific probiotics in the prevention of NEC or as adjuvant therapy along with antibiotics in *Helicobacter pylori* eradication. No single strain or combination of strains can be recommended for management of functional abdominal pain disorders, except for abdominal pain in irritable bowel syndrome, and in the treatment of children with functional constipation. Although probiotics are generally regarded as safe, the safety and efficacy of probiotics in children have to be considered in every individual patient. These products seem to be safe for healthy infants and older children. Centralized oversight and monitoring of probiotic products were recommended in the report drawn up by the Committee on

Nutrition of the ESPGHAN and the FAO of the United Nations' WHO.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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