Review Article

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Probiotics in Children: What Is the Evidence?

Iva Hojsak*,†,‡

*Children's Hospital Zagreb, †University of Zagreb, School of Medicine, Zagreb, ‡University Josip Juraj Strossmayer, School of Medicine Osijek, Osijek, Croatia

The number of papers discussing probiotics increases tremendously that limits the possibility for primary care physicians and clinicians to stay updated. Therefore, the aim of this paper will be to summarize available evidence of probiotic use in well-defined clinical indications of importance for pediatricians. Based on currently available evidence certain probiotic strains (_Lactobacillus rhamnosus_ GG [LGG] and _Saccharomyces boulardii_) have proven effect in the treatment of acute gastroenteritis and prevention of antibiotic associated diarrhea. Furthermore, LGG was proven to be effective in prevention of nosocomial diarrhea and respiratory tract infection in day care centers. In conclusion, not all probiotic strains have same efficacy for all clinical indications, therefore, only strains with proven efficacy and safety should be recommended.

Key Words: _Lactobacillus_, _Saccharomyces_, _Bifidobacterium_, Diarrhea, Infection

INTRODUCTION

Most widely used definition of probiotics was given by the Food and Agriculture Organization of the United Nations and the World Health Organization in 2002 [1]. That definition was accepted with minimal change by expert panel (International Scientific Association for Probiotics and Prebiotics) in 2014 stating that probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [2].

On the same document panel tried to emphasize the probiotic action, emphasizing that some of probiotics' effect can be attributed only to specific probiotic strain, but some effects can be ascribed to probiotics in general or certain species of probiotics [2]. Same recognition of clinical effectiveness was also approved and highlighted by European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Working Group (ESPGHAN WG) on pre- and probiotics. Stating that recommendations for probiotic use should always be strain specific and aim is to recommend only the strains which have proven efficacy by well-designed randomized controlled trials (RCTs).

There are many papers about probiotics produced on daily basis which makes clinical up-date on their effectiveness extremely difficult.

Therefore, the aim of this paper will be to summa-

rize available evidence of probiotic use in well-defined clinical indication including the treatment of acute gastroenteritis, prevention of antibiotic associated diarrhea and prevention of infections in children.

TREATMENT OF ACUTE GASTROENTERITIS

Acute gastroenteritis is usually defined as decrease in the stool consistency (loose or liquid) and/or an increase in the frequency (typically >3 stools/day), with or without vomiting or fever [3]. Diarrhea typically lasts less than 7 days and not longer than 14 days [3]. The incidence of acute gastroenteritis is still high, even in Europe, and it is estimated that the incidence in small children ranges from 0.5 to 1.9 episodes/child/year [3]. Major causes are still rotavirus, which decreases in countries with high rate of rotavirus vaccination, followed by norovirus [4]. The treatment strategy aims to treat and prevent dehydration, shorten duration of diarrhea and to prevent prolonged diarrhea. Therefore, the mainstay of treatment is rehydration which in majority of children can be provided orally by using oral rehydration solutions [3]. Yet, there is still no causal treatment. One treatment option is racecadotril, enkephalinase inhibitor which was proven to be effective in shortening the diarrhea [5]. Other well-defined treatment modalities include probiotics.

Recently, ESPGHAN WG on pre- and probiotic performed systematic review and provided guidelines on the use of different probiotic strains for the treatment of acute gastroenteritis [6]. Based on available, well designed RCTs, ESPGHAN WG recommended only two probiotic strains proved to be effective in at least two RCTs; these are *Lactobacillus rhamnosus* GG (LGG) and *Saccharomyces boulardii*.

Based on the Cochrane review from 2010 [7], LGG was investigated in 11 RCTs (n=2,072) and this meta-analysis found that use of LGG reduced the duration of diarrhea for mean of 27 hours (95% confidence interval [CI], −41 to −13). Subsequent systematic review performed by Szajewska et al. [8] in 2013 identified 15 RCTs (n=2,963). This review confirmed superiority of LGG in significantly decreasing duration of diarrhea comparing to placebo (mean difference [MD], −1.05 days; 95% CI, −1.7 to −0.4; based on 11 RCTs). However, there was no influence on stool volume (MD, 8.97 mL/g; 95% CI, −86.26 to 104.2; based on 2 RCTs). Regarding the dose, $\geq 10^{10}$ colony-forming units (CFU) was more effective than $< 10^{10}$ CFU [8].

Other strain with well-proven effect is *S. boulardii*. The above-mentioned Cochrane review found 6 RCTs (n=606) and reported reduced risk of diarrhea lasting ≥4 days (risk ratio [RR], 0.37; 95% CI, 0.2 to 0.65) if *S. boulardii* was used [7]. 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) [9]. None of the studies evaluated the influence on stool volume.

Finally, strain *Lactobacillus reuteri* ATCC 55730 had proven moderate clinical effect in treating acute gastroenteritis in children; however, this strain was found to carry transferable resistance trait for antibiotic resistance and was replaced by a new strain, *L. reuteri* DSM 17938 [10]. This, new strain *L. reuteri* DSM 17938 was investigated by 3 RCTs; two RCTs (n=196) were analyzed in systematic review from 2014 and showed significantly reduced diarrhea duration (MD, −32 hours; 95% CI, −41 to −24) [11]. Subsequently, one more RCT was published including 64 infants and children, showing similar results in the reduction of diarrhea duration [12].

Generally, after reviewing these results ESPGHAN WG on pre- and probiotics recommended the use of the following probiotic strains as an adjunct to rehydration therapy: LGG (quality of evidence: low; recommendation: strong), *S. boulardii* (quality of evidence: low; recommendation: strong) and *L. reuteri* DSM 17938 (quality of evidence: very low; recommendation: weak) [6].

It should be emphasized once again that systematic review of the literature did not found enough evidence (or evidence was negative) to recommend other probiotic strains.

For clinicians, it is of importance to know that probiotics have been proven mostly in watery (mainly viral) diarrhea and that their efficacy is more pronounced on the duration of diarrhea (study showed ability to shorten diarrhea for 1 day) than in stool volume [3,6]. Furthermore, when recommended they should be recommended only as an adjunct to rehydration and it is better to use them in the early course of disease [6].

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 [13]. It is more commonly caused by antibiotics that target anaerobic bacteria (e.g. clindamycin, penicillin, amoxicillin and clavulanic acid etc.) which cause significant disruption of the enteric microbiome [14,15]. Clinically, AAD may present as mild diarrhea, but it can also present as fulminant pseudomembranous colitis caused by *Clostridium difficile* [13]. Measures which can prevent AAD are limited mainly to reduction in antibiotic use, type of antibiotic prescribed and the use of probiotics.

Due to large number of studies and different recommendations available ESPGHAN WG on pre- and probiotics preformed systematic review with meta-analysis with aim to provide evidence based guidelines for every specific probiotic strain in the prevention of AAD [13]. This systematic review found only two probiotic strains with enough evidence (efficacy proven in more than 2 well-designed RCTs); these strains are LGG and *S. boulardii* [13].

LGG was investigated in 5 RCTs (n=445) and administration in children reduced the risk of AAD from 23% to 9.6% (RR, 0.48; 95% CI, 0.26 to 0.89), regardless of the reason for which antibiotics and probiotics were used [13]. However, only one trial [16] evaluated the effect of LGG in the prevention of *C. difficile*-associated diarrhea in children and found no effect.

Similarly, *S. boulardii* used in children 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) [13]. Furthermore, the administration of *S. boulardii* reduced the risk of *C. difficile*-associated diarrhea in children (2 RCTs, n=579; RR, 0.25; 95% CI, 0.08 to 0.73) [13].

However, there is a constant discussion whether probiotics should be used every time antibiotic is prescribed. The reasons for their routine use are the proven effect and the fact that AAD can be serious illness [13,17]. On contrary, reasons not to use them is usually related to their costs and fact that AAD is usually self-limited mild disease. There are certain groups of patients that would benefit the most from probiotic use including children of younger age, hospitalized children and children who experienced AAD (especially *C. difficile*-associated diarrhea) before [17]. Once again, the recent review identified only two strains to be effective in prevention of AAD; these are LGG (quality of evidence: moderate, recommendation: strong) and *S. boulardii* (quality of evidence: moderate, recommendation: strong) [13]. For prevention of *C. difficile*-associated diarrhea only *S. boulardii* showed efficacy (quality of evidence: moderate, recommendation: conditional) [13].

There is always a question when to administer probiotic in order not to be killed by antibiotic; there are no scientific evidence for that. However, some probiotic strains (like *S. boulardii*) are resistant to antibiotics used for bacterial infections. On the other hand, other strains (like LGG) were effective in RCTs when used for AAD, therefore their administration should follow the same scheme like in RCTs.

PREVENTION OF INFECTIONS

Infectious diseases are the most important cause of morbidity in children where respiratory and gastrointestinal (GI) infections encounter for majority of them [18]. Recurrent respiratory tract infections are common problem in preschool age, mainly due to the presence of unfavorable environmental conditions including early socialization in daycare centers and the physiologic immaturity of the immune

system [19]. There are two major settings where children acquire respiratory and GI infections and those are hospital and day care centers.

Prevention of infections in day care centers

Children who attend daycare centers have 2-3 times more infections than children who stay at home, they have more outpatient doctor and emergency room visits and increased usage of prescribed antibiotics [20]. Furthermore, they cause a substantial economic burden not only for child's family, but healthcare in general; their costs are estimated to be $1.8 billion per year in the United States [21]. Taking all that into account, together with possible complications, respiratory tract and GI infections are important health care problem for pediatricians who are facing a real task to discriminate the children who are at higher risk and try to offer preventive measures. These preventive measures usually include good hand hygiene, absenteeism of ill child from daycare center in order to prevent spreading of infection and vaccination for influenza and rotavirus [22]. However, all those measures often are ineffective leaving a place for possible new modalities, like probiotics. In the last two decades, there have been an increasing number of trials investigating the role of probiotics on the prevention of common infections in children.

As presented in Table 1, there are several trials which evaluated probiotics in the prevention of respiratory tract infection in children attending daycare centers [23-34]. Interestingly, majority of studies beyond infancy found positive effect on the lowering of respiratory tract infections [23,24,26-29,31]. Recent meta-analysis reviewed available literature and found that probiotics (in general) reduce the risk of respiratory tract infections (RR, 0.89; 95% CI, 0.82 to 0.96) [35]. Unfortunately, this meta-analysis included all age groups, was not strain specific and was not stratified based on the type of facility where probiotics were used. However, it was reported that although there was no effect on the duration of illness, absenteeism from the kindergarten was decreased [35].

Based on the presented results in Table 1, it can be concluded that probiotics could have a place in the prevention of upper respiratory tract infections. However, questions that remain are what strain to use, in which dose and when. Based on well-designed RCTs in children (Table 1), LGG was examined in 3 studies [24,26,27] involving all together 1,375 children and all studies reported positive effect on the lowering the incidence of respiratory tract infections. Other strain *Bifidobacterium animalis* subsp. *lactis* was evaluated in 4 RCTs [25,30,32,34] from which all found negative results.

The question is whether to recommend probiotic use routinely in all children who are at increased risk for respiratory infection. Based on currently available evidence, it seems prudent to use strains with proven efficacy in more than 2 RCT (which is LGG). However, there are no cost-effective analyses. Regression analysis determined that children who would benefit the most from the LGG use were children of younger age and with recurrent respiratory infections during winter months [26].

Majority of studies which investigated probiotic use in the prevention of respiratory tract infections also investigated the risk of acquiring GI infection (Table 1). Results from those studies are weak. There is no meta-analysis which assessed overall effect, however, based on literature search there are no 2 RCTs which investigated same probiotic strain and yielding positive results. Of note is that both studies investigating LGG found no effect [24,26], similarly is for *B. animalis* subsp. *lactis* investigated by other two studies [30,32].

All these results, however, should be interpreted with caution because most of them were performed in the winter period when the incidence of GI infections is much lower, and therefore someone can argue that the sample size was not powered enough to assess GI risk.

Nosocomial infections

Nosocomial, hospital-acquired or healthcare-associated infections, develop during a hospital stay and they are not present or incubating at the admission;

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

Author	n (age)	Probiotic (dose)	Effect on respiratory infection	Effect on gastrointestinal infection
Hatakka et al. [24] (2001)	571 (1-6 y)	LGG (1-2×10^8 CFU/day)	Lower number of upper respiratory tract infections Lower number of prescribed antibiotics	NS
Saavedra et al. [33] (2004)	118 (3-24 mo)	*Bifidobacterium animalis* subsp. *lactis* (Bb12) (10^7 CFU)+*Streptococcus thermophilus* (10^6 CFU)	Not significant difference in the incidence of upper respiratory tract infection Lower number of prescribed antibiotics	NS
Weizman et al. [34] (2005)	210 (4-10 mo)	*Bifidobacterium animalis* subsp. *lactis* (Bb12) or *Lactobacillus reuteri* 55730 (minimum 10^7 CFU)	Not significant difference in the incidence of upper respiratory tract infection *L. reuteri* group-lower number of prescribed antibiotics	Not assessed
Lin et al. [29] (2009)	1,062 (preschool children)	*Lactobacillus casei rhamnosus* (10^8 CFU), *Lactobacillus rhamnosus* T cell-1 (10^{10} CFU), multiple probiotic strains	Reduction in respiratory infection in the *L. casei rhamnosus* group Not significant for other strains	Multiple strain group showed reduction in gastrointestinal illness
Leyer et al. [28] (2009)	326 (3-5 y)	*Lactobacillus acidophilus* NCFM (10^{10} CFU) vs. *Lactobacillus acidophilus* NCFM+*Bifidobacterium animalis* subsp. *lactis* Bi-07 (10^{10} CFU)	Lower fiver and coughing episodes, lower antibiotic use in single and combination group	Not assessed
Hojsak et al. [26] (2010)	281 (1-7 y)	LGG (10^9 CFU)	Lower number of upper respiratory tract infections No difference in prescribed antibiotics	NS
Merenstein et al. [31] (2010)	638 (3-6 y)	*Lactobacillus casei* DN-114 001/CNCM I-1518 (10^8 CFU) (in yoghurt with *Streptococcus thermophilus* and *Lactobacillus bulgaricus*)	Lower number of upper respiratory tract infections Lower number of prescribed antibiotics	NS
Merenstein et al. [32] (2010)	182 (13 y)	*Bifidobacterium animalis* subsp. *lactis* (Bb12) (10^{10} CFU) (yoghurt containing also *Streptococcus thermophilus* and *Lactobacillus bulgaricus*)	NS	NS
Merenstein et al. [30] (2011)	172 (2-4 y)	*Bifidobacterium animalis* subsp. *lactis* (Bb12) (10^{10} CFU) (yoghurt containing also *Streptococcus thermophilus* and *Lactobacillus bulgaricus*)	NS	NS
Kumpu et al. [27] (2012)	523 (2-6 y)	LGG (6.7×10^5 to 1.9×10^6 CFU/mL)	Lower risk of respiratory infection in completed cases subgroup (recovery of LGG in fecal samples)	Not assessed
Gutierrez-Castrellon et al. [23] (2014)	336 (0.5-3 y)	*Lactobacillus reuteri* DSM 17938 (10^8 CFU)	Lower risk of respiratory tract infections	Lower risk of diarrhea
Hojsak et al. [25] (2016)	210 (1-7 y)	*Bifidobacterium animalis* subsp. *lactis* (Bb12) (10^9 CFU)	NS	NS

LGG: *Lactobacillus rhamnosus* GG, CFU: colony-forming units, NS: not significant.

infections that occur more than 48 hours after the admission are usually considered as nosocomial [36]. The incidence of nosocomial infections on pediatric wards even in developed countries is still high, ranging from 5% to 10% and GI and respiratory tract infections account for the majority of them [37]. Nosocomial infections have several negative impacts; they worsen the treatment outcome, could prolong the hospitalization, and significantly increase hospital costs [38]. Standard preventive measures, mainly hand hygiene, isolation of sick children and reduction in the number of hospitalized patients decreases infection spreading, but cannot successfully prevent them [39,40]. Therefore, there is a place for new strategies, one of which is the use of probiotics.

Recently, ESPGHAN WG on pre- and probiotics performed systematic review on the role of different probiotic strains in the prevention of nosocomial diarrhea [41]. This meta-analysis identified 8 RCTs out of which 3 investigated LGG. The administration of LGG reduced the risk of nosocomial diarrhea from 13.9% to 5.2% (2 RCTs, n=1823; RR, 0.35; 95% CI, 0.19 to 0.65) [41]. On contrary, *L. reuteri* DSM 17938 was investigated by two studies (same probiotic strain but different doses: 10^8 CFU/day [42] and 10^9 CFU/day [43]) and had negative results (RR, 1.11; 95% CI, 0.68 to 1.81) [41]. Based on the evidence ESPGHAN WG concludes that if probiotics for preventing nosocomial diarrhea in children are considered, LGG (at least 10^9 CFU/day, for the duration of hospital stay) should be used (quality of evidence: moderate, recommendation: strong) [41].

Due to lack of cost effectiveness, currently there is a need for identifying children in risk for acquiring nosocomial diarrhea. Based on regression analysis published in one of the RCTs [38] children who stay longer in hospital are especially prone to nosocomial infection, therefore this group of children would benefit the most.

On contrary to role of probiotics in the prevention of nosocomial diarrhea, we have only limited evidence of the role of probiotics in the prevention of nosocomial respiratory tract infection outside of intensive care unit. There are only two (although big)

RCTs. One RCT investigated LGG (n=742) at the dose of 10^9 CFU and found reduction in risk of upper respiratory tract infection [41]. Other study, performed at the same center used different probiotic strain, *B. animalis* subsp. *lactis* (Chr Hansen, Denmark) at the same dose, was not able to prove positive effect [44]. Authors also identified that children who stayed longer in the hospital and who were younger had higher chance of acquiring upper respiratory tract infections [41]. Although there is an evidence that some probiotic strain could have effect in the prevention of infection, still there is no enough evidence to recommend probiotics for the prevention of nosocomial respiratory tract infections.

CONCLUSION

Above mentioned evidence further demonstrates that not all probiotics have the same efficacy for every specific clinical indication. Based on currently available evidence certain probiotic strains (LGG and *S. boulardii*) have proven effect in the treatment of acute gastroenteritis and prevention of AAD. Furthermore, LGG was proven to be effective in prevention of nosocomial diarrhea and respiratory tract infection in day care centers.

Field of probiotics increases tremendously, thus it is hard for clinicians to follow the literature. Therefore, it is of utmost importance to recognize scientific authorities and to follow up their guidelines.

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