Monographic supplement series

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Probiotic *Lactobacillus rhamnosus* GG and respiratory illness in children

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KEYWORDS: Lactobacillus rhamnosus GG; respiratory infections; children.

ABSTRACT: Respiratory tract infections (RTIs) are the most common acute diseases in children. There are virtually no effective treatments or prophylaxes available for these infections. Increasing evidence shows that probiotics may be effective in the prevention of RTIs. Probiotic *Lactobacillus (L.) rhamnosus* GG is one of the most extensively studied probiotic bacterium. The purpose of this review is to summarize all the available data on the effects of L. rhamnosus GG on RTIs in children. To conclude, studies confirm that L. rhamnosus GG may be effective in RTIs by decreasing the risk or incidence of RTIs, alleviating symptoms or their duration, or decrease the numbers of prescribed antibiotics. However, more comparable trials investigating probiotic dose response and mechanisms of effects are necessary.

INTRODUCTION

Acute upper and lower respiratory tract infections (RTIs) are the most common diseases in children. These infections pose a considerable health and economic burden in terms of hospitalizations, medical costs, doctor's consultations, and absenteeism from work and school (1). The mean annual number of RTIs is approximately 5 in children under 5 years of age and 3 in older children (2-4). Children attending day care are especially at risk for acquiring RTIs (6, 7), as close physical contact among children in day care favour the transmission of infectious diseases. Acute upper RTIs may lead to complications such as otitis media (OM), which account for 80 percent of all infectious diseases diagnosed in general practice (8). The majority of acute RTIs are of viral origin. Viral RTIs can lead further to bacterial diseases, and mixed viral–bacterial infections are often associated with antibiotic treatment failure (9).

There are virtually no effective medications for the prevention of acute RTIs, and current symptomatic treatments for RTIs have limited advantage. In addition, as most acute RTIs are due to viral infection, antibiotic therapy offers no benefit. Currently, the only effective antivirals and vaccines for the prevention and treatment of respiratory virus infections are available against influenza viruses. Large varieties of other etiologic agents and increasing antibiotic and antiviral resistance challenge the development of efficient therapies. Consequently, it is of importance to find alternative and safe ways to reduce the risk of acute RTIs.

The mucosal surfaces of the respiratory and the gastrointestinal tract are the primary portals of entry for pathogenic micro-organisms. An effectively functioning immune system is important for the maintenance of physiological integrity and health and provides defense against infections.

The mucosal surfaces of the upper airways are functionally linked to mucosal surfaces of the gastrointestinal tract (gut associated lymphoid tissue, GALT). The intestinal normal microbiota is a metabolically active organ, which plays a significant role in the maturation and function of the immune system in the gut early in life (10).

The microbiota is continuously interacting with the environment, including other bacteria, the gut epithelium, and the mucosal and endocrine systems. Through these interactions the microbiota resists the colonisation of pathogens, participates in the elimination of foreign antigens, and regulates the immune responses.

Probiotics are defined as live microorganisms that confer a health benefit on the host (11).

The most common types of microbes used as probiotics are lactobacilli and bifidobacteria. Probiotics are likely to have an impact through gut mucosa by balancing the local microbiota by promoting regrowth of microbiota following antibiotic therapy (12), by inhibiting the growth of pathogenic microorganisms (13), and by enhancing local and systemic immune responses (14). They may also influence the composition and activity of microbiota in the intestinal contents. *Lactobacillus rhamnosus* GG (ATCC 53103) is one of the most extensively studied probiotic strains in humans and in experimental studies. Since its isolation from an adult human in 1985, it has gained a safe history of use in food products since 1990. The strain provides excellent survival in and transient colonization of the gastrointestinal tract, which is attributed to its adhesion capacity to the intestinal mucus and epithelial cells (15, 16).

This article reviews the current research regarding the effects of probiotic *Lactobacillus rhamnosus* GG on respiratory infections in children.

L. RHAMNOSUS GG IN THE PREVENTION OF RESPIRATORY INFECTIONS

Several trials in children have studied the effects of *L. rhamnosus* GG in the prevention of respiratory infections (Table 1).

Four randomized double blind placebo-controlled clinical trials have been conducted in healthy children attending day care. In Finland, 571 healthy children (1-6 years) from 18 day care centres were studied for 7 months in winter (17). Children received either *L. rhamnosus* GG in milk (1-2x10^8 cfu/day) or control milk together with their daily meals.

Study design and duration	Subjects	Probiotics used	Main findings: probiotic vs. placebo	Reference
R DB PC parallel 7 months	571 healthy children at day care centers in Finland (1-6 years)	*L. rhamnosus* GG in milk(on average10^8 cfu) 3 times daily	Respiratory symptoms, days: 25 vs. 27, p=0.22 Children with otitis media: 31% vs. 39%, p=0.08 RTIs with complications: 39% vs. 47%, p = 0.05 Antibiotic treatments: 44% vs. 54%, p = 0.03 (Age adjusted results: ns)	(16)
R DB PC parallel 7 months	523 healthy children at day care centers in Finland (2–6 years)	*L. rhamnosus* GG in milk (on average10^8 10^8 cfu) 3 times daily	Respiratory symptoms, days/month: 5.03 vs. 5.17, IRR 0.97; 95% CI: 0.94–1.00; p=0.098 4.71 vs. 5.67, IRR 0.83; 95% CI: 0.78–0.88; p<0.001 (completed cases) No. of respiratory symptom episodes/ month: 0.59 vs.0.55, IRR 1.06; 95% CI: 0.96–1.16; p=0.24 One health care visit with respiratory infection diagnosis: 48% vs 49%, p=0.89 Antibiotic treatments: 35% vs. 34% , p=0.80	(17)
R DB PC parallel 3 months	281 healthy children at day care centers in Croatia (2–6 years)	*L. rhamnosus* GG (10^9 cfu) in milk administred daily	Number of children with: RTIs: 43.2% vs. 67.6%, p<0.0010 Upper RTIs: 41.7% vs. 66.9%, p<0.001 Lower RTIs: 2.9% vs. 3.5%, p=0.759 Number of RTIs lasting >3 days: 28.1% vs. 49.3%, p<0.001	(18)
R DB PC parallel during hospital stay	742 hospitalized children in Croatia (over 12 months)	*L. rhamnosus* GG (10^9 cfu) in milk administered daily for the duration of hospitalization	Respiratory tract infections: 2.1% vs. 5.5%, RR: 0.38 (95% CI: 0.18–0.85); NNT: 30 (95% CI:16-159) Duration of respiratory infection >3 days: 2.1 vs. 5.2, RR: 0.40 (95%CI: 0.20–0.90); NNT: 33(95% CI:17–257) Duration of hospitalization, median: 5 vs. 4, p=0.1	(19)
R DB PC parallel 6 months	309 otitis prone children in Finland (10 months-6 years)	*L. rhamnosus* GG, *L. rhamnosus* LC705, *B. breve* 99, *P. freudenreichii* JS in capsules 8–9x10^9 cfu/capsule of each strain on capsule daily	Occurrence of AOM: 72% vs. 65%, p=ns Occurrence of recurrent respiratory infections: in the probiotic group (OR for ≥4 URIs: 0.56, 95%CI 0.31–0.99, p=0.046; OR for ≥6 URIs: 0.59, 95% CI 0.34 to 1.03, p =ns) Nasopharyngeal flora: At baseline or at 6 months: *Streptococcus pneumonial/Haemophilus influenza* p= ns *Moraxella catarrhalis* in probiotic group: p=0.028 After 3-6 months: HBoV DNA (studied in 152 children): 6.4% vs. 19.0%, p = 0.039	(20) (21)
R DB PC parallel 10–12 months	72 healthy newborns in Finland (<2 months)	*L. rhamnosus* GG + *Bifidobacterium lactis* Bb-12 10^{10} cfu in capsules supplemented to infant formula once a day	During first 7 months of life: Incidence of AOM: 22% vs. 50%, p=0.014 Antibiotic treatment: 31% vs. 60%, p = 0.015 Respiratory infection: 69% vs. 78%, p= 0.40 During the first 12 months of life: Incidence of AOM: 13% vs. 25%, p=ns Recurrent respiratory infections : 28% vs. 55%, p=0.022	(22)

Table 1. Reported effects of L. rhamnosus GG in respiratory tract infections in children. Abbreviations: R DB PC, randomized double-blind placebo controlled; IRR, incidence rate ratio; CI, confidence interval; RR, risk ratio; NNT, number needed to treat; ns, not significant.

Children receiving *L. rhamnosus* GG had fewer days of absence from day care because of illness when compared with controls (4.9 *vs.* 5.8 days, 16 percent difference, p=0.03). In addition, children in the *L. rhamnosus* GG group had fewer RTIs with complications (39 *vs.* 47, 17 percent difference, p=0.05) and less prescribed antibiotic treatments for RTIs (44 *vs.* 54, 19 percent difference, p=0.03). In addition, the occurrence of AOM was reduced by 21 percent in these children, but the difference between the groups was not statistically significant (children with OM: 31 *vs.* 39 percent, p = 0.08). However, after age adjustment, no significant differences were shown between the groups. The results suggest that *L. rhamnosus* GG may have positive impact on the attendance of children in daycare centres, and may reduce the cost to the parents and the associated public health issues.

Another study conducted in Finland with similar study setting included 523 children (2-6 years) (18).

There was no significant difference in the days with respiratory symptoms, the number of respiratory symptom episodes, health care visits due to respiratory infections, or prescribed antibiotic treatments between the study groups. Interestingly, however, when the study group analyzed a subgroup, where *L. rhamnosus* GG was analyzed in the fecal samples by PCR both before and at end of the intervention, children receiving *L. rhamnosus* GG had significantly less days with respiratory symptoms (4.71/month *vs.* 5.67/month, incidence rate ratio: 0.83, p<0.001).

These results highlight the importance to clarify the association between fecal recovery of a probiotic and the respiratory symptom prevalence. However, the effects of *L. rhamnosus* GG on RTIs may have been more pronounced with larger dose/concentration of probiotic in milk.

In Croatia, 281 children (2-6 years) attending day care were randomly allocated to receive either *L. rhamnosus* GG (10^9 cfu) in 100 ml of milk product or placebo for three months (19). When compared with placebo, children in the *L. rhamnosus* GG group had a significantly reduced risk of upper RTIs (RR 0.66, 95 percent CI 0.52-0.82, number needed to treat (NNT) 5, 95 percent CI 4-10), a reduced risk of RTIs lasting longer than 3 days (risk ratio (RR) 0.57, 95 percent CI 0.41-0.78, NNT 5, 95 percent CI 4-11), and a significantly lower number of days with respiratory symptoms (p< 0.001).

However, there was no risk reduction in regard to lower RTIs (RR 0.82, 95 percent CI 0.24-2.76). In contrast to other studies (16, 22) *L. rhamnosus* GG did not reduce antibiotic treatments (p= 0.12).

One study investigated the effects of *L. rhamnosus* GG in hospitalized children (20).

In Croatia, 742 hospitalized children (aged over 12 months) were randomly allocated to receive *L. rhamnosus* GG (10^9 cfu) in 100 ml of milk product or placebo during their hospital stay. In that study, children receiving *L. rhamnosus* GG, had significantly reduced risk for RTIs (RR: 0.38 [95 percent CI: 0.18–0.85]; NNT: 30 [95 percent CI: 16–159]), and episodes of RTIs that lasted longer than 3 days (RR: 0.4 [95 percent CI: 0.2–0.9]).

Groups did not differ, however, in the hospitalization duration (5 *vs.* 4, p=0.1).

The authors concluded that *L. rhamosus* GG can be recommended as a valid measure for decreasing the risk for nosocomial gastrointestinal and respiratory tract infections in paediatric facilities. Unlike other probiotic trials, this study involved a large number of patients.

Data is limited concerning the effects of probiotic mixtures versus single strain on the occurrence or outcome of RTIs. Only two studies have investigated the effects of *L. rhamnosus* GG in a combination with other probiotic bacteria on RTIs in the paediatric population.

First clinical trial was conducted with otitis-prone children in Finland (21). In that study 309 otitis-prone children (10 months-6 years) consumed either one capsule containing *L. rhamnosus* GG, *L. rhamnosus* LC705, *Bifidobacterium (B.) breve* 99 and *Propionibacterium freudenreichii* JS (8–9x10^9 cfu/capsule of each strain) or placebo daily for 24 weeks. In addition nasopharyngeal swab sample was collected at three times during the study. The results showed that probiotic intervention did not reduce the occurrence (probiotic *vs.* placebo: 72 *vs.* 65 percent, OR=1.48, *p*=n.s), the recurrence (≥ 3) of AOM episodes (18 *vs.* 17 percent, OR=1.04, *p*=n.s), or the median duration of AOM episodes (5.6 *vs.* 6.0 days, *p*= n.s). However, there was a tendency showing a reduction in the occurrence of recurrent RTIs in the probiotic group (OR for ≥ 4 URIs: 0.56: p=0.046; OR for ≥ 6 URIs: 0.59, p=n.s).

When the effects of probiotics were studied on nasopharyngeal carriage of bacterial pathogens, probiotics did not affect the carriage of *Streptococcus pneumoniae* or *Haemophilus influenzae*, but increased the prevalence of *Moraxella catarrhalis* (OR=1.79, p=0.028).

The authors concluded that in otitis-prone children the effect of probiotics is not sufficient to prevent AOM, as otitis-prone children are treated with several antibiotic courses, and experience increased nasopharyngel colonisation of otitis pathogens. However, the number of human bocavirus was reduced significantly in the nasopharynx of these children (p=0.039) as described by Lehtoranta et al 2012 (22), indicating that probiotics may be more effective against RTIs of viral origin.

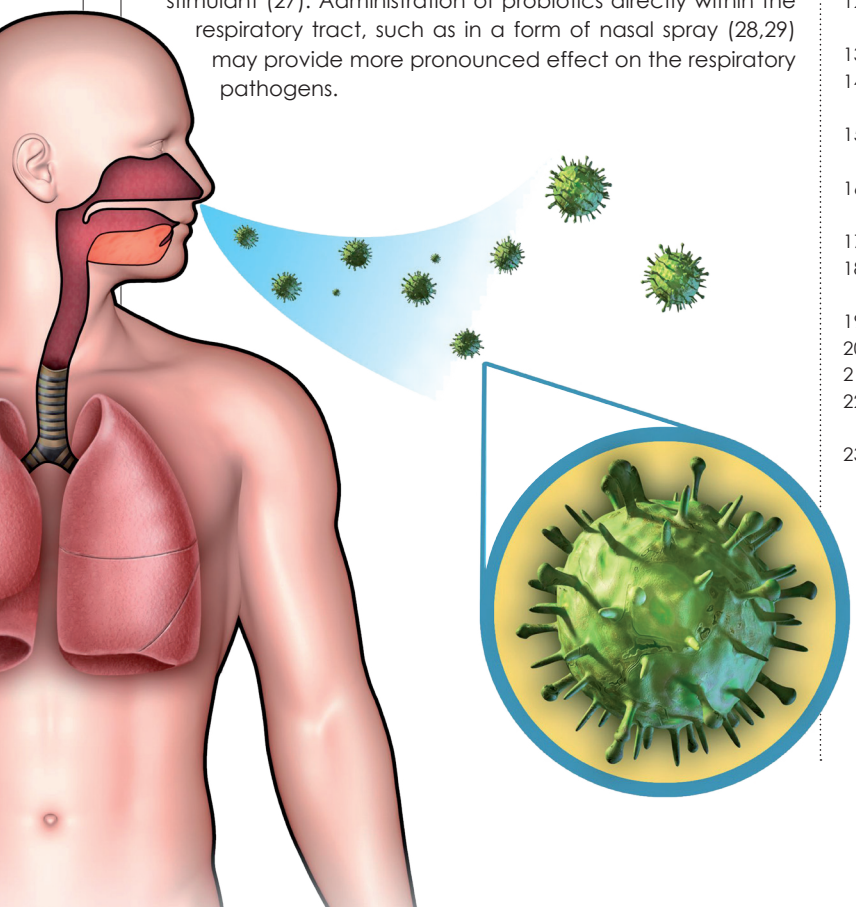
Another study investigated the effects of *L. rhamnosus* GG together with *B. lactis* Bb-12 on RTIs in healthy newborn infants (23). Altogether 72 infants requiring formula before the age of 2 months were randomized to get either a formula supplemented with *L. rhamnosus* GG and *B. lactis* Bb-12 (10^{10} cfu) or placebo. During the first 7 months of life, there were less incidences of AOM in the probiotic group (22 *vs.* 50 percent, p = 0.014) and less antibiotic treatments (31 *vs.* 60 percent, p=0.015).

In addition, probiotics reduced the incidence of recurrent respiratory infections during the first year of life (28 *vs.* 55 percent, p=0.022). The authors suggested that probiotics may offer safe means of reducing the risk of early AOM and antibiotic use, and the risk of recurrent respiratory infections during the first year of life.

Nevertheless, relatively small number of patients is a limitation of the study.

All studies show consistently that *L. rhamnosus* GG has potential for alleviating RTIs in children. The variable effects of *L. rhamnosus* GG between clinical trials, however, may be explained by different use of bacterial dose and matrices.

The beneficial effects of *L. rhamnosus* GG in RTIs are most likely mediated through the stimulation of the gut immune system, which may provide enhanced systemic protection of cells from infections. Several studies show that *L. rhamnosus* GG affects immune responses both specifically by stimulating antibody production and nonspecifically by enhancing phagocytosis of pathogens (24, 25). *L. rhamnosus* GG also modifies production of inflammatory and anti-inflammatory proteins. A recently discovered unique pilus structure of *L. rhamnosus* GG may provide improved adherence to the gut epithelium compared with other probiotic bacteria through mucus-binding ability (26), and possibly function as immune stimulant (27). Administration of probiotics directly within the respiratory tract, such as in a form of nasal spray (28,29) may provide more pronounced effect on the respiratory pathogens.



The studies conducted with *L. rhamnosus* GG in combination of other strains has limited value when assessing the effects of *L. rhamnosus* GG, as the effects of *L. rhamnosus* GG may be counteracted by other strains and any observed positive effect may be due to the other strains.

CONCLUSION

Respiratory infections cause a significant health burden on children.

Probiotic therapy may offer a safe alternative to prevent or alleviate these infections. Several trials show promising data on the effects of probiotic bacterium *Lactobacillus rhamnosus* GG in reducing the risk or alleviating the symptoms of respiratory tract infections. However, due to varied data available, more well designed clinical trials in children investigating probiotic dose response and the mechanisms of effects are necessary.

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