Lactobacillus rhamnosus GG Supplementation for Preventing Respiratory Infections in Children: *A Meta-analysis of Randomized, Placebo-controlled Trials*

SHAN LIU, PENGWEI HU, XIAOXIN DU, TAO ZHOU AND XIAOFANG PEI

From the Department of Public Health Microbiology, Department of Public Health Laboratory Technology, West China School of Public Health, Sichuan University, China.

Correspondence to: Xiaofang Pei, Professor of Public Health Microbiology, West China School of Public Health, Sichuan University, 16#, Section 3, Renmin Nan Lu, Chengdu, Sichuan 610041, China. xxpeiscu@163.com

Objective: To systematically review the effectiveness of administering *Lactobacillus rhamnosus* GG (LGG) for preventing respiratory infections in children.

Design: Systematic Review and Meta-analysis.

Data sources: Electronic databases and trial registries.

Results: Four RCTs involving 1805 participants met the inclusion criteria. Compared with placebo, LGG administration was associated with a reduced incidence of acute otitis media (four RCTs, n=1805, RR 0.76, 95% CI 0.64-0.91, fixed effects model, NNT 17, 95% CI 11-46), a reduced risk of upper respiratory infections (one RCT, n=281, RR 0.62, 95% CI 0.50-0.78, NNT 4, 95% CI 3-8) and antibiotic treatments (four RCTs, n=1805, RR 0.80, 95% CI 0.71-0.91, fixed effects model). There was no significant difference between the LGG and the control groups in the risk of overall respiratory infections and the incidence of lower respiratory infections. However, subgroup analysis of two studies on children older than 1 year showed significant reduction in the risk of overall respiratory infections (two RCTs, n=794, RR 0.73, 95% CI 0.57-0.92, random effects model, NNT 8, 95% CI 5-14). Adverse effects were similar in both groups. No serious adverse events were reported.

Conclusion: The administration of *Lactobacillus rhamnosus* GG compared with placebo has the potential to reduce the incidence of acute otitis media, the upper respiratory infections and antibiotic use in children.

Key words: *Children, LGG, Prevention. Probiotics, Respiratory infections.*

Respiratory (tract) infections are common among children and contribute substantially to pediatric morbidity and mortality worldwide. The inappropriate use of antibiotics for the treatment of these infections can cause side effects in children, including rash, diarrhea, and increased bacterial drug resistance rates [1]. Prevention of respiratory tract infections is an important publichealth challenge. A safe, relatively inexpensive, and effective intervention to prevent respiratory tract infections and its adverse effects to health would have significant public-health implications.

In this era of increasing antimicrobial resistance, use of probiotics in infection prevention has brought a new perspective. Probiotics have been defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [2]. One of the most studied probiotics is *Lactobacillus rhamnosus* GG (LGG), which influences the immune response both by stimulating antibody production and by improving the phagocytic activity of the blood leucocytes [3]. In children, there is now convincing data to support the use of LGG for the treatment of abdominal pain-related functional gastrointestinal disorders and the prevention of diarrhea [4]. Some studies show that probiotic strains can prevent respiratory infections [5]. However, evidence for the role of LGG in preventing respiratory tract infections in children is not clear.

We conducted a systematic analysis of data from all the currently available trials to evaluate the evidence for the efficacy of LGG in preventing respiratory infections in children.

METHODS

Inclusion and exclusion criteria: All randomized controlled trials to investigate the effect of LGG supplementation in the prevention of respiratory infections (as defined by the investigators) in children were included. Participants were the children aged 0 month to 18 years who were from community. The intervention was LGG, or LGG together with other probiotics at any form or dose compared with placebo or with no additional intervention. The primary outcome measure was the incidence of respiratory infections using the original investigator's definition, including the overall respiratory infections, the upper and lower

respiratory infections and acute otitis media. The secondary outcome measures were the incidence of antibiotic treatments and the adverse effects. We excluded studies of adults and studies with participants who are susceptible to infections. Only studies with >80% follow-up were included.

Search methods: We tried to identify all relevant trials irrespective of language or publication status (published, unpublished, in press and in progress). We systematically searched the major electronic databases (MEDLINE, EMBASE, ISI's Web of Science, the Cochrane Library and Chinese Publications) from their inception to September 2012 using the following terms with a topic-specific strategy: [respiratory infections OR respiratory tract infections] AND [probiotic(s) OR lactobacillus OR LGG OR L rhamnosus] AND [child(ren) OR infant(s) OR baby OR adolescent OR teenager]. Besides, two trial registries (ClinicalTrials.gov and EU Clinical Trials Register) were searched through their websites.

Selection of studies: Two authors (SL and PH) checked independently the titles and abstracts recognized via the search to identify the potentially eligible relevant publications and obtained the full articles. Then the articles were estimated by the same two authors utilizing an eligibility form based on the inclusion criteria. If there was an uncertainty whether the study should be included in the review, we attempted to contact the study author for clarification. All differences in opinion were resolved by further discussion or by discussion with a third author (XP). We excluded studies that did not meet the inclusion criteria and presented the reasons for their exclusion.

Data extraction and management: Data on author, year of publication, study methods, participants, interventions and outcome measures were extracted independently by two authors (SL and PH) according to a standardized data extraction form. Any disagreement among authors was resolved by discussion and review of the original publication. Data were then imported into the Cochrane Review Manager 5. For dichotomous outcomes, we extracted the total number of participants and the number of participants with the event for each group. For continuous outcomes, we extracted the total number of participants, geometric means and standard deviations. We compared the extracted data to identify errors.

Assessment of risk of bias in included studies: SL and PH independently assessed the risk of bias of the included trials using the current version of the Cochrane Handbook [6]. Any discrepancies were resolved by discussion. Randomization (sequence generation), blinding of participants and assessors, allocation concealment and incomplete data outcome were examined.

Data synthesis: We analyzed the data using Review Manager 5. For dichotomous data, the outcomes were analyzed as a comparison of proportions using risk ratio (RR) as a measure of effect. The mean difference was selected to represent the difference for continuous data. All results were presented with 95% confidence intervals (CI). Heterogeneity of effect sizes among the different trials was assessed by inspection of the forest plot using the chi-squared statistic and I^2 statistic. We combined the data using a fixed effect model. Where there was the heterogeneity (I^2>50%), and it was still appropriate to combine trials, we used the random effects model. To investigate heterogeneity, we analyzed subgroups according to the different ages of participants in some outcomes of the review. The effectiveness was also expressed as the "numbers needed to treat" (NNT) with a 95% CI to prevent a case of respiratory infections, which was calculated by STATSDIRECT statistical software (version 2.7.8, 2010-11-8; StatsDirect Ltd., Altrincham, UK).

RESULTS

The flowchart of article selection is shown in **Fig. 1**. A total of 1389 studies were identified from the primary electronic databases. After independent assessment of the titles and abstracts, 1378 were excluded as a result of duplicates (*n*=395), review articles (*n*=152), irrelevant (*n*=699), etc. Subsequently, authors independently reviewed the full texts of the remaining 11 articles and indentified that four studies met the inclusion criteria [7-10]. Excluded studies [11-17] are described in ***Web Table* I**.

The included four randomized placebo-controlled trials consisted of 2135 participants, with 1805 evaluated. Risk of bias assessment and the characteristics of the included trials are presented in ***Web Table* II**. All studies were based in European countries (Finland and Croatia) published during 2001 to 2010. The form of administration of probiotics was milk supplemented with probiotics or probiotics in capsules. In all studies, the probiotics intervention group contained LGG and was compared with placebo control group. In two studies, LGG was the only intervention [7, 8]. In the others, LGG was administered together with other probiotics (*L. rhamnosus* LC 705, *Bifidobacterium breve 99*, *Propionibacterium freudenreichii ssp shermanii* JS or *Bifidobacterium Bb-12*).The dose of LGG and duration of intervention varied **(*Web Table* II)**. One trial assessed the incidence of infections before the age of 7 months and the recurrent infections during the first year of life in its intervention period [10], while the remaining trials assessed the incidence of infections or other outcomes for the whole intervention period.

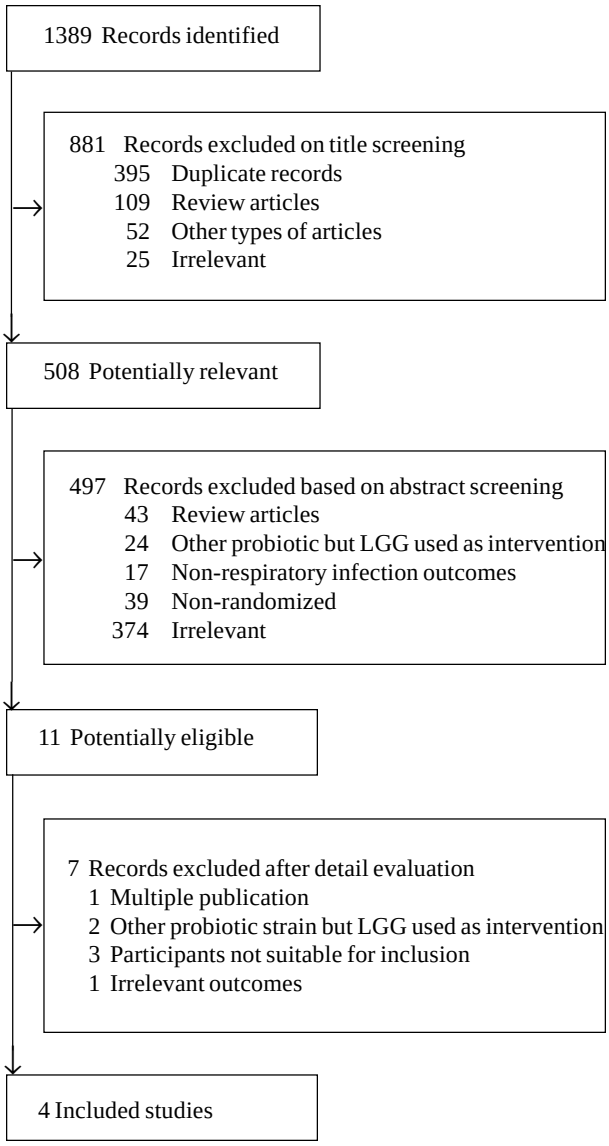

FIG. 1 *Flowchart of article selection.*

Primary outcomes: Compared with the placebo group, the pooled data in the LGG group had a significantly reduced risk of acute otitis media (four RCTs, *n*=1805, RR 0.76, 95% CI 0.64-0.91, fixed effects model, NNT 17, 95% CI 11-46) and a reduced risk of upper respiratory infections (one RCT, *n*=281, RR 0.62, 95% CI 0.50-0.78, NNT 4, 95% CI 3-8). Compared with the placebo group, children in the LGG group had no significant reduction in risk of the overall respiratory infections (four RCTs, *n*=1805, RR 0.84, 95% CI 0.67-1.05, random effects model) as well as no significant reduction in the risk of lower respiratory infections (one RCT, *n*=281, RR 0.82, 95% CI 0.22-2.98). Significant heterogeneity was found for the overall respiratory infections (χ^2=17.69, *P*=0.0005, I^2=83%). No significant heterogeneity was found for acute otitis media (χ^2=2.77, *P*=0.43, I^2=0%) (***Fig.* 2**). For a subgroup of children older than 1 year, the overall respiratory infections was reduced in those in the LGG group compared with those in the placebo group (two RCTs, *n*=794, RR 0.73, 95% CI 0.57-0.92, random effects model, NNT 8, 95% CI 5-14) with heterogeneity (χ^2=2.60, *P*=0.11, I^2=62%). For children younger than 2 months, there were no differences in the overall respiratory infections between the groups that received LGG or placebo (two RCTs, *n*=1011, RR 1.02, 95% CI 0.93-1.11, fixed effects model) and no heterogeneity (χ^2=0.94, *P*=0.33, I^2=0%).

Secondary outcomes: The pooled data showed a statistical significance for reducing antibiotic treatments in the LGG group compared with the placebo group (four RCTs, *n*=1805, RR 0.80, 95% CI 0.71-0.91, fixed effects model). No significant heterogeneity was detected (χ^2=3.16, *P*=0.37, I^2=5%) (***Fig.*2**). In two trials [7,8], no adverse effects were reported, and both products, LGG and placebo, were well tolerated. Three infants receiving placebo experienced vomiting, flatulence and increased fussing [10]. In the trial on newborn infants, the symptoms included abdominal discomfort, vomiting, crying, difficulty in swallowing the product and noncompliance with no difference between the LGG group and the placebo group [9].

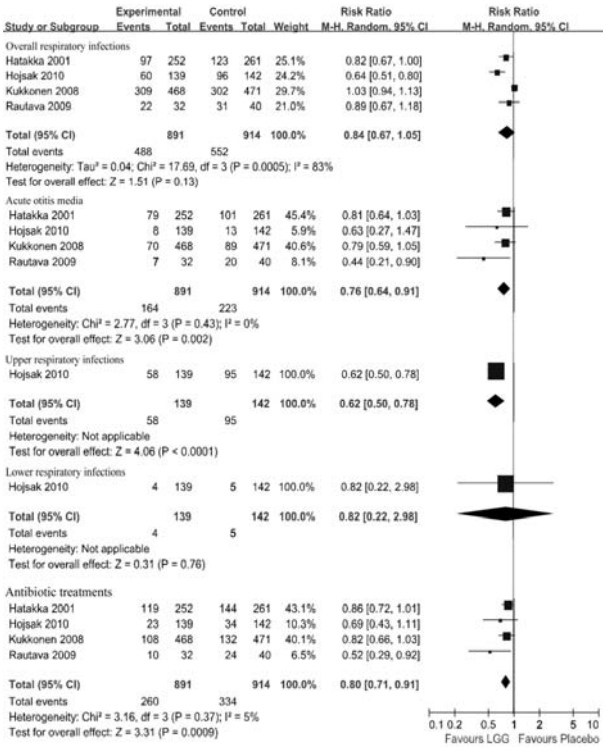

FIG.2 *Effect of Lactobacillus GG on respiratory infections.*

DISCUSSION

This meta-analysis of data from RCTs showed that the use of probiotic microorganism, LGG, compared with placebo among children was associated with a reduction in the incidence of acute otitis media, upper respiratory infections and antibiotic treatments. Subgroup analysis of two trials conducted in children older than 1 year showed significant reduction in the risk of overall respiratory infections. With respect to safety, no serious adverse effects were detected in the included studies. Adverse effects were similar in both groups.

Respiratory infections are generally considered to include infections of the lower and upper respiratory tract. However, the definitions of outcome measures among studies varied. In the trial by Hatakka, *et al*. [7], acute otitis media and sinusitis were reported as upper respiratory infections and acute bronchitis and pneumonia as lower respiratory infections [7]. One trial defined rhinitis, pharyngitis, sinusitis, otitis, common cold as upper respiratory tract infections and pneumonia, bronchitis, bronchiolitis as lower respiratory infections [8]. The other two trials did not provide a definition at all [9,10]. Our study indicated LGG may have a beneficial effect for preventing the upper respiratory infections. However, it did not have an effect on lower respiratory infections, perhaps due to the small number of infections affecting the lower respiratory tract (4 in LGG group and 5 in placebo group) [18,19]

To our knowledge, this is the only meta-analysis that examines the effects of LGG supplementation for the prevention of respiratory infections in children. In many countries, children experience three to six respiratory infections a year and 40% of them could even suffer from at least one episode of acute otitis media which is one of the most common bacterial infections and the main reason for antibiotic treatment in childhood [20-22]. Thus, a 5-10-% reduction in the incidence of acute otitis media and antibiotic use, which our results indicate is possible, could have important clinical, public health, and economic consequences.

Current data shows that consumption of LGG appears to be an effective strategy for reducing the risk of acute otitis media and upper respiratory infections in basically healthy children. In otitis-prone children, who experience nasopharyngeal colonisation of otitis pathogens, Hatakka, *et al*. [14] indicated that LGG treatment did not reduce the occurrence of acute otitis media. This analysis did not have the ability to evaluate the effect of LGG in preventing respiratory infections among children who have nasopharyngeal colonization with pathogens.

Contributors: LS searched literature, extracted data, conducted analysis and drafted manuscript. HPW searched literature, extracted data and conducted analysis. DXX and ZT checked the manuscript. PXF planned the study and contributed to manuscript writing.
Funding: None; *Competing interest*: None stated.

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