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REVIEW ARTICLE

Probiotics for prevention of atopic diseases in infants: systematic review and meta-analysis

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Abstract

Growing evidence underlines the pivotal role of infant gut colonization in the development of the immune system. The possibility to modify gut colonization through probiotic supplementation in childhood might prevent atopic diseases. The aim of the present systematic review and meta-analysis was to evaluate the effect of probiotic supplementation during pregnancy and early infancy in preventing atopic diseases. PubMed, Embase and Cochrane Library were searched for randomized controlled trials evaluating the use of probiotics during pregnancy or early infancy for prevention of allergic diseases. Fixed-effect models were used, and random-effects models where significant heterogeneity was present. Results were expressed as risk ratio (RR) with 95% confidence interval (CI). Seventeen studies, reporting data from 4755 children (2381 in the probiotic group and 2374 in the control group), were included in the meta-analysis. Infants treated with probiotics had a significantly lower RR for eczema compared to controls (RR 0.78 [95% CI: 0.69–0.89], $P = 0.0003$), especially those supplemented with a mixture of probiotics (RR 0.54 [95% CI: 0.43–0.68], $P < 0.00001$). No significant difference in terms of prevention of asthma (RR 0.99 [95% CI: 0.77–1.27], $P = 0.95$), wheezing (RR 1.02 [95% CI: 0.89–1.17], $P = 0.76$) or rhinoconjunctivitis (RR 0.91 [95% CI: 0.67–1.23], $P = 0.53$) was documented. The results of the present meta-analysis show that probiotic supplementation prevents infantile eczema, thus suggesting a new potential indication for probiotic use in pregnancy and infancy.

The incidence of paediatric atopic diseases such as asthma, rhinoconjunctivitis and eczema experienced a boost in the second half of the 20th century, and these diseases are now among the most important public health issues worldwide. The recent global report from the International Study of Asthma and Allergies in Childhood (1), which collected data from about 1 200 000 children in 98 countries, reported a similar prevalence of these diseases in developed and low-income countries. Specifically, this survey showed that in children aged 13–14 years, the prevalence of asthma, rhinoconjunctivitis and eczema was 14.1%, 14.6% and 7.3%,

Abbreviations
AD, atopic dermatitis; CI, confidence interval; RR, risk ratio; US, United States.

and in those aged 6–7 years, the prevalence was 11.7%, 8.5% and 7.9%, respectively.

Allergies cause an enormous burden for patients and society. In the United States (US), it is estimated that asthma causes annually 10.1 million days of school absence, 12.9 million contacts with a doctor and 200 000 hospitalizations, resulting in 1.9 million days of hospital admission (2). Allergic rhinitis, a chronic inflammatory disease of the upper airways, has a marked impact on the quality of life for US patients, and its economic burden is substantial, with an annual total direct medical cost of 3.4 billion US $, mostly attributable to prescription medications and outpatient visits (3). Atopic dermatitis (AD) has also a deep impact on family budget. An Italian study estimated an annual average cost of 1254 € (about 1540 US $) per family (4); in the US, the direct costs of AD were estimated in 900 million US$ (5), while in a Canadian study (6), the annual cost was estimated in about 282 Canadian $, 454 $ and 1242 $ per patients with mild, moderate and severe AD, and the total cost was about 1.4 billion $ per year.

In 1989, Strachan theorized the 'hygiene hypothesis', according to which the apparent rise in the prevalence of allergic diseases could be caused by a reduced exposure to micro-organisms, with a consequent alteration in the balance of the immune response (7). This theory evolved in the 1990s and, thanks to studies on animal models, scientists discovered a plausible mechanism involving the distinction of Th1 and Th2 lymphocyte populations. They recognized that 'natural immunity' to infections induces a Th1 pattern of cytokine release, potentially suppressing the Th2 immune responses involved in IgE-mediated allergy (8). However, not all the subsequent epidemiological and immunological studies confirmed this theory, and the expanding knowledge about a possible interaction between the intestinal microbiota and the human immune system through the interaction of dendritic and T-regulatory cells, bacterial metabolites and cytokines seems able to bridge these gaps (9).

Due to these crucial recent discoveries, a flourishing new area of research was identified with the aim of modifying gut colonization by pre- or probiotics supplementation.

Probiotics are live micro-organisms which, when ingested in adequate amounts, confer a health benefit to the host through an interaction with gut microbiota (10). In the last two decades, the use of probiotics for the prevention of allergies in children has been extensively investigated in many randomized controlled trials: these studies led to conflicting results, and this was probably due to methodological heterogeneity regarding the choice of probiotic strains, dose and timing, the clinical outcomes as well as the duration of supplementation. The position of the American Academy of Paediatrics regarding the possible role of probiotics in prevention and treatment of atopic diseases is cautious, claiming further confirmatory evidence before a strong recommendation for routine use can be stated (11). Recently, an *ad hoc* Task Force of the European Academy of Allergy and Clinical Immunology stated that there is still no evidence to support the use of probiotics for the prevention of food allergy and anaphylaxis (12).

The aim of the present systematic review and meta-analysis was to evaluate in detail the effect of probiotics in the setting of allergy prevention, with a focus on specific strains and on microbiological quality of currently available studies.

Methods

Literature search

A systematic review of all the studies reporting the use of probiotics for prevention of allergic diseases in the first years of life was conducted. The study protocol was designed jointly by the members of the Task Force on Probiotics of the Italian Society of Neonatology, in accordance with PRISMA guidelines (13).

An exhaustive search in the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed/), in the Embase database (http://www.embase.com/) and in the Cochrane Library database (http://www.thecochranelibrary.com/) was carried out. Search limits were set for studies written in English, involving only human subjects and published before 14 February 2014. The search string was built up combining all the terms related to allergic diseases and probiotics, using PubMed MeSH terms and free-text words and their combinations, obtained through the most proper Boolean operators, in order to be as comprehensive as possible. We have broadened our research looking for additional references also in SCOPUS (http://www.scopus.com/), ISI Web of Science (http://apps.webofknowledge.com/) and Google Scholar (https://scholar.google.it/).

The search strategies are reported in the flow chart in Fig. 1. The search was conducted by FM and GVZ: all the relevant studies were identified through abstracts reading, searching the reference lists of the papers retrieved and using 'snowballing' technique (14).

Inclusion criteria were the following: randomized or quasi-randomized controlled trials involving a paediatric population (<18 years of age) and reporting on AD, eczema, asthma, wheezing and/or rhinoconjunctivitis; enteral administration of any probiotic starting during pregnancy or within 3 months of age, compared to placebo or no treatment.

Data extraction and meta-analysis

Study details, including study population, characteristics of the intervention, use of placebo and outcome, were assessed independently by FM and GVZ and checked by DG.

Study quality was evaluated independently using the risk of bias tool as proposed by the Cochrane collaboration (chapter 8.5 of the Cochrane Handbook of Systematic Reviews) (15).

The association between probiotic use and allergic diseases was evaluated by meta-analyses, conducted by DG and AA, using the RevMan software (version 5.2.11) downloaded from the Cochrane website (http://tech.cochrane.org/revman/download).

For each outcome (eczema, asthma, wheezing, rhinoconjunctivitis), a specific meta-analysis was performed.

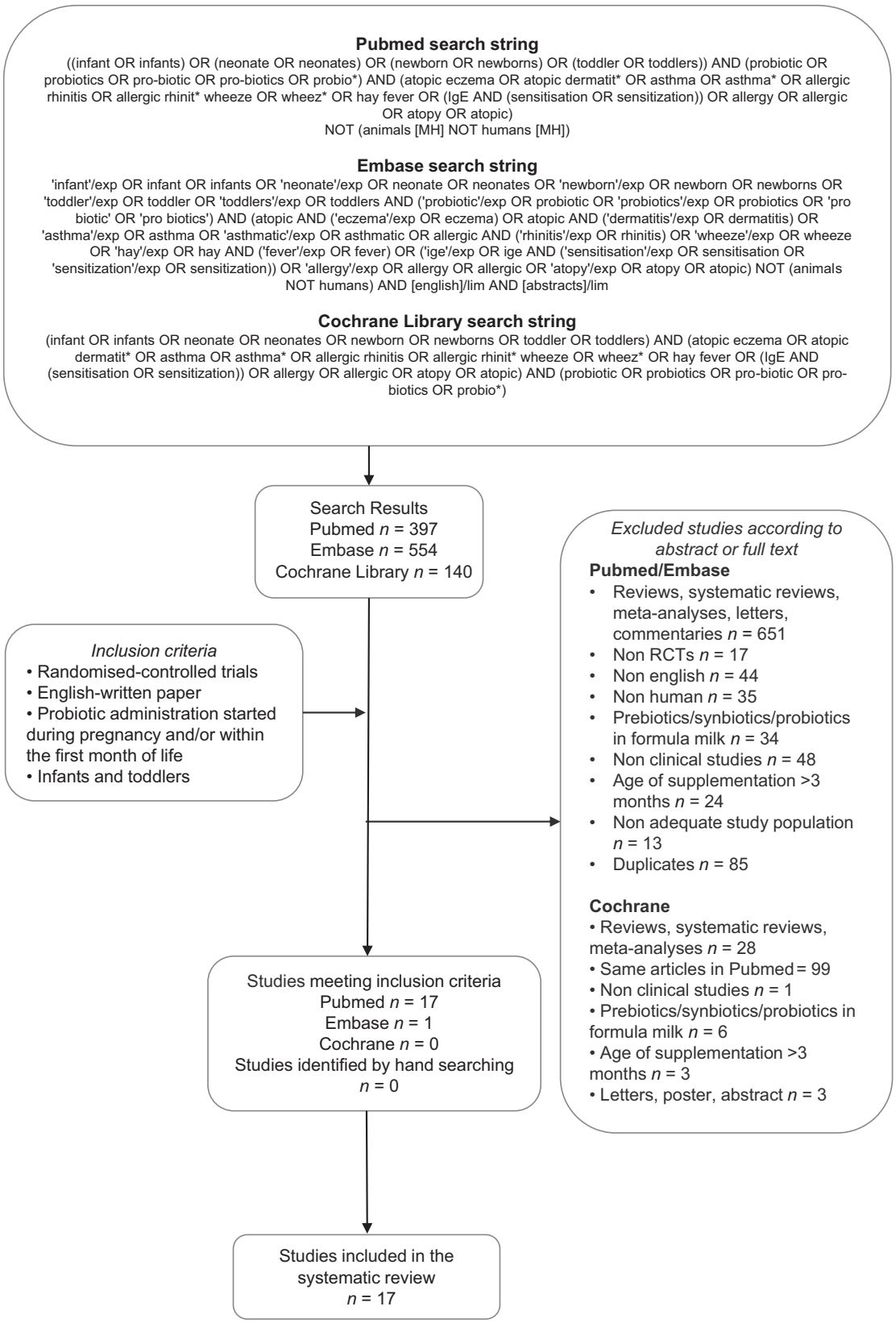

Figure 1 Flow chart of the search strategy and search results. The relevant number of papers at each point is given.

In addition, for the outcome eczema, sub-meta-analyses were conducted stratifying by probiotic strain and by age of onset of the first symptoms.

Microbiological quality of the included studies was evaluated by MLC and LM: studies were defined as having severe, moderate or minor microbiological flaws according to the evaluation of proper strain identification and microbiological assessment.

Results of meta-analyses were presented using forest plots, while funnel plots were used for investigating publication bias. Risk ratio (RR) was calculated using the Mantel–Haenszel method and reported with 95% confidence interval (CI). Fixed-effect models were used at first for the all the analyses. I^2 test for heterogeneity was performed: if a significant heterogeneity was found ($P < 0.05$ from the chi-squared test), a random-effects model was used instead. A random-effects model was also used when the number of studies was ≤5, because of the low power of heterogeneity tests in case of a limited number of studies (16).

Results

Literature search

Three hundred and ninety-seven papers were identified in the PubMed database, 211 articles were found in the Embase database and 140 in the Cochrane Library. Three hundred and forty-three additional articles were found in the general databases (SCOPUS, ISI and Google Scholar).

Duplicated papers were excluded at first. After a careful analysis of abstracts or full texts, studies were also excluded if probiotics were administered in children older than 3 months or if probiotics were administered in formula milk or with prebiotics. Seventeen studies were judged as suitable for inclusion in the meta-analyses (17–33). The characteristics of the included studies are described in Table 1. In all the studies, infants were considered as 'high risk' if they had one or more family members with eczema, asthma, gastrointestinal allergy, allergic urticaria or allergic rhinoconjunctivitis. Atopic dermatitis and eczema were diagnosed and defined as a pruritic, chronic or chronically relapsing noninfectious dermatitis with typical features and distribution (34). Clinical diagnosis of asthma, wheezing and rhinoconjunctivitis was made according to international guidelines. Wheeze was defined as an episode with obstructive airway symptoms. Asthma was defined as a chronic inflammatory disorder of the airways, usually associated with airway hyper-responsiveness and variable airflow obstruction, that is often reversible spontaneously or under treatment (34–36), needed to be doctor diagnosed through clinical symptoms and/or medication during the last 12 months and/or wheeze or nocturnal cough and a positive reversibility test and/or pathological Fraction of Exhaled Nitric Oxide (FENO) value. Asthma is often associated with rhinitis, an inflammation of the nasal mucosa and/or conjunctivitis, an inflammation of the conjunctiva (37). The diagnosis of rhinoconjunctivitis was based on standard ISAAC question and required the presence of itchy watery eyes and problem with sneezing or a runny or a blocked nose at least twice in contact with the same allergen and no signs of infection (34, 38). The outcomes reported in the studies included in meta-analysis were evaluated using a combination of questionnaires compiled by parents, follow-up visits by nurses and doctors, structured interview related to symptoms of allergic disease, physical examination, spirometry and skin prick tests.

Probiotics and eczema

Data from 4755 children (2381 in the probiotic group and 2374 in the control group) were analysed. Probiotic supplementation was started during pregnancy in all the studies except for the trials by Prescott et al. (25) and Taylor et al. (30), in which probiotics were administered to infants of atopic mothers within 48 h of delivery. Fewer children in the probiotic group developed eczema compared to those in the control group (672 [28.22%] *vs* 847 [35.67%], respectively). The RR was significantly lower in children treated with probiotics (RR 0.78 [95% CI 0.69–0.89], $P = 0.0003$). Heterogeneity among studies was moderate ($I^2 = 57\%$, $P < 0.0001$, Fig. 2), and for this reason, a random-effects model was used. The funnel plot did not show any clear asymmetry (Fig. 3). Number needed to treat was 13, which means that 13 infants needed to be supplemented with probiotics in order to prevent one case of eczema.

Probiotic strains were rather heterogeneous: in four studies (19, 23, 24, 27), different probiotic mixtures, containing both *Lactobacilli* and *Bifidobacteria*, were administered to pregnant women and infants, three studies (17, 21, 26) evaluated both a single strain of *Lactobacilli* and a single strain of *Bifidobacteria*, and the remaining 10 studies (18, 20, 22, 25, 28–33) evaluated different strains of *Lactobacilli*. Sub-meta-analyses showed a significant effect of probiotic mixtures' supplementation in the prevention of eczema (RR 0.54 [95% CI: 0.43–0.68], $P < 0.00001$, Fig. 4A). No significant effect of probiotic supplementation was documented in the studies using *Lactobacilli* (RR 0.90 [95% CI: 0.77–1.05], $P = 0.18$, Fig. 4B), and also in those using *Bifidobacteria* (RR 0.89 [95% CI: 0.73–1.08], $P = 0.23$, Fig. 4C). In Fig. 4A, the study by Kim et al. (23) and the study by Niers et al. (24) were reported three times each, because these studies reported the outcome at three different time points (3, 6 and 12 months of age and 3, 12 and 24 months of age, respectively). Similarly, data from the study by Rautava et al. (19) were reported twice as that study included two groups of patients, supplemented with different probiotic mixtures (*Lactobacillus rhamnosus LPR* + *Bifidobacterium longum BL999* and *Lactobacillus paracasei ST11* + *B. longum BL999*). Furthermore, in Fig. 4B, the studies by Boyle et al. (22) and Taylor et al. (30) were reported twice and the study by Ou et al. (20) was reported three times because all these studies evaluated the outcome at different time points (3 and 12 months of age, 6 and 12 months of age and 6, 18 and 36 months of age, respectively).

Table 1 Studies included in the systematic review and meta-analysis

Author, year	Study details	Study population Number of patients completed the follow-up Mean age	Intervention -Strain -Dose (D) -Start of treatment (S) -End of treatment (E)	Placebo	Outcomes evaluation	Follow-up
K. Wickens, 2013	Prospective Double blind Randomized Controlled	High-risk infants for atopy 144 placebo group 134 HN001 group 144 HN019 group Mean age 6.2 years	*Lactobacillus rhamnosus HN001* *Bifidobacterium animalis subsp lactis HN019* D: 6×10^9 cfu/day HN001 D: 9×10^9 cfu/day HN019 S: from gestational week 35 E: till 2 years in all infants	Not stated	Physical examination, skin prick test, blood examinations (total and sslgE), ISAAC questionnaire, spirometry	At 2–4–6 years of age
T. Abrahamsson, 2013	Prospective Double blind Randomized Controlled	High-risk infants for atopy 90 placebo group 94 *Lactobacillus reuteri* group Mean age 7 years	*L. reuteri ATCC 57730* D: 1×10^8 cfu/day S: from gestational week 36 E: till 12 months of age	Not stated	Physical examination, structured interviews, spirometry and measurement of fractional exhaled nitric oxide	At 2–7 years of age
S. Rautava, 2012	Prospective Double blind Randomized Controlled	High-risk infants for atopy 62 placebo 73 LPR + BL999 70 ST11 + BL999 Mean age 2 years	*L. rhamnosus LPR* and *Bifidobacterium longum BL999* *Lactobacillus paracasei ST11* and *B. longum BL999* D: 1×10^9 cfu/day each probiotic S: 2 months before delivery E: 2 months of age of breastfed infants	Dietary supplement without probiotics	Physical examination and skin prick test	At 1–3–6–12–24 months of age
C.-Y. Ou, 2012	Prospective Double blind Randomized Controlled	High-risk infants for atopy 65 LGG 63 placebo Mean age 36 months	*Lactobacillus GG* D: 1×10^{10} cfu/day S: from gestational week 24 E: 6 months of age of infants	Microcrystalline cellulose	ISAAC questionnaire, physical examination, blood examinations (total and sslgE)	At 6–18–36 months of age

Table 1 (continued)

Author, year	Study details	Study population Number of patients completed the follow-up Mean age	Intervention -Strain -Dose (D) -Start of treatment (S) -End of treatment (E)	Placebo	Outcomes evaluation	Follow-up
K. Wickens, 2012	Prospective Double blind Randomized Controlled	High-risk infants for atopy 143 placebo 136 HN001 146 HN019 Mean age 4 years	*L. rhamnosus HN001* *Bifidobacterium animalis subsp lactis HN019* D: 6×10^9 cfu/day D: 9×10^9 cfu/day S: from gestational week 35 E: till 2 years in all infants	Not stated	Physical examination, skin prick test, blood examinations (total and ssIgE), ISAAC questionnaire	At 2–4 years of age
R. J. Boyle, 2011	Prospective Double blind Randomized Controlled	High-risk infants for atopy 103 placebo 109 LGG Mean age 12 months	*L. rhamnosus GG (LGG)* D: 1.8×10^{10} cfu/day S: from 36 weeks of gestation E: until delivery	Maltodextrin	Physical examination, skin prick test only at 12 months, questionnaire about allergy and eczema symptoms	At 3–6–12 months of age
J.Y. Kim, 2010	Prospective Double blind Randomized Controlled	High-risk infants for atopy 35 placebo 33 probiotics Mean age 12 months	*Bifidobacterium bifidum BGN4, Bifidobacterium lactis AD011 and Lactobacillus acidophilus AD031* D: 1.6×10^9 cfu/day each strain S: from 8 weeks before the expected delivery E: from 4 to 6 months to infants	Maltodextrin	Physical examination, structured interviews, blood examinations (total and ssIgE) at 12 months	At 3–6–12 months of age
L. Niers, 2009	Prospective Double blind Randomized Controlled	High-risk infants for atopy 48 placebo 50 probiotics Mean age 2 years	*Bifidobacterium bifidum W23, Bifidobacterium lactis W52* *Lactobacillus lactis W58* D: 1×10^9 cfu/day each strain S: from 6 weeks before the expected delivery E: 12 months of age of infants	Rice starch and maltodextran	Physical examination, questionnaire completed by parents, total and ssIgE, skin prick test only at 24 months of age	At 3–12–24 months of age

Table 1 (continued)

Author, year	Study details	Study population Number of patients completed the follow-up Mean age	Intervention -Strain -Dose (D) -Start of treatment (S) -End of treatment (E)	Placebo	Outcomes evaluation	Follow-up
K. Wickens, 2008	Prospective Double blind Randomized Controlled	High-risk infants for atopy 150 placebo 144 HN001 152 HN019 Mean age 2 years	*L. rhamnosus HN001* D: 6×10^9 cfu/day *Bifidobacterium animalis subsp lactis HN019* D: 9×10^9 cfu/day S: from gestational week 35 E: 2 years of life	Dextran, salt and a yeast extract	Physical examination, structured interviews and skin prick test only at 24 months of age	At 3–6–12–18–24 months of age
A. Huurre, 2008	Prospective Double blind Randomized Controlled	High-risk infants for atopy 68 placebo 72 probiotics Mean age 12 months	*L. rhamnosus strain GG (ATCC 53103) and Bifidobacterium lactis Bb12* D: 1×10^{10} cfu/day each strain S: from the first trimester of pregnancy E: to the end of exclusive breastfeeding	Microcrystalline cellulose and dextrose anhydrate	Physical examination, skin prick test at 6–12 months,	At 1–6–12 months of age
M. V. Kopp, 2008	Prospective Double blind Randomized Controlled	High-risk infants for atopy 44 placebo 50 LGG Mean age 24 months	*Lactobacillus GG (American Type Culture Collection 53103)* D: 5×10^9 cfu twice daily S: 4 to 6 weeks before expected delivery E: 6 months of age	Microcrystalline cellulose	Physical examination, structured interviews; total IgE and sensitization to an inhalant allergen at 2 years of age	At 12–24 months of age
S. L. Prescott, 2008	Prospective Double blind Randomized Controlled	High-risk infants for atopy 76 placebo 77 L. acidophilus Mean age 2.5 years	*Lactobacillus acidophilus LAVRI-A1* D: 3×10^9 cfu/day S: from birth E: 6 months of age	Maltodextrin	Physical examination, structured interviews, skin prick test	At 2.5 years of age
M. F. Bottcher, 2008	Double blind, Randomized, placebo controlled	High-risk infants for atopy 51 *L. reuteri* 53 placebo Mean age 2 years	*L. reuteri strain (American Type Culture Collection 55730)* D: not stated S: from gestational week 36 until delivery E: 12 months of age	Not stated	Physical examination, structured interviews, skin prick test, venus blood sample	1–3–6–12–24 months of age

Table 1 (continued)

Author, year	Study details	Study population Number of patients completed the follow-up Mean age	Intervention -Strain -Dose (D) -Start of treatment (S) -End of treatment (E)	Placebo	Outcomes evaluation	Follow-up
T. R. Abrahamsson, 2007	Prospective Double blind Randomized Controlled	High-risk infants for atopy 93 placebo 95 *L. reuteri* Mean age 2 years	*L. reuteri (American Type Culture Collection 55730)* D: 1×10^8 cfu/day S: from gestational week 36 until delivery E: 12 months of age	Oil without bacteria	Physical examination, structured interviews, skin prick test (only at 6–12–24 months of age)	At 1–3–6–12–24 months of age
A. L. Taylor, 2007	Prospective Double blind Randomized Controlled	High-risk infants for atopy 89 placebo 89 LAVRI-A1 Mean age 12 months	*Lactobacillus acidophilus LAVRI-A1* D: 3×10^9 cfu/day S: from birth E: 6 months of age	Maltodextrin	Physical examination, structured interviews, skin prick test (only at 12 months of age)	At 1–6–12 months of age
S. Rautava, 2002	Prospective Double blind Randomized Controlled	High-risk infants for atopy 30 placebo 27 LGG Mean age 24 months	*L. rhamnosus strain GG (ATCC 53103)* D: 2×10^{10} cfu/day S: 4 weeks before the expected delivery E: 3 months of age	Microcrystalline cellulose;	Physical examination, structured interviews	At 3–6–12–18–24 months of age
M. Kalliomäki, 2001	Prospective Double blind Randomized Controlled	High-risk infants for atopy 68 placebo 64 LGG Mean age 24 months	*Lactobacillus GG* D: 1×10^{10} cfu/day S: 2–4 weeks before the expected delivery E: 6 months of age	Microcrystalline cellulose	Physical examination, structured interviews; skin prick tests at ages 6, 12 and 24 months; total and ssIgE at ages 3, 12 and 24 months	At 3–6–12–18–24 months of age

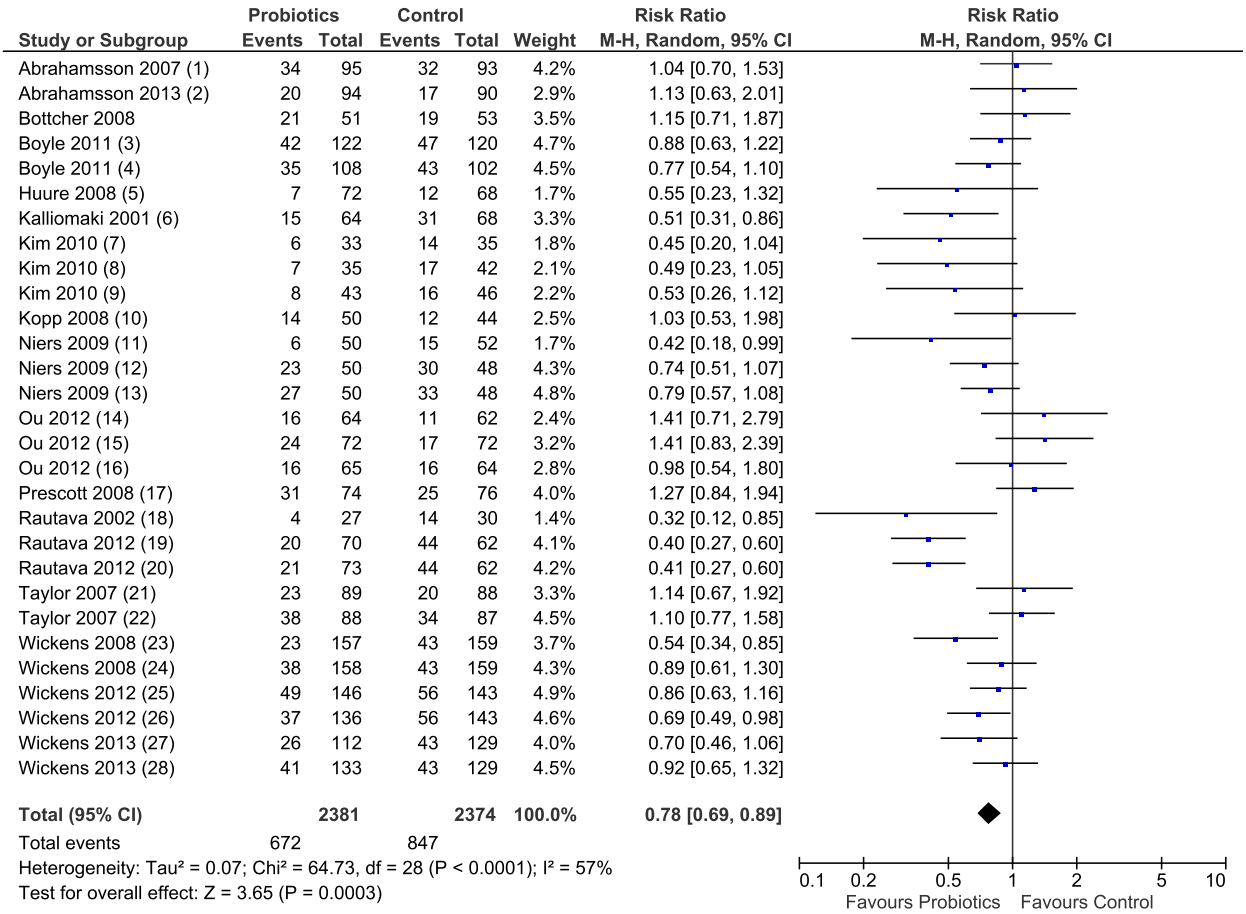

Figure 2 Forest plot showing the association between probiotics and eczema. M-H: Mantel–Haenszel method.

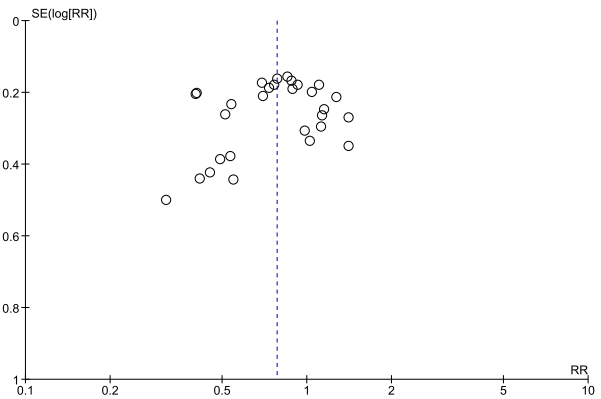

Figure 3 Funnel plot of the included studies reporting on eczema.

Age-specific sub-meta-analyses showed that probiotics prevented eczema in the first 24 months of life, with a partial loss of efficacy after 2 years of age. Stratified analysis of studies with different follow-up times showed a significant effect of probiotics at ≤12 months (RR 0.82 [95% CI: 0.71–0.95], $P = 0.008$, Fig. 5A (20, 22–24, 27, 30)), at 24 months (RR 0.70 [95% CI: 0.54–0.91], $P = 0.008$, Fig. 5B (19, 20, 24, 26, 28, 29, 31–33)), but not beyond 24 months (RR 0.88 [95% CI: 0.75–1.04], $P = 0.13$, Fig. 5C (5, 17, 18, 21, 25)).

Probiotics and asthma/wheezing

Eight studies (17, 18, 20–22, 25, 29, 30) reported on asthma or wheezing. Wheezing was defined as an episode of obstructive airway symptoms. On the other side, asthma diagnosis required a recurrence of symptoms with a clinical diagnosis according to the GINA criteria and the presence of symptoms and/or use of medications during the period prior the assessment. Sub-meta-analysis of the four studies (17, 18, 25, 29) reporting on asthma showed no significant effect of probiotic supplementation (RR 0.99 [95% CI: 0.77–1.27], $P = 0.95$; random-effects analysis). Data from the eight studies (17, 18, 20–22, 25, 29, 30) in which wheezing was evaluated showed similar results (RR 1.02 [95% CI: 0.89–1.17], $P = 0.76$; fixed-effect analysis).

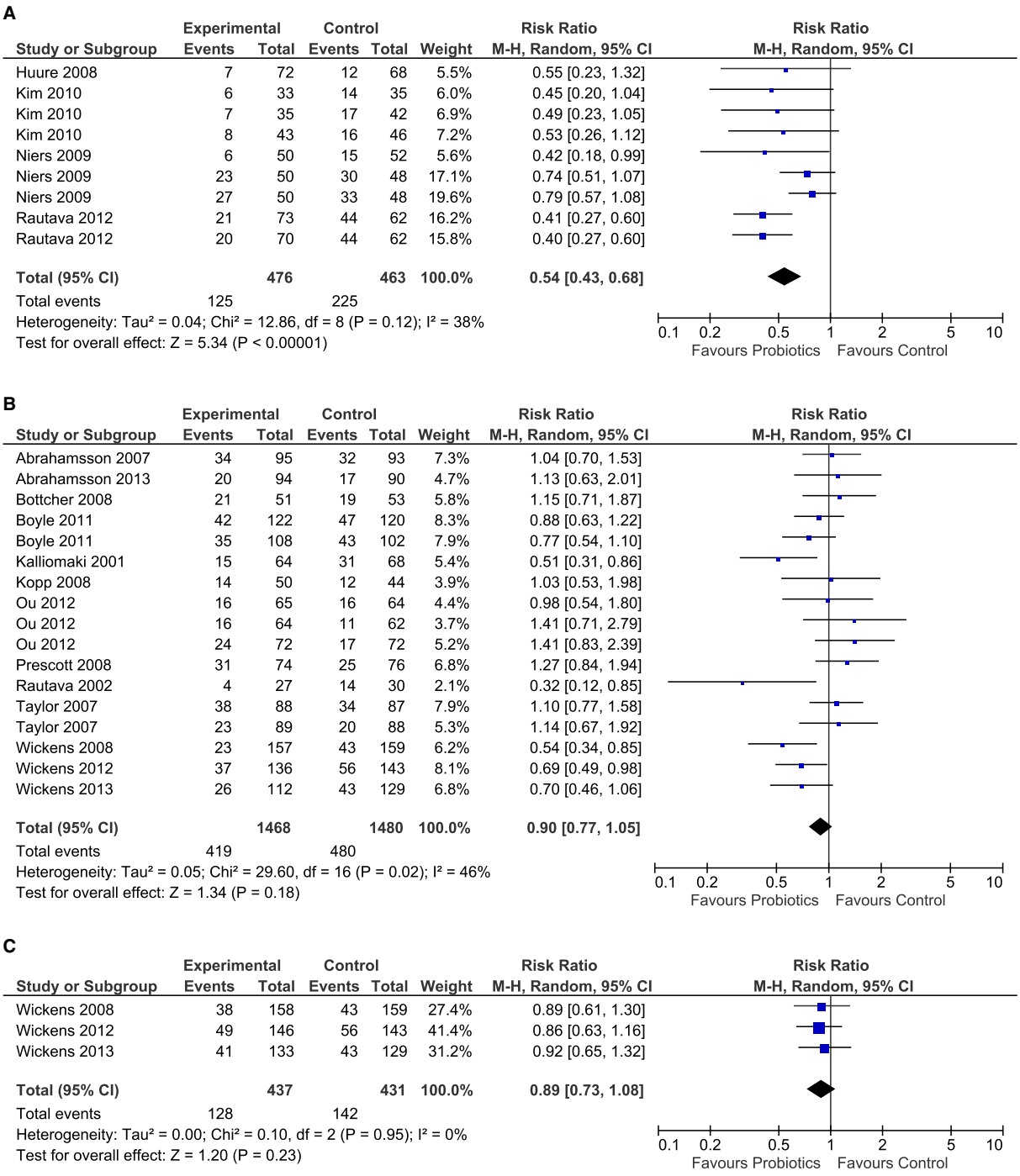

Figure 4 Forest plot showing the association between probiotics and eczema in the studies which used a probiotic mixture (A) or a single-strain probiotic product (B. *Lactobacilli*; C *Bifidobacteria*.). M-H: Mantel–Haenszel method.

Probiotics and rhinoconjunctivitis

Five studies (17, 18, 20, 21, 29) which evaluated the incidence of clinically diagnosed rhinoconjunctivitis showed no significant effect of probiotic supplementation (RR 0.91 [95% CI: 0.67–1.23], $P = 0.53$; random-effects analysis).

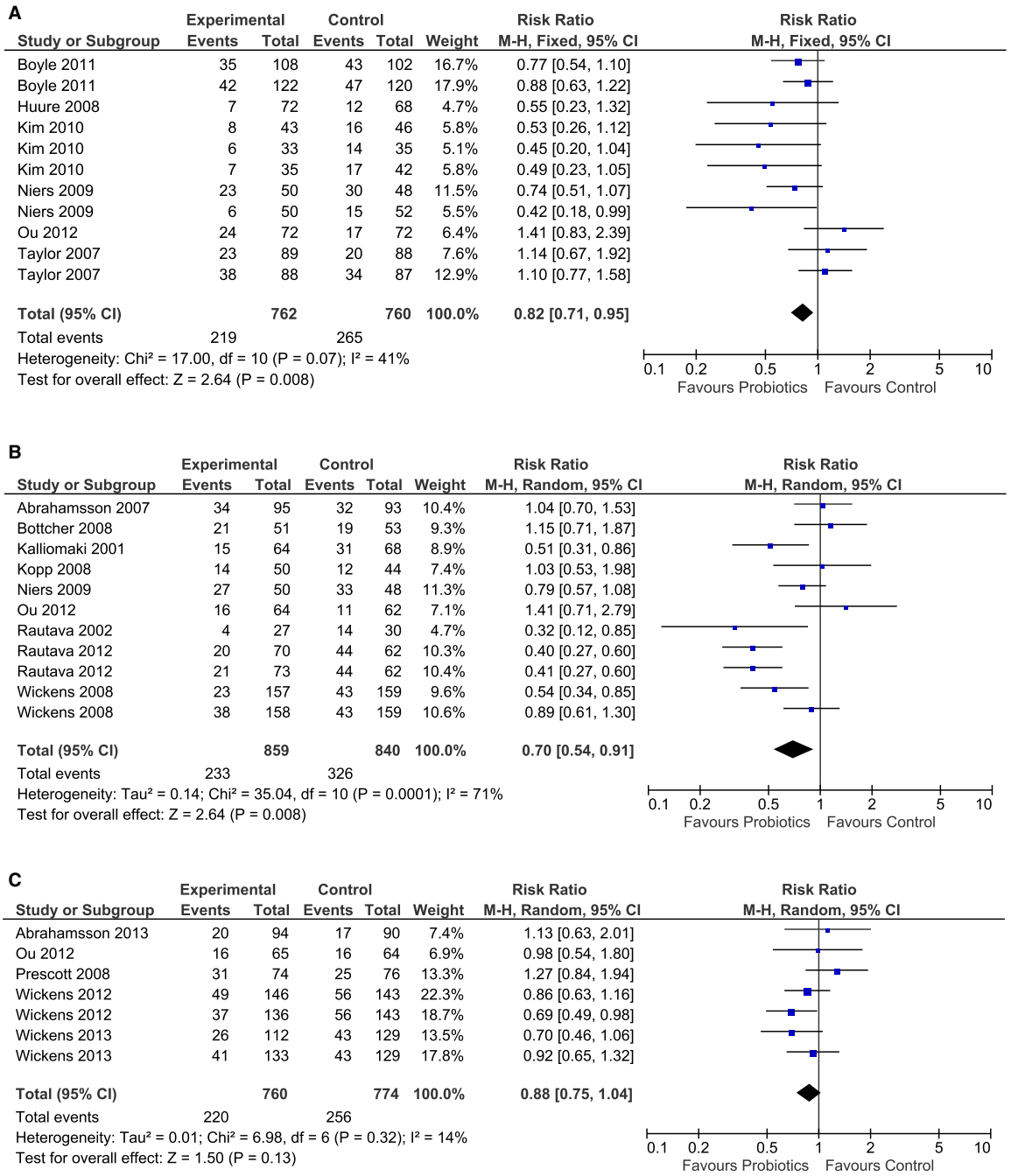

Figure 5 Forest plot showing the association between probiotics and eczema at ≤12 months of age (A), 24 months of age (B) and >2 years (C). M-H: Mantel–Haenszel method.

Study quality

Evaluation of the quality of the studies included in the meta-analysis according to the risk of bias tool as proposed by the Cochrane collaboration is shown in Table 2.

Microbiological quality

A microbiological assessment of the included studies was performed (Table 3). Thirteen studies (17–21, 23, 25, 27–32) were evaluated as having moderate microbiological flaws, one study (22) as having a minor microbiological flaw and

Table 2 Evaluation of the quality of the studies included in the meta-analysis according to the risk of bias tool as proposed by the Cochrane collaboration

Study	Random sequence generation	Allocation concealment	Blinding	Incomplete outcome data	Selective outcome reporting	Other sources of bias
K. Wickens, 2013	Low	Low	Unclear	High	Unclear	Low
T. Abrahamsson, 2013	Low	Low	Unclear	High	Unclear	Low
S. Rautava, 2012	Low	Low	Low	Low	Unclear	Low
C.-Y. Ou, 2012	Unclear	Unclear	Low	Low	Unclear	High
K. Wickens, 2012	Low	Low	Unclear	High	Unclear	Low
R. J. Boyle, 2011	Low	Low	Low	Low	Unclear	Low
J.Y. Kim, 2010	Low	Low	Low	Low	Unclear	Low
L. Niers, 2009	Low	Low	Low	Low	Unclear	High
K. Wickens, 2008	Low	Low	Low	Low	Unclear	Low
A. Huurre, 2008	Unclear	Unclear	Low	Unclear	Unclear	Low
M. V. Kopp, 2008	Low	Low	Low	Low	Unclear	Low
S. L. Prescott, 2008	Low	Low	Low	High	Unclear	Low
Bottcher 2008	Unclear	Unclear	Unclear	Low	Unclear	Low
T. R. Abrahamsson, 2007	Low	Low	Low	Low	Unclear	Low
A. L. Taylor, 2007	Low	Low	Low	Low	Unclear	Low
S. Rautava, 2002	Unclear	Unclear	Unclear	High	Unclear	Low
M. Kalliomäki, 2001	Low	Low	Low	Low	Unclear	Low

three studies (24, 26, 33) did not have any microbiological flaws. A clear identification of probiotic strains was always declared and six studies (22, 24, 26, 29, 30, 33) evaluated colonization assessment after probiotic supplementation.

Sensitivity analysis

Sensitivity testing after removal of each individual study was carried out. The analysis showed that the results of the meta-analyses did not change for the outcomes asthma, wheezing, rhinoconjunctivitis and atopic dermatitis after removal of each individual study.

In the eczema group, the sensitivity analyses showed, on the other hand:

- loss of significance after removing the study by Kim et al. (23) or the study by Niers et al. (24) in the eczema ≤1 year subgroup;
- loss of significance after removing the study by Rautava et al. (19) in the eczema >2 years subgroup.

In all the other meta-analyses for the eczema group, the results did not change after removal of each individual study.

Discussion

The present meta-analysis included randomized, double-blind, placebo-controlled trials of oral probiotic supplementation to pregnant and/or nursing mothers or to infants under 3 months of age for the prevention of atopic diseases. Data from included studies showed an overall benefit of probiotic supplementation for the prevention of eczema in high-risk infants. Strain-specific sub-meta-analyses showed that probiotic mixtures were effective in reducing the incidence of eczema, while no effect was documented for products containing *Lactobacilli* or *Bifidobacteria* alone.

In 2007, a first systematic review of the literature, regarding the use of probiotics for prevention of allergic disease and food hypersensitivity and including 12 trials, was performed (39). The authors concluded that there was insufficient evidence to recommend probiotic supplementation in infants aiming to prevent allergic diseases. However, studies included in that systematic review were heterogeneous in terms of study population (infants, children, preterm infants) and also type of supplementation (probiotics, prebiotics or symbiotics). Despite these limitations, the authors performed a sub-meta-analysis of the five less heterogeneous trials and observed a significant reduction of eczema in infants who were supplemented with probiotics.

Several randomized, double-blind, placebo-controlled trials were subsequently performed, and reviews and meta-analyses were conducted with the inclusion of the most recent studies.

A meta-analysis (40) of 18 randomized controlled trials, aiming to evaluate whether the incidence of AD and IgE-associated AD could be modified by the supplementation with probiotics during pregnancy and early infancy, showed a 20% statistically significant reduction in the incidence of these diseases following the use of probiotics. Similar results were found in a different meta-analysis (41) including six trials on prevention of AD. In addition, a sub-meta-analysis performed with the exclusion of one prevention study in which probiotics were administered only in the postnatal period showed a significant reduction in AD, suggesting that prenatal administration of probiotics could maximize the prophylactic potential of these products.

The results of these meta-analysis are consistent with the hygiene hypothesis, which shows that several factors beyond the Th1 and Th2 lymphocytes, such as dendritic and T-regulatory cells, metabolites such as short-chain fatty acids and long-chain fatty acids, chemokines and other regulatory

Table 3 Evaluation of the included studies according to their microbiological quality

Author, year	Probiotic strain	Strain identification	Microbiological assessment	Microbiological flaw
Wickens, 2013	*Lactobacillus rhamnosus HN001 Bifidobacterium animalis subsp lactis HN019*	Strains identified	No assessment of colonization	Moderate
Abrahamsson, 2013	*L. reuteri 55730*	Strains identified	No assessment of colonization	Moderate
Rautava, 2012	*L. rhamnosus LPR and B. longum BL999 Lactobacillus paracasei ST11 and B. longum BL999*	Strains identified	No assessment of colonization	Moderate
Ou, 2012	*L. rhamnosus GG*	Strains identified	No assessment of colonization	Moderate
Wickens, 2012	*L. rhamnosus HN001 Bifidobacterium animalis subsp lactis HN019*	Strains identified	No assessment of colonization	Moderate
Boyle, 2011	*L. rhamnosus GG*	Strains identified	Re-isolation of *L. rhamnosus* GG by count in plates	Minor
Kim, 2010	*Bifidobacterium bifidum BGN4, Bifidobacterium lactis AD011, Lactobacillus acidophilus AD031*	Strains identified	No assessment of colonization	Moderate
Niers, 2009	*Bifidobacterium bifidum W23, Bifidobacterium lactis W52, Lactobacillus lactis W58*	Strains identified	Assessment by DGGE (denaturing gradient gel electrophoresis) and qPCR (gender-specific primers)	Absent
Wickens, 2008	*L. rhamnosus HN001, Bifidobacterium. animalis subsp lactis strain HN019*	Strains identified	Assessment by qPCR (specie-specific primers)	Absent
Huurre, 2008	*L. rhamnosus strain GG Bifidobacterium lactis Bb12*	Strains identified	No assessment of colonization	Moderate
Kopp, 2008	*L. rhamnosus GG*	Strains identified	No assessment of colonization	Moderate
Bottcher, 2008	*L. reuteri strain (American Type Culture Collection 55730)*	Strains identified	Assessment of colonization of *L. reuteri* by count in plates with MRS agar	Absent
Abrahamsson, 2007	*L. reuteri ATCC 55730*	Strains identified	Assessment of colonization of *L. reuteri*, but not reported (referred in a separate communication)	Moderate
Taylor, 2007	*Lactobacillus acidophilus LAVRI-A1*	Strains identified	Assessment of colonization of lactobacilli and Bifidobacteria by plate count	Moderate
Prescott, 2008	*Lactobacillus acidophilus LAVRI-A1*	Strains identified	No assessment of colonization	Moderate
Rautava, 2002	*L. rhamnosus strain GG*	Strains identified	No assessment of colonization	Moderate
Kalliomaki, 2001	*L. rhamnosus strain GG*	Strains identified	No assessment of colonization	Moderate

factors, play an important role in the immune system development (42).

Furthermore, recent studies have shown that maternal microbial transfer to the offspring begins during pregnancy, providing a pioneer microbiome. Microbial DNA can be detected in amniotic fluid, placental and foetal membranes, umbilical cord blood and meconium (43). Thus, the close immunological interaction between the mother and her foetus creates the opportunity for the maternal microbiota to influence the offspring's immune development, and this may affect infant gut colonization patterns and subsequent susceptibility to allergic disease (44). Moreover, it is known that atopic children may have a different gut microbiota compared with nonatopic ones (45, 46); thus, an early probiotic administration may promote a healthier gut microbiota composition which, in turn, modulates the maturation of the immune response.

Despite the overall high quality of all included studies, previous reviews and meta-analyses proved to be rather heterogeneous, due to the inclusion of trials in which probiotics were supplemented in association with prebiotics or with formula milk. Another source of heterogeneity came from combining trials in which probiotics were administered during pregnancy or during the perinatal period and studies in which the treatment was administered to older children.

Recently, a good quality meta-analysis (47) of 14 randomized, double-blind, placebo-controlled trials showed a 31%

reduction in the incidence of eczema after probiotic supplementation. Ten of those 14 studies were also included in our literature review, with comparable results. Four studies were excluded from our review because in three studies (48–50) probiotics were administered with formula milk and in one trial (51) supplemented children were older than 3 months. In addition, when intervention trials were classified by the strain of probiotics used (*Lactobacilli*, *Bifidobacteria* or mixed strains), a sub-meta-analysis conducted by Dang et al. (47) showed results similar to ours, with a significant benefit of the supplementation with a mixture of probiotic strains. A possible explanation for this finding is that a mixture of different probiotic strains might be more effective in providing an ecological barrier than a single strain.

Data deriving from studies in which the preventive effect of probiotics on asthma, wheezing and rhinoconjunctivitis was evaluated showed only a weak, nonsignificant or even absent benefit. Our results are consistent with the literature. In a recent large meta-analysis performed on 25 trials (52), the authors concluded that a prenatal and/or early-life probiotic administration reduces the risk of atopic sensitization but may not reduce the risk of asthma/wheezing. In a different review, Tang and colleagues (53) reached the same conclusion, stating that there is no sufficient evidence to recommend a routine probiotic administration for the treatment or prevention of atopic diseases.

The strength of our meta-analysis is the inclusion of trials in which only probiotics were supplemented. This allowed analysing more homogeneous studies, even if a moderate heterogeneity, in terms of the starting point of supplementation during pregnancy, duration of treatment and type of administered strains, was present anyway.

In order to be as complete as possible, we decided to include also quasi-randomized studies. Quasi-randomization is defined as the process of allocation carried out on the basis of a pseudo-random sequence (e.g. odd/even hospital number or date of birth, alternation). We know that when such methods are used, the problem is that allocation is rarely concealed. We planned to use the assessment of the risk of bias in order to reduce this risk; however, none of the included studies was quasi-randomized, and thus, we can assume that no additional bias risk or heterogeneity has been added by different type of study inclusion.

Another strength of this review is the evaluation of methodological and, mainly, microbiological quality of included studies. Each study was assessed for quality using the risk of bias tool as proposed by the Cochrane collaboration, with a good proportion of well-conducted trials. Microbiological evaluation showed that the general quality of included studies was satisfactory, as a clear identification of the supplemented strain was performed in all the included studies and in six of them, the assessment of proper stool colonization was also carried out.

The studies included in the meta-analysis did not report any short-term adverse effect of probiotic supplementation, and several studies (54) confirmed the safety of probiotics use during pregnancy.

In conclusion, we could state that the prevention of infantile eczema represents a potential indication for probiotic use during pregnancy and early infancy. The aetiology and pathogenesis of infantile eczema and AD are not fully understood yet, and no effective treatment is available at the moment. For this reason, it is of capital importance to find an effective tool for the prevention of these diseases. Furthermore, given that AD is widely recognized as the earliest manifestation of atopy, the identification of AD as a target to stop the so-called *atopic march* (55) should become a priority in the future prevention strategies. Future research should aim at identifying the best time to begin probiotic supplementation, considering the fundamental impact of prenatal administration, the exact composition of probiotic mixtures and the duration of administration, in order to achieve the longest-term benefit.

Author contributions

All the authors, as part of the Task Force on Probiotics of the Italian Society of Neonatology, conceived and designed the study protocol. FM and GVZ performed the literature search and assessed study details, which were checked by DG. FM and GVZ also evaluated study quality. AA and DG performed the meta-analyses. MLC and LM evaluated microbiological quality of the included studies. FM and GVZ wrote the first draft of the paper, which was critically revised by all the other authors. All the authors gave final approval of the version to be submitted and agreed to be accountable for the whole paper.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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