Cochrane Library

Cochrane Database of Systematic Reviews

Probiotics to prevent necrotising enterocolitis in very preterm or very low birth weight infants (Review)

Sharif S, Meader N, Oddie SJ, Rojas-Reyes MX, McGuire W

Sharif S, Meader N, Oddie SJ, Rojas-Reyes MX, McGuire W.
Probiotics to prevent necrotising enterocolitis in very preterm or very low birth weight infants.
Cochrane Database of Systematic Reviews 2020, Issue 10. Art. No.: CD005496.
DOI: 10.1002/14651858.CD005496.pub5.

www.cochranelibrary.com

WILEY

TABLE OF CONTENTS

[Intervention Review]

Probiotics to prevent necrotising enterocolitis in very preterm or very low birth weight infants

Sahar Sharif[1], Nicholas Meader[1], Sam J Oddie[1,2], Maria Ximena Rojas-Reyes[3], William McGuire[1]

[1]Centre for Reviews and Dissemination, University of York, York, UK. [2]Bradford Neonatology, Bradford Teaching Hospitals NHS Foundation Trust, Bradford, UK. [3]Department of Clinical Epidemiology and Public Health, Institut de Recerca Hospital Santa Creu i Sant Pau, Barcelona, Spain

Contact address: William McGuire, william.mcguire@york.ac.uk.

Editorial group: Cochrane Neonatal Group.
Publication status and date: New search for studies and content updated (conclusions changed), published in Issue 10, 2020.

Citation: Sharif S, Meader N, Oddie SJ, Rojas-Reyes MX, McGuire W. Probiotics to prevent necrotising enterocolitis in very preterm or very low birth weight infants. *Cochrane Database of Systematic Reviews* 2020, Issue 10. Art. No.: CD005496. DOI: 10.1002/14651858.CD005496.pub5.

ABSTRACT

Background

Intestinal dysbiosis may contribute to the pathogenesis of necrotising enterocolitis (NEC) in very preterm or very low birth weight infants. Dietary supplementation with probiotics to modulate the intestinal microbiome has been proposed as a strategy to reduce the risk of NEC and associated mortality and morbidity.

Objectives

To determine the effect of supplemental probiotics on the risk of NEC and mortality and morbidity in very preterm or very low birth weight infants.

Search methods

We searched Cochrane Central Register of Controlled Trials (CENTRAL; 2020, Issue 2) in the Cochrane Library; MEDLINE Ovid (1946 to 17 Feb 2020), Embase Ovid (1974 to 17 Feb 2020), Maternity & Infant Care Database Ovid (1971 to 17 Feb 2020), the Cumulative Index to Nursing and Allied Health Literature (1982 to 18 Feb 2020). We searched clinical trials databases, conference proceedings, and the reference lists of retrieved articles for randomised controlled trials (RCTs) and quasi-RCTs.

Selection criteria

We included RCTs and quasi-RCTs comparing probiotic supplementation with placebo or no probiotics in very preterm or very low birth weight infants.

Data collection and analysis

We used the standard methods of Cochrane Neonatal. Two review authors separately evaluated trial quality, extracted data, and synthesised effect estimates using risk ratio (RR), risk difference (RD), and mean difference. We used the GRADE approach to assess the certainty of evidence for effects on NEC, all-cause mortality, late-onset infection, and severe neurodevelopmental impairment.

Main results

We included 56 trials in which 10,812 infants participated. Most trials were small (median sample size 149). Lack of clarity on methods to conceal allocation and mask caregivers or investigators were the main potential sources of bias in about half of the trials. Trials varied by the formulation of the probiotics. The most commonly used preparations contained *Bifidobacterium spp., Lactobacillus spp., Saccharomyces spp., and Streptococcus spp*. alone or in combinations.

Meta-analysis showed that probiotics may reduce the risk of NEC: RR 0.54, 95% CI 0.45 to 0.65 (54 trials, 10,604 infants; I^2 = 17%); RD -0.03, 95% CI -0.04 to -0.02; number needed to treat for an additional beneficial outcome (NNTB) 33, 95% CI 25 to 50. Evidence was assessed as low certainty because of the limitations in trials design, and the presence of funnel plot asymmetry consistent with publication bias. Sensitivity meta-analysis of trials at low risk of bias showed a reduced risk of NEC: RR 0.70, 95% CI 0.55 to 0.89 (16 trials, 4597 infants; I^2 = 25%); RD -0.02, 95% CI -0.03 to -0.01; NNTB 50, 95% CI 33 to 100. Meta-analyses showed that probiotics probably reduce mortality (RR 0.76, 95% CI 0.65 to 0.89; (51 trials, 10,170 infants; I^2 = 0%); RD -0.02, 95% CI -0.02 to -0.01; NNTB 50, 95% CI 50 to 100), and late-onset invasive infection (RR 0.89, 95% CI 0.82 to 0.97; (47 trials, 9762 infants; I^2 = 19%); RD -0.02, 95% CI -0.03 to -0.01; NNTB 50, 95% CI 33 to 100). Evidence was assessed as moderate certainty for both these outcomes because of the limitations in trials design. Sensitivity meta-analyses of 16 trials (4597 infants) at low risk of bias did not show an effect on mortality or infection. Meta-analysis showed that probiotics may have little or no effect on severe neurodevelopmental impairment (RR 1.03, 95% CI 0.84 to 1.26 (five trials, 1518 infants; I^2 = 0%). The certainty on this evidence is low because of limitations in trials design and serious imprecision of effect estimate. Few data (from seven of the trials) were available for extremely preterm or extremely low birth weight infants. Meta-analyses did not show effects on NEC, death, or infection (low-certainty evidence).

Authors' conclusions

Given the low to moderate level of certainty about the effects of probiotic supplements on the risk of NEC and associated morbidity and mortality for very preterm or very low birth weight infants, and particularly for extremely preterm or extremely low birth weight infants, further, large, high-quality trials are needed to provide evidence of sufficient quality and applicability to inform policy and practice.

PLAIN LANGUAGE SUMMARY

Probiotics for prevention of necrotising enterocolitis in very preterm or very low birthweight infants

Review question
Does giving very preterm or very low birth weight infants probiotics prevent necrotising enterocolitis?

Background
Very preterm infants (born more than eight weeks' early) and very low birth weight (less than 1.5 kg) are at risk of developing a severe bowel disorder, where a portion of the bowel becomes inflamed, infected, and dies, called necrotising enterocolitis. This condition is associated with death, serious infection, and long-term disability and developmental problems. One way to help prevent necrotising enterocolitis and associated conditions may be to add probiotics (dietary supplements containing potentially beneficial bacteria or yeasts) to milk feeds.

Study characteristics
The search is up to date as of 18 February 2020. We found 56 trials, with, in total, more than 10,000 infant participants. Trials were mostly small, and some had design flaws that might bias their findings.

Key results
Combined analyses showed that giving very preterm and very low birth weight infants probiotics may reduce the risk of necrotising enterocolitis, and probably reduces the risk of death and serious infection. There is no evidence of an effect on disability or developmental outcomes. Few trials provided data for extremely preterm infants (born more than 12 weeks' early) and extremely low birth weight (less than 1.0 kg), and these analyses did not show effects on necrotising enterocolitis, death and serious infection.

Certainty of evidence
The evidence for an effect on necrotising enterocolitis is "low-certainty" because of concerns that the effect could have been biased by small trials with unreliable methods.

SUMMARY OF FINDINGS

Summary of findings 1. Probiotics compared to control in very preterm or very low birth weight infants

Probiotics compared to control in very preterm or very low birth weight infants

Patient or population: very preterm or very low birth weight infants
Setting: neonatal care centres globally
Intervention: probiotics
Comparison: control

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Sensitivity analysis of trials at low risk of bias
	Risk with control	Risk with Probiotics				
Necrotising enterocolitis (before hospital discharge)	Study population		RR 0.54 (0.45 to 0.65)	10,604 (54 studies)	⊕⊕⊝⊝ Low[a,b]	Sensitivity meta-analysis of 16 trials (4597 infants) at low risk of bias showed a reduced risk of NEC: RR 0.70, 95% CI 0.55, 0.89 (I^2 = 25%)
	61 per 1000	33 per 1000 (27 to 40)				
Mortality (all-cause before hospital discharge)	Study population		RR 0.76 (0.65 to 0.89)	10,170 (51 studies)	⊕⊕⊕⊝ Moderate[a]	Sensitivity meta-analysis of 16 trials (4597 infants) at low risk of bias did not show an effect: RR 0.86, 95% CI 0.69, 1.07 (I^2 = 0%)
	65 per 1000	49 per 1000 (42 to 58)				
Invasive infection (before hospital discharge)	Study population		RR 0.89 (0.82 to 0.97)	9762 (47 studies)	⊕⊕⊕⊝ Moderate[a]	Sensitivity meta-analysis of 16 trials (4597 infants) at low risk of bias did not show an effect: RR 0.90, 95% CI 0.79, 1.02 (I^2 = 8%)
	173 per 1000	154 per 1000 (142 to 168)				
Severe neurodevelopmental impairment (18 months to 3 years)	Study population		RR 1.03 (0.84 to 1.26)	1518 (5 studies)	⊕⊕⊝⊝ Low[a,c]	Sensitivity meta-analysis of two trials (913 infants) at low risk of bias did not show an effect: RR 0.99, 95% CI 0.76, 1.27 (I^2 = 0%)
	194 per 1000	200 per 1000 (163 to 245)				

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio.

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.

Moderate certainty: we are moderately confident in the effect estimate; the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited; the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate; the true effect is likely to be substantially different from the estimate of effect.

[a]Downgraded one level for serious study limitations (high risk of bias due to uncertainty about methods used to generate random sequence, conceal allocation, and mask outcome assessment) in 12 trials
[b]Downgraded one level for serious publication bias (funnel plot asymmetry and statistical evidence consistent with trial size; trials favouring controls missing)
[c]Downgraded one level for serious imprecision of effect estimate (95% CI around estimate consistent with substantial harm or benefit)

Summary of findings 2. Probiotics compared to control in extremely preterm or extremely low birth weight infants

Probiotics compared to control in extremely preterm or extremely low birth weight infants

Patient or population: extremely preterm or extremely low birth weight infants
Setting: neonatal care centres globally
Intervention: probiotics
Comparison: control

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)
	Risk with control (extremely preterm or ELBW)	Risk with Probiotics			
Necrotising enterocolitis (before hospital discharge)	Study population		RR 0.90 (0.68 to 1.21)	1712 (8 studies)	⊕⊕⊝⊝ Low[a,b]
	100 per 1000	90 per 1000 (68 to 121)			
Mortality (before hospital discharge)	Study population		RR 0.91 (0.71 to 1.16)	1661 (6 studies)	⊕⊕⊝⊝ Low[a,b]
	137 per 1000	124 per 1000 (97 to 159)			
Invasive infection (before hospital discharge)	Study population		RR 0.90 (0.76 to 1.06)	1471 (6 studies)	⊕⊕⊝⊝ Low[a,b]
	282 per 1000	254 per 1000 (214 to 299)			

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio.

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate; the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited; the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate; the true effect is likely to be substantially different from the estimate of effect.

[a]Downgraded one level for serious study limitations due to high risk of bias (uncertainty about methods used to generate random sequence, conceal allocation, and mask assessments) in many trials

[b]Downgraded one level for serious imprecision of effect estimate (95% CI around estimate consistent with substantial harm or benefit)

BACKGROUND

The intestinal microbiome may play an important role in the pathogenesis of necrotising enterocolitis (NEC) (Embleton 2017). Probiotics are microorganisms that benefit the host by modulating the intestinal microbiome and promoting mucosal barrier functions and resistance to pathogens. Dietary supplementation with probiotics has been proposed as a strategy to reduce the risk of NEC and associated morbidity and mortality in very preterm or very low birth weight infants (VLBW) infants.

Description of the condition

Necrotising enterocolitis, a syndrome of acute intestinal necrosis of unknown aetiology, affects about 5% of very preterm or VLBW infants (Horbar 2012). The major risk predictors for NEC include being extremely preterm or extremely low birth weight (ELBW), and having evidence of intrauterine growth restriction or absent or reversed end-diastolic flow velocities in Doppler studies of the foetal aorta or umbilical artery (Samuels 2017). Infants who develop NEC experience more infections, have lower levels of nutrient intake, grow more slowly, and have longer durations of intensive care and hospital stay than gestation-comparable infants who do not (Battersby 2018; Berrington 2012). The associated mortality rate is about 20%, and infants who develop NEC, especially if associated with bloodstream infections, have a higher risk of neurodevelopmental problems and disability compared with their peers (Hickey 2018; Martin 2010).

The pathogenesis of NEC remains incompletely understood but is thought to involve intestinal dysbiosis, infection and inflammation (Eaton 2017; Mara 2018; Morgan 2011). Emerging evidence supports the theory that the intestinal microbiome affects the risk of developing NEC (Masi 2019; Olm 2019; Stewart 2012; Warner 2016). Most very preterm or VLBW infants who develop NEC have received enteral milk feeds. Feeding with human milk rather than cow's milk formula reduces the risk of NEC (Quigley M 2019). One putative mechanism for this protective effect is that “prebiotic” substances in human milk promote the growth of non-pathogenic probiotic microorganisms, predominantly lactobacilli and bifidobacteria, that modulate the intestinal microbiome and promote mucosal barrier functions (Embleton 2017; Granger 2020; Walsh 2019). Compared with human milk-fed term infants, however, very preterm or VLBW infants typically harbour fewer probiotic microorganisms and more potential pathogens such as enterococci and Enterobacteriaceae, which might be due to dysbiotic effects of enteral fasting and antibiotic exposure (Stewart 2017).

Given the putative role of probiotics in maintaining the structure, integrity, and function of the intestinal barrier, the possibility that supplemental probiotics might be effective in preventing NEC is of considerable research interest (Berrington 2019; Patel 2018).

Description of the intervention

The probiotic preparations used most commonly as enteral supplements contain one or more strains of bacteria (typically bifidobacteria or lactobacilli) or the fungus *Saccharomyces boulardii* (Thomas 2010). Other bacteria with probiotic properties include *Bacillus clausii, Enterococcus faecium,* and *Streptococcus thermophilus*. Exogenous probiotics can colonise the mucosal surface of the human gastrointestinal tract (Abdulkadir 2016; Zmora 2018). A range of probiotic supplements, as single- or multiple-strain preparations, are available commercially and have been used to prevent and treat infectious or inflammatory gastrointestinal conditions in adults. Despite biological plausibility and underpinning pre-clinical studies, however, evidence for benefit remains low certainty for most conditions (Bron 2017; Koretz 2018; Kunk 2019; Lerner 2019; Suez 2019). Furthermore, serious, unexpected adverse events and outcomes have been associated with probiotic supplementation for critically-ill adults (Besselink 2008; Boyle 2006).

Probiotics for very preterm infants

Policies and practices for the use of probiotic supplements to prevent NEC in very preterm or VLBW infants vary within and between countries (Duffield 2019; Viswanathan 2016). Parents have expressed willingness to consider use of probiotics for their very preterm or VLBW infants if evidence of benefit and safety exists (Sesham 2014). Enteral administration of commercially-available supplements of lyophilised probiotic microorganisms, usually multi-species preparations containing lactobacilli or bifidobacteria or both, is established in some settings (Robertson 2020). Routine use outwith trials, however, remains limited because of uncertainty about the optimal constitution of preparations (strains of microorganisms and dosing strategies), quality control and safety issues including contamination of products with potential pathogens, and national licensing processes and regulatory requirements (Berrington 2019; Fleming 2019; Pell 2019; van den Akker 2020; Vermeulen 2020). Although probiotic supplementation in immuno-competent adults is considered to be safe, exogenous probiotic microorganisms have been reported as causing bacteraemia or fungaemia in very preterm or VLBW infants (Bertelli 2015; Esaiassen 2016; Jenke 2012; Zbinden 2015).

How the intervention might work

Intestinal probiotic microorganisms are thought to exert their beneficial effects via several mechanisms. Probiotics may out-compete pathogens for nutrients and limit pathogen growth via production of inhibitory organic acids ("post-biotics") and antimicrobial compounds (Embleton 2017; Patel 2015). Infants supplemented with probiotics harbour fewer potential pathogens in the intestine (Alcon-Giner 2020). Other putative actions include stimulating differentiation and proliferation of enterocytes, enhancing expression of intestinal digestive enzymes, and improving intestinal mucosal barrier integrity (Bron 2017; Johnson-Henry 2016; Sanders 2019).

Why it is important to do this review

Necrotising enterocolitis and associated complications, particularly infections, are the commonest causes of mortality and serious morbidity beyond the early neonatal period in very preterm or VLBW infants (Berrington 2012). Since probiotic supplementation might reduce the risk of NEC, appraising and synthesising the trial evidence about the effectiveness and safety of probiotic supplementation could inform practice, policy, and research (Embleton 2016; Quigley E 2019). Currently, international policy statements that exist to guide practice do not make unconditional recommendations for use of any probiotic combination for very preterm or VLBW infants (Marchand 2012; van den Akker 2020).

OBJECTIVES

To determine the effect of supplemental probiotics on the risk of necrotising enterocolitis (NEC) and mortality and morbidity in very preterm or very low birth weight (VLBW) infants.

METHODS

Criteria for considering studies for this review

Types of studies

We included randomised controlled trials (RCTs) and quasi-RCTs.

Types of participants

We included very preterm (< 32 weeks' gestation) or extremely low birth weight (VLBW)(< 1500 g) infants (pre-specified analyses for extremely preterm (< 28 weeks' gestation) or extremely low birth weight (ELBW) (< 1000 g) infants).

Types of interventions

We included enteral administration of any probiotic or probiotic combination for at least one week compared to placebo or no treatment.

We categorised probiotic preparations at the genus level (*Bifidobacterium spp., Lactobacillus spp., Sacchromyces spp., Streptococcal spp.,* others, and combinations thereof).

Types of outcome measures

Primary outcomes

- Necrotising enterocolitis (NEC), confirmed at surgery or autopsy or diagnosed by at least two of the following clinical features (Walsh 1986):
  * abdominal radiograph showing pneumatosis intestinalis or gas in the portal venous system or free air in the abdomen;
  * abdominal distension with abdominal radiograph with gaseous distension or frothy appearance of bowel lumen (or both);
  * blood in stool;
  * lethargy, hypotonia or apnoea (or combination of these).
- All-cause mortality before discharge from hospital.

Secondary outcomes

- Late-onset invasive infection, as determined by culture of bacteria or fungus from blood or cerebrospinal fluid or from a normally sterile body space (> 48 hours after birth).
- Late-onset infection with the supplemented probiotic microorganism.
- Duration of hospitalisation (days).
- Neurodevelopmental impairment assessed by a validated test after 12 months' post-term: neurological evaluations, developmental scores, and classifications of disability, including cerebral palsy and auditory and visual impairment.

Search methods for identification of studies

We used the criteria and standard methods of Cochrane Neonatal.

Electronic searches

We used the standard search strategy of Cochrane Neonatal to search Cochrane Central Register of Controlled Trials (CENTRAL; 2020, Issue 2) in the Cochrane Library; MEDLINE Ovid (1946 to 17 Feb 2020), Embase Ovid (1974 to 17 Feb 2020), Maternity & Infant Care Database Ovid (1971 to 17 Feb 2020), the Cumulative Index to Nursing and Allied Health Literature (1982 to 18 Feb 2020), and clinical trials databases, and conference proceedings (see Appendix 1 for the full search strategies for each database). We searched clinical trials registries for ongoing or recently completed trials (clinicaltrials.gov; the World Health Organization's International Trials Registry and Platform, and the ISRCTN Registry).

Searching other resources

We searched the reference lists of any articles selected for inclusion in this review.

Data collection and analysis

We used the standard methods of Cochrane Neonatal.

Selection of studies

One review author (SS) screened titles and abstracts of all records identified by the search and coded records as "order" or "exclude". A second review author (WM) assessed all records coded as "order" and made the final decision about which records were ordered as full-text articles. SS and SO read the full texts and used a checklist to assess each article's eligibility for inclusion on the basis of pre-specified inclusion and exclusion criteria. WM checked these decisions.

Data extraction and management

Two review authors (SS and WM or SO) extracted data independently using a data collection form to aid extraction of information on design, methods, participants, interventions, outcomes, and treatment effects from each included study. We discussed disagreements until we reached consensus. If data from the trial reports were insufficient, we contacted trialists for further information.

Assessment of risk of bias in included studies

Two review authors (SS and WM or SO), independently assessed the risk of bias (low, high, or unclear) of all included trials using the Cochrane 'Risk of bias' tool (Higgins 2011) for the following domains.

- Sequence generation (selection bias).
- allocation concealment (selection bias).
- blinding of participants and personnel (performance bias).
- blinding of outcome assessment (detection bias).
- incomplete outcome data (attrition bias).

We resolved any disagreements by discussion or by a third assessor. See Appendix 2 for a description of risk of bias for each domain.

Measures of treatment effect

We analysed treatment effects in the individual trials using Review Manager 5 (Review Manager 2020), and reported risk ratios (RRs) and risk differences (RDs) for dichotomous data, and mean differences (MDs) for continuous data, with respective 95%

confidence intervals (CIs). We determined the number needed to treat for one additional beneficial outcome (NNTB) for analyses with a statistically significant difference in the RD.

Unit of analysis issues

The unit of analysis was the participating infant in individually-randomised trials. For cluster-randomised trials, we undertook analyses at the level of the individual while accounting for inter-cluster correlations in the data using methods recommended in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2019). Cross-over studies were not eligible for inclusion.

Dealing with missing data

We requested additional data from trial investigators when data on important outcomes were missing or were reported unclearly. If unavailable, we planned to undertake sensitivity analyses to assess the potential impact of missing outcome data.

Assessment of heterogeneity

We examined treatment effects in individual trials and heterogeneity between trial results by inspecting the forest plots if more than one trial was included in a meta-analysis. We calculated the I^2 statistic for each analysis to quantify inconsistency across studies and to describe the percentage of variability in effect estimates that may be due to heterogeneity rather than to sampling error. If we detected moderate (I^2 > 50%) or high (I^2 > 75%) heterogeneity, we planned to explore possible causes (differences in study design, participants, interventions, or outcome assessments).

Assessment of reporting biases

We assessed funnel plot asymmetry visually and with Harbord's modification of Egger's test in meta-analyses with data from more than nine trials contributing events (Harbord 2006).

Data synthesis

We used a fixed-effect model for meta-analysis (as per Cochrane Neonatal recommendations). When moderate or high heterogeneity existed, we planned to examine the potential causes in subgroup (see below) and sensitivity (by methodological quality) analyses.

Subgroup analysis and investigation of heterogeneity

We planned to undertake subgroup analyses by:

- genus of probiotics or combinations (*Bifidobacterium spp., Lactobacillus spp., Sacchromyces spp., Streptococcal spp.,* others, and combinations thereof);
- type of enteral feeding permitted for participating infants (human milk versus formula versus mixed).

Sensitivity analysis

We planned sensitivity analyses to determine how estimates were affected by including only studies at low risk of bias: (i) selection bias (adequate randomisation and allocation concealment), (ii) detection or performance bias (adequate masking of intervention and measurement), (iii) attrition bias (< 20% loss to follow-up for primary outcome assessment), and (iv) reporting bias (selective reporting).

Summary of findings and assessment of the certainty of the evidence

We used the GRADE approach, as outlined in the GRADE Handbook (Schünemann 2013), to assess the certainty of evidence of the following (clinically relevant) outcomes: NEC, all-cause mortality, late-onset infection, and severe neurodevelopmental impairment.

Three review authors (WM, MXRR and SO) independently assessed the certainty of the evidence for each of the outcomes above. We considered evidence from RCTs as high certainty but downgraded the evidence one level for serious (or two levels for very serious) limitations based upon the following: design (risk of bias), consistency across studies, directness of the evidence, precision of estimates, and presence of publication bias. We used the GRADEpro GDT Guideline Development Tool to create two 'Summary of findings' tables to report the certainty of the evidence.

The GRADE approach results in an assessment of the certainty of a body of evidence as one of four grades.

- High certainty: further research is very unlikely to change our confidence in the estimate of effect.
- Moderate certainty: further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
- Low certainty: further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
- Very low certainty: we are very uncertain about the estimate.

RESULTS

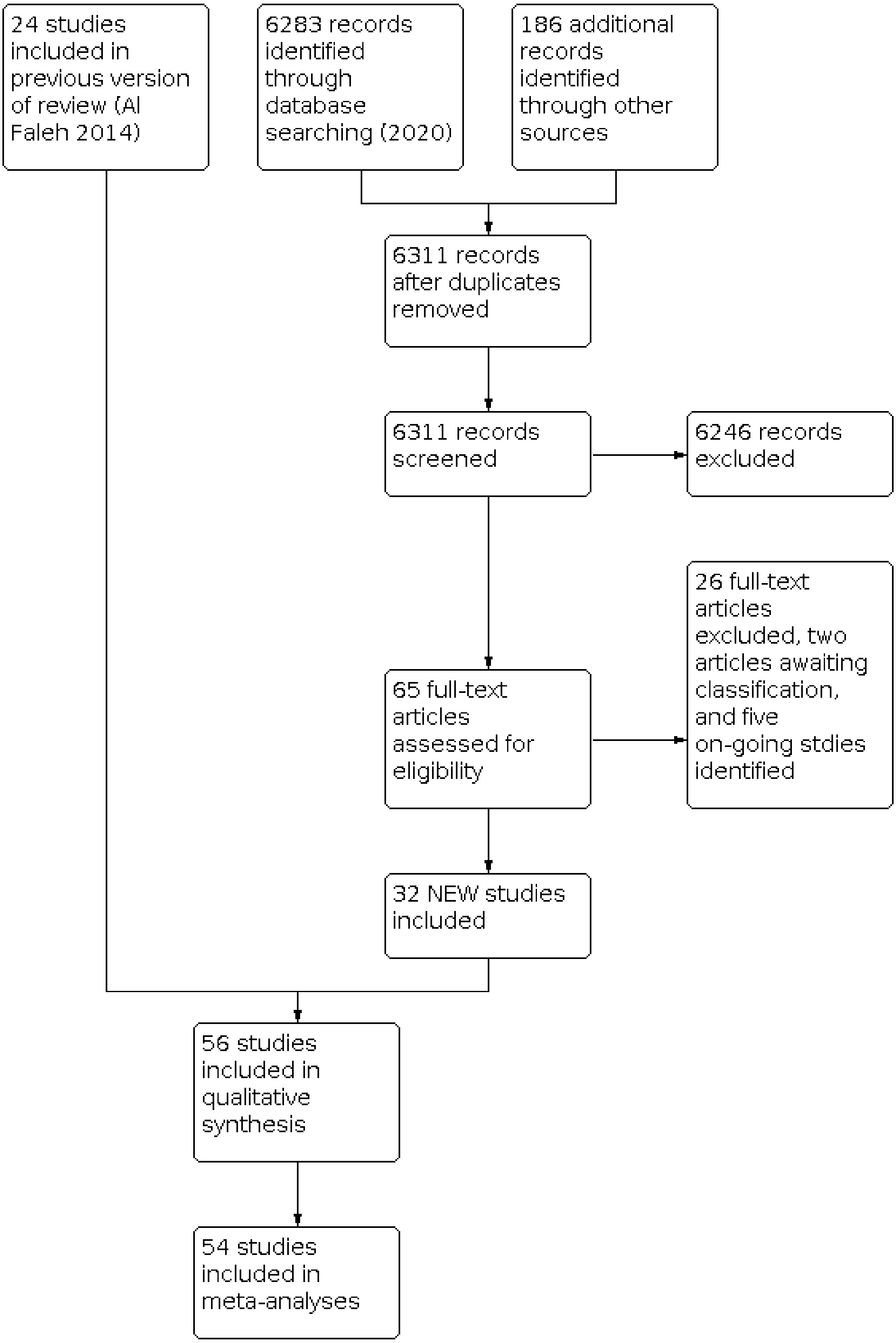
Description of studies

See: Characteristics of included studies; Characteristics of excluded studies; Characteristics of studies awaiting classification; Characteristics of ongoing studies

Results of the search

See Figure 1.

Figure 1. Study flow diagram: review update 2020



Included studies

See: Characteristics of included studies.

We included 56 trials. Most were conducted during the past 20 years (four trials pre-2000). Geographical spread was wide, though predominantly in Europe (23 trials) and Asia (23 trials). Only one trial took place in sub-Saharan Africa (Zeber-Lubecka 2016).

Most trials occurred in single centres. Nine were multicentre (Al-Hosni 2012; Costeloe 2015; Dani 2002; Dilli 2015; Hays 2015; Jacobs 2013; Lin 2008; Manzoni 2009; Totsu 2014).

In all but one of the trials, individual infants were allocated randomly to intervention or control groups. One trial, based in 19 neonatal units in Japan, used a cluster design, with the unit of randomisation being the neonatal unit (Totsu 2014).

Population

In total, 10,812 infants participated in the 56 included trials. The median number of participants in the trials was 149. Twenty-one trials enrolled fewer than 100 participants. Twenty trials enrolled between 100 and 199 participants. Twelve trials enrolled between 200 and 499 participants. Three trials enrolled 500 participants or more: Costeloe 2015 (N = 1310); Dani 2002 (N = 585); Jacobs 2013 (N = 1099).

Most trials enrolled only very preterm or VLBW infants, with average birth weight among participants typically 1000 g to 1200 g, and average gestation at birth 28 to 32 weeks'. Eight trials enrolled infants of gestational age up to 34 weeks', or birth weight up to 1800 g (Chandrashekar 2018; Dashti 2014; Fujii 2006; Hernandez-Enriquez 2016; Mohan 2006; Ren 2010; Strus 2018; Tewari 2015). Because the average gestation at birth was < 32 weeks', or the average birth weight < 1500 g, we included these trials.

Two trials restricted participation to extremely low birth weight (ELBW) infants (Al-Hosni 2012; Wejryd 2019). Four trials excluded infants who were born with birth weight below the 10th percentile for the reference population ("small-for-gestation") (Al-Hosni 2012; Hays 2015; Indrio 2017; Kitajima 1997). None of the trials specified exclusion of infants who had evidence of absent or reversed end-diastolic flow velocities detected on antenatal Doppler studies of the foetal aorta or umbilical artery.

In most trials, participating infants were permitted human milk or formula feeding. Seven trials enrolled infants who received human milk only (Roy 2014; Samanta 2009; Shadkam 2015; Shashidhar 2017; Tewari 2015; Van Niekerk 2014; Wejryd 2019), and five trials enrolled only formula-fed participants (Costalos 2003; Chrzanowska-Liszewska 2012; Indrio 2017; Reuman 1986; Stratiki 2007).

Interventions and comparisons

The probiotic preparations tested varied. Thirty-three trials used single-genus probiotics (most commonly, *Bifidobacterium spp.* or *Lactobacillus spp.*), and 23 used multi-genus combinations (most commonly, *Bifidobacterium spp.* plus *Lactobacillus spp.*). These were mostly commercially-available products supplied by the manufacturer for use in the trial.

- *Bifidobacterium spp.* (14 trials):
  - *B. breve* (Costeloe 2015; Fujii 2006; Hikaru 2010; Kitajima 1997; Li 2019; Patole 2014; Wang 2007);
  - *B. lactis* (Dilli 2015; Mihatsch 2010; Mohan 2006; Stratiki 2007);
  - *B. bifidum* (Totsu 2014);
  - *B. adolescentis* (Huang 2009);
  - B. *lactis*, or *B. longum*, or both (three intervention groups) (Hays 2015).
- *Lactobacillus spp.* 13 trials):
  - *L. rhamnosus* (Agarwal 2003; Chrzanowska-Liszewska 2012; Dani 2002; Manzoni 2006; Manzoni 2009; Millar 1993);
  - *L. reuteri* (Oncel 2014);
  - *L. acidophilus* (Reuman 1986).
- *Sacchromyces spp.* (four trials):
  - *Sacchromyces boulardii* (Costalos 2003; Demirel 2013; Serce 2013; Zeber-Lubecka 2016).
- *Bacillus spp.* (two trials):
  - *Bacillus clausii* (Tewari 2015);
  - *Bacillus coagulans** (Sari 2011).

(**Lactobacillus sporogenes* in report.)

- *Bifidobacterium spp.* plus *Lactobacillus spp.* (eight trials):
  - *B. breve* and *L. casei* (Yakult®) (Braga 2011);
  - *B. bifidum*, *B. longum*, *B. infantis*, *L. rhamnosus*, *L. paracasei* , *L. casei*, *L. acidophilus*, and *L.latis* (Cap TS6®) (Chowdhury 2016);
  - *B. bifidum* and *L. acidophilus* (Infloran®) (Lin 2005; Lin 2008; Saengtawesin 2014);
  - *B. longum* and *L. rhamnosus* (Rougé 2009);
  - *B. longum, B. bifidum, B. lactis* and *L. acidophilus* (Roy 2014);
  - *B. longum, B. bifidum, B.infantis* and *L. acidophilus* (Samanta 2009).
- *Bifidobacterium spp.* plus *Streptococcus spp.* (two trials):
  - *B. infantis*, *B. lactis* and *S. thermophilus* (Jacobs 2013);
  - *B. infantis*, *B. bifidum*** and *S. thermophilus* (Bin-Nun 2005).

(** *Lactobacillus bifidus* in report)

- *Bifidobacterium spp.* plus *Lactobacillus spp.* plus *Sacchromyces spp.* (four trials):
  - *B. infantis, L. rhamnosus, L. casei, L. plantarum, L acidophilus,* and *S. boulardii* (Dutta 2015);
  - *B. bifidum, L acidophilus,* and *S. boulardii* (Hariharan 2016);
  - *B. longum, L.acidophilus, L. rhamnosus,* and *S. boulardii* (Chandrashekar 2018; Shashidhar 2017).
- *Bifidobacterium spp.* plus *Lactobacillus spp.* plus *Streptococcus spp.* (five trials):

- *B longum*, *B. breve, L. acidophilu*s, *L. rhamnosus*, *L. bulgaricus*, *L. case*i, and *S. thermophilus* (Dashti 2014);
- *B. infantis, L. rhamnosus, L. casei, L. plantarum, L acidophilus,* and *S. thermophilus* (Fernández-Carrocera 2013);
- *B. infantis, L acidophilus,* and *Enterococcus faecium* (Kanic 2015);
- B*. infantis, L. acidophilus, Enterococcus faecium,* and *Bacillus cereus* (Ren 2010);
- *Bifidobacterium spp.* (not specified)*, L. acidophilus, L. delbrueckii.* and *S. thermophilus* (Rehman 2018).

Most trials initiated probiotic (and placebo if used) administration during the first week after birth, typically with the first enteral feed. The lyophilised probiotics were reconstituted in water or milk, and administered to supply 10^8 to 10^9 colony forming units per dose, once or twice daily via a gastric feeding tube. In most trials, the intervention period was at least six weeks, typically until 34 to 36 weeks' postmenstrual age, or until discharge from hospital. Eleven of the trials administered the intervention for a shorter period (from seven to 30 days) (Braga 2011; Costalos 2003; Dutta 2015; Huang 2009; Kitajima 1997; Millar 1993; Mohan 2006; Ren 2010; Reuman 1986; Shadkam 2015; Van Niekerk 2014). One trial continued the intervention until the infant reached 2000 g body weight (Totsu 2014).

Outcomes

Fifty-four trials reported the number of infants who developed NEC, and 51 trials reported mortality prior to hospital discharge. Forty-seven trials reported (or provided unpublished data) the number of infants with at least one episode of culture-confirmed infection. Other in-hospital outcomes reported included time to establish full enteral feeding, rate of weight gain, and duration of hospital stay (22 trials). Six trials reported neurodevelopmental or cognitive outcomes (Jacobs 2013; Lin 2005; Oncel 2014; Sari 2011; Totsu 2014; Patole 2014). Two trials did not report any of the review outcomes (Agarwal 2003; Li 2019).

Excluded studies

We excluded 26 reports of studies (Characteristics of excluded studies). The most common reasons for exclusion were ineligible population (most participants not very preterm, or VLBW), intervention (prebiotics or synbiotics) and design (not randomised). A further four screened articles were secondary reports for included trials.

Risk of bias in included studies

Methodological quality varied between the included trials (Risk of bias in included studies; Figure 2).

Figure 2. 'Risk of bias' summary: review authors' judgements about each risk of bias item for each included study.

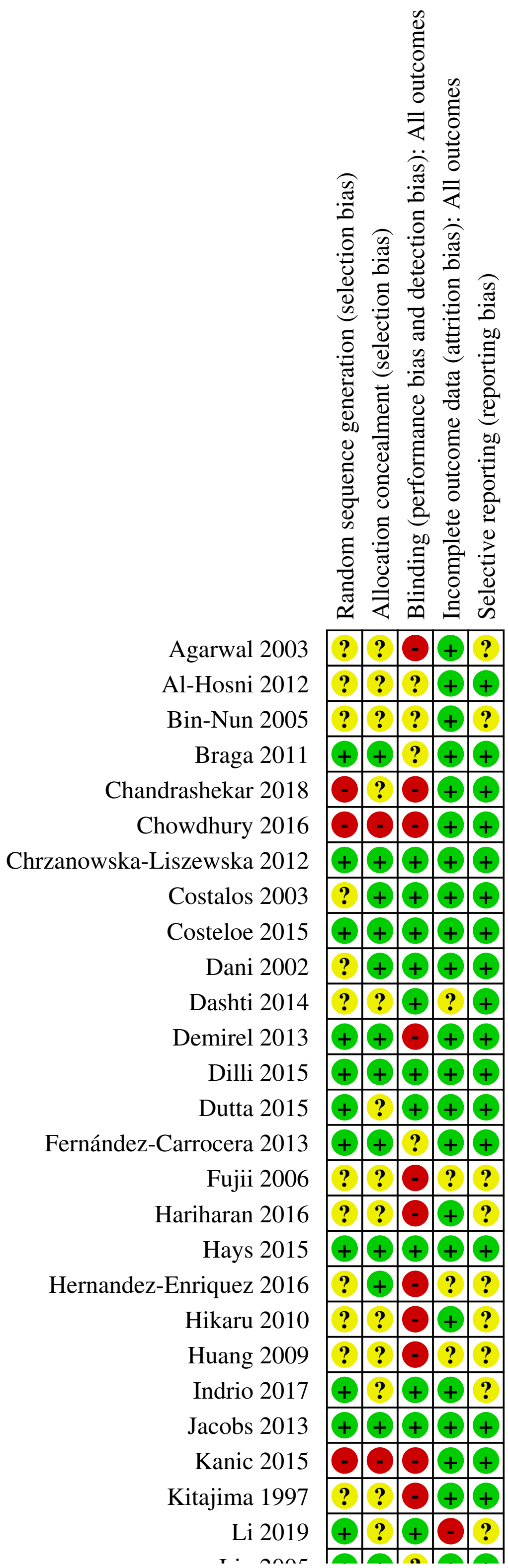
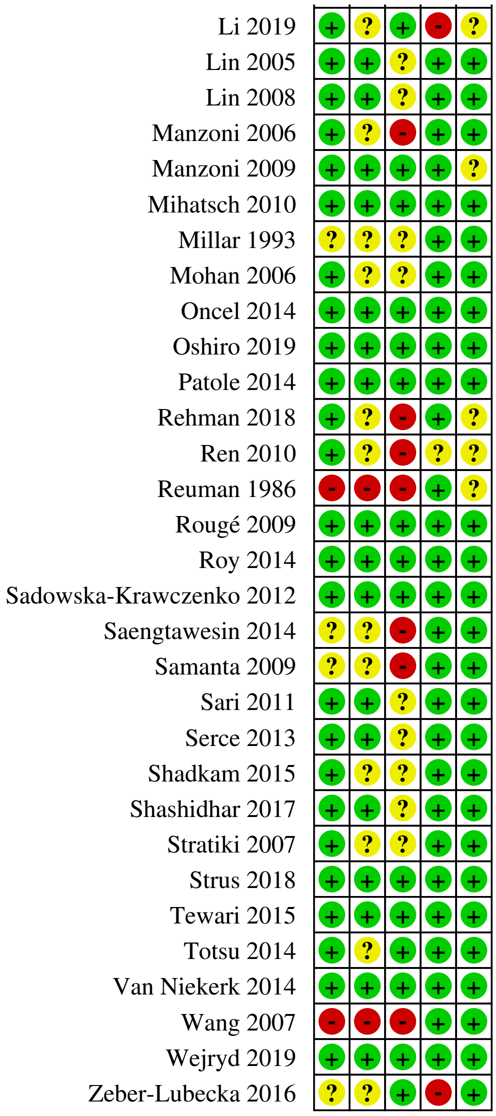


Figure 2. (Continued)



Allocation

Twenty-five of the 56 trials were assessed as being a low risk of selection bias. These employed adequate methods to generate the random sequence, typically computer-generated, and methods to conceal allocation, typically central or pharmacy allocation, or storage of allocation codes in sealed envelopes (we did not mandate that reports stated that envelopes were "opaque"). Randomisation and allocation concealment methods were not stated in 26 trial reports (unclear risk of bias), and in five "quasi-randomised" trials, alternate allocation was used (high risk of bias).

Blinding

Twenty-five trials were assessed as being a low risk of performance bias and detection bias. These were placebo-controlled (usually maltodextrin), or the report or investigators indicated that preparation of the intervention (mixing the probiotic in milk) was undertaken by staff who were not directly involved in other caregiving duties or outcome assessments (for example, pharmacy staff). In 13 trials, control infants received milk feeds without probiotic supplements, but it was unclear whether staff were aware of the group allocation (unclear risk of bias). Eighteen trials were at high risk of bias due to absence of any masking measures.

Incomplete outcome data

Attrition bias does not appear to be an issue in most trials (outcome data reported for > 80% of randomised cohorts).

Selective reporting

Most reports did not provide access to the trial protocol. It is unlikely, however, that reporting bias was an issue in most trials (low risk of bias) where the review primary and infant-important outcomes were reported. In trials where the aim was to assess surrogate outcomes such as stool colonisation or intestinal permeability, clinical outcome data were generally available from the investigators.

Effects of interventions

See: **Summary of findings 1** Probiotics compared to control in very preterm or very low birth weight infants; **Summary of findings 2** Probiotics compared to control in extremely preterm or extremely low birth weight infants

Comparison 1. Probiotics versus control

Primary outcomes

Necrotising enterocolitis

Meta-analysis of data from 54 trials (10,604 infants) showed a reduced risk of NEC (Analysis 1.1; Figure 3):

Figure 3. Forest plot of comparison: 1 Probiotics versus control, outcome: 1.1 Necrotising enterocolitis.

Study or Subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.1.1 Bifidobacterium spp.						
Costeloe 2015	61	650	66	660	20.0%	0.94 [0.67 , 1.31]
Dilli 2015	2	100	18	100	5.5%	0.11 [0.03 , 0.47]
Fujii 2006	0	11	0	8		Not estimable
Hays 2015	8	145	3	52	1.4%	0.96 [0.26 , 3.47]
Hikaru 2010	0	108	0	100		Not estimable
Huang 2009	0	95	3	88	1.1%	0.13 [0.01 , 2.53]
Kitajima 1997	0	45	0	46		Not estimable
Mihatsch 2010	2	91	4	89	1.2%	0.49 [0.09 , 2.60]
Mohan 2006	2	37	1	32	0.3%	1.73 [0.16 , 18.20]
Oshiro 2019	0	17	0	18		Not estimable
Patole 2014	0	77	1	76	0.5%	0.33 [0.01 , 7.95]
Stratiki 2007	0	41	3	36	1.1%	0.13 [0.01 , 2.36]
Totsu 2014	0	120	0	102		Not estimable
Wang 2007	0	22	0	22		Not estimable
Subtotal (95% CI)		**1559**		**1429**	**31.2%**	**0.72 [0.54 , 0.96]**
Total events:	75		99			
Heterogeneity: Chi² = 12.82, df = 7 (P = 0.08); I² = 45%						
Test for overall effect: Z = 2.27 (P = 0.02)						
1.1.2 Lactobacillus spp.						
Chrzanowska-Liszewska 2012	0	21	0	26		Not estimable
Dani 2002	4	295	8	290	2.5%	0.49 [0.15 , 1.61]
Hernandez-Enriquez 2016	1	24	5	20	1.7%	0.17 [0.02 , 1.31]
Indrio 2017	0	30	0	30		Not estimable
Manzoni 2006	1	39	2	41	0.6%	0.53 [0.05 , 5.57]
Manzoni 2009	0	238	5	247	1.7%	0.09 [0.01 , 1.70]
Millar 1993	0	10	0	10		Not estimable
Oncel 2014	8	200	10	200	3.1%	0.80 [0.32 , 1.99]
Reuman 1986	0	15	0	15		Not estimable
Sadowska-Krawczenko 2012	1	30	4	25	1.3%	0.21 [0.02 , 1.75]
Shadkam 2015	2	30	11	30	3.4%	0.18 [0.04 , 0.75]
Wejryd 2019	7	68	8	66	2.5%	0.85 [0.33 , 2.21]
Subtotal (95% CI)		**1000**		**1000**	**16.6%**	**0.45 [0.28 , 0.71]**
Total events:	24		53			
Heterogeneity: Chi² = 7.39, df = 7 (P = 0.39); I² = 5%						
Test for overall effect: Z = 3.44 (P = 0.0006)						
1.1.3 Sacchromyces spp.						
Costalos 2003	5	51	6	36	2.2%	0.59 [0.19 , 1.78]
Demirel 2013	6	135	7	136	2.1%	0.86 [0.30 , 2.50]
Serce 2013	7	104	7	104	2.1%	1.00 [0.36 , 2.75]
Zeber-Lubecka 2016	0	27	0	28		Not estimable
Subtotal (95% CI)		**317**		**304**	**6.4%**	**0.82 [0.44 , 1.50]**
Total events:	18		20			
Heterogeneity: Chi² = 0.50, df = 2 (P = 0.78); I² = 0%						
Test for overall effect: Z = 0.65 (P = 0.51)						
1.1.4 Bacillus spp.						
Sari 2011	6	110	10	111	3.0%	0.61 [0.23 , 1.61]
Tewari 2015	0	123	0	121		Not estimable
Subtotal (95% CI)		**233**		**232**	**3.0%**	**0.61 [0.23 , 1.61]**
Total events:	6		10			
Heterogeneity: Not applicable						
Test for overall effect: Z = 1.01 (P = 0.31)						

Risk Ratio
M-H, Fixed, 95% CI

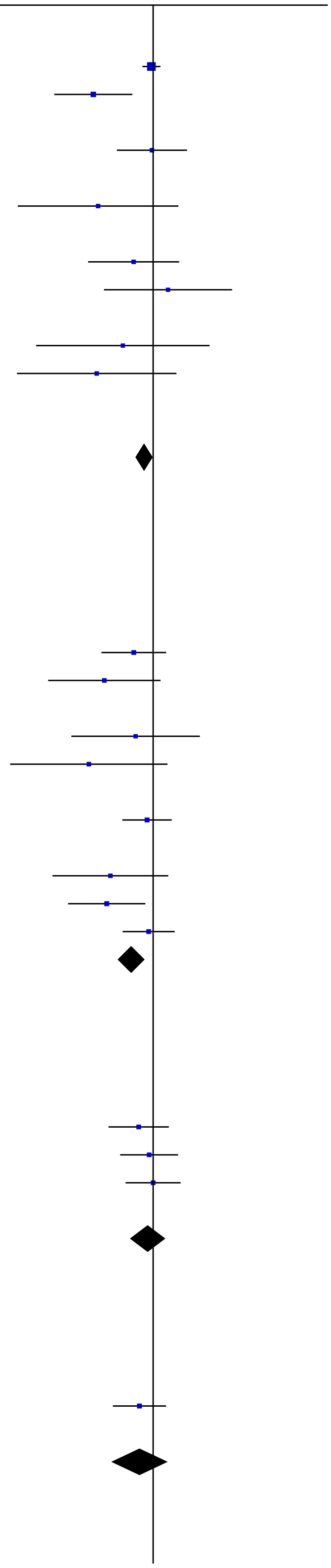
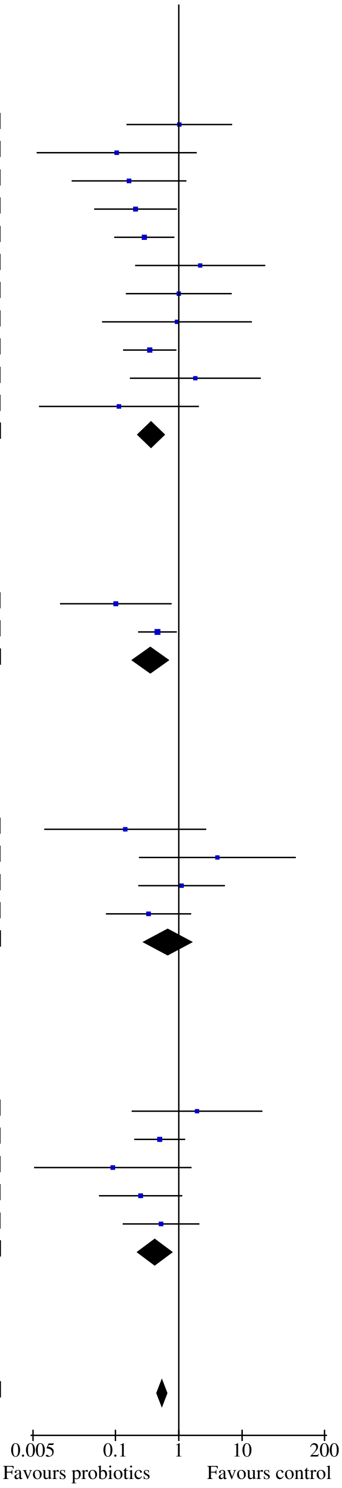


Figure 3. (Continued)

Heterogeneity: Not applicable
Test for overall effect: Z = 1.01 (P = 0.31)

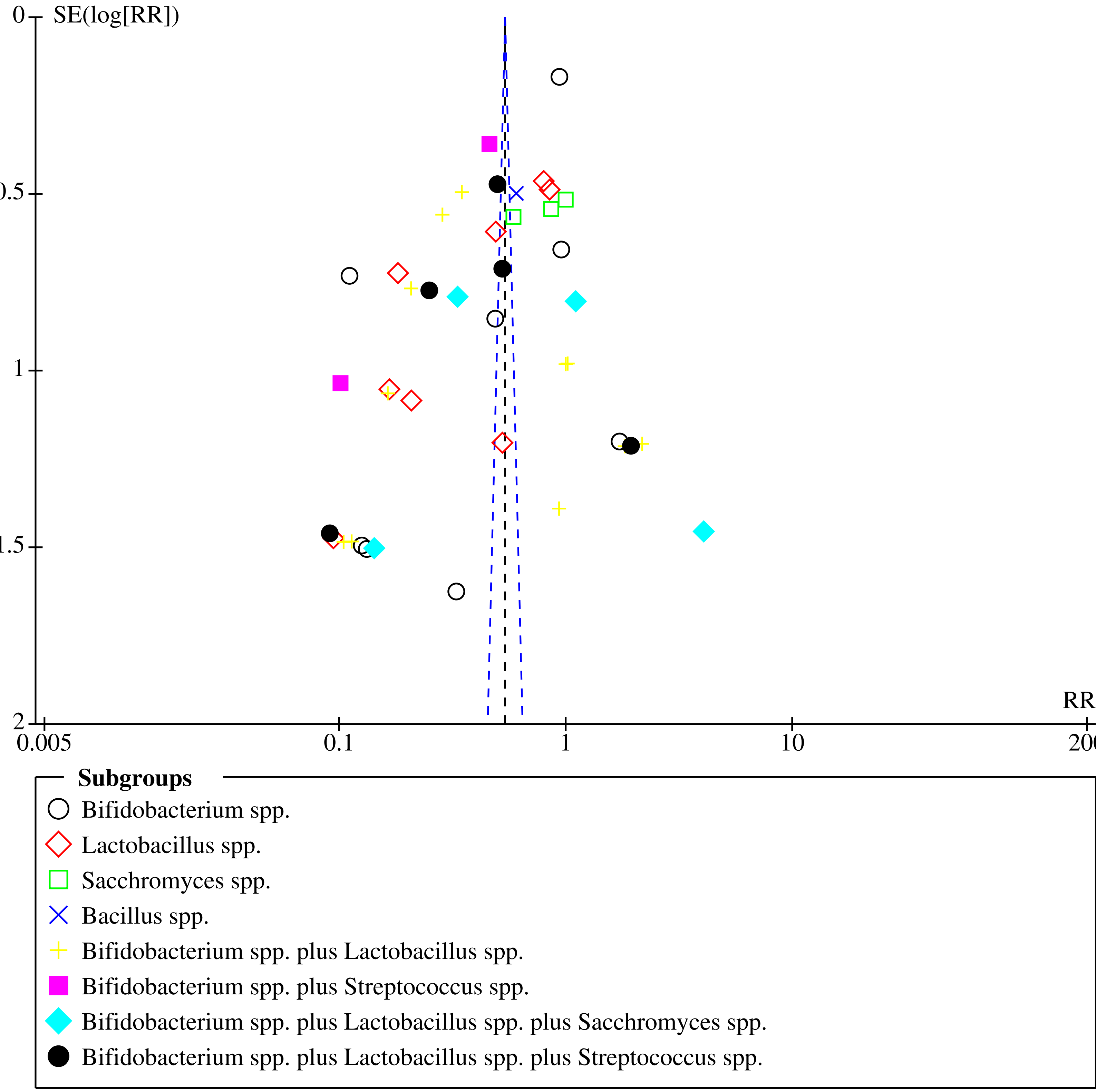
1.1.5 Bifidobacterium spp. plus Lactobacillus spp.						
Al-Hosni 2012	2	50	2	51	0.6%	1.02 [0.15 , 6.96]
Braga 2011	0	119	4	112	1.4%	0.10 [0.01 , 1.92]
Chowdhury 2016	1	60	6	59	1.9%	0.16 [0.02 , 1.32]
Lin 2005	2	180	10	187	3.0%	0.21 [0.05 , 0.94]
Lin 2008	4	217	14	217	4.3%	0.29 [0.10 , 0.85]
Rougé 2009	2	45	1	49	0.3%	2.18 [0.20 , 23.21]
Roy 2014	2	56	2	56	0.6%	1.00 [0.15 , 6.85]
Saengtawesin 2014	1	31	1	29	0.3%	0.94 [0.06 , 14.27]
Samanta 2009	5	91	15	95	4.5%	0.35 [0.13 , 0.92]
Strus 2018	2	80	1	73	0.3%	1.82 [0.17 , 19.71]
Van Niekerk 2014	0	91	4	93	1.4%	0.11 [0.01 , 2.08]
Subtotal (95% CI)		**1020**		**1021**	**18.6%**	**0.36 [0.23 , 0.59]**
Total events:	21		60			
Heterogeneity: Chi² = 9.19, df = 10 (P = 0.51); I² = 0%						
Test for overall effect: Z = 4.14 (P < 0.0001)						
1.1.6 Bifidobacterium spp. plus Streptococcus spp.						
Bin-Nun 2005	1	72	10	73	3.0%	0.10 [0.01 , 0.77]
Jacobs 2013	11	548	24	551	7.3%	0.46 [0.23 , 0.93]
Subtotal (95% CI)		**620**		**624**	**10.4%**	**0.36 [0.19 , 0.68]**
Total events:	12		34			
Heterogeneity: Chi² = 1.99, df = 1 (P = 0.16); I² = 50%						
Test for overall effect: Z = 3.12 (P = 0.002)						
1.1.7 Bifidobacterium spp. plus Lactobacillus spp. plus Sacchromyces spp.						
Chandrashekar 2018	0	70	3	70	1.1%	0.14 [0.01 , 2.72]
Dutta 2015	6	114	0	35	0.2%	4.07 [0.23 , 70.49]
Hariharan 2016	3	93	3	103	0.9%	1.11 [0.23 , 5.35]
Shashidhar 2017	2	49	6	49	1.8%	0.33 [0.07 , 1.57]
Subtotal (95% CI)		**326**		**257**	**4.0%**	**0.67 [0.28 , 1.58]**
Total events:	11		12			
Heterogeneity: Chi² = 3.76, df = 3 (P = 0.29); I² = 20%						
Test for overall effect: Z = 0.92 (P = 0.36)						
1.1.8 Bifidobacterium spp. plus Lactobacillus spp. plus Streptococcus spp.						
Dashti 2014	2	69	1	67	0.3%	1.94 [0.18 , 20.92]
Fernández-Carrocera 2013	6	75	12	75	3.7%	0.50 [0.20 , 1.26]
Kanic 2015	0	40	5	40	1.7%	0.09 [0.01 , 1.59]
Rehman 2018	2	73	8	73	2.4%	0.25 [0.05 , 1.14]
Ren 2010	3	80	5	70	1.6%	0.53 [0.13 , 2.12]
Subtotal (95% CI)		**337**		**325**	**9.7%**	**0.42 [0.22 , 0.77]**
Total events:	13		31			
Heterogeneity: Chi² = 3.39, df = 4 (P = 0.50); I² = 0%						
Test for overall effect: Z = 2.78 (P = 0.005)						
Total (95% CI)		**5412**		**5192**	**100.0%**	**0.54 [0.45 , 0.65]**
Total events:	180		319			
Heterogeneity: Chi² = 49.36, df = 41 (P = 0.17); I² = 17%						
Test for overall effect: Z = 6.80 (P < 0.00001)						
Test for subgroup differences: Chi² = 11.23, df = 7 (P = 0.13), I² = 37.7%						



- Risk ratio (RR) 0.54, 95% confidence interval (CI) 0.45 to 0.65 (I^2 = 17%);
- Risk difference (RD) -0.03, 95% CI -0.04 to -0.02;
- NNTB 33; 95% CI 25 to 50.

There was statistically significant evidence of funnel plot asymmetry consistent with trials favouring controls missing from the meta-analysis (Harbord's modified Egger test for bias -0.78, 95% CI -1.51 to -0.06; P = 0.04) (Figure 4).

Figure 4. Funnel plot of comparison: 1 Probiotics versus control, outcome: 1.1 Necrotising enterocolitis.



We assessed the certainty of evidence as "low" using GRADE approach, downgraded for serious study design limitations and serious risk of publication bias (Summary of findings 1).

Mortality

Meta-analysis of data from 51 trials (10,170 infants) showed a reduced risk of mortality (Analysis 1.2; Figure 5):

Figure 5. Forest plot of comparison: 1 Probiotics versus control, outcome: 1.2 Mortality.

Study or Subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.2.1 Bifidobacterium spp.						
Costeloe 2015	54	650	56	660	17.0%	0.98 [0.68 , 1.40]
Dilli 2015	3	100	12	100	3.7%	0.25 [0.07 , 0.86]
Fujii 2006	0	11	0	8		Not estimable
Hays 2015	3	145	1	52	0.4%	1.08 [0.11 , 10.11]
Hikaru 2010	0	108	4	100	1.4%	0.10 [0.01 , 1.89]
Kitajima 1997	0	45	2	46	0.8%	0.20 [0.01 , 4.14]
Mihatsch 2010	2	91	1	89	0.3%	1.96 [0.18 , 21.19]
Mohan 2006	0	37	0	32		Not estimable
Oshiro 2019	0	17	0	18		Not estimable
Patole 2014	0	77	0	76		Not estimable
Stratiki 2007	0	41	3	36	1.1%	0.13 [0.01 , 2.36]
Totsu 2014	2	120	0	102	0.2%	4.26 [0.21 , 87.65]
Subtotal (95% CI)		**1442**		**1319**	**24.9%**	**0.79 [0.58 , 1.09]**
Total events:	64		79			
Heterogeneity: Chi² = 10.68, df = 7 (P = 0.15); I² = 34%						
Test for overall effect: Z = 1.43 (P = 0.15)						
1.2.2 Lactobacillus spp.						
Chrzanowska-Liszewska 2012	0	21	0	26		Not estimable
Dani 2002	0	295	2	290	0.8%	0.20 [0.01 , 4.08]
Hernandez-Enriquez 2016	2	24	0	20	0.2%	4.20 [0.21 , 82.72]
Indrio 2017	0	30	0	30		Not estimable
Manzoni 2006	5	39	6	41	1.8%	0.88 [0.29 , 2.64]
Manzoni 2009	9	238	5	247	1.5%	1.87 [0.64 , 5.49]
Millar 1993	0	10	0	10		Not estimable
Oncel 2014	15	200	20	200	6.1%	0.75 [0.40 , 1.42]
Reuman 1986	1	15	3	15	0.9%	0.33 [0.04 , 2.85]
Sadowska-Krawczenko 2012	1	30	0	25	0.2%	2.52 [0.11 , 59.18]
Shadkam 2015	1	30	2	30	0.6%	0.50 [0.05 , 5.22]
Wejryd 2019	5	68	5	66	1.5%	0.97 [0.29 , 3.20]
Subtotal (95% CI)		**1000**		**1000**	**13.6%**	**0.91 [0.60 , 1.37]**
Total events:	39		43			
Heterogeneity: Chi² = 5.56, df = 8 (P = 0.70); I² = 0%						
Test for overall effect: Z = 0.46 (P = 0.65)						
1.2.3 Sacchromyces spp.						
Demirel 2013	5	135	5	136	1.5%	1.01 [0.30 , 3.40]
Serce 2013	5	104	4	104	1.2%	1.25 [0.35 , 4.52]
Zeber-Lubecka 2016	0	27	0	28		Not estimable
Subtotal (95% CI)		**266**		**268**	**2.7%**	**1.12 [0.46 , 2.70]**
Total events:	10		9			
Heterogeneity: Chi² = 0.06, df = 1 (P = 0.81); I² = 0%						
Test for overall effect: Z = 0.24 (P = 0.81)						
1.2.4 Bacillus spp.						
Sari 2011	3	110	3	111	0.9%	1.01 [0.21 , 4.89]
Tewari 2015	12	123	14	121	4.3%	0.84 [0.41 , 1.75]
Subtotal (95% CI)		**233**		**232**	**5.2%**	**0.87 [0.45 , 1.69]**
Total events:	15		17			
Heterogeneity: Chi² = 0.04, df = 1 (P = 0.84); I² = 0%						
Test for overall effect: Z = 0.41 (P = 0.69)						
1.2.5 Bifidobacterium spp. plus Lactobacillus spp.						
Al-Hosni 2012	3	50	4	51	1.2%	0.77 [0.18 , 3.25]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Risk Ratio
M-H, Fixed, 95% CI

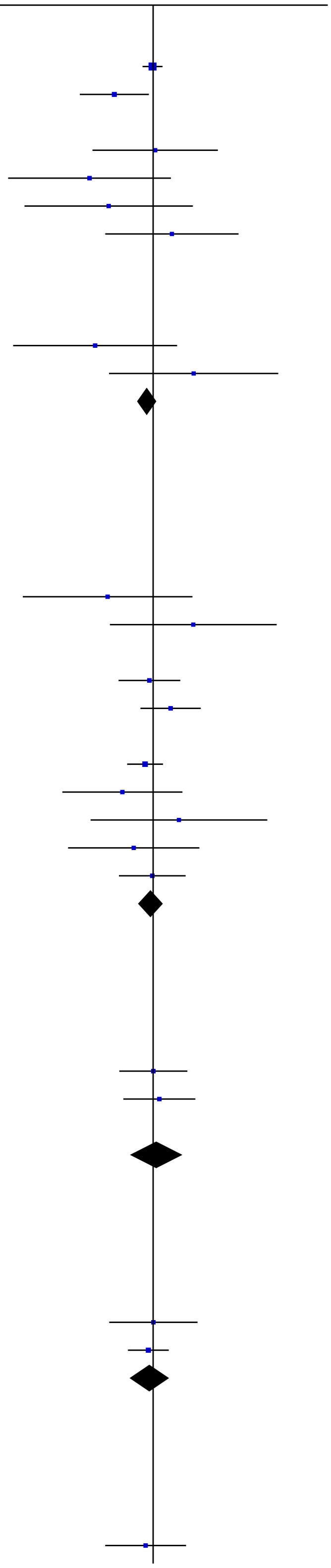
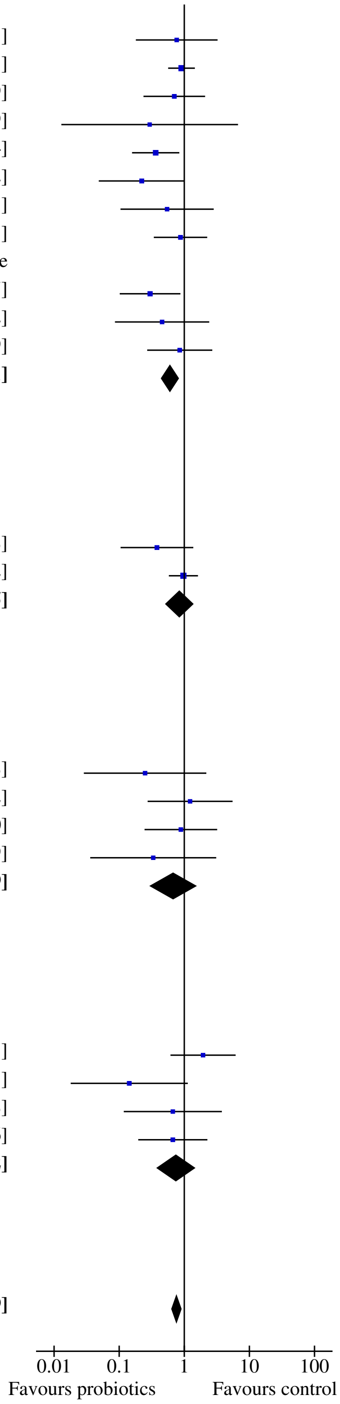


Figure 5. (Continued)

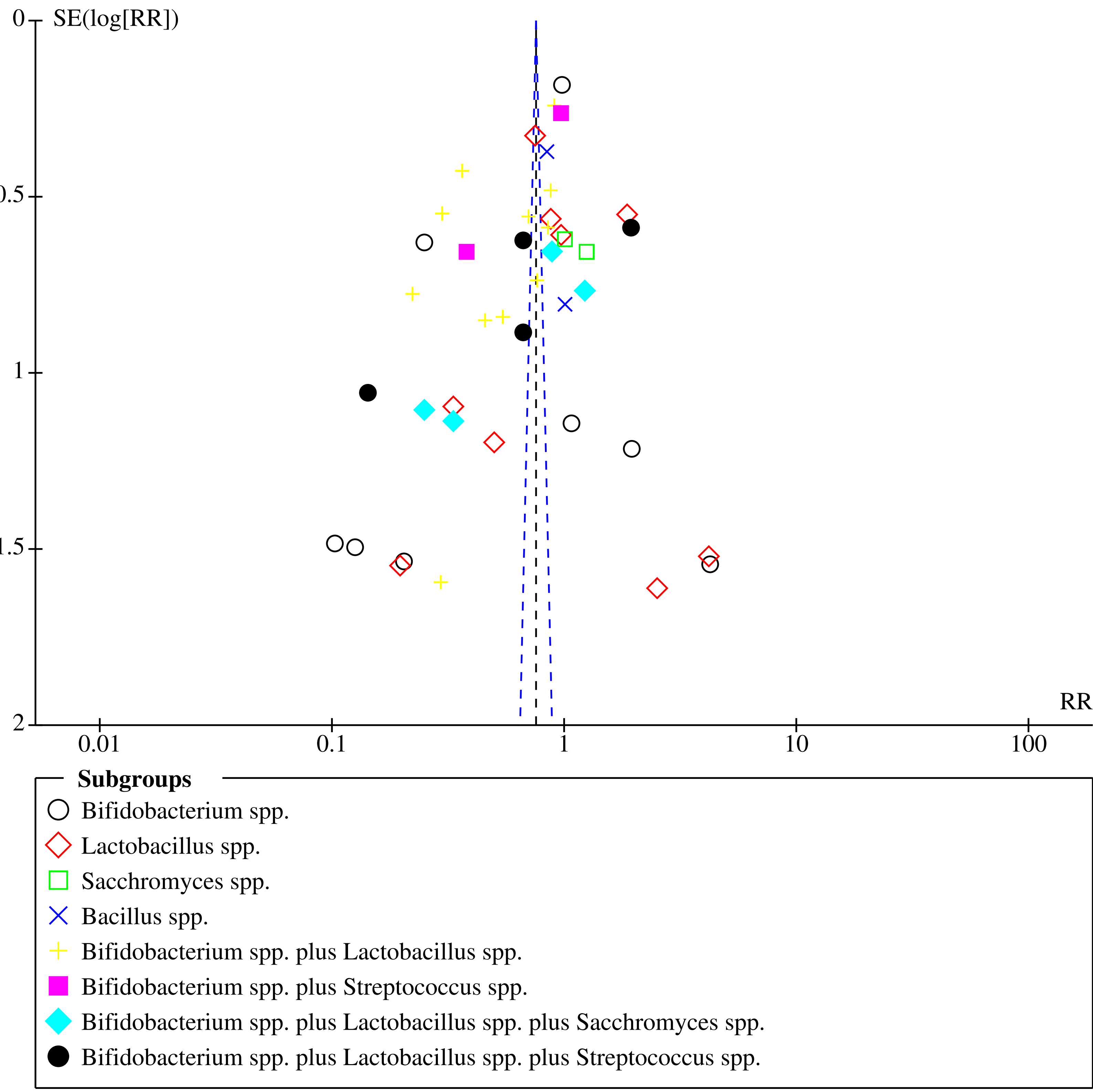
Study or subgroup	Probiotics events	Probiotics total	Control events	Control total	Weight	Risk ratio [95% CI]
1.2.5 Bifidobacterium spp. plus Lactobacillus spp.						
Al-Hosni 2012	3	50	4	51	1.2%	0.77 [0.18 , 3.25]
Braga 2011	26	119	27	112	8.5%	0.91 [0.56 , 1.45]
Chowdhury 2016	5	60	7	59	2.2%	0.70 [0.24 , 2.09]
Li 2019	0	16	1	14	0.5%	0.29 [0.01 , 6.69]
Lin 2005	7	180	20	187	6.0%	0.36 [0.16 , 0.84]
Lin 2008	2	217	9	217	2.7%	0.22 [0.05 , 1.02]
Rougé 2009	2	45	4	49	1.2%	0.54 [0.10 , 2.83]
Roy 2014	7	56	8	56	2.4%	0.88 [0.34 , 2.25]
Saengtawesin 2014	0	31	0	29		Not estimable
Samanta 2009	4	91	14	95	4.2%	0.30 [0.10 , 0.87]
Strus 2018	2	80	4	73	1.3%	0.46 [0.09 , 2.42]
Van Niekerk 2014	5	91	6	93	1.8%	0.85 [0.27 , 2.69]
Subtotal (95% CI)		**1036**		**1035**	**32.0%**	**0.60 [0.45 , 0.81]**
Total events:	63		104			
Heterogeneity: Chi² = 9.03, df = 10 (P = 0.53); I² = 0%						
Test for overall effect: Z = 3.40 (P = 0.0007)						
1.2.6 Bifidobacterium spp. plus Streptococcus spp.						
Bin-Nun 2005	3	72	8	73	2.4%	0.38 [0.11 , 1.38]
Jacobs 2013	27	548	28	551	8.5%	0.97 [0.58 , 1.62]
Subtotal (95% CI)		**620**		**624**	**11.0%**	**0.84 [0.52 , 1.35]**
Total events:	30		36			
Heterogeneity: Chi² = 1.76, df = 1 (P = 0.18); I² = 43%						
Test for overall effect: Z = 0.73 (P = 0.47)						
1.2.7 Bifidobacterium spp. plus Lactobacillus spp. plus Sacchromyces spp.						
Chandrashekar 2018	1	70	4	70	1.2%	0.25 [0.03 , 2.18]
Dutta 2015	8	114	2	35	0.9%	1.23 [0.27 , 5.52]
Hariharan 2016	4	93	5	103	1.4%	0.89 [0.25 , 3.20]
Shashidhar 2017	1	49	3	49	0.9%	0.33 [0.04 , 3.09]
Subtotal (95% CI)		**326**		**257**	**4.5%**	**0.67 [0.30 , 1.49]**
Total events:	14		14			
Heterogeneity: Chi² = 1.98, df = 3 (P = 0.58); I² = 0%						
Test for overall effect: Z = 0.98 (P = 0.33)						
1.2.8 Bifidobacterium spp. plus Lactobacillus spp. plus Streptococcus spp.						
Dashti 2014	8	69	4	67	1.2%	1.94 [0.61 , 6.15]
Fernández-Carrocera 2013	1	75	7	75	2.1%	0.14 [0.02 , 1.13]
Kanic 2015	2	40	3	40	0.9%	0.67 [0.12 , 3.78]
Rehman 2018	4	73	6	73	1.8%	0.67 [0.20 , 2.26]
Subtotal (95% CI)		**257**		**255**	**6.1%**	**0.74 [0.39 , 1.42]**
Total events:	15		20			
Heterogeneity: Chi² = 5.15, df = 3 (P = 0.16); I² = 42%						
Test for overall effect: Z = 0.90 (P = 0.37)						
Total (95% CI)		**5180**		**4990**	**100.0%**	**0.76 [0.65 , 0.89]**
Total events:	250		322			
Heterogeneity: Chi² = 37.21, df = 41 (P = 0.64); I² = 0%						
Test for overall effect: Z = 3.45 (P = 0.0006)						
Test for subgroup differences: Chi² = 4.40, df = 7 (P = 0.73), I² = 0%						



- RR 0.76, 95% CI 0.65 to 0.89 (I^2 = 0%);
- RD -0.02, 95% CI -0.02 to -0.01;
- NNTB 50; 95% CI 50 to 100.

There was some evidence of funnel plot asymmetry (Harbord's modified Egger test for bias -0.52, 95% CI -1.15 to 0.12, P = 0.11) (Figure 6).

Figure 6. Funnel plot of comparison: 1 Probiotics versus control, outcome: 1.2 Mortality.



We assessed the certainty of evidence as "moderate" using GRADE approach, downgraded for serious study design limitations (risk of bias in included trials) (Summary of findings 1).

Secondary outcomes

Invasive infection

Meta-analysis of data from 47 trials (9762 infants) showed a reduced risk of infection (Analysis 1.3):

- RR 0.89, 95% CI 0.82 to 0.97 (I^2 = 19%);
- RD -0.02, 95% CI -0.03 to -0.01;
- NNTB 50; 95% CI 33 to 100.

There was no evidence of funnel plot asymmetry (Harbord's modified Egger test for bias -0.07, 95% CI -0.86 to 0.73, P = 0.86).

We assessed the certainty of evidence as "moderate" using GRADE approach, downgraded for serious study design limitations (risk of bias in included trials).

Late-onset infection with the supplemented probiotic microorganism

None of the included studies reported invasive infection caused by the supplemented probiotic microorganisms.

Duration of birth hospitalisation

Meta-analysis of data from 22 trials (5458 infants) showed a shorter duration of hospitalisation (Analysis 1.4):

- MD -1.93 days, 95% CI -3.78 to -0.08 (I^2 = 26%).

There was no evidence of funnel plot asymmetry.

Two other trials reported data that could not be meta-analysed:

- Oncel 2014 reported shorter median duration of hospitalisation (38 versus 46 days);
- Tewari 2015 reported no difference in duration of hospitalisation.

Neurodevelopmental outcomes

Neurodevelopmental impairment

Five trials reported severe neurodevelopmental impairment (either motor, sensory, or cognitive) in surviving children. Three assessed children using Bayley Scales of Infant Development II (BSID-II) at 18 to 24 months (Oncel 2014; Sari 2011), or three years (Lin 2005) post-term. One trial assessed Bayley-III composite scales, Movement Assessment Battery for Children, and Wechsler Preschool and Primary Scale of Intelligence Full Scale Intelligence Quotient at two to five years (Jacobs 2013). One trial, undertaken in Japan, used the Kyoto Scale of Psychological Development 2001 (similar to the Bayley III scales) and physical examination to assess neurodevelopmental status at 18 months' post-term (Totsu 2014).

Completeness of neurodevelopmental follow-up assessment varied (balanced between groups in all trials):

- Lin 2005: 90%;
- Sari 2011: 84%;
- Totsu 2014: 73%;
- Oncel 2014: 68%;
- Jacobs 2013: 48%.

None of the individual trials, nor a meta-analysis of data from five trials (1518 infants) showed an effect (Analysis 1.5);

- RR 1.03, 95% CI 0.84 to 1.26 (I^2 = 0%).

We assessed the certainty of evidence as "low" using GRADE approach, downgraded for serious study design limitations (including attrition bias) and for serious imprecision of effect estimate.

Cerebral palsy

None of the individual trials, nor a meta-analysis of data from five trials (1512 infants) showed an effect (Analysis 1.6):

- RR 1.13, 95% CI 0.74 to 1.72 (I^2 = 18%).

Visual impairment

None of the individual trials, nor a meta-analysis of data from four trials (1356 infants) showed an effect (Analysis 1.7):

- RR 0.50, 95% CI 0.14 to 1.80 (I^2 = 0%).

Hearing impairment

None of the individual trials, nor a meta-analysis of data from four trials (1356 infants) showed an effect (Analysis 1.8):

- RR 0.46, 95% CI 0.18 to 1.17 (I^2 = 32%).

Cognitive performance

Patole 2014 assessed 42% of eligible participants aged three to five years using the Mullen's Scale of Early Learning tool. Analysis did not show an effect on the "early learning composite score" (Analysis 1.9):

- RR -1.00 (95% CI -6.38, 4.38).

Probiotics versus control in extremely preterm or ELBW infants

Two trials restricted participation to ELBW infants (Al-Hosni 2012; Wejryd 2019). Five trials reported subgroup data for extremely preterm or ELBW infants (Costeloe 2015; Jacobs 2013; Oncel 2014; Roy 2014; Tewari 2015; Wang 2007).

Necrotising enterocolitis

Meta-analysis of data from eight trials (1712 infants) did not show an effect (Analysis 2.1):

- RR 0.90, 95% CI 0.68 to 1.21 (I^2 = 0%).

We assessed the certainty of evidence as "low" using GRADE approach, downgraded one level for study limitations due to high risk of bias and one level for imprecision of effect estimate (Summary of findings 2).

Mortality

Meta-analysis of data from six trials (1661 infants) did not show an effect (Analysis 2.2):

- RR 0.91, 95% CI 0.71 to 1.16 (I^2 = 0%)

We assessed the certainty of evidence as "low" using GRADE approach, downgraded one level for serious study limitations due to high risk of bias and one level for serious imprecision of effect estimate (Summary of findings 2).

Invasive infection

Meta-analysis of data from six trials (1471 infants) did not show an effect (Analysis 2.3):

- RR 0.90, 95% CI 0.76 to 1.06 (I^2 = 0%)

We assessed the certainty of evidence as "low" using GRADE approach, downgraded one level for serious study limitations due to high risk of bias and one level for serious imprecision of effect estimate (Summary of findings 2).

Late-onset infection with the supplemented probiotic microorganism

None of the included studies reported invasive infection caused by the supplemented probiotic microorganisms.

Duration of birth hospitalisation

Analysis of data from one trial (22 infants) did not show an effect:

- MD -5.40 days, 95% CI -14.20 to 3.40)

Neurodevelopmental outcomes

None of the trials reports provided subgroup data for meta-analysis. Three reports stated that there was not an effect of probiotics on the rate of severe neurodevelopmental impairment in the extremely preterm or ELBW subgroup (Jacobs 2013; Sari 2011; Totsu 2014).

Subgroup comparison by genus of probiotics

Necrotising enterocolitis

There was some evidence of subgroup differences depending on genus of probiotics (Chi^2 = 11.23, df = 7 (P = 0.13), I^2 = 37.7%; Analysis 1.1; Figure 3). The largest effect size estimates favoured trials using combinations of:

- *Lactobacillus spp*.
- *Bifidobacterium spp*. plus *Lactobacillus spp*.
- *Bifidobacterium spp.* plus *Streptococcus spp.*
- *Bifidobacterium spp.* plus *Lactobacillus spp.* plus *Streptococcus spp*.

Mortality

There was no evidence of subgroup differences depending on genus of probiotics (Chi^2 = 4.40, df = 7 (P = 0.73), I^2 = 0%; Analysis 1.2; Figure 5).

Invasive infection

There was no evidence of subgroup differences depending on genus of probiotics (Chi^2 = 2.57, df = 7 (P = 0.92), I^2 = 0%; Analysis 1.3).

Duration of birth hospitalisation

There was no evidence of subgroup differences depending on genus of probiotics (Chi^2 = 2.56, df = 6 (P = 0.86), I^2 = 0%; Analysis 1.4).

Neurodevelopmental outcomes

Neurodevelopmental impairment

There was no evidence of subgroup differences depending on genus of probiotics (Chi^2 = 1.48, df = 4 (P = 0.83), I^2 = 0%; Analysis 1.5).

Cerebral palsy

There was no evidence of subgroup differences depending on genus of probiotics (Chi^2 = 3.66, df = 4 (P = 0.45), I^2 = 0%; Analysis 1.6).

Visual impairment

There was no evidence of subgroup differences depending on genus of probiotics (Chi^2 = 1.59, df = 2 (P = 0.45), I^2 = 0%; Analysis 1.7).

Hearing impairment

There was no evidence of subgroup differences depending on genus of probiotics (Chi^2 = 3.63, df = 3 (P = 0.30), I^2 = 17.4%; Analysis 1.8).

Subgroup comparison by type of enteral feed (human milk versus formula versus mixed)

Necrotising enterocolitis

There was no evidence of subgroup differences depending on the type of enteral feed (Chi^2 = 3.81, df = 2 (P = 0.15), I^2 = 47.6%; Analysis 3.1).

Mortality

There was no evidence of subgroup differences depending on the type of enteral feed (Chi^2 = 2.80, df = 2 (P = 0.25), I^2 = 28.7%; Analysis 3.2).

Invasive infection

There was no evidence of subgroup differences depending on the type of enteral feed (Chi^2 = 3.45, df = 2 (P = 0.18), I^2 = 42.0%; Analysis 3.3).

Duration of birth hospitalisation

There was no evidence of subgroup differences depending on the type of enteral feed (Chi^2 = 1.98, df = 2 (P = 0.37), I^2 = 0%; Analysis 3.4).

Neurodevelopmental outcomes

In all trials, participants may have received human milk, or formula, or both.

Sensitivity analyses by risk of bias

Necrotising enterocolitis

There was evidence of subgroup differences depending on risk bias (Chi^2 = 7.82, df = 2 (P = 0.02), I^2 = 74.4%). Sensitivity meta-analysis of 16 trials (4597 infants) at low risk of bias showed a reduced risk of NEC (Analysis 4.1):

- RR 0.70, 95% CI 0.55, 0.89 (I^2 = 25%);
- RD -0.02, 95% CI -0.03 to -0.01;
- NNTB 50; 95% CI 33 to 100.

Mortality

There was no evidence of subgroup differences depending on risk of bias (Chi^2 = 3.41, df = 2 (P = 0.18), I^2 = 41.3%). Sensitivity meta-analysis of 16 trials (4597 infants) at low risk of bias did not show an effect (Analysis 4.2):

- RR 0.86, 95% CI 0.69, 1.07 (I^2 = 0%);
- RD -0.01, 95% CI -0.03 to 0.00.

Invasive infection

There was some evidence of subgroup differences depending on risk of bias (Chi^2 = 4.62, df = 2 (P = 0.10), I^2 = 56.7%). Sensitivity meta-analysis of 16 trials (4597 infants) at low risk of bias did not show an effect (Analysis 4.3):

- RR 0.90, 95% CI 0.79, 1.02 (I^2 = 8%);
- RD -0.02, 95% CI -0.04 to 0.00.

Duration of birth hospitalisation

There was no evidence of subgroup differences depending on risk of selection bias (Chi^2 = 1.30, df = 2 (P = 0.52), I^2 = 0%). Sensitivity meta-analysis of six trials (2786 infants) at low risk of bias did not show an effect (Analysis 4.4):

- MD -2.44 days, 95% CI -5.76 to 1.29 (I^2 = 52%).

Neurodevelopmental outcomes

Neurodevelopmental impairment

There was no evidence of subgroup differences depending on risk of bias (Chi^2 = 0.30, df = 1 (P = 0.58), I^2 = 0%). Sensitivity meta-analysis of two trials (913 infants) at low risk of bias did not show an effect (Analysis 4.5):

- RR 0.99, 95% CI 0.76, 1.27 (I^2 = 0%);
- RD 0.00, 95% CI -0.05 to 0.05.

Cerebral palsy

There was no evidence of subgroup differences depending on risk of bias (Chi^2 = 0.01, df = 1 (P = 0.92), I^2 = 0%). Sensitivity meta-analysis of two trials (913 infants) at low risk of bias did not show an effect (Analysis 4.6):

- RR 1.14, 95% CI 0.68, 1.92 (I^2 = 0%);
- RD 0.01, 95% CI -0.02 to 0.04.

Visual impairment

There was no evidence of subgroup differences depending on risk of performance and detection bias (Chi^2 = 1.53, df = 1 (P = 0.22), I^2 = 34.6%). Sensitivity meta-analysis of two trials (913 infants) at low risk of bias did not show an effect (Analysis 4.7):

- RR 2.91, 95% CI 0.12, 71.21 (I^2 = not applicable);
- RD 0.00, 95% CI -0.01 to 0.01.

Hearing impairment

There was no evidence of subgroup differences depending on risk of performance and detection bias (Chi^2 = 1.96, df = 1 (P = 0.16), I^2 = 48.9%). Sensitivity meta-analysis of two trials (913 infants) at low risk of bias did not show an effect (Analysis 4.8):

- RR 0.30, 95% CI 0.09, 0.98 (I^2 = 60%); 0.30 [0.09, 0.98)
- RD -0.02, 95% CI -0.03 to -0.00.

DISCUSSION

Summary of main results

Meta-analyses of data from more than 50 trials, with more than 10,000 participants in total, show that enteral supplementation with probiotics may reduce the risk of NEC, and probably reduces mortality and the risk of late-onset invasive infection in very preterm or VLBW infants. Sensitivity meta-analyses of trials at low risk of bias did not show effects on mortality or infection. None of our included studies reported instances of invasive infection caused by the probiotic organisms being tested. Meta-analyses of data available from five trials do not show an effect on severe neurodevelopmental impairment. According to GRADE assessment, the certainty of the evidence in this review is low to moderate.

Overall completeness and applicability of evidence

Most of the trials were undertaken within the past 20 years in healthcare facilities internationally, but predominantly in Europe and Asia. Few data were available from trials conducted in sub-Saharan Africa. The findings should be applicable to current care practices for very preterm or VLBW infants including infants 'small for gestation' at birth (only four trials excluded such infants, and none defined evidence of abnormal end-diastolic flow velocities in fetal Doppler studies as an exclusion criterion). The average event rate for NEC in the control group was 6%, consistent with estimates from prevalence studies in very preterm of VLBW infants in high-income countries (Battersby 2018). We pre-specified a comparison including only data for extremely preterm or ELBW infants. Only two small trials, however, restricted participation to this population, and a further five trials reported subgroup data. Meta-analyses included fewer than 1800 infants, and did not show effects on any of the review outcomes. These estimates are imprecise due to few participants being included in meta-analyses. The wide confidence intervals around the point estimates do not rule out important benefits or harms in this subpopulation, and are consistent with the effects seen in the meta-analyses including the entire very preterm or VLBW population.

The review findings are likely to be broadly applicable to infants fed enterally with human milk or formula or both. Formula feeding increases risk of NEC and the risk-benefit balance of probiotic supplementation could differ between human milk- and formula-fed very preterm or VLBW infants (Quigley M 2019). Pre-specified subgroup analyses did not show differences in effect sizes between trials that permitted only human milk feeding for participants (seven trials), versus trials in which all infants received only formula (five trials), versus those trials in which infants could be fed with human milk or formula or both (42 trials). The reported data in trials that permitted human milk- or formula-feeding or both were insufficient to analyse subgroups effects at an infant level by type of enteral feeds received.

The main challenge in applying the findings of this review is the heterogeneity of the interventions tested. Subgroup analyses showed some evidence of differences in effect sizes depending on the genus of the probiotics used, with larger effects in trials that used combinations of bifidobacteria and lactobacilli (with or without *S. thermophilus*). Data from the only two large (> 1000 participants), high-quality trials support this interpretation (Costeloe 2015; Jacobs 2013). The largest trial of probiotic supplementation yet reported (N = 1310) showed that a single-strain preparation of *Bifidobacterium breve* is probably ineffective in reducing NEC (Costeloe 2015). Conversely, the combination of *Bifidobacterium infantis*, *Streptococcus thermophilus* and *B. lactis* used in the other large trial (N = 1099) is probably effective in reducing the risk of NEC (but not mortality or infection) (Jacobs 2013). These findings, although consistent with recent network analyses of different probiotic combinations, should be interpreted cautiously (Bi 2019; Morgan 2020; van den Akker 2018). As indirect comparisons are not randomised, any differences in effect between trials or groups of trials could be due to other factors, including methodological quality, types of participants, setting, and other practices and policies such as feeding protocols and antibiotic stewardship. Effect estimates may be confounded by species and strain level differences that affect how probiotic organisms interact with each other and endogenous microorganisms in the intestine of immature infants (Millar 2012). Consequently, the optimal probiotic composition or combination is unlikely to be determined reliably by analyses of the existing trial data.

Quality of the evidence

We assessed, using GRADE approach, the certainty of evidence as low or moderate for the pre-specified outcomes (Summary of findings 1; Summary of findings 2). About half of the trials had methodological quality weaknesses, including in measures used to conceal random allocation and to mask clinicians, parents, and caregivers to the intervention (Figure 2), increasing the risk of bias in outcomes assessment. Knowledge of the intervention group could have affected caregivers' or assessors' subjective perceptions of outcomes, for example, it may have influenced decisions on whether investigate or diagnose NEC or invasive infection.

Most of the included trials were small (median N = 149). The asymmetry evident in the funnel plot for the meta-analysis of the effect on NEC (and mortality to a lesser extent) was consistent with small-study bias (Figure 4). One explanation is publication bias - the tendency for articles that report "statistically significant" effects to be submitted and accepted for publication (Gale 2020). Publication bias, as well as other sources of small-study bias, has become increasingly evident as an important contributor to exaggerated effect size estimates in meta-analyses of interventions to improve outcomes in very preterm or VLBW infants (Ohlsson 2020; Pammi 2020). Another concern is that in many of the trials that aimed to assess the effect of probiotics on clinical outcomes, it is unclear from most reports how the sample size was defined, and whether trial "stopping rules" existed. If trial investigators were able to monitor accumulating outcome data until an effect on an outcome was detected, this may result a tendency to detect spurious effects that inflate the pooled estimate of effect sizes.

Attrition bias, due to loss of outcome data from randomised participants, was not a concern for the in-hospital outcomes (NEC, death, infection) assessed in this review. Completeness of long-term neurodevelopmental outcomes data, however, ranged from 48% to 90% between the trials that reported such assessments. The degree of incomplete "follow up" assessment was balanced across the intervention and control groups in each trial. Although this is reassuring with regard to the impact of attrition bias on effect estimates, some concern remains that the assessed population may not be representative of the entire cohort (Tin 1998). The findings in meta-analyses that probiotics does not affect neurodevelopmental outcomes are consequently of 'low-certainty'.

Potential biases in the review process

The main concern with meta-analysis of the effect on NEC is the possibility that the findings are subject to small-study biases, including publication bias. There may be a greater availability of data for inclusion in meta-analyses from trials which reported statistically significant or potentially important effects (Hopewell 2009). We attempted to minimise this threat by searching the proceedings of major international perinatal conferences to identify trial reports that were not published in full form in journals. We cannot be sure that other trials have been undertaken but not reported, and the concern remains that such trials are less likely than published trials to have detected statistically significant or clinically important effects.

We contacted trial investigators for unpublished data (Young 2011). In several cases, authors of "proof of concept" or exploratory trials that aimed primarily to assess whether probiotic administration affected intestinal (stool) colonisation patterns or permeability or immune function were able to provide unpublished clinical outcomes data for inclusion in meta-analyses.

We did not include any potential risk of bias due to the funding source of the included trials (where reported). In related contexts, such as manufacturers of breast milk substitutes funding infant feeding trials, this conflict is important to note (Cleminson 2015). We did not, however, consider this to be a substantial risk of bias here. Manufacturers of probiotic products supported some of the trials by supplying the intervention at no or low cost (noted in Characteristics of included studies), but we considered that they were unlikely to have a conflict of interest in the trial outcome for this relatively niche indication.

Agreements and disagreements with other studies or reviews

Our findings are broadly consistent with other recent systematic reviews of probiotics for preterm infants (summarised in Jarrett 2019). Our review differs from others in some key respects:

- we restricted the population of interest to very preterm and VLBW infants to enhance applicability to those infants at high risk of developing NEC and associated complications;
- we included trials that assessed probiotics only, and excluded trials that assessed prebiotics or synbiotics;
- we conducted genus-level subgroup analyses to explore for differences in effect sizes depending upon the probiotic or combination of probiotics assessed;
- we included formal statistical evaluation to assess the risk of small-study bias for the major outcomes;

- we pre-specified sensitivity analyses to determine how trial methodological quality affected effect sizes; and
- we included a formal GRADE assessment of the 'certainty' of the evidence at outcomes level to help inform policy, practice, and research (Gephart 2020).

AUTHORS' CONCLUSIONS

Implications for practice

Despite the quantity of trial evidence, and the effects shown on necrotising enterocolitis, mortality, and infection, uncertainty remains about how to interpret and apply the trial data of probiotic supplementation for very preterm or VLBW infants. As well as concern that effect size estimates are inflated by biases in the existing trials (including publication bias), the major barrier to implementing the findings is that existing analyses are not able to determine reliably the optimal constitution of probiotics (strains, doses, timing of introduction, duration of use) for routine prophylactic use. A variety of commercially-available probiotic preparations are in use in a minority of neonatal units internationally, but widespread use appears to be limited by availability and regulatory and licensing issues. Although the data from the included trials are reassuring with regard to safety, probiotic bacteraemia or fungaemia and other adverse effects have been reported in preterm infants (Bertelli 2015; Esaiassen 2016; Jenke 2012; Zbinden 2015). It remains unclear whether different strains or combinations have different safety profiles.

Implications for research

Given the uncertainty about whether (and which) probiotics affect important outcomes in very preterm or VLBW infants, consideration could be given to further assessment in randomised placebo-controlled trials. It is essential, firstly, for investigators to determine whether families and clinicians would support a trial of this intervention. Any planned trials should attempt to ensure that caregivers and assessors are masked to the intervention as investigation and diagnosis of important outcomes such as NEC, invasive infection and neurodevelopmental impairment can be subjective. While it may be appropriate to be broadly inclusive of very preterm and VLBW infant participants, trials should ensure sufficient power to assess effects in extremely preterm or ELBW infants, and to explore interactions with the type of enteral feed received.

A key concern in planning any trial is choosing the appropriate intervention to assess. Two options appear favourable. Firstly, a 'confirmatory' trial that uses the probiotic combination (*Bifidobacterium infantis*, *Streptococcus thermophilus* and *B. lactis*) already shown to be likely to reduce the risk of NEC in a large, high-quality trial in Australasia (Jacobs 2013). Alternatively, investigators may consider a pragmatic choice based on multi-strain products in established use in their regions (which provides some availability and quality control reassurances with regard to product integrity and safety). Furthermore, investigators could consider whether trials using 'synbiotics' (combinations of probiotics with 'prebiotics' such as human milk oligosaccharides and other milk glycans) are merited alongside trials, or as part of an adaptive design, of probiotics (Underwood 2019).

Unit of randomisation and analysis is another consideration. Although individual infant randomisation is preferred for statistical and analytical reasons, concern exists that cross-contamination of the trial organisms to infants in the control group will limit the power of the trial to detect an effect (as may have happened in Costeloe 2015). Randomising at the neonatal care centre level (cluster-RCT) obviates this problem, but inflates the sample size requirement considerably because of inter-cluster correlation of outcomes.

ACKNOWLEDGEMENTS

We thank all primary investigators who provided additional information about trial methods and outcomes.

We thank Cochrane Neonatal: Colleen Ovelman, Managing Editor, Jane Cracknell, Assistant Managing Editor, Carol Friesen, Information Specialist, and Roger Soll, Co-coordinating editor, who provided editorial and administrative support; and Kath Wright (CRD, York) for the electronic search strategy and database management.

We thank Yuan Chi for translating two trial reports (Huang 2009; Ren 2010).

We thank Mohan Pammi, Cochrane Neonatal Associate Editor, Robert Boyle and Sarah Hodgkinson (Cochrane Children and Families Network) for peer review.

Khalid AlFaleh, Jasim Anabrees, Dirk Bassler and Turki Al-Kharfi authored previous iterations of this review (AlFaleh 2005; Al Faleh 2008; Al Faleh 2011; Al Faleh 2014).

REFERENCES

References to studies included in this review

Agarwal 2003 *{published data only}*

Agarwal R, Sharma N, Chaudhry R, Deorari A, Paul VK, Gewolb IH, et al. Effects of oral Lactobacillus GG on enteric microflora in low-birth-weight neonates. *Journal of Pediatric Gastroenterology and Nutrition* 2003;**36**(3):397-402. [DOI: 10.1097/00005176-200303000-00019] [PMID: 12604982]

Al-Hosni 2012 *{published data only}*

* Al-Hosni M, Duenas M, Hawk M, Stewart LA, Borghese RA, Cahoon M, et al. Probiotics-supplemented feeding in extremely low-birth-weight infants. *Journal of Perinatology* 2012;**32**(4):253–9. [DOI: 10.1038/jp.2011.51] [PMID: 21546942]

Havranek T, Al-Hosni M, Armbrecht E. Probiotics supplementation increases intestinal blood flow velocity in extremely low birth weight preterm infants10.1038/jp.2012.37. *Journal of Perinatology* 2013;**33**(1):40-4. [DOI: 10.1038/jp.2012.37] [PMID: 22441111]

Bin-Nun 2005 *{published data only}*

Bin-Nun A, Bromiker R, Wilschanski M, Kaplan M, Rudensky B, Caplan M, et al. Oral probiotics prevent necrotizing enterocolitis in very low birth weight neonates. *Journal of Pediatrics* 2005;**147**(2):192-6. [DOI: 10.1016/j.jpeds.2005.03.054] [PMID: 16126048]

Braga 2011 *{published data only}*

Braga TD, da Silva GA, de Lira PI, de Carvalho Lima M. Efficacy of Bifidobacterium breve and Lactobacillus casei oral supplementation on necrotizing enterocolitis in very-low-birth-weight preterm infants: a double-blind, randomized, controlled trial. *American Journal of Clinical Nutrition* 2011;**93**(1):81–6. [DOI: 10.3945/ajcn.2010.29799] [PMID: 20980486]

Chandrashekar 2018 *{published data only}*

Chandrashekar GS, Shettigar S, Varghese TC. Role of probiotics in prevention of necrotizing enterocolitis in preterm neonates. *Indian Journal of Child Health* 2018;**5**(2):112-5. [DOI: 10.32677/IJCH.2018.v05.i02.010]

Chowdhury 2016 *{published data only}*

Chowdhury T, Ali MM, Hossain MM, Singh J, Yousuf AM, Yasmin F, et al. Efficacy of probiotics versus placebo in the prevention of necrotizing enterocolitis in preterm very low birth weight infants: a double-blind randomized controlled trial. *Journal of the College of Physicians and Surgeons Pakistan* 2016;**26**(9):770-4. [PMID: 27671183]

Chrzanowska-Liszewska 2012 *{published data only}*

Chrzanowska-Liszewska D, Seliga-Siwecka J, Kornacka MK. The effect of Lactobacillus rhamnosus GG supplemented enteral feeding on the microbiotic flora of preterm infants-double blinded randomized control trial. *Early Human Development* 2012;**88**(1):57–60. [DOI: 10.1016/j.earlhumdev.2011.07.002] [PMID: 22055271]

Costalos 2003 *{published data only}*

Costalos C, Skouteri V, Gounaris A, Sevastiadou S, Triandafilidou A, Ekonomidou C, et al. Enteral feeding of premature infants with Saccharomyces boulardii. *Early Human Development* 2003;**74**(2):89-96. [DOI: 10.1016/s0378-3782(03)00090-2] [PMID: 14580749]

Costeloe 2015 *{published data only}*

Costeloe K, Hardy P, Juszczak E, Wilks M, Millar MR. Bifidobacterium breve BBG-001 in very preterm infants:a randomised controlled phase 3 trial. *Lancet* 2015;**15**:01027-2. [DOI: 10.1016/S0140-6736(15)01027-2] [PMID: 26628328]

Dani 2002 *{published data only}*

Dani C, Biadaioli R, Bertini G, Martelli E, Rubaltelli FF. Probiotics feeding in prevention of urinary tract infection, bacterial sepsis and necrotizing enterocolitis in preterm infants. A prospective double-blind study. *Biology of the Neonate* 2002;**82**(2):103-8. [DOI: 10.1159/000063096] [PMID: 12169832]

Dashti 2014 *{published data only}*

Dashti AS, Afjey SA, Basiry A, Shirvani F, Seifi K, Taheri ZM. Prophylactic probiotics for prevention of necrotizing enterocolitis (NEC) in low birth weight neonates. *Archives of Pediatric Infectious Diseases* 2014;**1**(4):174-9. [DOI: 10.5812/pedinfect.11603]

Demirel 2013 *{published data only}*

Demirel G, Erdeve O, Celik IH, Dilmen U. Saccharomyces boulardii for prevention of necrotizing enterocolitis in preterm infants: a randomized, controlled study. *Acta Paediatrica* 2013;**102**(12):e560-5. [DOI: 10.1111/apa.12416] [PMID: 24028629]

Dilli 2015 *{published data only}*

Dilli D, Aydin B, Fettah ND, Ozyazıcı E, Beken S, Zenciroglu A, et al. The propre-save study: effects of probiotics and prebiotics alone or combined on necrotizing enterocolitis on very low birth weight infants. *Journal of Pediatrics* 2015;**166**:545-51. [DOI: 10.1016/j.jpeds.2014.12.004] [PMID: 25596096]

Dutta 2015 *{published data only}*

Dutta S, Ray P, Narang A. Comparison of stool colonization in premature infants by three dose regimes of a probiotic combination: a randomized controlled trial. *American Journal of Perinatology* 2015;**32**:733–40. [DOI: 10.1055/s-0034-1395473] [PMID: 25519197]

Fernández-Carrocera 2013 *{published data only}*

Fernández-Carrocera LA, Solis-Herrera A, Cabanillas-Ayón M, Gallardo-Sarmiento RB, García-Pérez CS, Montaño-Rodríguez R, et al. Double-blind, randomised clinical assay to evaluate the efficacy of probiotics in preterm newborns weighing less than 1500 g in the prevention of necrotising enterocolitis. *Archives of Diseases in Childhood. Fetal and Neonatal Edition* 2013;**98**(1):F5-9. [DOI: 10.1136/archdischild-2011-300435] [PMID: 22556209]

Fujii 2006 *{published data only}*

* Fujii T, Ohtsuka Y, Lee T, Kudo T, Shoji H, Sato H, et al. Bifidobacterium breve enhances transforming growth factor beta1 signaling by regulating Smad7 expression in preterm infants. *Journal of Pediatric Gastroenterology and Nutrition* 2006;**43**(1):83-8. [DOI: 10.1097/01.mpg.0000228100.04702.f8] [PMID: 16819382]

Li Y, Shimizu T, Hosaka A, Kaneko N, Ohtsuka Y, Yamashiro Y. Effects of bifidobacterium breve supplementation on intestinal flora of low birth weight infants. *Pediatrics International* 2004;**46**(5):509-15. [DOI: 10.1111/j.1442-200x.2004.01953.x] [PMID: 15491374]

Hariharan 2016 *{published data only}*

Hariharan D, Balasubramanian L, Kannappan V, Veluswami G. Probiotic supplementation in VLBW preterm infants improves feeding tolerance and reduces risk of gram negative sepsis. In: Journal of Pediatric Gastroenterology and Nutrition. Vol. 62. 49th Annual Meeting of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition, 2016:655.

Hays 2015 *{published data only}*

Hays S, Jacquot A, Gauthier H, Kempf C, Beissel A, Pidoux O, et al. Probiotics and growth in preterm infants: a randomized controlled trial. *Clinical Nutrition* 2015;**35**(4):802-11. [DOI: 10.1016/j.clnu.2015.06.006] [PMID: 26220763]

Hernandez-Enriquez 2016 *{published data only}*

Hernandez-Enriquez NP, Rosas-Sumano AB, Monzoy-Ventre MA, Galicia-Flores L. Lactobacillus reuteri DSM 17938 in preventing necrotizing enterocolitis in preterm newborns. Pilot study of efficacy and safety [Lactobacillus reuteri DSM 17938 en la prevención de enterocolitis necrosante en recién nacidos prematuros. Estudio piloto de eficacia y seguridad]. *Revista Mexicana de Pediatría* 2016;**83**(2):37-43.

Hikaru 2010 *{published data only}*

Hikaru U, Koichi S, Yayoi S, Hiromichi S, Hiroaki S, Yoshikazu O. Bifidobacteria prevents preterm infants from developing infection and sepsis. *International Journal of Probiotics and Prebiotics* 2010;**5**(1):33-6.

Huang 2009 *{published data only}*

Huang B, Yang H, Huang X. Probiotics supplementation for prevention of necrotizing enterocolitis in very low-birth-weight neonates: a randomized, controlled trial. *Journal of Guangdong Medical College* 2009;**27**:37-9.

Indrio 2017 *{published data only}*

Indrio F, Riezzo G, Tafuri S, Ficarella M, Carlucci B, Bisceglia M, et al. Probiotic supplementation in preterm: feeding intolerance and hospital cost. *Nutrients* 2017;**9**(9):965. [DOI: 10.3390/nu9090965] [PMID: 28858247]

Jacobs 2013 *{published data only}*

* Jacobs SE, Tobin JM, Opie GF, Donath S, Tabrizi SN, Pirotta M, et al. Probiotic effects on late-onset sepsis in very preterm infants: a randomized controlled trial. *Pediatrics* 2013;**132**(6):1055-62. [DOI: 10.1542/peds.2013-1339] [PMID: 24249817]

Plummer EL, Bulach DM, Murray GL, Jacobs SE, Tabrizi SN, Garland SM, ProPrems Study Group. Gut microbiota of preterm infants supplemented with probiotics: sub-study of the ProPrems trial. *BMC Microbiology* 2018;**18**(1):184. [DOI: 10.1186/s12866-018-1326-1] [PMID: 30424728]

Kanic 2015 *{published data only}*

Kanic Z, Turk DM, Burja S, Kanic V, Dinevski D. Influence of a combination of probiotics on bacterial infections in very low birthweight newborns. *Wiener Klinische Wochenschrift* 2015;**127**:S210-5. [DOI: 10.1007/s00508-015-0845-0] [DOI: 26373743]

Kitajima 1997 *{published data only}*

Kitajima H, Sumida Y, Tanaka R, Yuki N, Takayama H, Fujimura M. Early administration of Bifidobacterium breve to preterm infants: randomised controlled trial. *Archives of Disease in Childhood. Fetal and Neonatal Edition* 1997;**76**(2):F101-7. [DOI: 10.1136/fn.76.2.f101] [PMID: 9135288]

Li 2019 *{published data only}*

Li YF, Zhu CR, Gong XL, Li HL, Xiong LK, Wang KJ, et al. Beneficial effects of probiotic treatment on gut microbiota in very low birth weight infants. *Gastroenterology Research and Practice* 2019;**3682836**:eCollection 2019. [DOI: 10.1155/2019/3682836] [PMID: 31772570]

Lin 2005 *{published data only}*

Chou IC, Kuo HT, Chang JS, Wu SF, Chiu HY, Su BH, et al. Lack of effects of oral probiotics on growth and neurodevelopmental outcomes in preterm very low birth weight infants. *Journal of Pediatrics* 2010;**156**(3):393-6. [DOI: 10.1016/j.jpeds.2009.09.051] [PMID: 19914635]

* Lin HC, Su BH, Chen AC, Lin TW, Tsai CH, Yeh TF, et al. Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. *Pediatrics* 2005;**115**(1):1-4. [DOI: 10.1542/peds.2004-1463] [PMID: 15629973]

Lin 2008 *{published data only}*

Lin HC, Hsu CH, Chen HL, Chung MY, Hsu JF, Lien RI, et al. Oral probiotics prevent necrotizing enterocolitis in very low birth weight preterm infants: a multicenter, randomized, controlled trial. *Pediatrics* 2008;**122**(4):693–700. [DOI: 10.1542/peds.2007-3007] [PMID: 18829790]

Manzoni 2006 *{published data only}*

Manzoni P, Mostert M, Leonessa ML, Priolo C, Farina D, Monetti C, et al. Oral supplementation with Lactobacillus casei subspecies rhamnosus prevents enteric colonization by Candida species in preterm neonates: a randomized study. *Clinical Infectious Diseases* 2006;**42**(12):1735-42. [DOI: 10.1086/504324] [PMID: 16705580]

Manzoni 2009 *{published data only}*

Manzoni P, Meyer M, Stolfi I, Rinaldi M, Cattani S, Pugni L, et al. Bovine lactoferrin supplementation for prevention of necrotizing enterocolitis in very-low-birth-weight neonates: a randomized clinical trial. *Early Human Development*

2014;**90**(Suppl 1):S60-5. [DOI: 10.1016/S0378-3782(14)70020-9] [PMID: 24709463]

* Manzoni P, Rinaldi M, Cattani S, Pugni L, Romeo MG, Messner H, et al, Italian Task Force for the Study and Prevention of Neonatal Fungal Infections, Italian Society of Neonatology. Bovine lactoferrin supplementation for prevention of late-onset sepsis in very low-birth-weight neonates: a randomized trial. *JAMA* 2009;**302**(13):1421-8. [DOI: 10.1001/jama.2009.1403] [PMID: 19809023]

Manzoni P, Sánchez RG, Meyer M, Stolfi I, Pugni L, Messner H, et al, Italian Task Force for the Study, and Prevention of Neonatal Fungal Infections and the Italian Society of Neonatology. Exposure to gastric acid inhibitors increases the risk of infection in preterm very low birth weight infants but concomitant administration of lactoferrin counteracts this effect. *Journal of Pediatrics* 2018;**193**:62-7.e1. [DOI: 10.1016/j.jpeds.2017.09.080] [PMID: 29198543]

Mihatsch 2010 *{published data only}*

Mihatsch WA, Vossbeck S, Eikmanns B, Hoegel J, Pohlandt F. Effect of Bifidobacterium lactis on the incidence of nosocomial infections in very-low-birth-weight infants: a randomized controlled trial. *Neonatology* 2010;**98**(2):156–63. [DOI: 10.1159/000280291] [PMID: 20234140]

Millar 1993 *{published data only}*

* Millar MR, Bacon C, Smith SL, Walker V, Hall MA. Enteral feeding of premature infants with Lactobacillus GG. *Archives of Disease in Childhood* 1993;**69**(5 Spec No):483-7. [DOI: 10.1136/adc.69.5_spec_no.483] [PMID: 8285750]

Stansbridge EM, Walker V, Hall MA, Smith SL, Millar MR, Bacon C, et al. Effects of feeding premature infants with Lactobacillus GG on gut fermentation. *Archives of Disease in Childhood* 1993;**69**(5 Spec No):488-92. [DOI: 10.1136/adc.69.5_spec_no.488] [PMID: 8285751]

Mohan 2006 *{published and unpublished data}*

Mohan R, Koebnick C, Schildt J, Schmidt S, Mueller M, Possner M, et al. Effects of Bifidobacterium lactis Bb12 supplementation on intestinal microbiota of preterm infants: a double-blind, placebo-controlled, randomized study. *Journal of Clinical Microbiology* 2006;**44**(11):4025–31. [DOI: 10.1128/JCM.00767-06] [PMID: 16971641]

Oncel 2014 *{published data only}*

Akar M, Eras Z, Oncel MY, Arayici S, Guzoglu N, Canpolat FE, et al. Impact of oral probiotics on neurodevelopmental outcomes in preterm infants. *Journal of Maternal-Fetal & Neonatal Medicine* 2017;**30**(4):411-5. [DOI: 10.1080/14767058.2016.1174683] [PMID: 27045204]

* Oncel MY, Sari FN, Arayici S, Guzoglu N, Erdeve O, Uras N, et al. Lactobacillus reuteri for the prevention of necrotising enterocolitis in very low birth weight infants: a randomised controlled trial. *Archives of Disease in Childhood Fetal & Neonatal Edition* 2014;**99**(2):F110–5. [DOI: 10.1136/archdischild-2013-304745] [PMID: 24309022]

Oshiro 2019 *{published data only}*

Oshiro T, Nagata S, Wang C, Takahashi T, Tsuji H, Asahara T, et al. Bifidobacterium supplementation of colostrum and breast milk enhances weight gain and metabolic responses associated with microbiota establishment in very-preterm infants. *Biomedicine Hub* 2019;**4**(3):1-10. [DOI: 10.1159/000502935] [PMID: 31993433]

Patole 2014 *{published data only}*

Agrawal S, Pestell CF, Granich J, Rao S, Nathan E, Wray JA, et al. Difficulties in developmental follow-up of preterm neonates in a randomised-controlled trial of Bifidobacterium breve M16-V—Experience from Western Australia. *Early Human Development* 2020;**151**:105165. [DOI: 10.1016/j.earlhumdev.2020.105165] [PMID: 32871454]

* Patole S, Keil AD, Chang A, Nathan E, Doherty D, Simmer K, et al. Effect of Bifidobacterium breve M-16V supplementation on fecal bifidobacteria in preterm neonates - a randomised double blind placebo controlled trial. *PLOS One* 2014;**9**(3):e89511. [DOI: 10.1371/journal.pone.008951] [PMID: 24594833]

Rehman 2018 *{published data only}*

Rehman SU, Iqbal A, Ali W. Role of probiotics in reducing frequency of necrotizing enterocolitis in preterm neonates. *Pakistan Pediatric Journal* 2018;**42**(3):171-6.

Ren 2010 *{published data only}*

Ren B. Preventive effect of Bifidobacterium tetravaccine tablets in premature infants with necrotizing enterocolitis. *Journal of Pediatric Pharmacy* 2010;**16**(2):24-5.

Reuman 1986 *{published data only}*

Reuman PD, Duckworth DH, Smith KL, Kagan R, Bucciarelli RL, Ayoub EM. Lack of effect of Lactobacillus on gastrointestinal bacterial colonization in premature infants. *Pediatric Infectious Disease* 1986;**5**(6):663-8. [DOI: 10.1097/00006454-198611000-00013] [PMID: 3099269]

Rougé 2009 *{published data only}*

Rougé C, Piloquet H, Butel MJ, Berger B, Rochat F, Ferraris L, et al. Oral supplementation with probiotics in very low-birth-weight preterm infants: a randomized,double-blind, placebo-controlled trial. *American Journal of Clinical Nutrition* 2009;**89**(6):1828-35. [DOI: 10.3945/ajcn.2008.26919] [PMID: 19369375]

Roy 2014 *{published data only}*

Roy A, Chaudhuri J, Sarkar D, Ghosh P, Chakraborty S. Role of enteric supplementation of probiotics on late-onset sepsis by Candida species in preterm low birth weight neonates: a randomized, double blind, placebo-controlled trial. *North American Journal of Medical Sciences* 2014;**6**:50-7. [DOI: 10.4103/1947-2714.125870] [PMID: 24678479]

Sadowska-Krawczenko 2012 *{published data only}*

Sadowska-Krawczenko IK, Polak P, Wietlicka-Piszcz A, Szajewska H. Lactobacilllus rhamnosus ATC A07FA for preventing necrotizing enterocolitis in very-low-birth-weight preterm infants: a randomized controlled trial (preliminary results) [Ocena skutecznoci Lactobacillus rhamnosus

ATC A07FAw zapobieganiu martwiczego zapalenia jelit wczeniaków z bardzoma urodzeniow mas ciaa: badanie z randomizacj (wstpnewyniki)]. *Polish Journal of Pediatrics* 2012;**87**(2):139-45.

Saengtawesin 2014 *{published data only}*

Saengtawesin V, Tangpolkaiwalsak R, Kanjanapattankul W. Effect of oral probiotics supplementation in the prevention of necrotizing enterocolitis among very low birth weight preterm infants. *Journal of the Medical Association of Thailand* 2014;**97**(Suppl. 6):S20-5. [PMID: 25391168]

Samanta 2009 *{published data only}*

Samanta M, Sarkar M, Ghosh P, Ghosh JK, Sinha MK, Chatterjee S. Prophylactic probiotics for prevention of necrotizing enterocolitis in very low birth weight newborns. *Journal of Tropical Pediatrics* 2009;**55**(2):128-31. [DOI: 10.1093/tropej/fmn091] [PMID: 18842610]

Sari 2011 *{published data only}*

* Sari FN, Dizdar EA, Oguz S, Erdeve O, Uras N, Dilmen U. Oral probiotics: Lactobacillus sporogenes for prevention of necrotizing enterocolitis in very low-birth weight infants: a randomized, controlled trial. *European Journal of Clinical Nutrition* 2011;**65**(4):434-9. [DOI: 10.1038/ejcn.2010.278] [PMID: 21245887]

Sari FN, Eras Z, Dizdar EA, Erdeve O, Oguz SS, Uras N, et al. Do oral probiotics affect growth and neurodevelopmental outcomes in very low-birth-weight preterm infants? *American Journal of Perinatology* 2012;**29**(8):579-86. [DOI: 10.1055/s-0032-1311981] [DOI: 10.1055/s-0032-1311981] [PMID: 22566113]

Serce 2013 *{published data only}*

* Serce O, Benzer D, Gursoy T, Karatekin G, Ovali F. Efficacy of saccharomyces boulardii on necrotizing enterocolitis or sepsis in very low birth weight infants: a randomised controlled trial. *Early Human Development* 2013;**89**(12):1033–6. [DOI: 10.1016/j.earlhumdev.2013.08.013] [PMID: 24041815]

Serce O, Gursoy T, Ovali F, Karatekin G. Effects of Saccharomyces boulardii on neonatal hyperbilirubinemia: a randomized controlled trial. *American Journal of Perinatology* 2015;**30**(2):137-42. [DOI: 10.1055/s-0034-1376390] [PMID: 24915562]

Shadkam 2015 *{published data only}*

Shadkam MN, Jalalizadeh F, Nasiriani K. Effects of probiotic lactobacillus reuteri (DSM 17938) on the incidence of necrotizing enterocolitis in very low birth weight premature infants. *Iranian Journal of Neonatology* 2015;**6**(4):15-20. [DOI: 10.22038/IJN.2015.6143]

Shashidhar 2017 *{published data only}*

Shashidhar A, Suman Rao PN, Nesargi S, Bhat S, Chandrakala BS. Probiotics for promoting feed tolerance in very low birth weight neonates - a randomized controlled trial. *Indian Pediatrics* 2017;**54**(5):363-7. [DOI: 10.1007/s13312-017-1106-2] [PMID: 28368269]

Stratiki 2007 *{published data only}*

Stratiki Z, Costalos C, Sevastiadou S, Kastanidou O, Skouroliakou M, Giakoumatou A, et al. The effect of a bifidobacteria supplemented bovine milk on intestinal permeability of preterm infants. *Early Human Development* 2007;**83**(9):575–9. [DOI: 10.1016/j.earlhumdev.2006.12.002] [PMID: 17229535]

Strus 2018 *{published data only}*

Strus M, Helwich E, Lauterbach R, Rzepecka-Węglarz B, Nowicka K, Wilińska M, et al. Effects of oral probiotic supplementation on gut Lactobacillus and Bifidobacterium populations and the clinical status of low-birth-weight preterm neonates: a multicenter randomized, double-blind,placebo-controlled trial. *Infection and Drug Resistance* 2018;**11**:1557-71. [DOI: 10.2147/IDR.S166348] [PMID: 30288066]

Tewari 2015 *{published data only}*

Tewari VV, Dubey SK, Gupta G. Bacillus clausii for prevention of late-onset sepsis in preterm infants: a randomized controlled trial. *Journal of Tropical Pediatrics* 2015;**61**:377–85. [DOI: 10.1093/tropej/fmv050] [PMID: 26246087]

Totsu 2014 *{published data only}*

Totsu S, Terahara M, Kusuda S. Probiotics and the development of very low birthweight infants: follow-up study of a randomised trial. *BMJ Paediatrics Open* 2018;**2**(1):e000256. [DOI: 10.1136/bmjpo-2018-000256] [PMID: 29687082]

* Totsu S, Yamasaki C, Terahara M, Uchiyama A, Kusuda S, Probiotics Study Group in Japan. Bifidobacterium and enteral feeding in preterm infants: cluster-randomized trial. *Pediatrics International* 2014;**56**(5):714-9. [DOI: 10.1111/ped.12330] [PMID: 24617812]

Van Niekerk 2014 *{published data only}*

Van Niekerk E, Kirsten GF, Nel DG, Blaauw R. Probiotics, feeding tolerance, and growth: a comparison between HIV-exposed and unexposed very low birth weight infants. *Nutrition* 2014;**30**(6):645-53. [DOI: 10.1016/j.nut.2013.10.024] [PMID: 24613436]

Wang 2007 *{published data only}*

Wang C, Shoji H, Sato H, Nagata S, Ohtsuka Y, Shimizu T, et al. Effects of oral administration of bifidobacterium breve on fecal lactic acid and short-chain fatty acids in low birth weight infants. *Journal of Pediatric Gastroenterology and Nutrition* 2007;**44**(2):252-7. [DOI: 10.1097/01.mpg.0000252184.89922.5f] [PMID: 17255840]

Wejryd 2019 *{published data only}*

Wejryd E, Marchini G, Frimmel V, Jonsson B, Abrahamsson T. Probiotics promoted head growth in extremely low birthweight infants in a double-blind placebo-controlled trial. *Acta Paediatrica* 2019;**108**(1):62-9. [DOI: 10.1111/apa.14497] [PMID: 29999201]

Zeber-Lubecka 2016 *{published data only}*

Zeber-Lubecka N, Kulecka M, Ambrozkiewicz F, Paziewska A, Lechowicz M, Konopka E, et al. Effect of Saccharomyces boulardii and mode of delivery on the early development of the gut microbial community in preterm infants. *PLOS One* 2016;**11**(2):e0150306. [DOI: 10.1371/journal.pone.0150306] [PMID: 26918330]

References to studies excluded from this review

Arora 2017 *{published data only}*

Arora S, Khurana MS, Saini R. To study the role of probiotics in the prevention of necrotizing enterocolitis in preterm neonates. *International Journal of Contempary Pediatrics* 2017;**4**(5):6. [DOI: 10.18203/2349-3291.ijcp20173787]

Awad 2010 *{published data only}*

Awad H, Mokhtar H, Imam SS, Gad GI, Hafez H, Aboushady N. Comparison between killed and living probiotic usage versus placebo for the prevention of necrotizing enterocolitis and sepsis in neonates. *Pakistan Journal of Biological Sciences* 2010;**13**(6):253-62. [DOI: 10.3923/pjbs.2010.253.262] [PMID: 20506712]

Chi 2019 *{published data only}*

Chi C, Xue Y, Liu R, Wang Y, Lv N, Zeng H, et al. Effects of a formula with a probiotic Bifidobacterium lactis supplement on the gut microbiota of low birth weight infants. *European Journal of Nutrition* 2019;**59**(4):1493–503. [DOI: 10.1007/s00394-019-02006-4] [PMID: 31197506]

Dasopoulou 2015 *{published data only}*

Dasopoulou M, Briana DD, Boutsikou T, Karakasidou E, Roma E, Costalos C, et al. Motilin and gastrin secretion and lipid profile in preterm neonates following prebiotics supplementation: a double-blind randomized controlled study. *Journal of Parenteral and Enteral Nutrition* 2015;**39**(3):359-68. [DOI: 10.1177/0148607113510182] [PMID: 24233255]

Deng 2010 *{published data only}*

Deng J, Chen K. Early minimal feeding combined with probiotics to prevent necrotizing enterocolitis in preterm infant. *Chinese Journal of Modern Drug Application* 2010;**4**(6):13-4.

Denkel 2016 *{published data only}*

Denkel LA, Schwab F, Garten L, Geffers C, Gastmeier P, Piening B. Protective effect of dual-strain probiotics in preterm infants: a multi-center time series analysis. *PLOS One* 2016;**11**(6):e0158136. [DOI: 10.1371/journal.pone.0158136] [PMID: 27332554]

Di 2010 *{published data only}*

Di M, Li X. Effects of Bifidobacterium supplementation for prevention of necrotizing enterocolitis in preterm infants: a randomized, controlled trial. *Zhong Guo She Qu Yi Shi* 2010;**231**:69.

Dongol-Singh 2017 *{published data only}*

Dongol Singh S, Klobassa DS, Resch B, Urlesberger B, Shrestha RP. Placebo controlled introduction of prophylactic supplementation of probiotics to decrease the incidence of necrotizing enterocolitis at Dhulikhel Hospital in Nepal. *Kathmandu University Medical Journal* 2017;**15**(60):319-23. [PMID: 30580349]

Hua 2014 *{published data only}*

Hua X-T, Tang J, Mu D-Z. Effect of oral administration of probiotics on intestinal colonization with drug resistant bacteria in preterm infants [口服益生菌对早产儿肠道耐药菌定植的影响]. *Chinese Journal of Contempoary Pediatrics* 2014;**16**(6):606-9. [PMID: 24927436]

Hussain 2016 *{published data only}*

Hussain M, Jabeen S, Subhani RU. Role of probiotics in prevention of necrotizing enterocolitis in preterm low birth weight neonates. *Pakistan Journal of Medicine and Health Sciences* 2016;**10**:455-9.

Kaban 2019 *{published data only}*

Kaban RK, Hegar B, Rohsiswatmo R, Handryastuti S, Amelia N, Muktiarti D, et al. Lactobacillus reuteri DSM 17938 improves feeding intolerance in preterm infants. *Pediatric Gastroenterology, Hepatology & Nutrition* 2019;**22**(6):545-53. [DOI: 10.5223/pghn.2019.22.6.545] [PMID: 31777720]

Ke 2008 *{published data only}*

Ke D, Su Z, Li L. Effects of Bifido supplement for prevention of necrotizing enterocolitis in preterm infants: a randomized controlled trial. *Chinese Pediatric Emergency Medicine* 2008;**12**:69-71.

Koksal 2015 *{published data only}*

Köksal N, Varal İ, Özkan H, Bagcı O, Doğan P. Effect of probiotic support on feeding intolerance and mortality at preterm infants. In: Journal of Perinatal Medicine. Vol. 43. 2015:P-0612.

Moles 2015 *{published data only}*

Moles L, Escribano E, De Andres J, Montes MT, Rodriguez JM, Jimenez E, et al. Administration of Bifidobacterium breve PS12929 and Lactobacillus salivarius PS12934, two strains isolated from human milk, to very low and extremely low birth weight preterm infants: A pilot study. *Journal of Immunology Research* 2015;**2015**:538171. [DOI: 10.1155/2015/538171] [PMID: 25759843]

Partty 2013 *{published data only}*

Partty A, Luoto R, Kalliomaki M, Salminen S, Isolauri E. Effects of early prebiotic and probiotic supplementation on development of gut microbiota and fussing and crying in preterm infants: a randomized, double-blind, placebo-controlled trial. *Journal of Pediatrics* 2013;**163**:1272-7. [DOI: 10.1016/j.jpeds.2013.05.035] [PMID: 23915796]

Qiao 2017 *{published data only}*

Qiao LX, Zhu WY, Zhang HY, Wang H. Effect of early administration of probiotics on gut microflora and feeding in pre-term infants: arandomized controlled trial. *Journal of Maternal-Fetal & Neonatal Medicine* 2017;**30**(1):13-6. [DOI: 10.3109/14767058.2016.1163674] [PMID: 26956782]

Rojas 2012 *{published data only}*

Rojas MA, Lozano JM, Rojas MX, Rodriguez VA, Rondon MA, Bastidas JA, et al. Prophylactic probiotics to prevent death and nosocomial infection in preterm infants. *Pediatrics* 2012 Nov;**130**(5):e1113-20. [DOI: 10.1542/peds.2011-3584] [PMID: 23071204]

Romeo 2011 *{published data only}*

Romeo MG, Romeo DM, Trovato L, Oliveri S, Palermo F, Cota F, et al. Role of probiotics in the prevention of the enteric colonization by Candida in preterm newborns: incidence of late-onset sepsis and neurological outcome. *Journal of Perinatology* 2011;**31**(1):63–9. [DOI: 10.1038/jp.2010.57] [PMID: 20410904]

Shujie 2011 *{published data only}*

Shujie Y, Haiying Y, Bin G, Shu X, Xianglan D, Jiang W. The clinical application value of endangered preterm infants given earlier amounts of micro feedings and adding probiotics. *Journal of Pediatric Pharmacy* 2011;**17**:21-4.

Sinha 2015 *{published data only}*

Sinha A, Gupta SS, Chellani H, Maliye C, Kumari V, Arya S, et al. Role of probiotics VSL#3 in prevention of suspected sepsis in low birthweight infants in India: a randomised controlled trial. *BMJ Open* 2015;**5**(7):e006564. [DOI: 10.1136/bmjopen-2014-006564] [PMID: 26163028]

Thanhaeuser 2014 *{published data only}*

Thanhaeuser M, Repa A, Weber M, Endress D, Kreissl A, Binder C, et al. Probiotics (infloran) for NEC prevention: Influence of enteral nutrition. In: Archives of Disease in Childhood. 5th Congress of the European Academy of Paediatric Societies, EAPS 2014, Barcelona, Spain. Vol. 99. 2014:A176-7.

Uhlemann 1999 *{published data only}*

Uhlemann M, Heine W, Mohr C, Plath C, Pap S. Effects of oral administration of bifidobacteria on intestinal microflora in premature and newborn infants newborn infants. *Zeitschrift fur Geburtshilfe und Neonatologie* 1999;**203**(5):213-7. [PMID: 10596415]

Underwood 2014 *{published data only}*

Underwood MA, Kalanetra KM, Bokulich NA, Mirmiran M, Barile D, Tancredi DJ, et al. Prebiotic oligosaccharides in premature infants. *Journal of Pediatric Gastroenterology and Nutrition* 2014;**58**(3):352-60. [DOI: 10.1097/MPG.0000000000000211] [PMID: 24135979]

Xu 2016 *{published data only}*

Xu L, Wang Y, Wang Y, Fu J, Sun M, Mao Z et al. A double-blinded randomized trial on growth and feeding tolerance with Saccharomyces boulardii CNCMI-745 in formula-fed preterm infants. *Jornal de Pediatria* 2016;**92**(3):296-301. [DOI: 10.1016/j.jped.2015.08.013] [PMID: 26946967]

Zhou 2012 *{published data only}*

Zhou N. The observation of effect of probiotics in the prevention of neonatal necrotizing enterocolitis. *Chinese Journal of Ethnomedicine and Ethnopharmacy* 2012;**21**:81.

Zhuang 2007 *{published data only}*

Zhuang X-Y, Li X-Y, Gao X-X, Su L-D. Relative factors of neonatal necrotizing enterocolitis and preventive effect of microeco-preparation. *Journal of Applied Clinical Pediatrics* 2006;**22**:1392-3.

References to studies awaiting assessment

Coleta 2013 *{published data only}*

Coleta E, Gheonea M, Sarbu M. Oral supplementation with probiotics in premature infants-a randomised clinical trial. In: Intensive Care Medicine. 24th Annual Meeting of the European Society of Paediatric and Neonatal Intensive Care edition. Vol. 39. Rotterdam, Netherlands, 2013:S113.

Punnahitananda 2006 *{unpublished data only}***ISRCTN39142169**

Punnahitananda S, Thaithumyanon P, Soongsawang K. Nosocomial infection and necrotizing enterocolitis in preterm neonates treated with Lactobacillus acidophilus and Bifidobacterium infantis in a neonatal intensive care unit: a randomized controlled study. In: 14th Congress of the Federation of Asia Oceania Perinatal Societies. Bangkok, Thailand, 2006.

References to ongoing studies

Marisen 2019 *{published data only}*

Marisen J, Hais A, Meyer C, Van Rossum T, Bunte LM, Frommhold D, et al. Efficacy of Bifidobacterium longum, B. infantis and Lactobacillus acidophilus probiotics to prevent gut dysbiosis in preterm infants of 28+0-32+6 weeks of gestation: a randomised, placebo-controlled, double-blind, multicentre trial: the PRIMAL Clinical Study protocol. *BMJ Open* 2019;**9**(11):e032617. [DOI: 10.1136/bmjopen-2019-032617] [PMID: 31753895]

NCT00977912 *{unpublished data only}*

NCT00977912. Necrotizing enterocolitis (Nec) and B. Lactis in premature babies [Prevention of NEC in preterm Infants with B. lactis]. clinicaltrials.gov/ct2/show/NCT00977912 (first received 16 September 2009).

NCT01181791 *{unpublished data only}*

NCT01181791. Effects of Lactobacillus reuteri in premature infants (reuteri) [Pilot study to evaluate the effects of Lactobacillus reuteri in preterm newborns]. clinicaltrials.gov/ct2/show/NCT01181791 (first received 13 August 2010).

NCT01375309 *{unpublished data only}*

NCT01375309. Bifidobacterium supplementation for very low birth weight infants (Bifido(RCT)) [Effect of bifidobacterium bifidum supplementation on morbidity of very low birth weight infants]. clinicaltrials.gov/ct2/show/NCT01375309 (first received 17 June 2011).

NCT04541771 *{published data only}*

NCT04541771. The role of Lactobacillus reuteri in preventing necrotizing enterocolitis (NEC) in pre-term infants (NEC) [The role of Lactobacillus reuteri (L. reuteri) in preventing necrotizing enterocolitis (NEC) in pre-term infants less than 34 weeks of gestation]. clinicaltrials.gov/ct2/show/NCT04541771 (first received 9 September 2020).

Additional references

Abdulkadir 2016

Abdulkadir B, Nelson A, Skeath T, Marrs EC, Perry JD, Cummings SP, et al. Routine use of probiotics in preterm infants: longitudinal impact on the microbiome and metabolome. *Neonatology* 2016;**109**(4):239-47. [DOI: 10.1159/000442936] [PMID: 26859305]

Alcon-Giner 2020

Alcon-Giner C, Dalby MJ, Caim S, , Ketskemety J, Shaw A, Sim K, et al. Microbiota supplementation with Bifidobacterium and Lactobacillus modifies the preterm infant gut microbiota and metabolome: an observational study. *Cell Reports Medicine* 2020;**1**(5):100077. [DOI: 10.1016/j.xcrm.2020.100077] [PMID: 32904427]

Battersby 2018

Battersby C, Santhalingam T, Costeloe K, Modi N. Incidence of neonatal necrotising enterocolitis in high-income countries: a systematic review. *Archives of Disease in Childhood Fetal and Neonatal Edition* 2018;**103**(2):F182-9. [DOI: 10.1136/archdischild-2017-313880] [PMID: 29317459]

Berrington 2012

Berrington JE, Hearn RI, Bythell M, Wright C, Embleton ND. Deaths in preterm infants: changing pathology over 2 decades. *Journal of Pediatrics* 2012;**160**(1):49-53. [DOI: 10.1016/j.jpeds.2011.06.046] [PMID: 21868028]

Berrington 2019

Berrington JE, Zalewski S. The future of probiotics in the preterm infant. *Early Human Deveopment* 2019;**135**:75-81. [DOI: 10.1016/j.earlhumdev.2019.05.008] [PMID: 31130262]

Bertelli 2015

Bertelli C, Pillonel T, Torregrossa A, Prod'hom G, Fischer CJ, Greub G, et al. Bifidobacterium longum bacteremia in preterm infants receiving probiotics. *Clinical Infectious Diseases* 2015;**60**(6):924-7. [DOI: 10.1093/cid/ciu946] [PMID: 25472946]

Besselink 2008

Besselink MG, van Santvoort HC, Buskens E, Boermeester MA, van Goor H, Timmerman HM, Dutch Acute Pancreatitis Study Group. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. *Lancet* 2008;**371**(9613):651-9.

Bi 2019

Bi LW, Yan BL, Yang QY, Li MM, Cui HL. Which is the best probiotic treatment strategy to prevent the necrotizing enterocolitis in premature infants: a network meta-analysis revealing the efficacy and safety. *Medicine* 2019;**98**(41):e17521. [DOI: 10.1097/MD.0000000000017521] [PMID: 31593123]

Boyle 2006

Boyle RJ, Robins-Browne RM, Tang ML. Probiotic use in clinical practice: what are the risks? *American Journal of Clinical Nutrition* 2006;**83**(6):1446-7.

Bron 2017

Bron PA, Kleerebezem M, Brummer R, Cani PD, Mercenier A, MacDonald TT, et al. Can probiotics modulate human disease by impacting intestinal barrier function? *British Journal of Nutrition* 2017;**117**(1):93-107. [DOI: 10.1017/S0007114516004037] [PMID: 28102115]

Cleminson 2015

Cleminson J, Oddie S, Renfrew MJ, McGuire W. Being baby friendly: evidence-based breastfeeding support. *Archives of Disease in Childhood- Fetal and Neonatal Edition* 2015;**100**(2):F173-8.

Duffield 2019

Duffield SD, Clarke P. Current use of probiotics to prevent necrotising enterocolitis. *Archives of Disease in Childhood Fetal and Neonatal Edition* 2019;**104**(2):F228. [DOI: 10.1136/archdischild-2018-316199] [PMID: 30464004]

Eaton 2017

Eaton S, Rees CM, Hall NJ. Current research on the epidemiology, pathogenesis, and management of necrotizing enterocolitis. *Neonatology* 2017;**111**(4):423-30. [DOI: 10.1159/000458462] [PMID: 28538238]

Embleton 2016

Embleton ND, Zalewski S, Berrington JE. Probiotics for prevention of necrotizing enterocolitis and sepsis in preterm infants. *Current Opinion in Infectious Diseases* 2016;**29**(3):256-61. [DOI: 10.1097/QCO.0000000000000269] [PMID: 27023404]

Embleton 2017

Embleton ND, Berrington JE, Dorling J, Ewer AK, Juszczak E, Kirby JA, et al. Mechanisms affecting the gut of preterm infants in enteral feeding trials. *Frontiers in Nutrition* 2017;**4**:14. [DOI: 10.3389/fnut.2017.00014] [PMID: 28534028]

Esaiassen 2016

Esaiassen E, Cavanagh P, Hjerde E, Simonsen GS, Stoen R, Klingenberg C. Bifidobacterium longum subspecies infantis bacteremia in 3 extremely preterm infants receiving probiotics. *Emerging Infectious Diseases* 2016;**22**(9):1664-6. [DOI: 10.3201/eid2209.160033] [PMID: 27532215]

Fleming 2019

Fleming PF, Berrington JE, Jacobs SE. Addressing safety concerns of probiotic use in preterm babies. *Early Human Development* 2019;**135**:72-4. [DOI: 10.1016/j.earlhumdev.2019.05.016] [PMID: 31155280]

Gale 2020

Gale C, McGuire W, Juszczak E. Randomised controlled trials for informing perinatal care. *Neonatology* 2020;**117**(1):8-14. [DOI: 10.1159/000499881] [PMID: 31137030]

Gephart 2020

Gephart SM, Underwood MA, Rosito S, Kim JH, Caplan M. Grading the evidence to identify strategies to modify risk for necrotizing enterocolitis. *Pediatric Research* 2020;**88**(Suppl 1):41-7. [DOI: 10.1038/s41390-020-1079-z] [PMID: 32855512]

GRADEpro GDT [Computer program]

McMaster University (developed by Evidence Prime) GRADEpro GDT. Version accessed 8 May 2018. Hamilton (ON): McMaster University (developed by Evidence Prime). Available at gradepro.org.

Granger 2020

Granger CL, Embleton ND, Palmer JM, Lamb CA, Berrington JE, Stewart CJ. Maternal breast milk, infant gut microbiome, and the impact on preterm infant health. *Acta Paediatrica* 2020;**00**:1-8. [DOI: 10.1111/apa.15534]

Harbord 2006

Harbord RM, Egger M, Sterne JA. A modified test for small-study effects in meta-analyses of controlled trials with binary endpoints. *Statistics in Medicine* 2006;**25**(20):3443-57. [DOI: 10.1002/sim.2380] [PMID: 16345038]

Hickey 2018

Hickey M, Georgieff M, Ramel S. Neurodevelopmental outcomes following necrotizing enterocolitis. *Seminars in Fetal and Neonatal Medicine* 2018;**23**(6):426-32. [DOI: 10.1016/j.siny.2018.08.005] [PMID: 30145060]

Higgins 2011

Higgins JP, Altman DG, Sterne JA: on behalf of the Cochrane Statistical Methods Group and the Cochrane Bias Methods Group. Chapter 8: Assessing risk of bias in included studies. In: Higgins JP, Green S, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011. Available from handbook.cochrane.org.

Higgins 2019

Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, et al (editors). Cochrane Handbook for Systematic Reviews of Interventions version 6.1 (updated September 2020). Cochrane, 2020. Available from www.training.cochrane.org/handbook.

Hopewell 2009

Hopewell S, Loudon K, Clarke MJ, Oxman AD, Dickersin K. Publication bias in clinical trials due to statistical significance or direction of trial results. *Cochrane Database of Systematic Reviews* 2009, Issue 1. Art. No: MR000006. [DOI: 10.1002/14651858.MR000006.pub3]

Horbar 2012

Horbar JH, Carpenter JH, Badger GJ, Kenny MJ, Soll RF, Morrow KA, et al. Mortality and neonatal morbidity among infants 501 to 1500 grams from 2000 to 2009. *Pediatrics* 2012;**129**(6):1019-26. [DOI: 10.1542/peds.2011-3028] [PMID: 22614775]

Jarrett 2019

Jarrett P, Meczner A, Costeloe K, Fleming P. Historical aspects of probiotic use to prevent necrotising enterocolitis in preterm babies. *Early Human Development* 2019;**135**:51-7. [DOI: 10.1016/j.earlhumdev.2019.05.015] [PMID: 31153726]

Jenke 2012

Jenke A, Ruf EM, Hoppe T, Heldmann M, Wirth S. Bifidobacterium septicaemia in an extremely low-birthweight infant under probiotic therapy. *Archives of Disease in Childhood-Fetal and Neonatal Edition* 2012;**97**(3):F217-8. [DOI: 10.1136/archdischild-2011-300838] [PMID: 22058179]

Johnson-Henry 2016

Johnson-Henry KC, Abrahamsson TR, Wu RY, Sherman PM. Probiotics, prebiotics, and synbiotics for the prevention of necrotizing enterocolitis. *Advances in Nutrition* 2016;**7**(5):928-37. [DOI: 10.3945/an.116.012237] [PMID: 27633108]

Koretz 2018

Koretz RL. Probiotics in gastroenterology: How pro Is the evidence in adults? *American Journal of Gastroenterology* 2018;**113**(8):1125-36. [DOI: 10.1038/s41395-018-0138-0] [PMID: 29915396]

Kunk 2019

Kunk D. Probiotics: elixir or empty promise. *Lancet Gastroenterology & Hepatology* 2019;**4**(2):81. [DOI: 10.1016/S2468-1253(18)30415-1] [PMID: 30647011]

Lerner 2019

Lerner A, Shoenfeld Y, Matthias T. Probiotics: if it does not help it does not do any harm. Really? *Microorganisms* 2019;**7**(4):104. [10.3390/microorganisms7040104]

Mara 2018

Mara MA, Good M, Weitkamp JH. Innate and adaptive immunity in necrotizing enterocolitis. In: Seminars in Fetal and Neonatal Medicine. Vol. 23. Elsevier, 2018:394-9. [DOI: 10.1016/j.siny.2018.08.002]

Marchand 2012

Marchand V, Canadian Paediatric Society, Nutrition and Gastroenterology Committee. Using probiotics in the paediatric population. *Paediatrics and Child Health* 2012;**17**(10):575-6.

Martin 2010

Martin CR, Dammann O, Allred EN, Patel S, O'Shea TM, Kuban KC, et al. Neurodevelopment of extremely preterm infants who had necrotizing enterocolitis with or without late bacteremia. *Journal of Pediatrics* 2010;**157**(5):751-6. [DOI: 10.1016/j.jpeds.2010.05.042] [PMID: 20598317]

Masi 2019

Masi AC, Stewart CJ. The role of the preterm intestinal microbiome in sepsis and necrotising enterocolitis. *Early Human Development* 2019;**138**:104854. [DOI: 10.1016/j.earlhumdev.2019.104854] [PMID: 31481262]

Millar 2012

Millar M, Wilks M, Fleming P, Costeloe K. Should the use of probiotics in the preterm be routine? *Archives of Disease in Childhood - Fetal and Neonatal Edition* 2012;**97**(1):F70. [DOI: 10.1136/adc.2009.178939] [PMID: 20870904]

Morgan 2011

Morgan JA, Young L, McGuire W. Pathogenesis and prevention of necrotizing enterocolitis. *Current Opinion in Infectious Diseases* 2011;**24**(3):183-9. [DOI: 10.1097/QCO.0b013e328345d5b5] [PMID: 21455063]

Morgan 2020

Morgan RL, Preidis GA, Kashyap PC, Weizman AV, Sadeghirad B, McMaster Probiotic, Prebiotic, and Synbiotic Work Group. Probiotics reduce mortality and morbidity in preterm, low-birth-weight infants: a systematic review and network meta-analysis of randomized trials. *Gastroenterology* 2020;**159**(2):467-80. [DOI: 10.1053/j.gastro.2020.05.096] [PMID: 32592699]

Ohlsson 2020

Ohlsson A, Lacy JB. Intravenous immunoglobulin for suspected or proven infection in neonates. *Cochrane Database of Systematic Reviews* 2020, Issue 1. Art. No: CD001239. [DOI: 10.1002/14651858.CD001239.pub6]

Olm 2019

Olm MR, Bhattacharya N, Crits-Christoph A, Firek BA, Baker R, Song YS, et al. Necrotizing enterocolitis is preceded by increased gut bacterial replication, Klebsiella, and fimbriae-encoding bacteria. *Science Advances* 2019;**5**(12):eaax5727. [DOI: 10.1126/sciadv.aax5727] [PMID: 31844663]

Pammi 2020

Pammi M, Suresh G. Enteral lactoferrin supplementation for prevention of sepsis and necrotizing enterocolitis in preterm infants. *Cochrane Database of Systematic Reviews* 2020, Issue 3. Art. No: CD007137. [DOI: 10.1002/14651858.CD007137.pub6]

Patel 2015

Patel RM, Denning PW. Intestinal microbiota and its relationship with necrotising enterocolitis. *Pediatric Research* 2015;**78**(3):232-8. [DOI: 10.1038/pr.2015.97] [PMID: 25992911]

Patel 2018

Patel RM, Underwood MA. Probiotics and necrotizing enterocolitis. *Seminars in Pediatric Surgery* 2018;**27**(1):39-46. [DOI: 10.1053/j.sempedsurg.2017.11.008] [PMID: 29275816]

Pell 2019

Pell LG, Loutet MG, Roth DE, Sherman PM. Arguments against routine administration of probiotics for NEC prevention. *Current Opinion in Pediatrics* 2019;**31**(2):195-201. [DOI: 10.1097/MOP.0000000000000730] [PMID: 30624281]

Quigley E 2019

Quigley EM. Prebiotics and probiotics in digestive health. *Clinical Gastroenterology and Hepatology* 2019;**17**(2):333-44. [DOI: 10.1016/j.cgh.2018.09.028] [PMID: 30267869]

Quigley M 2019

Quigley M, Embleton ND, McGuire W. Formula versus donor breast milk for feeding preterm or low birth weight infants. *Cochrane Database of Systematic Reviews* 2019, Issue 7. Art. No: CD002971. [DOI: 10.1002/14651858.CD002971.pub5]

Review Manager 2020 [Computer program]

The Cochrane Collaboration Review Manager 5 (RevMan 5). Version 5.4. Copenhagen: The Cochrane Collaboration, 2020.

Robertson 2020

Robertson C, Savva GM, Clapuci R, Jones J, Maimouni H, Brown E, et al. Incidence of necrotising enterocolitis before and after introducing routine prophylactic Lactobacillus and Bifidobacterium probiotics.. *Archives of Disease in Childhood-Fetal and Neonatal Edition* 2020;**105**(4):380-6. [DOI: 10.1136/archdischild-2019-317346] [PMID: 31666311]

Samuels 2017

Samuels N, van de Graaf RA, de Jonge RC, Reiss IKM, Vermeulen MJ. Risk factors for necrotizing enterocolitis in neonates: a systematic review of prognostic studies. *BMC Pediatrics* 2017;**17**(1):105. [DOI: 10.1186/s12887-017-0847-3] [PMID: 28410573]

Sanders 2019

Sanders ME, Merenstein DJ, Reid G, Gibson GR, Rastall RA. Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. *Nature Reviews Gastroenterology & Hepatology* 2019;**16**(10):605-16. [DOI: 10.1038/s41575-019-0173-3] [PMID: 31296969]

Schünemann 2013

Schünemann H, Brożek J, Guyatt G, Oxman A, editor(s). Handbook for grading the quality of evidence and the strength of recommendations using the GRADE approach (updated October 2013). GRADE Working Group, 2013. Available from gdt.guidelinedevelopment.org/app/handbook/handbook.html.

Sesham 2014

Sesham R, Oddie S, Embleton ND, Clarke P. Probiotics for preterm neonates: parents' perspectives and present prevalence. *Archives of Disease in Childhood-Fetal and Neonatal Edition* 2014;**99**(4):F345. [DOI: 10.1136/archdischild-2014-306344] [PMID: 24723695]

Stewart 2012

Stewart CJ, Marrs EC, Magorrian S, Nelson A, Lanyon C, Perry JD, et al. The preterm gut microbiota: changes associated with necrotizing enterocolitis and infection. *Acta Paediatrica* 2012;**101**(11):1121-7. [DOI: 10.1111/j.1651-2227.2012.02801.x] [PMID: 22845166]

Stewart 2017

Stewart CJ, Embleton ND, Marrs EC, Smith DP, Fofanova T, Nelson A, et al. Longitudinal development of the gut microbiome and metabolome in preterm neonates with late onset sepsis and healthy controls. *Microbiome* 2017;**5**(1):75. [DOI: 10.1186/s40168-017-0295-1] [PMID: 28701177]

Suez 2019

Suez J, Zmora N, Segal E, Elinav E. The pros, cons, and many unknowns of probiotics. *Nature Medicine* 2019;**25**(5):716-29. [DOI: 10.1038/s41591-019-0439-x] [PMID: 31061539]

Thomas 2010

Thomas DW, Greer FR, American Academy of Pediatrics Committee on Nutrition, American Academy of Pediatrics Section on Gastroenterology, Hepatology, and Nutrition. Probiotics and prebiotics in pediatrics. *Pediatrics* 2010;**126**(6):1217-31. [DOI: 10.1542/peds.2010-2548] [PMID: 21115585]

Tin 1998

Tin W, Fritz S, Wariyar U, Hey E. Outcome of very preterm birth: children reviewed with ease at 2 years differ from those followed up with difficulty. *Archives of Disease in Childhood-Fetal and Neonatal Edition* 1998;**79**(2):F83-7. [DOI: 10.1136/fn.79.2.f83] [PMID: 9828731]

Underwood 2019

Underwood MA. Probiotics and human milk oligosaccharides in premature infants. *Neoreviews* 2019;**20**(1):e1-1. [DOI: 10.1542/neo.20-1-e1] [PMID: 31261069]

van den Akker 2018

van den Akker CH, van Goudoever JB, Szajewska H, Embleton ND, Hojsak I, Reid D, et al, ESPGHAN Working Group for Probiotics, Prebiotics & Committee on Nutrition. Probiotics for preterm infants: a strain-specific systematic review and network meta-analysis. *Journal of Pediatric Gastroenterology and Nutrition* 2018;**67**(1):103-22. [DOI: 10.1097/MPG.0000000000001897] [PMID: 29384838]

van den Akker 2020

van den Akker CH, van Goudoever JB, Shamir R, Domellof M, Embleton ND, Hojsak I, et al. Probiotics and preterm infants: a position paper by the European Society for Paediatric Gastroenterology Hepatology and Nutrition Committee on Nutrition and the European Society for Paediatric Gastroenterology Hepatology and Nutrition Working Group for Probiotics and Prebiotics. *Journal of Pediatric Gastroenterology and Nutrition* 2020;**70**(5):664-80. [DOI: 10.1097/MPG.0000000000002655] [PMID: 32332478]

Vermeulen 2020

Vermeulen MJ, Luijendijk A, van Toledo L, van Kaam AH, Reiss IK. Quality of probiotic products for preterm infants: contamination and missing strains. *Acta Paediatrica* 2020;**109**(2):276-9. [DOI: 10.1111/apa.14976] [PMID: 31423636]

Viswanathan 2016

Viswanathan S, Lau C, Akbari H, Hoyen C, Walsh MC. Survey and evidence based review of probiotics used in very low birth weight preterm infants within the United States. *Journal of Perinatology* 2016;**36**(12):1106-11. [DOI: 10.1038/jp.2016.144] [PMID: 27583387]

Walsh 1986

Walsh MC, Kliegman RM. Necrotizing enterocolitis: treatment based on staging criteria. *Pediatric Clinics of North America* 1986;**33**(1):179-201. [DOI: 10.1016/s0031-3955(16)34975-6] [PMID: 3081865]

Walsh 2019

Walsh V, McGuire W. Immunonutrition for Preterm Infants. *Neonatology* 2019;**115**(4):398-405. [DOI: 10.1159/000497332] [PMID: 30974431]

Warner 2016

Warner BB, Deych E, Zhou Y, Hall-Moore C, Weinstock GM, Sodergren E, et al. Gut bacteria dysbiosis and necrotising enterocolitis in very low birthweight infants: a prospective case-control study. *Lancet* 2016;**387**(10031):1928-36. [DOI: 10.1016/S0140-6736(16)00081-7] [PMID: 26969089]

Young 2011

Young T, Hopewell S. Methods for obtaining unpublished data. *Cochrane Database of Systematic Reviews* 2011, Issue 11. Art. No: MR000027. [DOI: 10.1002/14651858.MR000027.pub2] [PMID: 22071866]

Zbinden 2015

Zbinden A, Zbinden R, Berger C, Arlettaz R. Case series of Bifidobacterium longum bacteremia in three preterm infants on probiotic therapy. *Neonatology* 2015;**107**(1):56-9. [DOI: 10.1159/000367985] [PMID: 25402825]

Zmora 2018

Zmora N, Zilberman-Schapira G, Suez J, Mor U, Dori-Bachash M, Bashiardes S, et al. Personalized gut mucosal colonization resistance to empiric probiotics is associated with unique host and microbiome features. *Cell* 2018;**174**(6):1388-405. [DOI: 10.1016/j.cell.2018.08.041] [PMID: 30193112]

References to other published versions of this review

Al Faleh 2008

Alfaleh K, Bassler D. Probiotics for prevention of necrotizing enterocolitis in preterm infants. *Cochrane Database of Systematic Reviews* 2008, Issue 1. Art. No: CD005496. [DOI: 10.1002/14651858.CD005496.pub2]

Al Faleh 2011

Alfaleh K, Anabrees J, Bassler D, Al-Kharfi T. Probiotics for prevention of necrotizing enterocolitis in preterm infants. *Cochrane Database of Systematic Reviews* 2011, Issue 3. Art. No: CD005496. [DOI: 10.1002/14651858.CD005496.pub3]

Al Faleh 2014

AlFaleh K, Anabrees J. Probiotics for prevention of necrotizing enterocolitis in preterm infants. *Cochrane Database of Systematic Reviews* 2014, Issue 4. Art. No: CD005496. [DOI: 10.1002/14651858.CD005496.pub4]

AlFaleh 2005

AlFaleh KM, Bassler D. Probiotics for prevention of mortality and morbidity in preterm infants. *Cochrane Database of Systematic Reviews* 2005, Issue 4. Art. No: CD005496. [DOI: 10.1002/14651858.CD005496]

* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Agarwal 2003

Study characteristics	
Methods	RCT
Participants	39 VLBW infants
Interventions	Probiotics (N = 24): *Lactobacillus rhamnosus* GG once daily with human milk or formula for 21 days or discharge from hospital Control (N = 15): unsupplemented milk feeds
Outcomes	• Stool colonisation patterns (NEC, death, infection not reported)
Notes	India (1999 to 2000) Funding: UK National Institute for Health (Fogarty Grant TW-00601) and Conagra Foods Inc., USA (supplied intervention)

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Not described
Allocation concealment (selection bias)	Unclear risk	Not described
Blinding (performance bias and detection bias) All outcomes	High risk	Unmasked
Incomplete outcome data (attrition bias) All outcomes	Low risk	Unlikely
Selective reporting (reporting bias)	Unclear risk	No clinical outcomes reported

Al-Hosni 2012

Study characteristics	
Methods	RCT
Participants	101 ELBW infants (appropriate for gestational age)
Interventions	Probiotic (N = 50): *Lactobacillus rhamnosus GG* (LGG) and *Bifidobacterium infantis* added to the 1st milk feed and continued once daily until discharge or until 34 weeks' postmenstrual age

Al-Hosni 2012 *(Continued)*

	Control (N = 51): unsupplemented milk feeds
Outcomes	• Weight gain • NEC • Death • Infection
Notes	USA (2009 to 2011) Funding: not stated

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Not described
Allocation concealment (selection bias)	Unclear risk	Not described
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Unsupplemented milk feeds- not placebo-controlled
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Low risk	Unlikely

Bin-Nun 2005

Study characteristics

Methods	RCT
Participants	145 VLBW infants
Interventions	Probiotics (N = 72): "*Lactobacillus bifidus"* (likely *Bifidobactrium bifidum*), *Streptococcus thermophilus,* and *B. infantis* added to expressed breast milk or formula enteral feeds daily until 36 weeks' postmenstrual age Control (N = 73): unsupplemented milk feeds
Outcomes	• NEC • Death • Infection • Time to full enteral feeds
Notes	Israel (2001 to 2004) Funding: Solgar, Wyeth (manufacturer of intervention)

Risk of bias

Bin-Nun 2005 *(Continued)*

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Not described
Allocation concealment (selection bias)	Unclear risk	Not described
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Unsupplemented milk feeds- not placebo-controlled
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Unclear risk	Data published in an abstract form on two previous occasions at the Society of Pediatrics Research (SPR 2003, 2005) with different inclusion criteria and clinical outcomes

Braga 2011

Study characteristics	
Methods	RCT
Participants	231 VLBW infants (birth weight 750 g to 1500 g)
Interventions	Probiotics (N = 119): *Lactobacillus casei* and *Bifidobacterium breve* (Yakult - LB) in human milk once daily until day 30 or hospital discharge Control (N = 112): unsupplemented milk feeds
Outcomes	• NEC • Death • Infection • Days to full enteral feeds • Duration hospital stay
Notes	Brazil (2007 to 2008) Funding: public/state. External Study Committee terminated trial early (quote:) "for a clear benefit" after enrolment of 231 infants

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Low risk	Sealed envelope with group allocation

Braga 2011 *(Continued)*

Blinding (performance bias and detection bias) All outcomes	Unclear risk	Unsupplemented milk feeds- not placebo-controlled
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Low risk	Unlikely

Chandrashekar 2018

Study characteristics	
Methods	Quasi-RCT
Participants	145 preterm infants of gestation < 34 weeks' (most participants were very preterm or VLBW)
Interventions	Probitics (N = 72): *Lactobacillus acidophilus, L. rhamnosus, Bifidobacterium longum,* and *Saccharomyces boulardii* with human milk or formula feeds until discharge from hospital Control (N = 73): unsupplemented milk feeds (no placebo)
Outcomes	• NEC • Death • Infection • Duration of hospitalisation
Notes	India (2014 to 2015) Funding: not stated

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	High risk	Quote: "Simple random sampling method"
Allocation concealment (selection bias)	Unclear risk	Not described
Blinding (performance bias and detection bias) All outcomes	High risk	Unmasked
Incomplete outcome data (attrition bias) All outcomes	Low risk	Near-complete (5 participants withdrawn pre-analysis)
Selective reporting (reporting bias)	Low risk	Unlikely

Chowdhury 2016

Study characteristics	
Methods	RCT
Participants	119 VLBW Infants (28 to 33 weeks' gestation)
Interventions	Probiotics (N = 60): (quote:) "Cap TS6" containing *Lactobacillus rhamnosus GG*, *L. paracasei* , *L. casei*, *L. acidophilus*, *Lactococcus latis*, *Bifidobacterium bifidum*, *B. longum*, *B. infantis*) in human milk once daily until discharge Control (N = 59): unsupplemented milk feeds
Outcomes	• NEC • Death • Infection days to achieve full enteral feeding • Length of hospital stay
Notes	Bangladesh (2012 to 2015) Funding: not stated

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	High risk	First allocation by lottery, and subsequent by alternate allocation
Allocation concealment (selection bias)	High risk	Unconcealed
Blinding (performance bias and detection bias) All outcomes	High risk	Unmasked
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Low risk	Unlikely

Chrzanowska-Liszewska 2012

Study characteristics	
Methods	RCT
Participants	47 very preterm infants (birth weight > 1000 g)
Interventions	Probiotics (N = 21): *Lactobacillus rhamnosus GG*, added to formula, once daily until day 42 Control (N = 26): maltodextrin placebo added to formula
Outcomes	• Microflora of stool measured on day 7, 21, and 42

Chrzanowska-Liszewska 2012 *(Continued)*

	• NEC • Death • Infection (courtesy of investigators)
Notes	Poland (2008 to 2009) Funding: not stated

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Low risk	Coded capsules containing probiotics or placebo
Blinding (performance bias and detection bias) All outcomes	Low risk	Masked
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Low risk	Unlikely

Costalos 2003

Study characteristics

Methods	RCT
Participants	87 formula-fed infants of gestational age at birth 28 to 32 weeks.
Interventions	Probiotics (N = 51): *Saccharomyces boulardii* added to formula every 12 hours during the 1st week of life when enteral feed are tolerated for 30 days Control (N = 36): maltodextrin placebo
Outcomes	• NEC • Death • Infection • Weight gain
Notes	Greece (period of study: not specified) Funding: not stated

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Not described

Costalos 2003 *(Continued)*

Allocation concealment (selection bias)	Low risk	Cards with allocation in sealed envelopes
Blinding (performance bias and detection bias) All outcomes	Low risk	Masked
Incomplete outcome data (attrition bias) All outcomes	Low risk	Near-complete (5 infants with incomplete data were not included in analyses)
Selective reporting (reporting bias)	Low risk	Unlikely

Costeloe 2015

Study characteristics	
Methods	RCT
Participants	1310 infants born before 31 weeks' gestation
Interventions	Probiotics (N = 650): *Bifidobacterium breve BBG-001* once daily until 36 weeks' postmenstrual age or discharge from hospital Control (N = 660): corn starch placebo
Outcomes	• NEC • Death • Infection
Notes	UK (24 neonatal units; 2010 to 2013) Funding: by UK National Institute for Health Research Health Technology Assessment programme (ISRCTN 05511098)

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Low risk	Web-based
Blinding (performance bias and detection bias) All outcomes	Low risk	Masked
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete

Costeloe 2015 *(Continued)*

Selective reporting (reporting bias)	Low risk	No

Dani 2002

Study characteristics	
Methods	RCT
Participants	585 VLBW infants (or < 33 weeks' gestation at birth)
Interventions	Probiotics (N = 295): *Lactobacillus rhamnosus GG* added to milk (human or formula) feeds once daily until hospital discharge Control (N = 290): maltodextrin placebo
Outcomes	• NEC • Death • Infection • Duration hospitalisation
Notes	Italy (12 centres; study period not specified) Funding: not stated

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Not described
Allocation concealment (selection bias)	Low risk	Sealed envelope containing allocation
Blinding (performance bias and detection bias) All outcomes	Low risk	Masked (placebo-controlled)
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Low risk	Unlikely

Dashti 2014

Study characteristics	
Methods	RCT
Participants	136 preterm infants of birth weight 700 g to 1800 g (most participants very preterm or VLBW)

Dashti 2014 *(Continued)*

Interventions	Probiotics (N = 69): *Lactobacillus acidophilus*, *L. rhamnosus*, *L. bulgaricus*, *L. casei*, *Streptococcus thermophilus*, *Bifidobacterium longum*, *B. breve* added to milk feeds once daily until hospital discharge Control (N = 67): placebo powder (not described)
Outcomes	• NEC • Death • (infection data sought from investigators July 2020)
Notes	Iran (2010 to 2011) Funding: not stated

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Not described
Allocation concealment (selection bias)	Unclear risk	Not described
Blinding (performance bias and detection bias) All outcomes	Low risk	Masked (placebo-controlled)
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Complete
Selective reporting (reporting bias)	Low risk	Unlikely

Demirel 2013

Study characteristics

Methods	RCT
Participants	271 VLBW infants (gestational age ≤ 32 weeks at birth)
Interventions	Probiotics (N = 135): *Saccharomyces boulardii* added to human milk or formula once a day, starting with the 1st feed, until hospital discharge Control (N = 136): unsupplemented milk
Outcomes	• NEC • Death • Infection
Notes	Turkey (2011) Funding: not stated ClinicalTrials.gov Identifier: NCT01315821

Demirel 2013 *(Continued)*

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Low risk	Allocations sealed in opaque, sequentially-numbered envelopes
Blinding (performance bias and detection bias) All outcomes	High risk	Unmasked
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Low risk	Unlikely

Dilli 2015

Study characteristics	
Methods	RCT
Participants	200 very preterm or VLBW infants
Interventions	Probiotics (N = 100): *Bifidobacterium lactis* added to human milk or formula once daily for 8 weeks (or hospital discharge) Control (N= 100): maltodextrin powder placebo
Outcomes	• NEC • Death • Infection • Length of hospital stay
Notes	Turkey (5 centres: 2011 to 2014) Funding: not stated NB. This was a 4-arm RCT- 2 other groups were prebiotic (N = 100) and synbiotic (n + 100)

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Low risk	Sealed opaque envelopes

Dilli 2015 *(Continued)*

Blinding (performance bias and detection bias) All outcomes	Low risk	Masked (placebo-controlled)
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Low risk	Unlikely

Dutta 2015

Study characteristics	
Methods	RCT
Participants	149 infants (27 to 33 weeks' gestation at birth)
Interventions	Probiotics (N = 114): *Lactobacillus acidophilus, L. rhamnosus, Bifidobacterium longum, and Saccharomyces boulardii* (3 groups: (quote:) "low-dose" (10^9) for 21 days or quote:) "high-dose" (10^{10}) 2 times daily with human milk or formula feeds for 14 or 21 days Control (N = 35): maltodextrin placebo for 21 days
Outcomes	• Probiotic stool colonisation • NEC • Mortality • Infection
Notes	India (study period not stated) Funding: Aristo Pharmaceuticals Pvt Ltd, Madhya Pradesh, India provided the sachets of probiotics and placebo free of cost

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Unclear risk	Not described
Blinding (performance bias and detection bias) All outcomes	Low risk	Masked
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Low risk	Unlikely

Fernández-Carrocera 2013

Study characteristics		
Methods	RCT	
Participants	150 VLBW infants	
Interventions	Probiotics (N = 75): *Lactobacillus rhamnosus, L. casei, L. plantarum, L acidophilus, Bifidobacteruim infantis*, and *Streptococcus thermophilus* added to human milk or formula (duration intervention not stated) Control (N = 75): unsupplemented milk feeds	
Outcomes	• NEC • Death • Infection	
Notes	Mexico (2007 to 2010) Funding: not stated	
Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Random number table
Allocation concealment (selection bias)	Low risk	Staff unable to predict allocation by number
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Unsupplemented milk feeds- not placebo-controlled
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Low risk	Unlikely

Fujii 2006

Study characteristics	
Methods	Quasi-RCT
Participants	19 preterm infants (most very preterm or VLBW)
Interventions	Probiotics group (N = 11): *Bifidobacterium breve* 2 times daily with human milk or formula feeds until hospital discharge Control (N = 8): unsupplemented milk feeds
Outcomes	• Cytokine levels in plasma • NEC

Fujii 2006 *(Continued)*

	• Death • Infection
Notes	Japan (2000 to 2002) Published: 2004 Funding: Morinaja Milk industry and Meiji Dairies (manufactured intervention)

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Not described
Allocation concealment (selection bias)	Unclear risk	Not described
Blinding (performance bias and detection bias) All outcomes	High risk	Unmasked
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Complete
Selective reporting (reporting bias)	Unclear risk	Unclear

Hariharan 2016

Study characteristics

Methods	RCT
Participants	196 very preterm infants with birth weight < 1250 g
Interventions	Probiotics (N = 93): *Lactobacillus acidophilus, Bifidobacterium bifidum, Saccharomyces boulardii* 2 times daily in milk feeds for 6 weeks Control (N = 103): unsupplemented feeds
Outcomes	• NEC • Death • Infection
Notes	India (study period not stated) Funding: Not stated

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Not described

Hariharan 2016 *(Continued)*

Allocation concealment (selection bias)	Unclear risk	Not described
Blinding (performance bias and detection bias) All outcomes	High risk	Unmasked
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Unclear risk	Unclear

Hays 2015

Study characteristics	
Methods	RCT
Participants	199 very preterm infants (gestation at birth 25 to 31 weeks), and birth weight 700 g to 1600 g that was appropriate for gestational age
Interventions	Probiotics (3 groups: N = 145): *Bifidobacterium lactis*, or *B. longum*, or both once daily in sterile water for 4 to 6 weeks (depending on gestation at birth) Control (N = 52): maltodextrin placebo
Outcomes	• NEC • Death • Infection
Notes	France (three centres: 2007 to 2010) Funding: Nestle France (Marne-la-Vallee, France) and Nestec (Vevey, Switzerland)

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Low risk	Consecutively numbered, sealed, opaque envelopes
Blinding (performance bias and detection bias) All outcomes	Low risk	Masked (placebo-controlled)
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete

Hays 2015 *(Continued)*

Selective reporting (reporting bias)	Low risk	Unlikely

Hernandez-Enriquez 2016

Study characteristics	
Methods	RCT
Participants	44 preterm infants < 34 weeks' gestation or ≤ 1550 g birth weight (most infants very preterm or VLBW)
Interventions	Intervention (N = 24): *Lactobacillus reuteri* once daily for 1st 10 days after birth Control (N = 20): placebo (sterile water)
Outcomes	• NEC • Death • Infection (data courtesy of investigators)
Notes	Mexico (2012 to 2013) Funding: not stated

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Quote: "Simple randomisation sequence"
Allocation concealment (selection bias)	Low risk	Seaed opaque envelopes
Blinding (performance bias and detection bias) All outcomes	High risk	Unmasked
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Complete
Selective reporting (reporting bias)	Unclear risk	Unlikely

Hikaru 2010

Study characteristics	
Methods	RCT
Participants	208 VLBW infants

Hikaru 2010 *(Continued)*

Interventions	Probiotics (N = 108): *Bifidobacterium breve* in human milk or formula once daily until discharge from the intensive care unit Control (N = 100): unsupplemented milk feeds
Outcomes	• Infection. (NEC not reported)
Notes	Japan (2001 to 2013) Funding: Morinaga Milk Industry Co. Ltd. (supplied *Bifidobacterium breve* preparation)

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Not described
Allocation concealment (selection bias)	Unclear risk	Not described
Blinding (performance bias and detection bias) All outcomes	High risk	Unmasked
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Unclear risk	Unclear

Huang 2009

Study characteristics

Methods	RCT
Participants	183 VLBW infants who survived 7 days after birth and began enteral feeding
Interventions	Probiotics (N = 95): *Bifidobacterium adolescentis* twice daily with milk feeds daily for 7 days Control (N = 88): unsupplemented milk feeds
Outcomes	NEC (unclear whether death or infection assessed)
Notes	China (single centre, study dates not stated) Translation from Chinese courtesy of Yuan Chi

Risk of bias

Bias	Authors' judgement	Support for judgement

Huang 2009 *(Continued)*

Random sequence generation (selection bias)	Unclear risk	Not described
Allocation concealment (selection bias)	Unclear risk	Not described
Blinding (performance bias and detection bias) All outcomes	High risk	Unmasked
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Unable to assess
Selective reporting (reporting bias)	Unclear risk	Mortality and infection not reported

Indrio 2017

Study characteristics	
Methods	RCT
Participants	60 preterm infants of gestational age 28 to 32 weeks' at birth
Interventions	Probiotics (N = 30): *Lactobacillus reuteri* DSM 17938 suspended in sunflower and medium-chain triglyceride oils, given once daily until day 30 Control (N = 30): identical oils without probiotics
Outcomes	• NEC • Death • Infection • Duration of hospital stay (data courtesy of personal communication from investigators)
Notes	Italy (2011 to 2012) Funding: University of Bari, Italy ClinicalTrials.gov no. NCT00985816

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Unclear risk	Not described
Blinding (performance bias and detection bias) All outcomes	Low risk	Masked (placebo-controlled)

Indrio 2017 *(Continued)*

Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Unclear risk	Unlikely

Jacobs 2013

Study characteristics	
Methods	RCT
Participants	1099 very preterm VLBW infants
Interventions	Probiotics (N = 548): *Bifidobacterium infantis*, *Streptococcus thermophilus* and *B. lactis* once daily in human milk or formula until discharge from hospital or term corrected age. Control (N = 551): maltodextrin powder placebo
Outcomes	• NEC • Death • Infection • Infection with a probiotic species • Duration of birth hospitalisation • Major neurodevelopmental impairment comprised any of: moderate or severe cerebral palsy, Bayley-III Motor Composite Scale < –2SD (or Movement Assessment Battery for Children < 15th centile if > 42 months' post-term), Bayley-III Composite Cognitive or Language Scales <–2 SD (or Wechsler Preschool and Primary Scale of Intelligence Full Scale Intelligence Quotient <–2 SD if > 42 months' post-term), blindness or deafness
Notes	Australasia (10 centres; 2007 to 2011) Funding: National Health and Research Medical Council, Australia

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Low risk	Central allocation
Blinding (performance bias and detection bias) All outcomes	Low risk	Masked (placebo-controlled)
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete for in hospital outcomes (Neurodevelopmental assessment = 48%)
Selective reporting (reporting bias)	Low risk	Unlikely

Kanic 2015

Study characteristics	
Methods	RCT
Participants	80 VLBW infants
Interventions	Probiotics (N = 40): *Lactobacillus acidophilus, Enterococcus faecium, Bifidobacterium infantis* 2 times daily with milk feeds until discharge from hospital Control (N = 40): unsupplemented milk feeds
Outcomes	• NEC • Death • Infection • Duration of birth hospitalisation
Notes	Slovenia (2008 to 2011) Funding: not stated

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	High risk	Alternate allocation (quote: "quasi-randomised")
Allocation concealment (selection bias)	High risk	Unconcealed
Blinding (performance bias and detection bias) All outcomes	High risk	Unmasked
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Low risk	Unlikely

Kitajima 1997

Study characteristics	
Methods	RCT
Participants	91 VLBW infants
Interventions	Probiotics (N = 45): *Bifidobacterium breve* in distilled water once daily for 28 days Control (N = 46): distilled water
Outcomes	• Probiotic colonisation of stool

Kitajima 1997 *(Continued)*

	(NEC, death, infection- data courtesy of investigators)	
Notes	Japan (1990 to 1991) Funding: not stated	
Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Not described
Allocation concealment (selection bias)	Unclear risk	Not described
Blinding (performance bias and detection bias) All outcomes	High risk	Unmasked
Incomplete outcome data (attrition bias) All outcomes	Low risk	Near-complete (4 participants not included in analyses)
Selective reporting (reporting bias)	Low risk	Data

Li 2019

Study characteristics		
Methods	RCT	
Participants	30 VLBW infants	
Interventions	Probiotics (N = 16): *Lactobacillus plantarum*, *Bifidobacterium longum*, *B. bifidum* once daily with milk feeds until 36 weeks' postmenstrual age. Control (N = 14): 5% glucose solution	
Outcomes	• Change of gut microbiota • Correlation of gut microbial composition • Levels of cytokines (NEC, death, infection not reported (author contacted in May 2020))	
Notes	China (2014 to 2015) Funding: not stated	
Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer-generated

Li 2019 *(Continued)*

Allocation concealment (selection bias)	Unclear risk	Quote: "Concealed by the principal investigator according to sequential numbers"
Blinding (performance bias and detection bias) All outcomes	Low risk	Masked (intervention and control solutions identical)
Incomplete outcome data (attrition bias) All outcomes	High risk	> 50% outcome data unreported
Selective reporting (reporting bias)	Unclear risk	Unable to determine

Lin 2005

Study characteristics	
Methods	RCT
Participants	367 VLBW infants
Interventions	Probiotics (N = 180): *Lactobacillus acidophilus* and *Bifidobacterium. infantis (*Infloran®) 2 times daily with human milk until discharge from hospital Control (N = 187): unsupplemented milk feeds (no placebo)
Outcomes	• NEC • Death • Infection • Duration of hospitalisation • Neurodevelopmental impairment at aged 3 years, defined as 1 or more of: BSID-II MDI < 70, PDI < 70, bilateral blindness, bilateral hearing impairment requiring amplification, or moderate or severe cerebral palsy (requiring ambulatory assistance)
Notes	Taiwan (1999 to 2003) Funding: Research Department of China Medical University Hospital

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Random-number table
Allocation concealment (selection bias)	Low risk	Opaque, sequentially numbered, sealed envelopes
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Unsupplemented milk feeds- not placebo-controlled (investigators aware of allocation)
Incomplete outcome data (attrition bias) All outcomes	Low risk	Near-complete (90% for neurodevelopmental assessments)

Lin 2005 *(Continued)*

Selective reporting (reporting bias)	Low risk	Unlikely

Lin 2008

Study characteristics	
Methods	RCT
Participants	434 VLBW infants
Interventions	Probiotics (N = 217): *Bifidobacterium bifidum* and *Lactobacillus acidophilus*, added to human milk or formula 2 times daily for 6 weeks Control (N = 217): unsupplemented milk feeds
Outcomes	• NEC • Death • Infection
Notes	Taiwan (7 centres: 2005 to 2007) Funding: National Science Council of Taiwan ClinicalTrials.gov Identifier: NCT00540033

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Low risk	Allocated centrally
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Unsupplemented milk feeds- not placebo-controlled
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Low risk	Unlikely

Manzoni 2006

Study characteristics	
Methods	RCT
Participants	80 VLBW infants

Manzoni 2006 *(Continued)*

Interventions	Probiotics (N = 39): *Lactoacillus casei subspecies rhamnosus* with human milk until 6 weeks or hospital discharge Control (N = 41): unsupplemented milk feeds
Outcomes	• NEC • Death • Infection
Notes	Italy (2004 to 2005) Funding: not stated

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Unclear risk	Not described.
Blinding (performance bias and detection bias) All outcomes	High risk	Unmasked
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Low risk	Unlikely

Manzoni 2009

Study characteristics

Methods	RCT
Participants	485 VLBW infants
Interventions	Probiotics (N = 238): *Lactoacillus casei subspecies rhamnosus* with human milk or formula until 4 (VLBW) or 6 (ELBW) weeks plus bovine lactoferrin (100 mg/day) Control (N = 247): bovine lactoferrin alone (All doses including placebo were diluted in prepared milk so as to maintain masking)
Outcomes	• NEC • Death • Infection
Notes	Italy (11 centres: 2007 to 2008) Funding: Dicofarm SpA (manufacturer of intervention)

Risk of bias

Manzoni 2009 *(Continued)*

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Low risk	Pharmacy allocation (remote)
Blinding (performance bias and detection bias) All outcomes	Low risk	Masked
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Unclear risk	Data for invasive infection in complete cohort not reported in primary publication (available to derive from later publications)

Mihatsch 2010

Study characteristics	
Methods	RCT
Participants	180 VLBW infants (< 30 weeks' gestation)
Interventions	Probiotics (N = 91): *Bifidobacterium lactis* BB12 mixed with powdered fortifier in human milk or formula once daily for 6 weeks Control (N = 89): powdered fortifier placebo
Outcomes	• NEC • Death • Infection
Notes	Germany (2000 to 2003) Funding: Nestlé AG, Frankfurt, Germany

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Low risk	Sealed envelopes
Blinding (performance bias and detection bias) All outcomes	Low risk	Masked (placebo-controlled)
Incomplete outcome data (attrition bias)	Low risk	Complete

Mihatsch 2010 *(Continued)*

All outcomes		
Selective reporting (reporting bias)	Low risk	Unlikely

Millar 1993

Study characteristics	
Methods	RCT
Participants	20 infants < 33 weeks' gestation (most participants very preterm or VLBW)
Interventions	Probiotics (N = 10): *Lactobacillus rhamnosus GG* mixed with human milk or formula 2 times daily for 14 days, starting with 1st feed Control (N = 10): unsupplemented milk feeds
Outcomes	• Stool colonisation • Invasive infection (NEC, death (courtesy of investigators))
Notes	UK (1991 to 1992) Funding: Wessex Medical Trust

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Not described
Allocation concealment (selection bias)	Unclear risk	Not described
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Unsupplemented milk feeds- not placebo-controlled
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Low risk	Unlikely

Mohan 2006

Study characteristics	
Methods	RCT
Participants	69 preterm infants (most participants were very preterm or VLBW)

Mohan 2006 *(Continued)*

Interventions	Probiotics (N = 37): *Bifidobacterium lactis* in milk feeds from 1st day after birth for 21 days Control (N = 32): unsupplemented milk feeds
Outcomes	• No clinical outcomes were presented in the published data (NEC, death, infection (courtesy of investigators))
Notes	Germany (2003 to 2005) Funding: Nestlé, Konolfingen, Switzerland

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Unclear risk	Central allocation (web-based)
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Unsupplemented milk feeds- not placebo-controlled
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Low risk	Unlikely

Oncel 2014

Study characteristics

Methods	RCT
Participants	424 VLBW infants (and gestational age ≤ 32 weeks' at birth)
Interventions	Probiotics (N = 213) *Lactobacillus reuteri* DSM 17938 once daily with milk feeds until discharge from hospital Placebo (N = 211): placebo containing only oil base
Outcomes	• NEC • Death • Infection • Culture-proven infection with *L reuteri* • (duration of hospitalisation- presented as median/range) • Neurodevelopmental impairment at 18 to 24 months, defined as 1 or more of: BSID-II MDI < 70, PDI < 70, moderate-to-severe cerebral palsy, bilateral hearing impairment, or bilateral blindness
Notes	Turkey (2012 to 2013) Funding: not stated

Oncel 2014 *(Continued)*

	ClinicalTrials.gov Identifier: NCT01531179

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Low risk	Opaque, sequentially numbered sealed envelopes
Blinding (performance bias and detection bias) All outcomes	Low risk	Masked
Incomplete outcome data (attrition bias) All outcomes	Low risk	Near-complete (8 participants withdrawn by family) for in hospital outcomes (Neurodevelopmental assessment = 68%)
Selective reporting (reporting bias)	Low risk	Unlikely

Oshiro 2019

Study characteristics

Methods	RCT
Participants	35 VLBW infants
Interventions	Probiotics (N = 17): *Bifidobacterium breve* BBG-01 in human milk feeds once daily during the hospital stay Control (N = 18): placebo
Outcomes	• NEC • Death • Infection • Weight gain
Notes	Japan (2015 to 2017) Funding: Yakult Honsha Company, Japan (manufacturer of intervention) Additional data via personal communication: Dr Yuichiro Yamashiro UMIN Registration No. UMIN000005412

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated

Oshiro 2019 *(Continued)*

Allocation concealment (selection bias)	Low risk	Sealed, opaque envelopes
Blinding (performance bias and detection bias) All outcomes	Low risk	Masked (probiotic added to milk by dieticians who were not involved in the care of the infant)
Incomplete outcome data (attrition bias) All outcomes	Low risk	Masked
Selective reporting (reporting bias)	Low risk	Unlikely

Patole 2014

Study characteristics	
Methods	RCT
Participants	159 VLBW infants (< 33 weeks' gestation at birth)
Interventions	Probiotics (N = 79): *Bifidobacterium breve* M-16V in milk feeds once daily until term equivalent Control (N = 80): maltodextrin placebo
Outcomes	Probiotic colonisation of stool NEC, death, infection, blood culture-positive sepsis by *B. breve* M-16V (neurodevelopmental outcomes- Agrawal 2020)
Notes	Australia (2009 to 2012) Funding: Morinaga Milk Industry Company, Japan supplied the product free for the trial

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Low risk	Opaque, sealed, coded envelopes
Blinding (performance bias and detection bias) All outcomes	Low risk	Masked (placebo-controlled)
Incomplete outcome data (attrition bias) All outcomes	Low risk	Near-complete (6 infants withdrawn)
Selective reporting (reporting bias)	Low risk	Unlikely

Rehman 2018

Study characteristics	
Methods	RCT
Participants	146 VLBW preterm infants (gestational age at birth > 26 weeks')
Interventions	Probiotics (N = 70): *Bifidobacterium spp* (not specified), *Lactobacilli acidophilhis, Streptococcus thermophilus, L. delbrueckii* with human milk or formula 2 times daily until hospital discharge Control (N = 70): unsupplemented milk feeds
Outcomes	• NEC • Death (data courtesy of investigators)
Notes	Pakistan (2014) Funding: not stated

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Unclear risk	Not described
Blinding (performance bias and detection bias) All outcomes	High risk	Unmasked
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Unclear risk	Infection not reported

Ren 2010

Study characteristics	
Methods	RCT
Participants	150 preterm infants (most participants were very preterm)
Interventions	Probiotics (N = 79): *Bifidobacterium infantis, Lactobacillus acidophilus, Bacillus cereus,* and *Enterococcus faecalis* in milk feeds twice daily from day 7 after birth for 7 days (route translated as "oral or nasal"- presumed to refer to oro-gastric or naso-gastric tube)

Ren 2010 *(Continued)*

	Control (N = 80): unsupplemented milk feeds	
Outcomes	NEC	
Notes	China (single centre, 2006-2008) Translation from Chinese courtesy of Yuan Chi	
Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	"Drawing lots"
Allocation concealment (selection bias)	Unclear risk	Safeguards unclear
Blinding (performance bias and detection bias) All outcomes	High risk	Unmasked
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Unable to assess
Selective reporting (reporting bias)	Unclear risk	Mortality and infection not reported

Reuman 1986

Study characteristics		
Methods	Quasi-RCT	
Participants	30 very preterm infants (birth weight < 2000 g)	
Interventions	Probiotics (N = 15): *Lactobacillus acidophilus* in formula daily for 28 days Control (N = 15): unsupplemented formula feeds	
Outcomes	• Stool colonisation • NEC • Death • Duration of hospitalisation • Rate of weight gain	
Notes	US (early 1980s) Funding: not stated	
Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**

Reuman 1986 *(Continued)*

Random sequence generation (selection bias)	High risk	Random number charts and the last digit of patient's chart number, then alternate allocation of next participant
Allocation concealment (selection bias)	High risk	Unconcealed
Blinding (performance bias and detection bias) All outcomes	High risk	Unmasked
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Unclear risk	Infection not reported

Rougé 2009

Study characteristics		
Methods	RCT	
Participants	94 very preterm or VLBW infants	
Interventions	Probiotics (N = 45): *Lactobacillus rhamnosus* GG and *Bifidobacterium longum* with human milk or formula once daily until discharge from hospital Control (N = 49): maltodextrin placebo	
Outcomes	• NEC • Death • Infection • Duration of hospital stay	
Notes	France (2005 to 2007) Funding: Programme Hospitalier de Recherche Clinique of the French Ministry of Health	
Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Low risk	Centrally allocated
Blinding (performance bias and detection bias) All outcomes	Low risk	Masked (placebo-controlled)
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete

Rougé 2009 *(Continued)*

Selective reporting (reporting bias)	Low risk	Unlikely

Roy 2014

Study characteristics	
Methods	RCT
Participants	112 preterm VLBW infants
Interventions	Probiotics (N = 56): *Lactobacillus acidophilus, Bifidobacterium longum, B. bifidum, B. lactis 2 times* daily with human milk for 6 weeks or until discharged from hospital Control (N = 56): sterile water as (quote:) "placebo"
Outcomes	• NEC • Death • Infection
Notes	India (2012 to 2013) Funding: none

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Low risk	Centrally allocated
Blinding (performance bias and detection bias) All outcomes	Low risk	Masked
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Low risk	Unlikely

Sadowska-Krawczenko 2012

Study characteristics	
Methods	RCT
Participants	55 very preterm or VLBW infants

Sadowska-Krawczenko 2012 *(Continued)*

Interventions	Probiotics (N = 30): *Lactobacillus rhamnosus* 2 times daily in 2 mL of 5% dextrose until discharge from hospital Control (N = 25): maltodextrin placebo
Outcomes	• NEC • Death • Infection
Notes	Poland (2008 to 2009) Funding: Biomed Lublin, Poland supplied the intervention

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Low risk	Central allocation
Blinding (performance bias and detection bias) All outcomes	Low risk	Masked (placebo-controlled)
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Low risk	Unlikely

Saengtawesin 2014

Study characteristics

Methods	RCT
Participants	60 VLBW infants with gestational age ≤ 34 weeks' at birth
Interventions	Probiotics (N = 31): *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (Infloran®) once daily with human milk or formula until 6 weeks or hospital discharge Control (N = 29): unsupplemented milk feeds
Outcomes	• NEC • Death • Infection • Probiotic (quote:) "sepsis" • Duration of hospitalisation
Notes	Thailand (2012 to 2013)

Saengtawesin 2014 *(Continued)*

	Funding: Queen Sirikit National Institute of Child Health, Perinatal Society of Thailand and DKSH (Thailand) Limited

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Not described
Allocation concealment (selection bias)	Unclear risk	Not described
Blinding (performance bias and detection bias) All outcomes	High risk	Unmasked
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Low risk	Unlikely

Samanta 2009

Study characteristics

Methods	RCT
Participants	186 very preterm or VLBW infants
Interventions	Probiotics (N = 91): *Bifidobacteria infantis, B. bifidum, B. longum* and *Lactobacillus acidophilus* with human milk 2 times daily until hospital discharge Control (N = 95): unsupplemented human milk feeds
Outcomes	• NEC • Death • Infection • Duration of hospital stay
Notes	India (2007 to 2008) Funding: not stated

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Not described.
Allocation concealment (selection bias)	Unclear risk	Not described.

Samanta 2009 *(Continued)*

Blinding (performance bias and detection bias) All outcomes	High risk	Unmasked
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Low risk	Unlikely

Sari 2011

Study characteristics

Methods	RCT
Participants	221 VLBW infants (gestational age < 33 weeks' at birth)
Interventions	Probiotics (N = 110): *Lactobacillus sporogenes* in human milk or formula once daily until discharge from hospital Control (N = 111): unsupplemented milk feeds
Outcomes	• NEC • Death • Infection • Rate of weight gain • Neurodevelopmental impairment at 18 to 24 months' post-term, defined as one or more of: BSID-II MDI < 70, PDI < 70, cerebral palsy, bilateral blindness, or hearing impairment requiring amplification in both ears
Notes	Turkey (2008 to 2009) Funding: not stated

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Low risk	Opaque, sequentially numbered, sealed envelopes
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Caregivers masked, investigators not masked
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete for in hospital outcomes (Neurodevelopmental assessment = 84%)
Selective reporting (reporting bias)	Low risk	Unlikely

Serce 2013

Study characteristics	
Methods	RCT
Participants	208 very preterm or VLBW infants
Interventions	Probiotics (N = 104): *Saccharomyces boulardii* in human milk or formula once daily until discharge from hospital Control (N = 104): unsupplemented milk feeds
Outcomes	• NEC • Death • Infection • Rate of weight gain • Duration of hospitalisation • Culture proven *Saccharomyces boulardii* (quote:) "sepsis"
Notes	Turkey (2010 to 2011) Funding: Biocodex supplied the intervention

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Low risk	Opaque, sequentially-numbered, sealed envelopes.
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Unsupplemented milk feeds- not placebo-controlled
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Low risk	Unlikely

Shadkam 2015

Study characteristics	
Methods	RCT
Participants	60 preterm infants born between 28 to 34 weeks' gestation and birth weight 1000 g to 1800 g (most participants were very preterm or VLBW)
Interventions	Probiotics (N = 30): *Lactobacillus reuteri* DSM 17938 2 times daily with human milk until full enteral feeding was reached (about 2 weeks)

Shadkam 2015 *(Continued)*

	Control (N =30): unsupplemented milk feeds
Outcomes	• NEC • Death • Infection
Notes	Iran (2012 to 2013) Funding: Shahid Sadughi University, Iran

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	States that random allocation software was used
Allocation concealment (selection bias)	Unclear risk	No information on concealment
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Unsupplemented milk feeds- not placebo-controlled
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Low risk	Unlikely

Shashidhar 2017

Study characteristics

Methods	RCT
Participants	104 VLBW infants
Interventions	Probiotics (N = 52): *Lactobacillus acidophilus, L. rhamnosus, Bifidobacterium longum* and *Saccharomyces boulardii* (Darolac) once daily in human milk until discharge from hospital Control (N = 52): unsupplemented milk feeds
Outcomes	• NEC • Death • Duration of hospital stay
Notes	India (2012 to 2013) Funding: not stated

Risk of bias

Bias	Authors' judgement	Support for judgement

Shashidhar 2017 *(Continued)*

Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Low risk	Sequentially-numbered, opaque. sealed envelopes
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Unsupplemented milk feeds- not placebo-controlled
Incomplete outcome data (attrition bias) All outcomes	Low risk	Near-complete (3 infants in each group withdrawn)
Selective reporting (reporting bias)	Low risk	Unlikely

Stratiki 2007

Study characteristics

Methods	RCT
Participants	77 preterm infants with gestation at birth > 26 weeks' (most participants were very preterm or VLBW)
Interventions	Probiotics (N = 41): *Bifidobacterium lactis* supplemented formula for 30 days Control (N = 36): unsupplemented formula feeds
Outcomes	• Stool colonisation • Intestinal permeability • NEC • Death • Infection • Rate of weight gain
Notes	Greece (2004 to 2005) Funding: Nestlé, Vevey provide the *B. lactis* supplemented formula

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Random numbers generator
Allocation concealment (selection bias)	Unclear risk	Not described
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Unsupplemented milk feeds- not placebo-controlled
Incomplete outcome data (attrition bias)	Low risk	Near-complete (3 infants not included in analyses)

Stratiki 2007 *(Continued)*

All outcomes		
Selective reporting (reporting bias)	Low risk	Unlikely

Strus 2018

Study characteristics		
Methods	RCT	
Participants	181 preterm infants ≤ 34 weeks' gestation and birth weight 750 g to 1800 g (most participants were very preterm or VLBW)	
Interventions	Probiotics (N = 90): *Lactobacillus rhamnosus* KL53A and *Bifidobacterium breve* PB04 in milk feeds for 6 weeks or until hospital discharge Control (N = 91): maltodextrin placebo	
Outcomes	• Stool colonisation • NEC • Death • Infection	
Notes	Poland (2012 to 2013) Funding: IBSS BIOMED S.A., Krakow, Poland ClinicalTrials.gov no. NCT02073214	

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer-generated sequence
Allocation concealment (selection bias)	Low risk	Centrally allocated
Blinding (performance bias and detection bias) All outcomes	Low risk	Masked (placebo-controlled)
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Low risk	Unlikely

Tewari 2015

Study characteristics	
Methods	RCT
Participants	244 preterm infants < 34 weeks' gestation at birth (most participants were very preterm or VLBW)
Interventions	Probiotics (N = 121): *Bacillus clausii* 3 times daily with human milk for 6 weeks, or until discharge or death or occurrence of late-onset invasive infection Control (N= 123): sterile water placebo (probiotic and the placebo were identical in appearance)
Outcomes	NEC, death, infection, duration of hospital stay
Notes	India (2012 to 14) Funding: Enterogermina, Sanofi-Aventis, Italy supplied intervention

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Low risk	Web-based
Blinding (performance bias and detection bias) All outcomes	Low risk	Masked
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Low risk	Unlikely

Totsu 2014

Study characteristics	
Methods	Cluster-RCT
Participants	283 VLBW infants in 19 neonatal centres
Interventions	Probiotics (N = 10 centres; 153 infants*): *Bifidobacterium bifidum* with human milk or formula feeds 2 times daily until infant reached 2000 g body weight Control (N = 9 centres; 130 infants*): maltodextrin placebo *Inter-cluster correlation correction of data for inclusion in meta-analyses achieved by dividing numerators and denominator by the design effect (1.2779): Probiotics: adjusted N = 120 for in hospital outcomes; N = 80 for neurodevelopmental assessment outcomes

Totsu 2014 *(Continued)*

	Control: adjusted N = 102 for in hospital outcomes; N = 82 for neurodevelopmental assessment outcomes
Outcomes	• NEC • Death • Infection • Duration of hospital stay • Rate of weight gain • Neurodevelopmental impairment at 18 months, defined as Kyoto Scale of Psychological Development 2001 developmental quotient < 70, hearing (bilateral aids) or visual impairment, cerebral palsy (Gross Motor Function Classification System level II or greater)
Notes	Japan (19 centres: 2010 to 2011) Funding: Meiji, Tokyo, Japan

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer-generated (stratified by (quote: "patient volume" of centre)
Allocation concealment (selection bias)	Unclear risk	Not described
Blinding (performance bias and detection bias) All outcomes	Low risk	Masked (placebo-controlled)
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete for in hospital outcomes (Neurodevelopmental assessment = 73%)
Selective reporting (reporting bias)	Low risk	Unlikely

Van Niekerk 2014

Study characteristics	
Methods	RCT
Participants	184 VLBW infants (< 1250 g)
Interventions	Probiotics (N = 91): *Lactobacillus rhamnosus* GG and *Bifidobacterium infantis* daily with human milk feeds for 4 weeks Control (N = 93): MCT oil placebo in milk feeds
Outcomes	• NEC • Death • Infection
Notes	South Africa (2011 to 2012)

Van Niekerk 2014 *(Continued)*

	Funding: National Research Foundation, Nestle Nutrition Institute Africa, Medical Research Council and the Faculty of Medicine and Health Sciences, Stellenbosch University ClinicalTrials.gov no. NCT01868737

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Independent statistician-generated
Allocation concealment (selection bias)	Low risk	Pharmacy allocation (stratified by maternal HIV status)
Blinding (performance bias and detection bias) All outcomes	Low risk	Masked (placebo-controlled)
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Low risk	Unlikely

Wang 2007

Study characteristics

Methods	Quasi-RCT
Participants	44 VLBW infants
Interventions	Probiotics (N = 22): *Bifidobacterium breve* in milk feeds 2 times daily until hospital discharge Control (N = 33): unsupplemented milk feeds
Outcomes	• Short chain fatty acid and faecal lactic acid concentration • Infection (NEC (courtesy of investigators))
Notes	Japan (2001 to 2004) Funding: intervention provided by Morinaga Milk Industry, Kanagawa, Japan

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	High risk	Alternate allocation
Allocation concealment (selection bias)	High risk	Unconcealed

Wang 2007 *(Continued)*

Blinding (performance bias and detection bias) All outcomes	High risk	Unmasked
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete
Selective reporting (reporting bias)	Low risk	Unlikely (did not aim to assess clinical outcomes)

Wejryd 2019

Study characteristics	
Methods	RCT
Participants	141 ELBW infants (of gestation born < 28 weeks')
Interventions	Probiotics (N = 72): *Lactobacillus reuteri* DSM 17938 once daily with human milk until 36 weeks' postmenstrual age Control (N = 69): maltodextrin placebo
Outcomes	• Time to full enteral feeds • NEC • Death • Infection
Notes	Sweden (10 centres: 2012 to 2015) Funding: Swedish Research Council, the Swedish Society for Medical Research, the Swedish Society of Medicine, the Research Council for the South-East Sweden, ALF Grants, Region Ostergotland, the Ekhaga Foundation, and BioGaia AB ClinicalTrials.gov no. NCT01603368

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Low risk	Centrally coded by sequential study number
Blinding (performance bias and detection bias) All outcomes	Low risk	Masked (placebo-controlled)
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete

Wejryd 2019 *(Continued)*

Selective reporting (reporting bias)	Low risk	Unlikely

Zeber-Lubecka 2016

Study characteristics	
Methods	RCT
Participants	55 preterm infant < 33 weeks' gestation (most participants were very preterm or VLBW)
Interventions	Probiotics (N = 28): *Saccharomyces boulardii* once daily with human milk or formula feeds for six weeks Control (N = 27): maltodextrin placebo
Outcomes	• Stool microbiomic structure (NEC, death, infection- no events courtesy investigators)
Notes	Poland (study period not stated) Funding: The National Science Centre, Poland

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Not described (quote: "randomly divided")
Allocation concealment (selection bias)	Unclear risk	Not described
Blinding (performance bias and detection bias) All outcomes	Low risk	Masked (placebo-controlled)
Incomplete outcome data (attrition bias) All outcomes	High risk	Missing data from each group (10 from probiotics and 6 from placebo) – not accounted for
Selective reporting (reporting bias)	Low risk	Unlikely (primary aim to study intestinal microbiome)

BBG-01: Bifidobacterium breve;**BSID:** the Bayley Scales of Infant Development; **ELBW:** extremely low birth weight; **g:** gram(s); **HIV:** human immunodeficiency virus; **LGG:** *Lactobacillus rhamnosus* GG; **MCT:** medium chain triglycerides; **MDI:** Mental Developmental Index; **NEC:** necrotising enterocolitis; **PDI:** Psychomotor Development Index; **RCT:** randomised controlled trial; **SD:** standard deviation; **VLBW**: very low birth weight.

Characteristics of excluded studies *[ordered by study ID]*

Study	**Reason for exclusion**
Arora 2017	Most participants not very preterm or VLBW.

Study	Reason for exclusion
Awad 2010	Most participants not very preterm or VLBW.
Chi 2019	Not an RCT.
Dasopoulou 2015	RCT of *pre*biotics.
Deng 2010	Most participants not very preterm or VLBW.
Denkel 2016	Not an RCT.
Di 2010	Most participants not very preterm or VLBW.
Dongol-Singh 2017	Most participants not very preterm or VLBW.
Hua 2014	Most participants not very preterm or VLBW.
Hussain 2016	Most participants not very preterm or VLBW.
Kaban 2019	Most participants not very preterm or VLBW.
Ke 2008	Most participants not very preterm or VLBW.
Koksal 2015	RCT of *syn*biotics
Moles 2015	A pilot study with including 5 infants.
Partty 2013	Most participants not very preterm or VLBW.
Qiao 2017	Most participants not very preterm or VLBW.
Rojas 2012	Most participants not very preterm or VLBW.
Romeo 2011	Most participants not very preterm or VLBW.
Shujie 2011	Most participants not very preterm or VLBW.
Sinha 2015	Most participants not very preterm or VLBW.
Thanhaeuser 2014	Not an RCT.
Uhlemann 1999	Most participants not very preterm or VLBW.
Underwood 2014	RCT of *pre*biotics
Xu 2016	Most participants not very preterm or VLBW.
Zhou 2012	Most participants not very preterm or VLBW.
Zhuang 2007	Most participants not very preterm or VLBW.

RCT: randomised controlled trial; **VLBW:** very low birth weight

Characteristics of studies awaiting classification *[ordered by study ID]*

Coleta 2013

Methods	Randomised controlled trial
Participants	60 preterm infants
Interventions	Probiotics (N = 31): human milk with *Lactobacillus reuteri* Control (N = 21): human milk alone
Outcomes	Efficacy of probiotics on digestive tolerance to enteral feeding
Notes	Romania (study period not stated) Unlikley to have been reported fully (unable to contact investigators)

Punnahitananda 2006

Methods	RCT
Participants	VLBW infants
Interventions	*Lactobacillus acidophilus* and *Bifidobacterium infantis*
Outcomes	Late-onset infection, NEC, feeding tolerance, time to full enteral feeding
Notes	Data presented at 14th Congress of the Federation of Asia Oceania Perinatal Societies, 2006, Bangkok,Thailand (report not available)

NEC: necrotising enterocolitis; **RCT:** randomised controlled trial; **VLBW:** very low birth weight

Characteristics of ongoing studies *[ordered by study ID]*

Marisen 2019

Study name	Efficacy of *Bifidobacterium longum*, *B. infantis* and *Lactobacillus acidophilus* probiotics to prevent gut dysbiosis in preterm infants of 28- 32 weeks' gestation: a randomised, placebo-controlled, double-blind, multicentre trial: the PRIMAL Clinical Study protocol
Methods	RCT
Participants	Preterm infants (28 to 32 weeks')
Interventions	*Bifidobacterium longum, B. infantis,* and *Lactobacillus acidophilus*
Outcomes	Stool colonisation
Starting date	2020
Contact information	Christoph Hartel, Department of Paediatrics, University of Lübeck, Germany
Notes	Trial registration number: DRKS00013197

NCT00977912

Study name	Necrotizing enterocolitis (Nec) and B. Lactis in premature babies
Methods	RCT
Participants	VLBW infants
Interventions	*B. lactis* for 6 weeks
Outcomes	NEC, antibiotic administration, stool microbiology
Starting date	November 2009
Contact information	Dr Peter Cooper, University of Witwatetersrand & Charlotte Maxek Johannestburg Academic Hospital, Zambia
Notes	(Quote:) "Terminated" in 2013 - unlikely to have been completed (not reported)

NCT01181791

Study name	Effects of Lactobacillus reuteri in premature infants (reuteri)
Methods	RCT
Participants	VLBW infant
Interventions	*Lactobacillus reuteri* during hospitalisation
Outcomes	Time to reach full enteral feeds, stool colonisation and Intestinal immunological response
Starting date	2010
Contact information	Teresa del Moral, University of Miami
Notes	Chile (Quote:) "Terminated" because of slow recruitment- unlikely to have been reported

NCT01375309

Study name	Bifidobacterium supplementation for very low birth weight infants (Bifido(RCT))
Methods	RCT
Participants	VLBW infants
Interventions	*Bifidobacterium bifidum* (duration not clear)
Outcomes	Time to full enteral feeding, weight gain, NEC
Starting date	2011
Contact information	Satoshi Kusuda, Professor of Neonatology, Tokyo Women's Medical University

NCT01375309 *(Continued)*

Notes	(Quote:) "Completed" 2012 - unlikely to have been reported

NCT04541771

Study name	The role of Lactobacillus reuteri in preventing necrotizing enterocolitis (NEC) in pre-term infants (NEC)
Methods	RCT
Participants	Preterm infants (28 to 34 weeks')
Interventions	*Lactobacillus reuteri* until 35 weeks' of gestation or discharged from hospital
Outcomes	NEC, infection
Starting date	2020
Contact information	Dr Summera Tabasum, The Children Complex & The Institute of Child Health, Multan
Notes	ClinicalTrials.gov identifier: NCT04541771

NEC: necrotising enterocolitis; **RCT:** randomised controlled trial; **VLBW:** very low birth weight

DATA AND ANALYSES

Comparison 1. Probiotics versus control

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.1 Necrotising enterocolitis	54	10604	Risk Ratio (M-H, Fixed, 95% CI)	0.54 [0.45, 0.65]
1.1.1 Bifidobacterium spp.	14	2988	Risk Ratio (M-H, Fixed, 95% CI)	0.72 [0.54, 0.96]
1.1.2 Lactobacillus spp.	12	2000	Risk Ratio (M-H, Fixed, 95% CI)	0.45 [0.28, 0.71]
1.1.3 Sacchromyces spp.	4	621	Risk Ratio (M-H, Fixed, 95% CI)	0.82 [0.44, 1.50]
1.1.4 Bacillus spp.	2	465	Risk Ratio (M-H, Fixed, 95% CI)	0.61 [0.23, 1.61]
1.1.5 Bifidobacterium spp. plus Lactobacillus spp.	11	2041	Risk Ratio (M-H, Fixed, 95% CI)	0.36 [0.23, 0.59]
1.1.6 Bifidobacterium spp. plus Streptococcus spp.	2	1244	Risk Ratio (M-H, Fixed, 95% CI)	0.36 [0.19, 0.68]
1.1.7 Bifidobacterium spp. plus Lactobacillus spp. plus Sacchromyces spp.	4	583	Risk Ratio (M-H, Fixed, 95% CI)	0.67 [0.28, 1.58]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.1.8 Bifidobacterium spp. plus Lactobacillus spp. plus Streptococcus spp.	5	662	Risk Ratio (M-H, Fixed, 95% CI)	0.42 [0.22, 0.77]
1.2 Mortality	51	10170	Risk Ratio (M-H, Fixed, 95% CI)	0.76 [0.65, 0.89]
1.2.1 Bifidobacterium spp.	12	2761	Risk Ratio (M-H, Fixed, 95% CI)	0.79 [0.58, 1.09]
1.2.2 Lactobacillus spp.	12	2000	Risk Ratio (M-H, Fixed, 95% CI)	0.91 [0.60, 1.37]
1.2.3 Sacchromyces spp.	3	534	Risk Ratio (M-H, Fixed, 95% CI)	1.12 [0.46, 2.70]
1.2.4 Bacillus spp.	2	465	Risk Ratio (M-H, Fixed, 95% CI)	0.87 [0.45, 1.69]
1.2.5 Bifidobacterium spp. plus Lactobacillus spp.	12	2071	Risk Ratio (M-H, Fixed, 95% CI)	0.60 [0.45, 0.81]
1.2.6 Bifidobacterium spp. plus Streptococcus spp.	2	1244	Risk Ratio (M-H, Fixed, 95% CI)	0.84 [0.52, 1.35]
1.2.7 Bifidobacterium spp. plus Lactobacillus spp. plus Sacchromyces spp.	4	583	Risk Ratio (M-H, Fixed, 95% CI)	0.67 [0.30, 1.49]
1.2.8 Bifidobacterium spp. plus Lactobacillus spp. plus Streptococcus spp.	4	512	Risk Ratio (M-H, Fixed, 95% CI)	0.74 [0.39, 1.42]
1.3 Invasive infection	47	9762	Risk Ratio (M-H, Fixed, 95% CI)	0.89 [0.82, 0.97]
1.3.1 Bifidobacterium spp.	12	2736	Risk Ratio (M-H, Fixed, 95% CI)	0.84 [0.70, 1.02]
1.3.2 Lactobacillus spp.	11	1970	Risk Ratio (M-H, Fixed, 95% CI)	0.96 [0.76, 1.21]
1.3.3 Sacchromyces spp.	4	621	Risk Ratio (M-H, Fixed, 95% CI)	0.84 [0.58, 1.22]
1.3.4 Bacillus spp.	2	465	Risk Ratio (M-H, Fixed, 95% CI)	1.00 [0.67, 1.51]
1.3.5 Bifidobacterium spp. plus Lactobacillus spp.	10	1913	Risk Ratio (M-H, Fixed, 95% CI)	0.92 [0.78, 1.08]
1.3.6 Bifidobacterium spp. plus Streptococcus spp.	2	1244	Risk Ratio (M-H, Fixed, 95% CI)	0.92 [0.72, 1.17]
1.3.7 Bifidobacterium spp. plus Lactobacillus spp. plus Sacchromyces spp.	4	583	Risk Ratio (M-H, Fixed, 95% CI)	0.79 [0.53, 1.18]
1.3.8 Bifidobacterium spp. plus Lactobacillus spp. plus Streptococcus spp.	2	230	Risk Ratio (M-H, Fixed, 95% CI)	0.79 [0.63, 1.00]
1.4 Duration of birth hospitalisation (days)	22	5458	Mean Difference (IV, Random, 95% CI)	-1.93 [-3.78, -0.08]

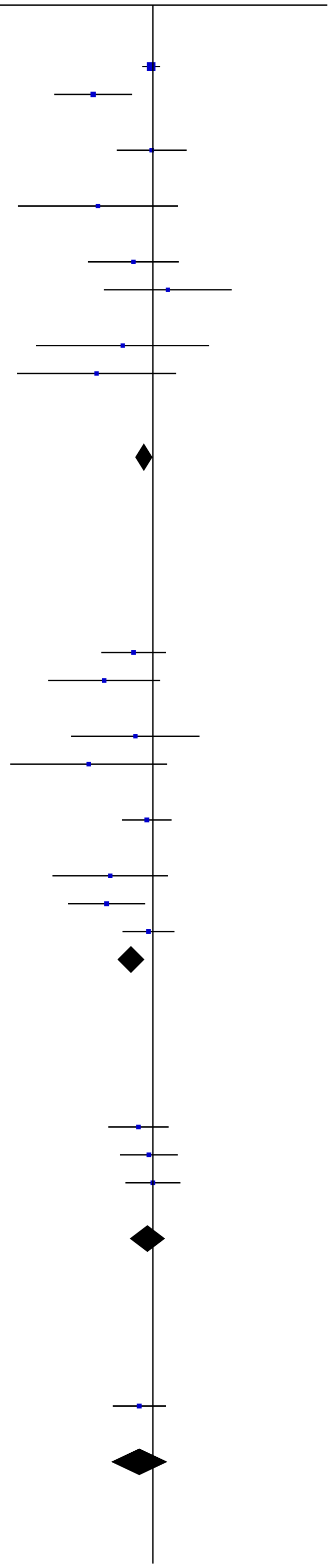
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.4.1 Bifidobacterium spp.	4	1945	Mean Difference (IV, Random, 95% CI)	-1.05 [-6.55, 4.45]
1.4.2 Lactobacillus spp.	4	217	Mean Difference (IV, Random, 95% CI)	-1.95 [-10.81, 6.90]
1.4.3 Sacchromyces spp.	2	470	Mean Difference (IV, Random, 95% CI)	-2.88 [-8.06, 2.29]
1.4.4 Bifidobacterium spp. plus Lactobacillus spp.	7	1265	Mean Difference (IV, Random, 95% CI)	-1.74 [-5.22, 1.73]
1.4.5 Bifidobacterium spp. plus Streptococcus spp.	1	1044	Mean Difference (IV, Random, 95% CI)	-3.00 [-6.28, 0.28]
1.4.6 Bifidobacterium spp. plus Lactobacillus spp. plus Sacchromyces spp.	2	231	Mean Difference (IV, Random, 95% CI)	-5.65 [-11.68, 0.38]
1.4.7 Bifidobacterium spp. plus Lactobacillus spp. plus Streptococcus spp.	2	286	Mean Difference (IV, Random, 95% CI)	1.69 [-6.73, 10.11]
1.5 Severe neurodevelopmental impairment	5	1518	Risk Ratio (M-H, Fixed, 95% CI)	1.03 [0.84, 1.26]
1.5.1 Bifidobacterium spp.	1	162	Risk Ratio (M-H, Fixed, 95% CI)	0.77 [0.34, 1.72]
1.5.2 Lactobacillus spp.	1	249	Risk Ratio (M-H, Fixed, 95% CI)	1.01 [0.69, 1.48]
1.5.3 Bacillus spp.	1	174	Risk Ratio (M-H, Fixed, 95% CI)	1.09 [0.58, 2.07]
1.5.4 Bifidobacterium spp. plus Streptococcus spp.	1	664	Risk Ratio (M-H, Fixed, 95% CI)	0.97 [0.69, 1.36]
1.5.5 Bifidobacterium spp. plus Lactobacillus spp.	1	269	Risk Ratio (M-H, Fixed, 95% CI)	1.27 [0.81, 1.98]
1.6 Cerebral palsy	5	1512	Risk Ratio (M-H, Fixed, 95% CI)	1.13 [0.74, 1.72]
1.6.1 Bifidobacterium spp.	1	156	Risk Ratio (M-H, Fixed, 95% CI)	0.38 [0.10, 1.36]
1.6.2 Lactobacillus spp.	1	249	Risk Ratio (M-H, Fixed, 95% CI)	0.92 [0.40, 2.08]
1.6.3 Bacillus spp.	1	174	Risk Ratio (M-H, Fixed, 95% CI)	2.05 [0.38, 10.88]
1.6.4 Bifidobacterium spp. plus Streptococcus spp.	1	664	Risk Ratio (M-H, Fixed, 95% CI)	1.32 [0.67, 2.58]
1.6.5 Bifidobacterium spp. plus Lactobacillus spp.	1	269	Risk Ratio (M-H, Fixed, 95% CI)	2.28 [0.62, 8.41]
1.7 Visual impairment	4	1356	Risk Ratio (M-H, Fixed, 95% CI)	0.50 [0.14, 1.80]
1.7.1 Bifidobacterium spp.	1	174	Risk Ratio (M-H, Fixed, 95% CI)	0.51 [0.05, 5.54]
1.7.2 Lactobacillus spp.	1	249	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.7.3 Bifidobacterium spp. plus Streptococcus spp.	1	664	Risk Ratio (M-H, Fixed, 95% CI)	2.91 [0.12, 71.21]
1.7.4 Bifidobacterium spp. plus Lactobacillus spp.	1	269	Risk Ratio (M-H, Fixed, 95% CI)	0.21 [0.02, 1.89]
1.8 Hearing impairment	4	1356	Risk Ratio (M-H, Fixed, 95% CI)	0.46 [0.18, 1.17]
1.8.1 Bifidobacterium spp.	1	174	Risk Ratio (M-H, Fixed, 95% CI)	1.02 [0.07, 16.10]
1.8.2 Lactobacillus spp.	1	249	Risk Ratio (M-H, Fixed, 95% CI)	3.02 [0.12, 73.52]
1.8.3 Bifidobacterium spp. plus Streptococcus spp.	1	664	Risk Ratio (M-H, Fixed, 95% CI)	0.18 [0.04, 0.79]
1.8.4 Bifidobacterium spp. plus Lactobacillus spp.	1	269	Risk Ratio (M-H, Fixed, 95% CI)	1.71 [0.16, 18.64]
1.9 Continuous early learning composite measure	1	52	Mean Difference (IV, Fixed, 95% CI)	-1.00 [-6.38, 4.38]

Analysis 1.1. Comparison 1: Probiotics versus control, Outcome 1: Necrotising enterocolitis

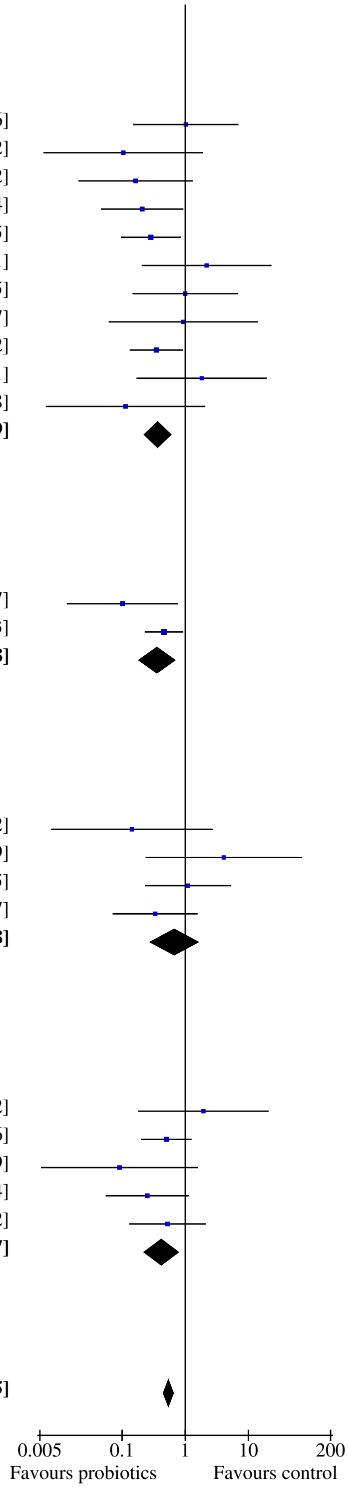
Study or Subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.1.1 Bifidobacterium spp.						
Costeloe 2015	61	650	66	660	20.0%	0.94 [0.67 , 1.31]
Dilli 2015	2	100	18	100	5.5%	0.11 [0.03 , 0.47]
Fujii 2006	0	11	0	8		Not estimable
Hays 2015	8	145	3	52	1.4%	0.96 [0.26 , 3.47]
Hikaru 2010	0	108	0	100		Not estimable
Huang 2009	0	95	3	88	1.1%	0.13 [0.01 , 2.53]
Kitajima 1997	0	45	0	46		Not estimable
Mihatsch 2010	2	91	4	89	1.2%	0.49 [0.09 , 2.60]
Mohan 2006	2	37	1	32	0.3%	1.73 [0.16 , 18.20]
Oshiro 2019	0	17	0	18		Not estimable
Patole 2014	0	77	1	76	0.5%	0.33 [0.01 , 7.95]
Stratiki 2007	0	41	3	36	1.1%	0.13 [0.01 , 2.36]
Totsu 2014	0	120	0	102		Not estimable
Wang 2007	0	22	0	22		Not estimable
Subtotal (95% CI)		**1559**		**1429**	**31.2%**	**0.72 [0.54 , 0.96]**
Total events:	75		99			
Heterogeneity: Chi² = 12.82, df = 7 (P = 0.08); I² = 45%						
Test for overall effect: Z = 2.27 (P = 0.02)						
1.1.2 Lactobacillus spp.						
Chrzanowska-Liszewska 2012	0	21	0	26		Not estimable
Dani 2002	4	295	8	290	2.5%	0.49 [0.15 , 1.61]
Hernandez-Enriquez 2016	1	24	5	20	1.7%	0.17 [0.02 , 1.31]
Indrio 2017	0	30	0	30		Not estimable
Manzoni 2006	1	39	2	41	0.6%	0.53 [0.05 , 5.57]
Manzoni 2009	0	238	5	247	1.7%	0.09 [0.01 , 1.70]
Millar 1993	0	10	0	10		Not estimable
Oncel 2014	8	200	10	200	3.1%	0.80 [0.32 , 1.99]
Reuman 1986	0	15	0	15		Not estimable
Sadowska-Krawczenko 2012	1	30	4	25	1.3%	0.21 [0.02 , 1.75]
Shadkam 2015	2	30	11	30	3.4%	0.18 [0.04 , 0.75]
Wejryd 2019	7	68	8	66	2.5%	0.85 [0.33 , 2.21]
Subtotal (95% CI)		**1000**		**1000**	**16.6%**	**0.45 [0.28 , 0.71]**
Total events:	24		53			
Heterogeneity: Chi² = 7.39, df = 7 (P = 0.39); I² = 5%						
Test for overall effect: Z = 3.44 (P = 0.0006)						
1.1.3 Sacchromyces spp.						
Costalos 2003	5	51	6	36	2.2%	0.59 [0.19 , 1.78]
Demirel 2013	6	135	7	136	2.1%	0.86 [0.30 , 2.50]
Serce 2013	7	104	7	104	2.1%	1.00 [0.36 , 2.75]
Zeber-Lubecka 2016	0	27	0	28		Not estimable
Subtotal (95% CI)		**317**		**304**	**6.4%**	**0.82 [0.44 , 1.50]**
Total events:	18		20			
Heterogeneity: Chi² = 0.50, df = 2 (P = 0.78); I² = 0%						
Test for overall effect: Z = 0.65 (P = 0.51)						
1.1.4 Bacillus spp.						
Sari 2011	6	110	10	111	3.0%	0.61 [0.23 , 1.61]
Tewari 2015	0	123	0	121		Not estimable
Subtotal (95% CI)		**233**		**232**	**3.0%**	**0.61 [0.23 , 1.61]**
Total events:	6		10			
Heterogeneity: Not applicable						
Test for overall effect: Z = 1.01 (P = 0.31)						

Risk Ratio M-H, Fixed, 95% CI



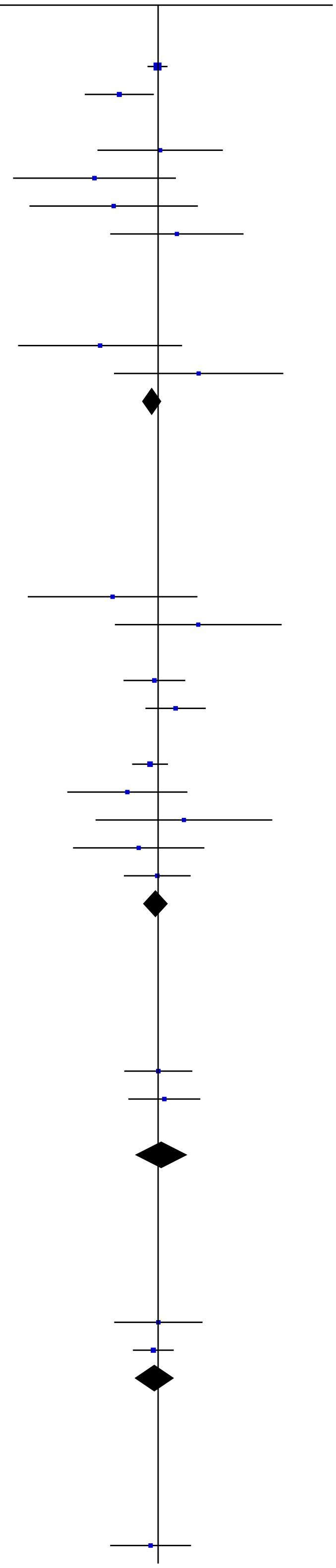
Analysis 1.1. (Continued)

Heterogeneity: Not applicable						
Test for overall effect: Z = 1.01 (P = 0.31)						
1.1.5 Bifidobacterium spp. plus Lactobacillus spp.						
Al-Hosni 2012	2	50	2	51	0.6%	1.02 [0.15 , 6.96]
Braga 2011	0	119	4	112	1.4%	0.10 [0.01 , 1.92]
Chowdhury 2016	1	60	6	59	1.9%	0.16 [0.02 , 1.32]
Lin 2005	2	180	10	187	3.0%	0.21 [0.05 , 0.94]
Lin 2008	4	217	14	217	4.3%	0.29 [0.10 , 0.85]
Rougé 2009	2	45	1	49	0.3%	2.18 [0.20 , 23.21]
Roy 2014	2	56	2	56	0.6%	1.00 [0.15 , 6.85]
Saengtawesin 2014	1	31	1	29	0.3%	0.94 [0.06 , 14.27]
Samanta 2009	5	91	15	95	4.5%	0.35 [0.13 , 0.92]
Strus 2018	2	80	1	73	0.3%	1.82 [0.17 , 19.71]
Van Niekerk 2014	0	91	4	93	1.4%	0.11 [0.01 , 2.08]
Subtotal (95% CI)		**1020**		**1021**	**18.6%**	**0.36 [0.23 , 0.59]**
Total events:	21		60			
Heterogeneity: Chi² = 9.19, df = 10 (P = 0.51); I² = 0%						
Test for overall effect: Z = 4.14 (P < 0.0001)						
1.1.6 Bifidobacterium spp. plus Streptococcus spp.						
Bin-Nun 2005	1	72	10	73	3.0%	0.10 [0.01 , 0.77]
Jacobs 2013	11	548	24	551	7.3%	0.46 [0.23 , 0.93]
Subtotal (95% CI)		**620**		**624**	**10.4%**	**0.36 [0.19 , 0.68]**
Total events:	12		34			
Heterogeneity: Chi² = 1.99, df = 1 (P = 0.16); I² = 50%						
Test for overall effect: Z = 3.12 (P = 0.002)						
1.1.7 Bifidobacterium spp. plus Lactobacillus spp. plus Sacchromyces spp.						
Chandrashekar 2018	0	70	3	70	1.1%	0.14 [0.01 , 2.72]
Dutta 2015	6	114	0	35	0.2%	4.07 [0.23 , 70.49]
Hariharan 2016	3	93	3	103	0.9%	1.11 [0.23 , 5.35]
Shashidhar 2017	2	49	6	49	1.8%	0.33 [0.07 , 1.57]
Subtotal (95% CI)		**326**		**257**	**4.0%**	**0.67 [0.28 , 1.58]**
Total events:	11		12			
Heterogeneity: Chi² = 3.76, df = 3 (P = 0.29); I² = 20%						
Test for overall effect: Z = 0.92 (P = 0.36)						
1.1.8 Bifidobacterium spp. plus Lactobacillus spp. plus Streptococcus spp.						
Dashti 2014	2	69	1	67	0.3%	1.94 [0.18 , 20.92]
Fernández-Carrocera 2013	6	75	12	75	3.7%	0.50 [0.20 , 1.26]
Kanic 2015	0	40	5	40	1.7%	0.09 [0.01 , 1.59]
Rehman 2018	2	73	8	73	2.4%	0.25 [0.05 , 1.14]
Ren 2010	3	80	5	70	1.6%	0.53 [0.13 , 2.12]
Subtotal (95% CI)		**337**		**325**	**9.7%**	**0.42 [0.22 , 0.77]**
Total events:	13		31			
Heterogeneity: Chi² = 3.39, df = 4 (P = 0.50); I² = 0%						
Test for overall effect: Z = 2.78 (P = 0.005)						
Total (95% CI)		**5412**		**5192**	**100.0%**	**0.54 [0.45 , 0.65]**
Total events:	180		319			
Heterogeneity: Chi² = 49.36, df = 41 (P = 0.17); I² = 17%						
Test for overall effect: Z = 6.80 (P < 0.00001)						
Test for subgroup differences: Chi² = 11.23, df = 7 (P = 0.13), I² = 37.7%						



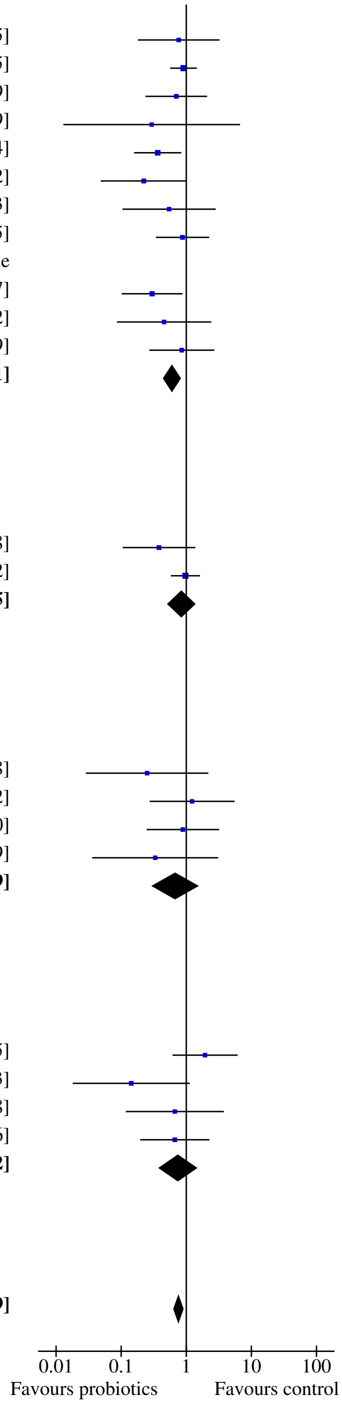
Analysis 1.2. Comparison 1: Probiotics versus control, Outcome 2: Mortality

Study or Subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.2.1 Bifidobacterium spp.						
Costeloe 2015	54	650	56	660	17.0%	0.98 [0.68 , 1.40]
Dilli 2015	3	100	12	100	3.7%	0.25 [0.07 , 0.86]
Fujii 2006	0	11	0	8		Not estimable
Hays 2015	3	145	1	52	0.4%	1.08 [0.11 , 10.11]
Hikaru 2010	0	108	4	100	1.4%	0.10 [0.01 , 1.89]
Kitajima 1997	0	45	2	46	0.8%	0.20 [0.01 , 4.14]
Mihatsch 2010	2	91	1	89	0.3%	1.96 [0.18 , 21.19]
Mohan 2006	0	37	0	32		Not estimable
Oshiro 2019	0	17	0	18		Not estimable
Patole 2014	0	77	0	76		Not estimable
Stratiki 2007	0	41	3	36	1.1%	0.13 [0.01 , 2.36]
Totsu 2014	2	120	0	102	0.2%	4.26 [0.21 , 87.65]
Subtotal (95% CI)		**1442**		**1319**	**24.9%**	**0.79 [0.58 , 1.09]**
Total events:	64		79			
Heterogeneity: Chi² = 10.68, df = 7 (P = 0.15); I² = 34%						
Test for overall effect: Z = 1.43 (P = 0.15)						
1.2.2 Lactobacillus spp.						
Chrzanowska-Liszewska 2012	0	21	0	26		Not estimable
Dani 2002	0	295	2	290	0.8%	0.20 [0.01 , 4.08]
Hernandez-Enriquez 2016	2	24	0	20	0.2%	4.20 [0.21 , 82.72]
Indrio 2017	0	30	0	30		Not estimable
Manzoni 2006	5	39	6	41	1.8%	0.88 [0.29 , 2.64]
Manzoni 2009	9	238	5	247	1.5%	1.87 [0.64 , 5.49]
Millar 1993	0	10	0	10		Not estimable
Oncel 2014	15	200	20	200	6.1%	0.75 [0.40 , 1.42]
Reuman 1986	1	15	3	15	0.9%	0.33 [0.04 , 2.85]
Sadowska-Krawczenko 2012	1	30	0	25	0.2%	2.52 [0.11 , 59.18]
Shadkam 2015	1	30	2	30	0.6%	0.50 [0.05 , 5.22]
Wejryd 2019	5	68	5	66	1.5%	0.97 [0.29 , 3.20]
Subtotal (95% CI)		**1000**		**1000**	**13.6%**	**0.91 [0.60 , 1.37]**
Total events:	39		43			
Heterogeneity: Chi² = 5.56, df = 8 (P = 0.70); I² = 0%						
Test for overall effect: Z = 0.46 (P = 0.65)						
1.2.3 Sacchromyces spp.						
Demirel 2013	5	135	5	136	1.5%	1.01 [0.30 , 3.40]
Serce 2013	5	104	4	104	1.2%	1.25 [0.35 , 4.52]
Zeber-Lubecka 2016	0	27	0	28		Not estimable
Subtotal (95% CI)		**266**		**268**	**2.7%**	**1.12 [0.46 , 2.70]**
Total events:	10		9			
Heterogeneity: Chi² = 0.06, df = 1 (P = 0.81); I² = 0%						
Test for overall effect: Z = 0.24 (P = 0.81)						
1.2.4 Bacillus spp.						
Sari 2011	3	110	3	111	0.9%	1.01 [0.21 , 4.89]
Tewari 2015	12	123	14	121	4.3%	0.84 [0.41 , 1.75]
Subtotal (95% CI)		**233**		**232**	**5.2%**	**0.87 [0.45 , 1.69]**
Total events:	15		17			
Heterogeneity: Chi² = 0.04, df = 1 (P = 0.84); I² = 0%						
Test for overall effect: Z = 0.41 (P = 0.69)						
1.2.5 Bifidobacterium spp. plus Lactobacillus spp.						
Al-Hosni 2012	3	50	4	51	1.2%	0.77 [0.18 , 3.25]



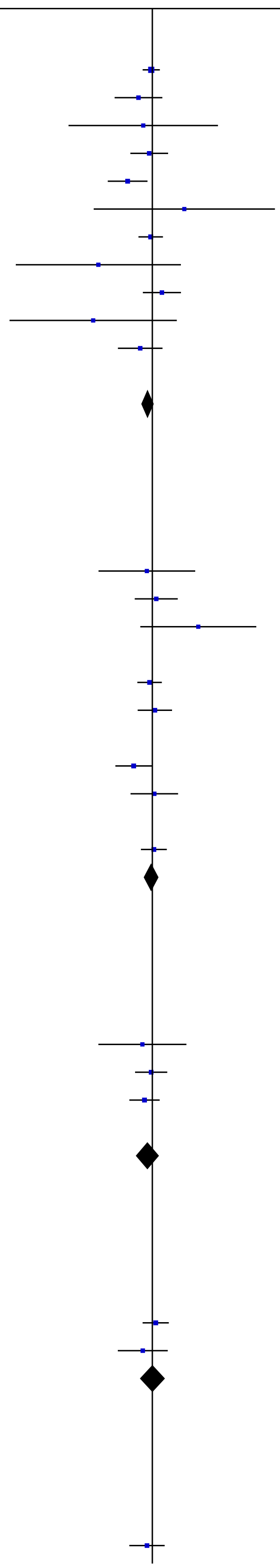
Analysis 1.2. (Continued)

Study or subgroup	Probiotics events	Probiotics N	Control events	Control N	Weight	Risk Ratio
1.2.5 Bifidobacterium spp. plus Lactobacillus spp.						
Al-Hosni 2012	3	50	4	51	1.2%	0.77 [0.18 , 3.25]
Braga 2011	26	119	27	112	8.5%	0.91 [0.56 , 1.45]
Chowdhury 2016	5	60	7	59	2.2%	0.70 [0.24 , 2.09]
Li 2019	0	16	1	14	0.5%	0.29 [0.01 , 6.69]
Lin 2005	7	180	20	187	6.0%	0.36 [0.16 , 0.84]
Lin 2008	2	217	9	217	2.7%	0.22 [0.05 , 1.02]
Rougé 2009	2	45	4	49	1.2%	0.54 [0.10 , 2.83]
Roy 2014	7	56	8	56	2.4%	0.88 [0.34 , 2.25]
Saengtawesin 2014	0	31	0	29		Not estimable
Samanta 2009	4	91	14	95	4.2%	0.30 [0.10 , 0.87]
Strus 2018	2	80	4	73	1.3%	0.46 [0.09 , 2.42]
Van Niekerk 2014	5	91	6	93	1.8%	0.85 [0.27 , 2.69]
Subtotal (95% CI)		**1036**		**1035**	**32.0%**	**0.60 [0.45 , 0.81]**
Total events:	63		104			
Heterogeneity: Chi² = 9.03, df = 10 (P = 0.53); I² = 0%						
Test for overall effect: Z = 3.40 (P = 0.0007)						
1.2.6 Bifidobacterium spp. plus Streptococcus spp.						
Bin-Nun 2005	3	72	8	73	2.4%	0.38 [0.11 , 1.38]
Jacobs 2013	27	548	28	551	8.5%	0.97 [0.58 , 1.62]
Subtotal (95% CI)		**620**		**624**	**11.0%**	**0.84 [0.52 , 1.35]**
Total events:	30		36			
Heterogeneity: Chi² = 1.76, df = 1 (P = 0.18); I² = 43%						
Test for overall effect: Z = 0.73 (P = 0.47)						
1.2.7 Bifidobacterium spp. plus Lactobacillus spp. plus Sacchromyces spp.						
Chandrashekar 2018	1	70	4	70	1.2%	0.25 [0.03 , 2.18]
Dutta 2015	8	114	2	35	0.9%	1.23 [0.27 , 5.52]
Hariharan 2016	4	93	5	103	1.4%	0.89 [0.25 , 3.20]
Shashidhar 2017	1	49	3	49	0.9%	0.33 [0.04 , 3.09]
Subtotal (95% CI)		**326**		**257**	**4.5%**	**0.67 [0.30 , 1.49]**
Total events:	14		14			
Heterogeneity: Chi² = 1.98, df = 3 (P = 0.58); I² = 0%						
Test for overall effect: Z = 0.98 (P = 0.33)						
1.2.8 Bifidobacterium spp. plus Lactobacillus spp. plus Streptococcus spp.						
Dashti 2014	8	69	4	67	1.2%	1.94 [0.61 , 6.15]
Fernández-Carrocera 2013	1	75	7	75	2.1%	0.14 [0.02 , 1.13]
Kanic 2015	2	40	3	40	0.9%	0.67 [0.12 , 3.78]
Rehman 2018	4	73	6	73	1.8%	0.67 [0.20 , 2.26]
Subtotal (95% CI)		**257**		**255**	**6.1%**	**0.74 [0.39 , 1.42]**
Total events:	15		20			
Heterogeneity: Chi² = 5.15, df = 3 (P = 0.16); I² = 42%						
Test for overall effect: Z = 0.90 (P = 0.37)						
Total (95% CI)		**5180**		**4990**	**100.0%**	**0.76 [0.65 , 0.89]**
Total events:	250		322			
Heterogeneity: Chi² = 37.21, df = 41 (P = 0.64); I² = 0%						
Test for overall effect: Z = 3.45 (P = 0.0006)						
Test for subgroup differences: Chi² = 4.40, df = 7 (P = 0.73), I² = 0%						



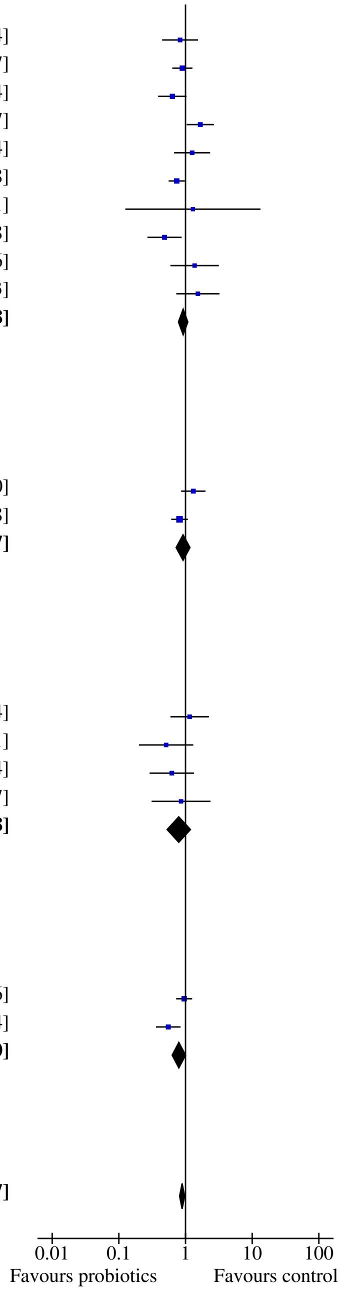
Analysis 1.3. Comparison 1: Probiotics versus control, Outcome 3: Invasive infection

Study or Subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.3.1 Bifidobacterium spp.						
Costeloe 2015	73	650	77	660	9.1%	0.96 [0.71 , 1.30]
Dilli 2015	8	100	13	100	1.5%	0.62 [0.27 , 1.42]
Fujii 2006	1	11	1	8	0.1%	0.73 [0.05 , 9.97]
Hays 2015	25	145	10	52	1.8%	0.90 [0.46 , 1.74]
Hikaru 2010	10	108	22	100	2.7%	0.42 [0.21 , 0.84]
Kitajima 1997	1	45	0	46	0.1%	3.07 [0.13 , 73.32]
Mihatsch 2010	28	91	29	89	3.5%	0.94 [0.61 , 1.45]
Oshiro 2019	0	17	3	18	0.4%	0.15 [0.01 , 2.72]
Patole 2014	17	77	12	76	1.4%	1.40 [0.72 , 2.73]
Stratiki 2007	0	41	3	36	0.4%	0.13 [0.01 , 2.36]
Totsu 2014	10	120	13	102	1.7%	0.65 [0.30 , 1.43]
Wang 2007	0	22	0	22		Not estimable
Subtotal (95% CI)		**1427**		**1309**	**22.8%**	**0.84 [0.70 , 1.02]**
Total events:	173		183			
Heterogeneity: Chi² = 11.65, df = 10 (P = 0.31); I² = 14%						
Test for overall effect: Z = 1.73 (P = 0.08)						
1.3.2 Lactobacillus spp.						
Chrzanowska-Liszewska 2012	2	21	3	26	0.3%	0.83 [0.15 , 4.49]
Dani 2002	14	295	12	290	1.4%	1.15 [0.54 , 2.44]
Hernandez-Enriquez 2016	6	24	1	20	0.1%	5.00 [0.66 , 38.15]
Indrio 2017	0	30	0	30		Not estimable
Manzoni 2006	19	39	22	41	2.6%	0.91 [0.59 , 1.40]
Manzoni 2009	20	238	19	247	2.2%	1.09 [0.60 , 1.99]
Millar 1993	0	10	0	10		Not estimable
Oncel 2014	13	200	25	200	3.0%	0.52 [0.27 , 0.99]
Sadowska-Krawczenko 2012	9	30	7	25	0.9%	1.07 [0.47 , 2.46]
Shadkam 2015	0	30	0	30		Not estimable
Wejryd 2019	25	68	23	66	2.8%	1.05 [0.67 , 1.66]
Subtotal (95% CI)		**985**		**985**	**13.3%**	**0.96 [0.76 , 1.21]**
Total events:	108		112			
Heterogeneity: Chi² = 6.77, df = 7 (P = 0.45); I² = 0%						
Test for overall effect: Z = 0.37 (P = 0.71)						
1.3.3 Sacchromyces spp.						
Costalos 2003	3	51	3	36	0.4%	0.71 [0.15 , 3.30]
Demirel 2013	20	135	21	136	2.5%	0.96 [0.55 , 1.69]
Serce 2013	19	104	25	104	3.0%	0.76 [0.45 , 1.29]
Zeber-Lubecka 2016	0	27	0	28		Not estimable
Subtotal (95% CI)		**317**		**304**	**5.9%**	**0.84 [0.58 , 1.22]**
Total events:	42		49			
Heterogeneity: Chi² = 0.40, df = 2 (P = 0.82); I² = 0%						
Test for overall effect: Z = 0.91 (P = 0.36)						
1.3.4 Bacillus spp.						
Sari 2011	29	110	26	111	3.1%	1.13 [0.71 , 1.78]
Tewari 2015	8	123	11	121	1.3%	0.72 [0.30 , 1.72]
Subtotal (95% CI)		**233**		**232**	**4.4%**	**1.00 [0.67 , 1.51]**
Total events:	37		37			
Heterogeneity: Chi² = 0.81, df = 1 (P = 0.37); I² = 0%						
Test for overall effect: Z = 0.01 (P = 0.99)						
1.3.5 Bifidobacterium spp. plus Lactobacillus spp.						
Al-Hosni 2012	13	50	16	51	1.9%	0.83 [0.45 , 1.54]



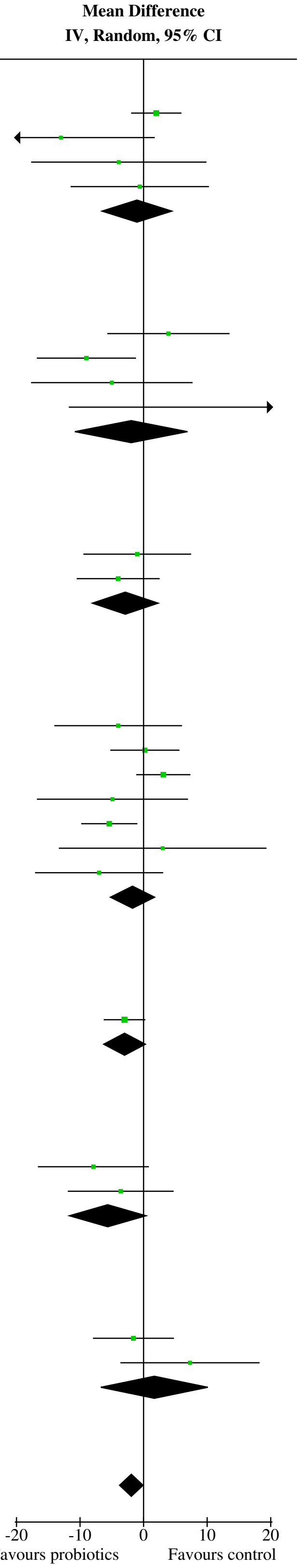
Analysis 1.3. (Continued)

1.3.5 Bifidobacterium spp. plus Lactobacillus spp.						
Al-Hosni 2012	13	50	16	51	1.9%	0.83 [0.45 , 1.54]
Braga 2011	40	119	42	112	5.2%	0.90 [0.63 , 1.27]
Lin 2005	22	180	36	187	4.2%	0.63 [0.39 , 1.04]
Lin 2008	40	217	24	217	2.9%	1.67 [1.04 , 2.67]
Rougé 2009	15	45	13	49	1.5%	1.26 [0.67 , 2.34]
Roy 2014	31	56	42	56	5.0%	0.74 [0.56 , 0.98]
Saengtawesin 2014	2	31	1	20	0.1%	1.29 [0.13 , 13.31]
Samanta 2009	13	91	28	95	3.3%	0.48 [0.27 , 0.88]
Strus 2018	12	80	8	73	1.0%	1.37 [0.59 , 3.16]
Van Niekerk 2014	15	91	10	93	1.2%	1.53 [0.73 , 3.23]
Subtotal (95% CI)		**960**		**953**	**26.2%**	**0.92 [0.78 , 1.08]**
Total events:	203		220			
Heterogeneity: Chi² = 19.10, df = 9 (P = 0.02); I² = 53%						
Test for overall effect: Z = 1.00 (P = 0.32)						
1.3.6 Bifidobacterium spp. plus Streptococcus spp.						
Bin-Nun 2005	31	72	24	73	2.8%	1.31 [0.86 , 2.00]
Jacobs 2013	72	548	89	551	10.6%	0.81 [0.61 , 1.08]
Subtotal (95% CI)		**620**		**624**	**13.4%**	**0.92 [0.72 , 1.17]**
Total events:	103		113			
Heterogeneity: Chi² = 3.40, df = 1 (P = 0.07); I² = 71%						
Test for overall effect: Z = 0.70 (P = 0.48)						
1.3.7 Bifidobacterium spp. plus Lactobacillus spp. plus Sacchromyces spp.						
Chandrashekar 2018	15	70	13	70	1.5%	1.15 [0.59 , 2.24]
Dutta 2015	10	114	6	35	1.1%	0.51 [0.20 , 1.31]
Hariharan 2016	9	93	16	103	1.8%	0.62 [0.29 , 1.34]
Shashidhar 2017	6	49	7	49	0.8%	0.86 [0.31 , 2.37]
Subtotal (95% CI)		**326**		**257**	**5.3%**	**0.79 [0.53 , 1.18]**
Total events:	40		42			
Heterogeneity: Chi² = 2.46, df = 3 (P = 0.48); I² = 0%						
Test for overall effect: Z = 1.13 (P = 0.26)						
1.3.8 Bifidobacterium spp. plus Lactobacillus spp. plus Streptococcus spp.						
Fernández-Carrocera 2013	42	75	44	75	5.2%	0.95 [0.72 , 1.26]
Kanic 2015	16	40	29	40	3.5%	0.55 [0.36 , 0.84]
Subtotal (95% CI)		**115**		**115**	**8.7%**	**0.79 [0.63 , 1.00]**
Total events:	58		73			
Heterogeneity: Chi² = 4.53, df = 1 (P = 0.03); I² = 78%						
Test for overall effect: Z = 1.96 (P = 0.05)						
Total (95% CI)		**4983**		**4779**	**100.0%**	**0.89 [0.82 , 0.97]**
Total events:	764		829			
Heterogeneity: Chi² = 50.79, df = 41 (P = 0.14); I² = 19%						
Test for overall effect: Z = 2.69 (P = 0.007)						
Test for subgroup differences: Chi² = 2.57, df = 7 (P = 0.92), I² = 0%						



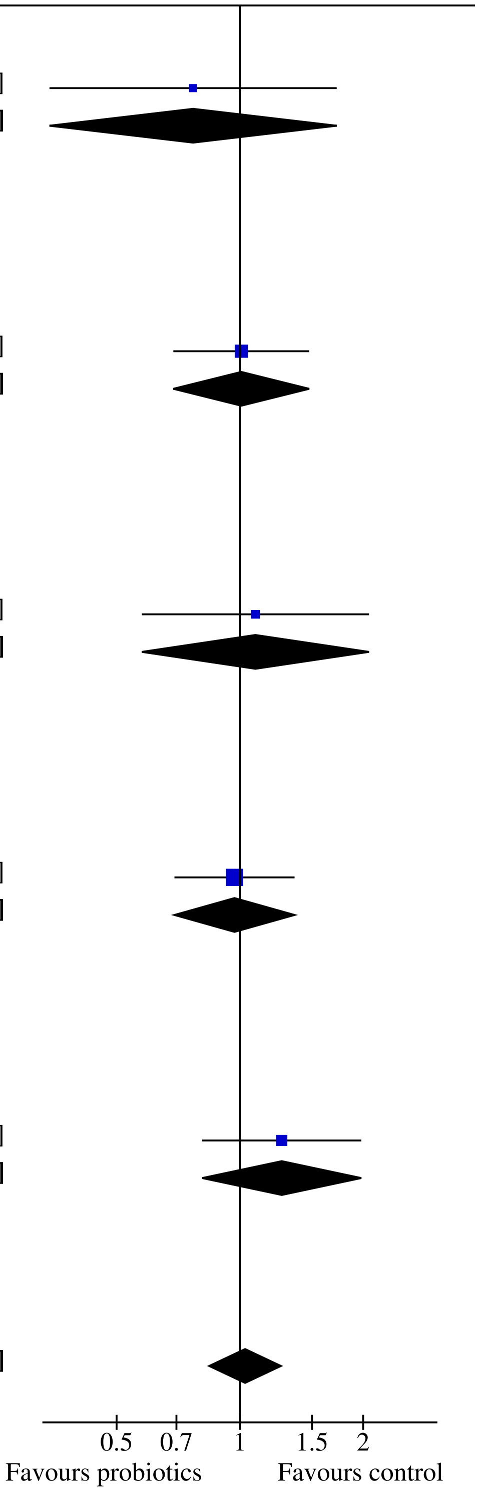
Analysis 1.4. Comparison 1: Probiotics versus control, Outcome 4: Duration of birth hospitalisation (days)

Study or Subgroup	Probiotics Mean	Probiotics SD	Probiotics Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
1.4.1 Bifidobacterium spp.								
Costeloe 2015	68	37	647	66	36	657	10.6%	2.00 [-1.96 , 5.96]
Dilli 2015	37	38	100	50	65	100	1.5%	-13.00 [-27.76 , 1.76]
Hikaru 2010	91.8	54.1	108	95.7	47.4	100	1.7%	-3.90 [-17.70 , 9.90]
Totsu 2014	92.3	44.5	119	92.9	40.2	114	2.5%	-0.60 [-11.48 , 10.28]
Subtotal (95% CI)			**974**			**971**	**16.2%**	**-1.05 [-6.55 , 4.45]**
Heterogeneity: Tau² = 10.02; Chi² = 4.19, df = 3 (P = 0.24); I² = 28%								
Test for overall effect: Z = 0.37 (P = 0.71)								
1.4.2 Lactobacillus spp.								
Chrzanowska-Liszewska 2012	49.9	18	21	46	15	26	3.1%	3.90 [-5.72 , 13.52]
Indrio 2017	13.4	13	30	22.4	17.5	30	4.4%	-9.00 [-16.80 , -1.20]
Manzoni 2006	30	28	39	35	30	41	1.9%	-5.00 [-17.71 , 7.71]
Reuman 1986	59.4	56.4	15	38.7	30.6	15	0.3%	20.70 [-11.77 , 53.17]
Subtotal (95% CI)			**105**			**112**	**9.8%**	**-1.95 [-10.81 , 6.90]**
Heterogeneity: Tau² = 39.89; Chi² = 6.37, df = 3 (P = 0.09); I² = 53%								
Test for overall effect: Z = 0.43 (P = 0.67)								
1.4.3 Sacchromyces spp.								
Demirel 2013	55	33.1	135	56	38	136	3.9%	-1.00 [-9.48 , 7.48]
Serce 2013	39	24	99	43	23	100	5.8%	-4.00 [-10.53 , 2.53]
Subtotal (95% CI)			**234**			**236**	**9.6%**	**-2.88 [-8.06 , 2.29]**
Heterogeneity: Tau² = 0.00; Chi² = 0.30, df = 1 (P = 0.58); I² = 0%								
Test for overall effect: Z = 1.09 (P = 0.27)								
1.4.4 Bifidobacterium spp. plus Lactobacillus spp.								
Chowdhury 2016	16	21	52	20	28	44	2.9%	-4.00 [-14.05 , 6.05]
Lin 2005	46.7	27.1	180	46.5	26.1	187	7.4%	0.20 [-5.25 , 5.65]
Lin 2008	46.4	24.2	217	43.3	21	217	9.8%	3.10 [-1.16 , 7.36]
Rougé 2009	60.7	28.8	45	65.6	30	49	2.2%	-4.90 [-16.79 , 6.99]
Roy 2014	25.8	9.2	49	31.2	12.7	48	9.4%	-5.40 [-9.82 , -0.98]
Saengtawesin 2014	60	32	31	57	27	20	1.2%	3.00 [-13.34 , 19.34]
Samanta 2009	17	18	31	24	39	95	2.9%	-7.00 [-17.08 , 3.08]
Subtotal (95% CI)			**605**			**660**	**35.8%**	**-1.74 [-5.22 , 1.73]**
Heterogeneity: Tau² = 7.68; Chi² = 9.81, df = 6 (P = 0.13); I² = 39%								
Test for overall effect: Z = 0.98 (P = 0.33)								
1.4.5 Bifidobacterium spp. plus Streptococcus spp.								
Jacobs 2013	71	28	521	74	26	523	12.5%	-3.00 [-6.28 , 0.28]
Subtotal (95% CI)			**521**			**523**	**12.5%**	**-3.00 [-6.28 , 0.28]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.79 (P = 0.07)								
1.4.6 Bifidobacterium spp. plus Lactobacillus spp. plus Sacchromyces spp.								
Chandrashekar 2018	15.6	23.6	69	23.5	27.9	66	3.7%	-7.90 [-16.64 , 0.84]
Shashidhar 2017	27.6	18.5	48	31.2	22.9	48	4.0%	-3.60 [-11.93 , 4.73]
Subtotal (95% CI)			**117**			**114**	**7.7%**	**-5.65 [-11.68 , 0.38]**
Heterogeneity: Tau² = 0.00; Chi² = 0.49, df = 1 (P = 0.49); I² = 0%								
Test for overall effect: Z = 1.84 (P = 0.07)								
1.4.7 Bifidobacterium spp. plus Lactobacillus spp. plus Streptococcus spp.								
Dashti 2014	27.2	18.4	69	28.8	19.5	67	6.0%	-1.60 [-7.98 , 4.78]
Fernández-Carrocera 2013	59.3	35.6	75	52	32.8	75	2.5%	7.30 [-3.66 , 18.26]
Subtotal (95% CI)			**144**			**142**	**8.5%**	**1.69 [-6.73 , 10.11]**
Heterogeneity: Tau² = 18.69; Chi² = 1.89, df = 1 (P = 0.17); I² = 47%								
Test for overall effect: Z = 0.39 (P = 0.69)								
Total (95% CI)			**2700**			**2758**	**100.0%**	**-1.93 [-3.78 , -0.08]**
Heterogeneity: Tau² = 4.35; Chi² = 28.21, df = 21 (P = 0.13); I² = 26%								
Test for overall effect: Z = 2.05 (P = 0.04)								
Test for subgroup differences: Chi² = 2.56, df = 6 (P = 0.86), I² = 0%								



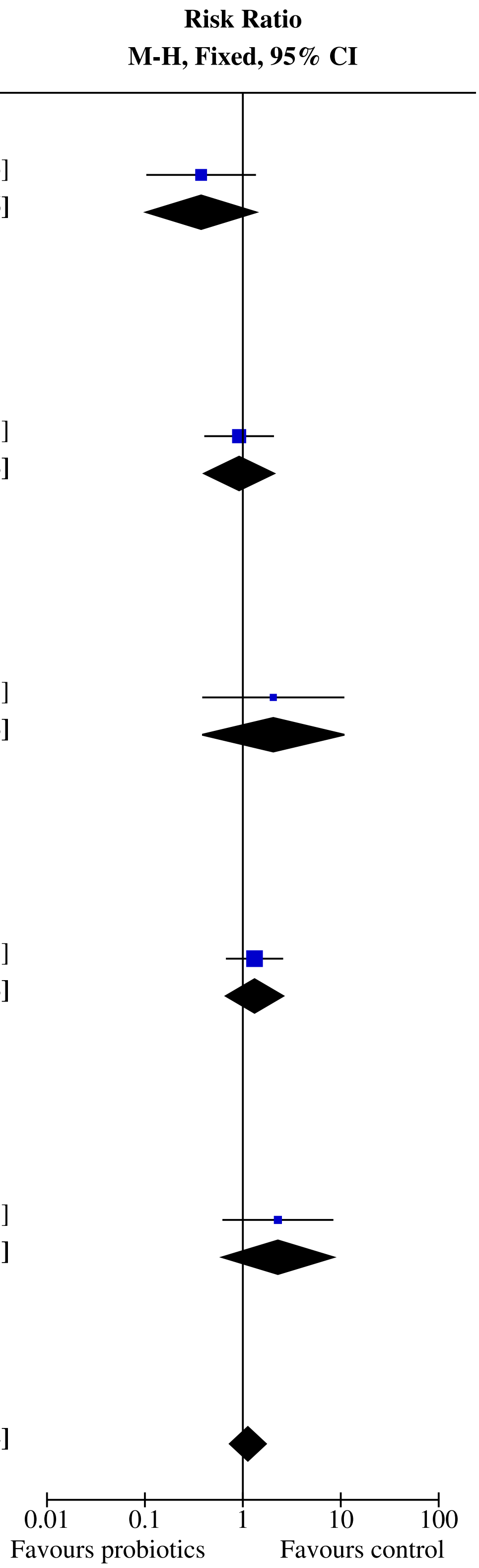
Analysis 1.5. Comparison 1: Probiotics versus control, Outcome 5: Severe neurodevelopmental impairment

Study or Subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.5.1 Bifidobacterium spp.						
Totsu 2014	9	80	12	82	8.0%	0.77 [0.34 , 1.72]
Subtotal (95% CI)		**80**		**82**	**8.0%**	**0.77 [0.34 , 1.72]**
Total events:	9		12			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.64 (P = 0.52)						
1.5.2 Lactobacillus spp.						
Oncel 2014	37	124	37	125	25.0%	1.01 [0.69 , 1.48]
Subtotal (95% CI)		**124**		**125**	**25.0%**	**1.01 [0.69 , 1.48]**
Total events:	37		37			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.04 (P = 0.97)						
1.5.3 Bacillus spp.						
Sari 2011	16	86	15	88	10.1%	1.09 [0.58 , 2.07]
Subtotal (95% CI)		**86**		**88**	**10.1%**	**1.09 [0.58 , 2.07]**
Total events:	16		15			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.27 (P = 0.79)						
1.5.4 Bifidobacterium spp. plus Streptococcus spp.						
Jacobs 2013	56	337	56	327	38.6%	0.97 [0.69 , 1.36]
Subtotal (95% CI)		**337**		**327**	**38.6%**	**0.97 [0.69 , 1.36]**
Total events:	56		56			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.17 (P = 0.86)						
1.5.5 Bifidobacterium spp. plus Lactobacillus spp.						
Lin 2005	37	145	25	124	18.3%	1.27 [0.81 , 1.98]
Subtotal (95% CI)		**145**		**124**	**18.3%**	**1.27 [0.81 , 1.98]**
Total events:	37		25			
Heterogeneity: Not applicable						
Test for overall effect: Z = 1.03 (P = 0.30)						
Total (95% CI)		**772**		**746**	**100.0%**	**1.03 [0.84 , 1.26]**
Total events:	155		145			
Heterogeneity: Chi² = 1.48, df = 4 (P = 0.83); I² = 0%						
Test for overall effect: Z = 0.29 (P = 0.78)						
Test for subgroup differences: Chi² = 1.48, df = 4 (P = 0.83), I² = 0%						



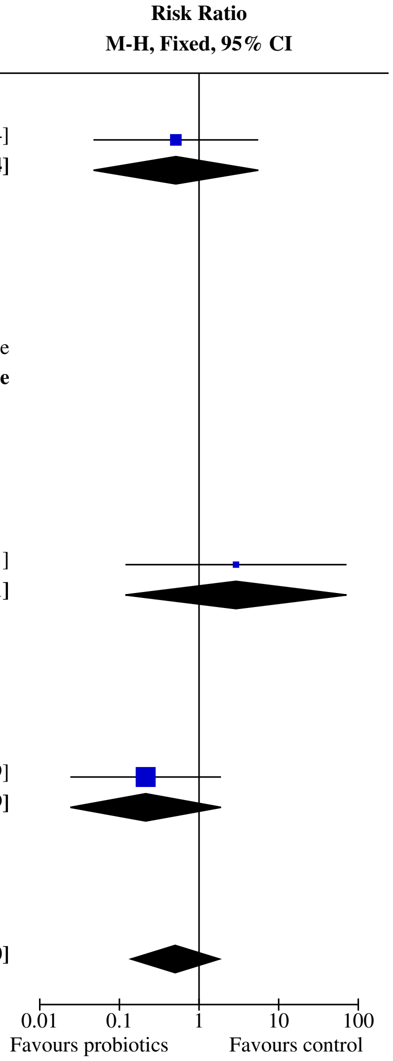
Analysis 1.6. Comparison 1: Probiotics versus control, Outcome 6: Cerebral palsy

Study or Subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.6.1 Bifidobacterium spp.						
Totsu 2014	3	78	8	78	20.8%	0.38 [0.10 , 1.36]
Subtotal (95% CI)		**78**		**78**	**20.8%**	**0.38 [0.10 , 1.36]**
Total events:	3		8			
Heterogeneity: Not applicable						
Test for overall effect: Z = 1.49 (P = 0.14)						
1.6.2 Lactobacillus spp.						
Oncel 2014	10	124	11	125	28.5%	0.92 [0.40 , 2.08]
Subtotal (95% CI)		**124**		**125**	**28.5%**	**0.92 [0.40 , 2.08]**
Total events:	10		11			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.21 (P = 0.83)						
1.6.3 Bacillus spp.						
Sari 2011	4	86	2	88	5.2%	2.05 [0.38 , 10.88]
Subtotal (95% CI)		**86**		**88**	**5.2%**	**2.05 [0.38 , 10.88]**
Total events:	4		2			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.84 (P = 0.40)						
1.6.4 Bifidobacterium spp. plus Streptococcus spp.						
Jacobs 2013	19	337	14	327	37.0%	1.32 [0.67 , 2.58]
Subtotal (95% CI)		**337**		**327**	**37.0%**	**1.32 [0.67 , 2.58]**
Total events:	19		14			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.80 (P = 0.42)						
1.6.5 Bifidobacterium spp. plus Lactobacillus spp.						
Lin 2005	8	145	3	124	8.4%	2.28 [0.62 , 8.41]
Subtotal (95% CI)		**145**		**124**	**8.4%**	**2.28 [0.62 , 8.41]**
Total events:	8		3			
Heterogeneity: Not applicable						
Test for overall effect: Z = 1.24 (P = 0.22)						
Total (95% CI)		**770**		**742**	**100.0%**	**1.13 [0.74 , 1.72]**
Total events:	44		38			
Heterogeneity: Chi² = 4.86, df = 4 (P = 0.30); I² = 18%						
Test for overall effect: Z = 0.55 (P = 0.59)						
Test for subgroup differences: Chi² = 4.86, df = 4 (P = 0.30), I² = 17.7%						



Analysis 1.7. Comparison 1: Probiotics versus control, Outcome 7: Visual impairment

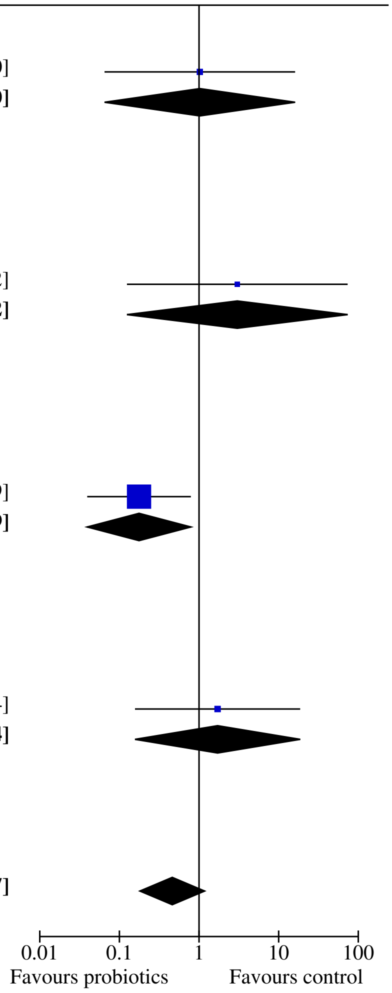
Study or Subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.7.1 Bifidobacterium spp.						
Sari 2011	1	86	2	88	29.1%	0.51 [0.05 , 5.54]
Subtotal (95% CI)		**86**		**88**	**29.1%**	**0.51 [0.05 , 5.54]**
Total events:	1		2			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.55 (P = 0.58)						
1.7.2 Lactobacillus spp.						
Oncel 2014	0	124	0	125		Not estimable
Subtotal (95% CI)		**124**		**125**		**Not estimable**
Total events:	0		0			
Heterogeneity: Not applicable						
Test for overall effect: Not applicable						
1.7.3 Bifidobacterium spp. plus Streptococcus spp.						
Jacobs 2013	1	337	0	327	7.5%	2.91 [0.12 , 71.21]
Subtotal (95% CI)		**337**		**327**	**7.5%**	**2.91 [0.12 , 71.21]**
Total events:	1		0			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.66 (P = 0.51)						
1.7.4 Bifidobacterium spp. plus Lactobacillus spp.						
Lin 2005	1	145	4	124	63.4%	0.21 [0.02 , 1.89]
Subtotal (95% CI)		**145**		**124**	**63.4%**	**0.21 [0.02 , 1.89]**
Total events:	1		4			
Heterogeneity: Not applicable						
Test for overall effect: Z = 1.39 (P = 0.17)						
Total (95% CI)		**692**		**664**	**100.0%**	**0.50 [0.14 , 1.80]**
Total events:	3		6			
Heterogeneity: Chi² = 1.75, df = 2 (P = 0.42); I² = 0%						
Test for overall effect: Z = 1.06 (P = 0.29)						
Test for subgroup differences: Chi² = 1.75, df = 2 (P = 0.42), I² = 0%						



Analysis 1.8. Comparison 1: Probiotics versus control, Outcome 8: Hearing impairment

Study or Subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.8.1 Bifidobacterium spp.						
Sari 2011	1	86	1	88	7.2%	1.02 [0.07 , 16.10]
Subtotal (95% CI)		**86**		**88**	**7.2%**	**1.02 [0.07 , 16.10]**
Total events:	1		1			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.02 (P = 0.99)						
1.8.2 Lactobacillus spp.						
Oncel 2014	1	124	0	125	3.6%	3.02 [0.12 , 73.52]
Subtotal (95% CI)		**124**		**125**	**3.6%**	**3.02 [0.12 , 73.52]**
Total events:	1		0			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.68 (P = 0.50)						
1.8.3 Bifidobacterium spp. plus Streptococcus spp.						
Jacobs 2013	2	337	11	327	81.3%	0.18 [0.04 , 0.79]
Subtotal (95% CI)		**337**		**327**	**81.3%**	**0.18 [0.04 , 0.79]**
Total events:	2		11			
Heterogeneity: Not applicable						
Test for overall effect: Z = 2.27 (P = 0.02)						
1.8.4 Bifidobacterium spp. plus Lactobacillus spp.						
Lin 2005	2	145	1	124	7.9%	1.71 [0.16 , 18.64]
Subtotal (95% CI)		**145**		**124**	**7.9%**	**1.71 [0.16 , 18.64]**
Total events:	2		1			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.44 (P = 0.66)						
Total (95% CI)		**692**		**664**	**100.0%**	**0.46 [0.18 , 1.17]**
Total events:	6		13			
Heterogeneity: Chi² = 4.39, df = 3 (P = 0.22); I² = 32%						
Test for overall effect: Z = 1.62 (P = 0.10)						
Test for subgroup differences: Chi² = 4.35, df = 3 (P = 0.23), I² = 31.1%						

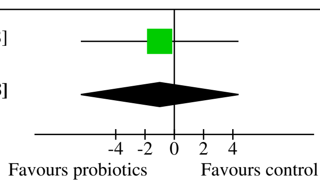
Risk Ratio M-H, Fixed, 95% CI



Analysis 1.9. Comparison 1: Probiotics versus control, Outcome 9: Continuous early learning composite measure

Study or Subgroup	Probiotics Mean	Probiotics SD	Probiotics Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Fixed, 95% CI
Patole 2014	96	9.6	26	97	10.2	26	100.0%	-1.00 [-6.38 , 4.38]
Total (95% CI)			**26**			**26**	**100.0%**	**-1.00 [-6.38 , 4.38]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 0.36 (P = 0.72)								
Test for subgroup differences: Not applicable								

Mean Difference IV, Fixed, 95% CI



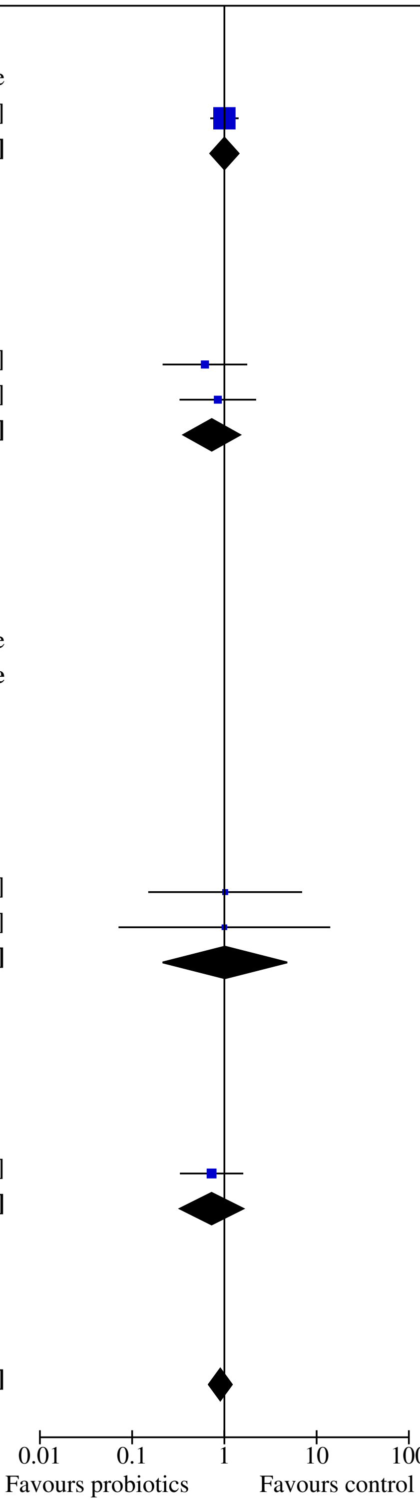
Comparison 2. Probiotics versus control (extremely preterm or ELBW)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2.1 Necrotising enterocolitis	8	1712	Risk Ratio (M-H, Fixed, 95% CI)	0.90 [0.68, 1.21]
2.1.1 Bifidobacterium spp.	2	665	Risk Ratio (M-H, Fixed, 95% CI)	1.00 [0.70, 1.43]
2.1.2 Lactobacillus spp.	2	330	Risk Ratio (M-H, Fixed, 95% CI)	0.73 [0.36, 1.48]
2.1.3 Bacillus spp.	1	120	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
2.1.4 Bifidobacterium spp. plus Lactobacillus spp.	2	123	Risk Ratio (M-H, Fixed, 95% CI)	1.01 [0.21, 4.79]
2.1.5 Bifidobacterium spp. plus Streptococcus spp.	1	474	Risk Ratio (M-H, Fixed, 95% CI)	0.73 [0.33, 1.60]
2.2 Mortality	6	1661	Risk Ratio (M-H, Fixed, 95% CI)	0.91 [0.71, 1.16]
2.2.1 Bifidobacterium spp.	1	474	Risk Ratio (M-H, Fixed, 95% CI)	0.98 [0.60, 1.61]
2.2.2 Lactobacillus spp.	2	330	Risk Ratio (M-H, Fixed, 95% CI)	0.78 [0.42, 1.42]
2.2.3 Bacillus clausii	1	120	Risk Ratio (M-H, Fixed, 95% CI)	0.86 [0.36, 2.08]
2.2.4 Bifidobacterium spp. plus Lactobacillus spp.	1	100	Risk Ratio (M-H, Fixed, 95% CI)	0.75 [0.18, 3.18]
2.2.5 Bifidobacterium spp. plus Streptococcus spp.	1	637	Risk Ratio (M-H, Fixed, 95% CI)	0.94 [0.65, 1.35]
2.3 Invasive infection	6	1471	Risk Ratio (M-H, Fixed, 95% CI)	0.90 [0.76, 1.06]
2.3.1 Bifidobacterium spp.	2	642	Risk Ratio (M-H, Fixed, 95% CI)	1.00 [0.73, 1.37]
2.3.2 Lactobacillus spp.	1	134	Risk Ratio (M-H, Fixed, 95% CI)	1.05 [0.67, 1.66]
2.3.3 Bacillus clausii	1	120	Risk Ratio (M-H, Fixed, 95% CI)	0.80 [0.43, 1.47]
2.3.4 Bifidobacterium spp. plus Lactobacillus spp.	1	101	Risk Ratio (M-H, Fixed, 95% CI)	0.83 [0.45, 1.54]
2.3.5 Bifidobacterium spp. plus Streptococcus spp.	1	474	Risk Ratio (M-H, Fixed, 95% CI)	0.82 [0.64, 1.06]
2.4 Duration of birth hospitalisation (days)	1	22	Mean Difference (IV, Random, 95% CI)	-5.40 [-14.20, 3.40]
2.4.1 Bifidobacterium spp. plus Lactobacillus spp.	1	22	Mean Difference (IV, Random, 95% CI)	-5.40 [-14.20, 3.40]

Analysis 2.1. Comparison 2: Probiotics versus control (extremely preterm or ELBW), Outcome 1: Necrotising enterocolitis

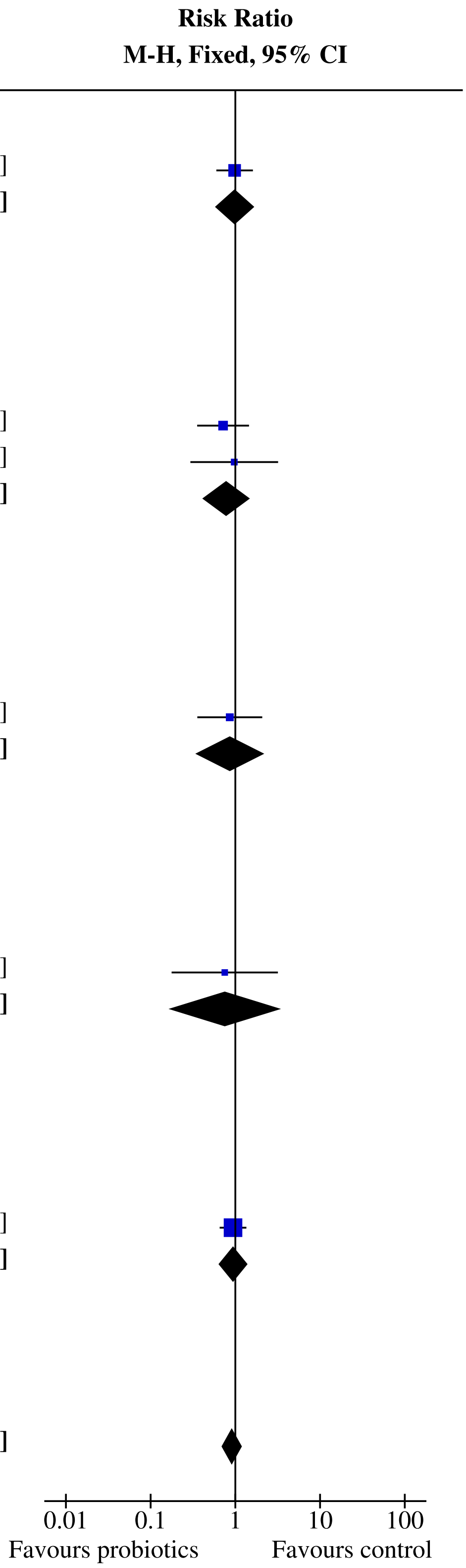
Study or Subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
2.1.1 Bifidobacterium spp.						
Wang 2007	0	11	0	11		Not estimable
Costeloe 2015	50	312	53	331	60.5%	1.00 [0.70 , 1.43]
Subtotal (95% CI)		**323**		**342**	**60.5%**	**1.00 [0.70 , 1.43]**
Total events:	50		53			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.00 (P = 1.00)						
2.1.2 Lactobacillus spp.						
Oncel 2014	5	93	9	103	10.1%	0.62 [0.21 , 1.77]
Wejryd 2019	7	68	8	66	9.6%	0.85 [0.33 , 2.21]
Subtotal (95% CI)		**161**		**169**	**19.6%**	**0.73 [0.36 , 1.48]**
Total events:	12		17			
Heterogeneity: Chi² = 0.20, df = 1 (P = 0.66); I² = 0%						
Test for overall effect: Z = 0.87 (P = 0.38)						
2.1.3 Bacillus spp.						
Tewari 2015	0	61	0	59		Not estimable
Subtotal (95% CI)		**61**		**59**		**Not estimable**
Total events:	0		0			
Heterogeneity: Not applicable						
Test for overall effect: Not applicable						
2.1.4 Bifidobacterium spp. plus Lactobacillus spp.						
Al-Hosni 2012	2	50	2	51	2.3%	1.02 [0.15 , 6.96]
Roy 2014	1	11	1	11	1.2%	1.00 [0.07 , 14.05]
Subtotal (95% CI)		**61**		**62**	**3.5%**	**1.01 [0.21 , 4.79]**
Total events:	3		3			
Heterogeneity: Chi² = 0.00, df = 1 (P = 0.99); I² = 0%						
Test for overall effect: Z = 0.02 (P = 0.99)						
2.1.5 Bifidobacterium spp. plus Streptococcus spp.						
Jacobs 2013	10	235	14	239	16.3%	0.73 [0.33 , 1.60]
Subtotal (95% CI)		**235**		**239**	**16.3%**	**0.73 [0.33 , 1.60]**
Total events:	10		14			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.79 (P = 0.43)						
Total (95% CI)		**841**		**871**	**100.0%**	**0.90 [0.68 , 1.21]**
Total events:	75		87			
Heterogeneity: Chi² = 1.16, df = 5 (P = 0.95); I² = 0%						
Test for overall effect: Z = 0.69 (P = 0.49)						
Test for subgroup differences: Chi² = 0.98, df = 3 (P = 0.81), I² = 0%						

Risk Ratio M-H, Fixed, 95% CI



Analysis 2.2. Comparison 2: Probiotics versus control (extremely preterm or ELBW), Outcome 2: Mortality

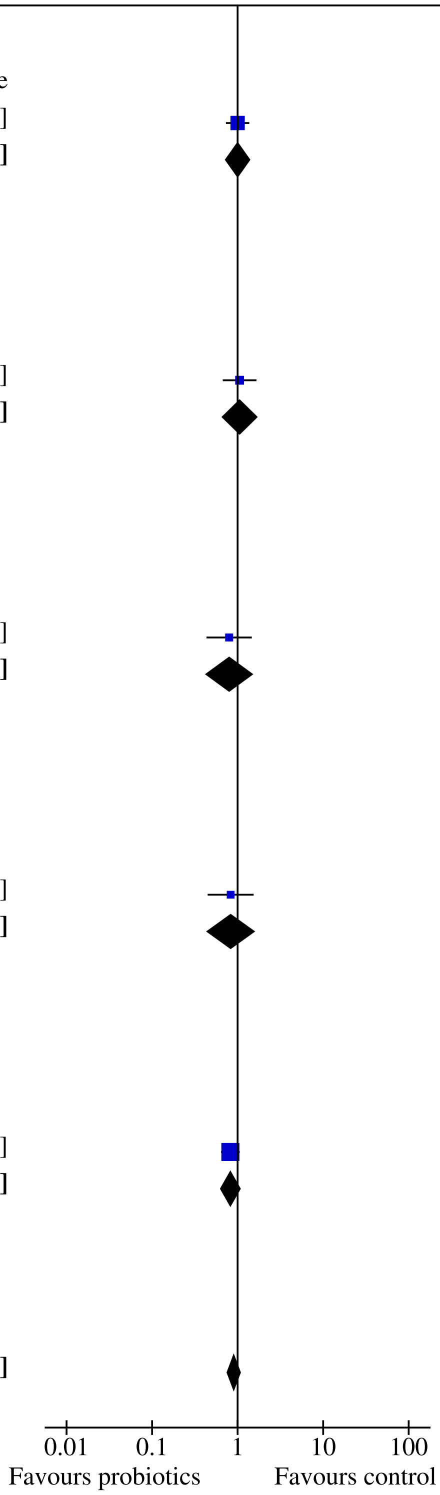
Study or Subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
2.2.1 Bifidobacterium spp.						
Jacobs 2013	27	235	28	239	24.6%	0.98 [0.60 , 1.61]
Subtotal (95% CI)		**235**		**239**	**24.6%**	**0.98 [0.60 , 1.61]**
Total events:	27		28			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.08 (P = 0.94)						
2.2.2 Lactobacillus spp.						
Oncel 2014	11	93	17	103	14.3%	0.72 [0.35 , 1.45]
Wejryd 2019	5	68	5	66	4.5%	0.97 [0.29 , 3.20]
Subtotal (95% CI)		**161**		**169**	**18.8%**	**0.78 [0.42 , 1.42]**
Total events:	16		22			
Heterogeneity: Chi² = 0.18, df = 1 (P = 0.67); I² = 0%						
Test for overall effect: Z = 0.82 (P = 0.41)						
2.2.3 Bacillus clausii						
Tewari 2015	8	61	9	59	8.1%	0.86 [0.36 , 2.08]
Subtotal (95% CI)		**61**		**59**	**8.1%**	**0.86 [0.36 , 2.08]**
Total events:	8		9			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.34 (P = 0.74)						
2.2.4 Bifidobacterium spp. plus Lactobacillus spp.						
Al-Hosni 2012	3	50	4	50	3.5%	0.75 [0.18 , 3.18]
Subtotal (95% CI)		**50**		**50**	**3.5%**	**0.75 [0.18 , 3.18]**
Total events:	3		4			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.39 (P = 0.70)						
2.2.5 Bifidobacterium spp. plus Streptococcus spp.						
Costeloe 2015	46	306	53	331	45.0%	0.94 [0.65 , 1.35]
Subtotal (95% CI)		**306**		**331**	**45.0%**	**0.94 [0.65 , 1.35]**
Total events:	46		53			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.34 (P = 0.73)						
Total (95% CI)		**813**		**848**	**100.0%**	**0.91 [0.71 , 1.16]**
Total events:	100		116			
Heterogeneity: Chi² = 0.65, df = 5 (P = 0.99); I² = 0%						
Test for overall effect: Z = 0.78 (P = 0.44)						
Test for subgroup differences: Chi² = 0.46, df = 4 (P = 0.98), I² = 0%						



Analysis 2.3. Comparison 2: Probiotics versus control (extremely preterm or ELBW), Outcome 3: Invasive infection

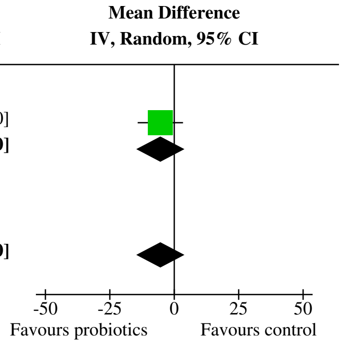
Study or Subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
2.3.1 Bifidobacterium spp.						
Wang 2007	0	11	0	11		Not estimable
Costeloe 2015	63	315	61	305	30.0%	1.00 [0.73 , 1.37]
Subtotal (95% CI)		**326**		**316**	**30.0%**	**1.00 [0.73 , 1.37]**
Total events:	63		61			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.00 (P = 1.00)						
2.3.2 Lactobacillus spp.						
Wejryd 2019	25	68	23	66	11.3%	1.05 [0.67 , 1.66]
Subtotal (95% CI)		**68**		**66**	**11.3%**	**1.05 [0.67 , 1.66]**
Total events:	25		23			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.23 (P = 0.82)						
2.3.3 Bacillus clausii						
Tewari 2015	14	61	17	59	8.4%	0.80 [0.43 , 1.47]
Subtotal (95% CI)		**61**		**59**	**8.4%**	**0.80 [0.43 , 1.47]**
Total events:	14		17			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.73 (P = 0.46)						
2.3.4 Bifidobacterium spp. plus Lactobacillus spp.						
Al-Hosni 2012	13	50	16	51	7.7%	0.83 [0.45 , 1.54]
Subtotal (95% CI)		**50**		**51**	**7.7%**	**0.83 [0.45 , 1.54]**
Total events:	13		16			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.59 (P = 0.55)						
2.3.5 Bifidobacterium spp. plus Streptococcus spp.						
Jacobs 2013	72	235	89	239	42.7%	0.82 [0.64 , 1.06]
Subtotal (95% CI)		**235**		**239**	**42.7%**	**0.82 [0.64 , 1.06]**
Total events:	72		89			
Heterogeneity: Not applicable						
Test for overall effect: Z = 1.51 (P = 0.13)						
Total (95% CI)		**740**		**731**	**100.0%**	**0.90 [0.76 , 1.06]**
Total events:	187		206			
Heterogeneity: Chi² = 1.61, df = 4 (P = 0.81); I² = 0%						
Test for overall effect: Z = 1.23 (P = 0.22)						
Test for subgroup differences: Chi² = 1.60, df = 4 (P = 0.81), I² = 0%						

Risk Ratio M-H, Fixed, 95% CI



Analysis 2.4. Comparison 2: Probiotics versus control (extremely preterm or ELBW), Outcome 4: Duration of birth hospitalisation (days)

Study or Subgroup	Probiotics Mean	Probiotics SD	Probiotics Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
2.4.1 Bifidobacterium spp. plus Lactobacillus spp.								
Roy 2014	28.8	9.2	11	34.2	11.7	11	100.0%	-5.40 [-14.20 , 3.40]
Subtotal (95% CI)			**11**			**11**	**100.0%**	**-5.40 [-14.20 , 3.40]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.20 (P = 0.23)								
Total (95% CI)			**11**			**11**	**100.0%**	**-5.40 [-14.20 , 3.40]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.20 (P = 0.23)								
Test for subgroup differences: Not applicable								



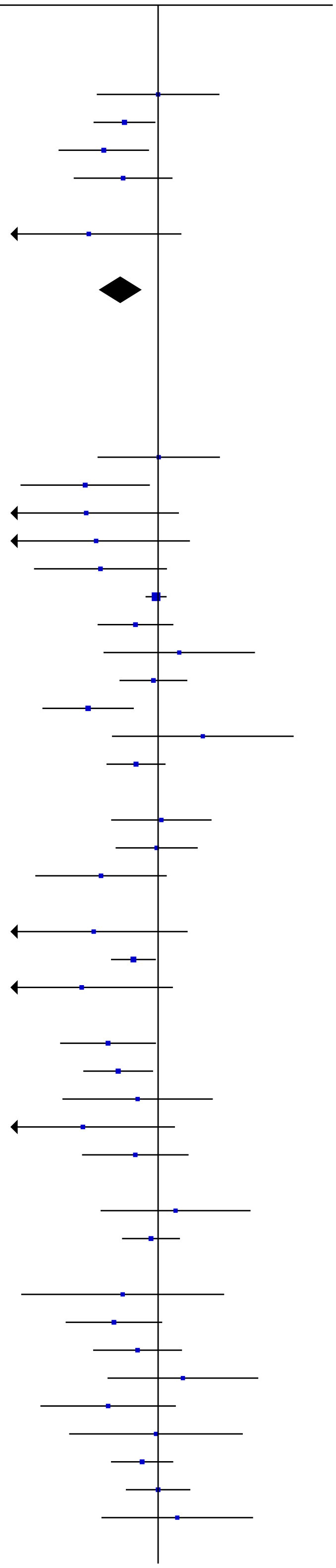
Comparison 3. Subgroup analysis by type of feeding

Outcome or subgroup title	No. of studies	No. of partici-pants	Statistical method	Effect size
3.1 Necrotising enterocolitis	54	10604	Risk Ratio (M-H, Fixed, 95% CI)	0.54 [0.45, 0.65]
3.1.1 Human milk only	8	986	Risk Ratio (M-H, Fixed, 95% CI)	0.30 [0.16, 0.57]
3.1.2 Mixed- human milk or formula or both	42	9364	Risk Ratio (M-H, Fixed, 95% CI)	0.58 [0.48, 0.70]
3.1.3 Formula only	4	254	Risk Ratio (M-H, Fixed, 95% CI)	0.43 [0.16, 1.18]
3.2 Mortality	51	10271	Risk Ratio (M-H, Fixed, 95% CI)	0.76 [0.65, 0.89]
3.2.1 Human milk only	8	986	Risk Ratio (M-H, Fixed, 95% CI)	0.64 [0.41, 1.00]
3.2.2 Mixed- human milk or formula or both	40	9118	Risk Ratio (M-H, Fixed, 95% CI)	0.79 [0.67, 0.94]
3.2.3 Formula only	3	167	Risk Ratio (M-H, Fixed, 95% CI)	0.22 [0.04, 1.21]
3.3 Invasive infection	47	9762	Risk Ratio (M-H, Fixed, 95% CI)	0.89 [0.82, 0.97]
3.3.1 Human milk only	8	986	Risk Ratio (M-H, Fixed, 95% CI)	0.76 [0.59, 0.96]
3.3.2 Mixed- human milk or formula or both	36	8552	Risk Ratio (M-H, Fixed, 95% CI)	0.91 [0.83, 1.00]
3.3.3 Formula only	3	224	Risk Ratio (M-H, Fixed, 95% CI)	0.41 [0.11, 1.49]
3.4 Duration of birth hospitalisation (days)	22	5458	Mean Difference (IV, Random, 95% CI)	-1.93 [-3.78, -0.08]
3.4.1 Human milk only	4	366	Mean Difference (IV, Random, 95% CI)	-3.95 [-7.70, -0.21]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
3.4.2 Mixed- human milk or formualor both	16	5002	Mean Difference (IV, Random, 95% CI)	-1.00 [-2.84, 0.85]
3.4.3 Formula only	2	90	Mean Difference (IV, Random, 95% CI)	1.50 [-26.33, 29.32]

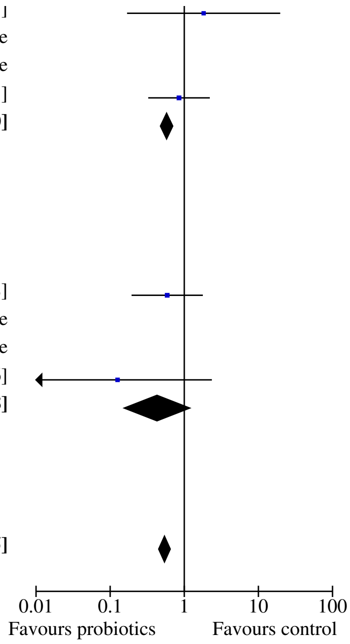
Analysis 3.1. Comparison 3: Subgroup analysis by type of feeding, Outcome 1: Necrotising enterocolitis

Study or Subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
3.1.1 Human milk only						
Chrzanowska-Liszewska 2012	0	21	0	26		Not estimable
Roy 2014	2	56	2	56	0.6%	1.00 [0.15 , 6.85]
Samanta 2009	5	91	15	95	4.5%	0.35 [0.13 , 0.92]
Shadkam 2015	2	30	11	30	3.4%	0.18 [0.04 , 0.75]
Shashidhar 2017	2	49	6	49	1.8%	0.33 [0.07 , 1.57]
Tewari 2015	0	123	0	121		Not estimable
Van Niekerk 2014	0	91	4	93	1.4%	0.11 [0.01 , 2.08]
Zeber-Lubecka 2016	0	27	0	28		Not estimable
Subtotal (95% CI)		**488**		**498**	**11.7%**	**0.30 [0.16 , 0.57]**
Total events:	11		38			
Heterogeneity: Chi² = 2.50, df = 4 (P = 0.64); I² = 0%						
Test for overall effect: Z = 3.68 (P = 0.0002)						
3.1.2 Mixed- human milk or formula or both						
Al-Hosni 2012	2	50	2	51	0.6%	1.02 [0.15 , 6.96]
Bin-Nun 2005	1	72	10	73	3.0%	0.10 [0.01 , 0.77]
Braga 2011	0	119	4	112	1.4%	0.10 [0.01 , 1.92]
Chandrashekar 2018	0	70	3	70	1.1%	0.14 [0.01 , 2.72]
Chowdhury 2016	1	60	6	59	1.9%	0.16 [0.02 , 1.32]
Costeloe 2015	61	650	66	660	20.0%	0.94 [0.67 , 1.31]
Dani 2002	4	295	8	290	2.5%	0.49 [0.15 , 1.61]
Dashti 2014	2	69	1	67	0.3%	1.94 [0.18 , 20.92]
Demirel 2013	6	135	7	136	2.1%	0.86 [0.30 , 2.50]
Dilli 2015	2	100	18	100	5.5%	0.11 [0.03 , 0.47]
Dutta 2015	6	114	0	35	0.2%	4.07 [0.23 , 70.49]
Fernández-Carrocera 2013	6	75	12	75	3.7%	0.50 [0.20 , 1.26]
Fujii 2006	0	11	0	8		Not estimable
Hariharan 2016	3	93	3	103	0.9%	1.11 [0.23 , 5.35]
Hays 2015	8	145	3	52	1.4%	0.96 [0.26 , 3.47]
Hernandez-Enriquez 2016	1	24	5	20	1.7%	0.17 [0.02 , 1.31]
Hikaru 2010	0	108	0	100		Not estimable
Huang 2009	0	95	3	88	1.1%	0.13 [0.01 , 2.53]
Jacobs 2013	11	548	24	551	7.3%	0.46 [0.23 , 0.93]
Kanic 2015	0	40	5	40	1.7%	0.09 [0.01 , 1.59]
Kitajima 1997	0	45	0	46		Not estimable
Lin 2005	2	180	10	187	3.0%	0.21 [0.05 , 0.94]
Lin 2008	4	217	14	217	4.3%	0.29 [0.10 , 0.85]
Manzoni 2006	1	39	2	41	0.6%	0.53 [0.05 , 5.57]
Manzoni 2009	0	238	5	247	1.7%	0.09 [0.01 , 1.70]
Mihatsch 2010	2	91	4	89	1.2%	0.49 [0.09 , 2.60]
Millar 1993	0	10	0	10		Not estimable
Mohan 2006	2	37	1	32	0.3%	1.73 [0.16 , 18.20]
Oncel 2014	8	200	10	200	3.1%	0.80 [0.32 , 1.99]
Oshiro 2019	0	17	0	18		Not estimable
Patole 2014	0	77	1	76	0.5%	0.33 [0.01 , 7.95]
Rehman 2018	2	73	8	73	2.4%	0.25 [0.05 , 1.14]
Ren 2010	3	80	5	70	1.6%	0.53 [0.13 , 2.12]
Rougé 2009	2	45	1	49	0.3%	2.18 [0.20 , 23.21]
Sadowska-Krawczenko 2012	1	30	4	25	1.3%	0.21 [0.02 , 1.75]
Saengtawesin 2014	1	31	1	29	0.3%	0.94 [0.06 , 14.27]
Sari 2011	6	110	10	111	3.0%	0.61 [0.23 , 1.61]
Serce 2013	7	104	7	104	2.1%	1.00 [0.36 , 2.75]
Strus 2018	2	80	1	73	0.3%	1.82 [0.17 , 19.71]
Totsu 2014	0	120	0	102		Not estimable



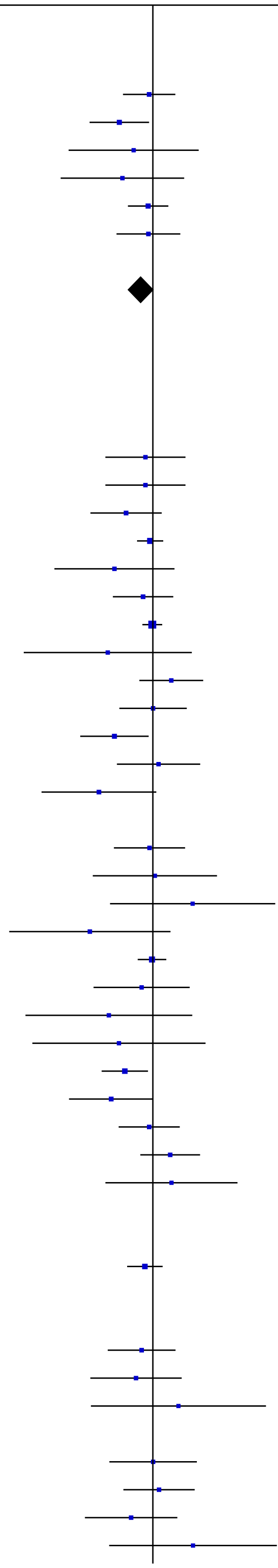
Analysis 3.1. (Continued)

Strus 2018	2	80	1	73	0.5%	1.82 [0.17 , 19.71]
Totsu 2014	0	120	0	102		Not estimable
Wang 2007	0	22	0	22		Not estimable
Wejryd 2019	7	68	8	66	2.5%	0.85 [0.33 , 2.21]
Subtotal (95% CI)		**4787**		**4577**	**85.0%**	**0.58 [0.48 , 0.70]**
Total events:	164		272			
Heterogeneity: Chi² = 41.64, df = 34 (P = 0.17); I² = 18%						
Test for overall effect: Z = 5.71 (P < 0.00001)						
3.1.3 Formula only						
Costalos 2003	5	51	6	36	2.2%	0.59 [0.19 , 1.78]
Indrio 2017	0	30	0	30		Not estimable
Reuman 1986	0	15	0	15		Not estimable
Stratiki 2007	0	41	3	36	1.1%	0.13 [0.01 , 2.36]
Subtotal (95% CI)		**137**		**117**	**3.3%**	**0.43 [0.16 , 1.18]**
Total events:	5		9			
Heterogeneity: Chi² = 0.99, df = 1 (P = 0.32); I² = 0%						
Test for overall effect: Z = 1.64 (P = 0.10)						
Total (95% CI)		**5412**		**5192**	**100.0%**	**0.54 [0.45 , 0.65]**
Total events:	180		319			
Heterogeneity: Chi² = 49.36, df = 41 (P = 0.17); I² = 17%						
Test for overall effect: Z = 6.80 (P < 0.00001)						
Test for subgroup differences: Chi² = 3.81, df = 2 (P = 0.15), I² = 47.6%						



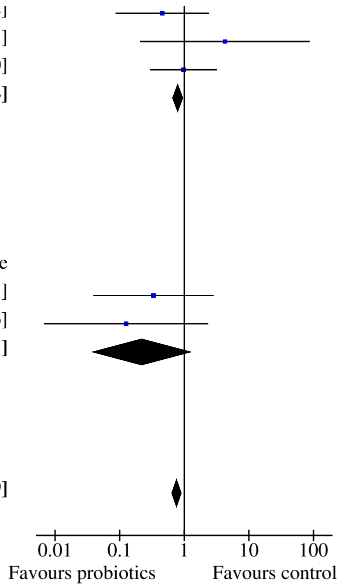
Analysis 3.2. Comparison 3: Subgroup analysis by type of feeding, Outcome 2: Mortality

Study or Subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
3.2.1 Human milk only						
Chrzanowska-Liszewska 2012	0	21	0	26		Not estimable
Roy 2014	7	56	8	56	2.4%	0.88 [0.34 , 2.25]
Samanta 2009	4	91	14	95	4.1%	0.30 [0.10 , 0.87]
Shadkam 2015	1	30	2	30	0.6%	0.50 [0.05 , 5.22]
Shashidhar 2017	1	49	3	49	0.9%	0.33 [0.04 , 3.09]
Tewari 2015	12	123	14	121	4.3%	0.84 [0.41 , 1.75]
Van Niekerk 2014	5	91	6	93	1.8%	0.85 [0.27 , 2.69]
Zeber-Lubecka 2016	0	27	0	28		Not estimable
Subtotal (95% CI)		**488**		**498**	**14.1%**	**0.64 [0.41 , 1.00]**
Total events:	30		47			
Heterogeneity: Chi² = 3.52, df = 5 (P = 0.62); I² = 0%						
Test for overall effect: Z = 1.98 (P = 0.05)						
3.2.2 Mixed- human milk or formula or both						
Al-Hosni 2012	3	50	4	51	1.2%	0.77 [0.18 , 3.25]
Al-Hosni 2012	3	50	4	51	1.2%	0.77 [0.18 , 3.25]
Bin-Nun 2005	3	72	8	73	2.4%	0.38 [0.11 , 1.38]
Braga 2011	26	119	27	112	8.4%	0.91 [0.56 , 1.45]
Chandrashekar 2018	1	70	4	70	1.2%	0.25 [0.03 , 2.18]
Chowdhury 2016	5	60	7	59	2.1%	0.70 [0.24 , 2.09]
Costeloe 2015	54	650	56	660	16.8%	0.98 [0.68 , 1.40]
Dani 2002	0	295	2	290	0.8%	0.20 [0.01 , 4.08]
Dashti 2014	8	69	4	67	1.2%	1.94 [0.61 , 6.15]
Demirel 2013	5	135	5	136	1.5%	1.01 [0.30 , 3.40]
Dilli 2015	3	100	12	100	3.6%	0.25 [0.07 , 0.86]
Dutta 2015	8	114	2	35	0.9%	1.23 [0.27 , 5.52]
Fernández-Carrocera 2013	1	75	7	75	2.1%	0.14 [0.02 , 1.13]
Fujii 2006	0	11	0	8		Not estimable
Hariharan 2016	4	93	5	103	1.4%	0.89 [0.25 , 3.20]
Hays 2015	3	145	1	52	0.4%	1.08 [0.11 , 10.11]
Hernandez-Enriquez 2016	2	24	0	20	0.2%	4.20 [0.21 , 82.72]
Hikaru 2010	0	108	4	100	1.4%	0.10 [0.01 , 1.89]
Jacobs 2013	27	548	28	551	8.4%	0.97 [0.58 , 1.62]
Kanic 2015	2	40	3	40	0.9%	0.67 [0.12 , 3.78]
Kitajima 1997	0	45	2	46	0.7%	0.20 [0.01 , 4.14]
Li 2019	0	16	1	14	0.5%	0.29 [0.01 , 6.69]
Lin 2005	7	180	20	187	5.9%	0.36 [0.16 , 0.84]
Lin 2008	2	217	9	217	2.7%	0.22 [0.05 , 1.02]
Manzoni 2006	5	39	6	41	1.8%	0.88 [0.29 , 2.64]
Manzoni 2009	9	238	5	247	1.5%	1.87 [0.64 , 5.49]
Mihatsch 2010	2	91	1	89	0.3%	1.96 [0.18 , 21.19]
Millar 1993	0	10	0	10		Not estimable
Mohan 2006	0	37	0	32		Not estimable
Oncel 2014	15	200	20	200	6.0%	0.75 [0.40 , 1.42]
Oshiro 2019	0	17	0	18		Not estimable
Patole 2014	0	77	0	76		Not estimable
Rehman 2018	4	73	6	73	1.8%	0.67 [0.20 , 2.26]
Rougé 2009	2	45	4	49	1.2%	0.54 [0.10 , 2.83]
Sadowska-Krawczenko 2012	1	30	0	25	0.2%	2.52 [0.11 , 59.18]
Saengtawesin 2014	0	31	0	29		Not estimable
Sari 2011	3	110	3	111	0.9%	1.01 [0.21 , 4.89]
Serce 2013	5	104	4	104	1.2%	1.25 [0.35 , 4.52]
Strus 2018	2	80	4	73	1.3%	0.46 [0.09 , 2.42]
Totsu 2014	2	120	0	102	0.2%	4.26 [0.21 , 87.65]



Analysis 3.2. (Continued)

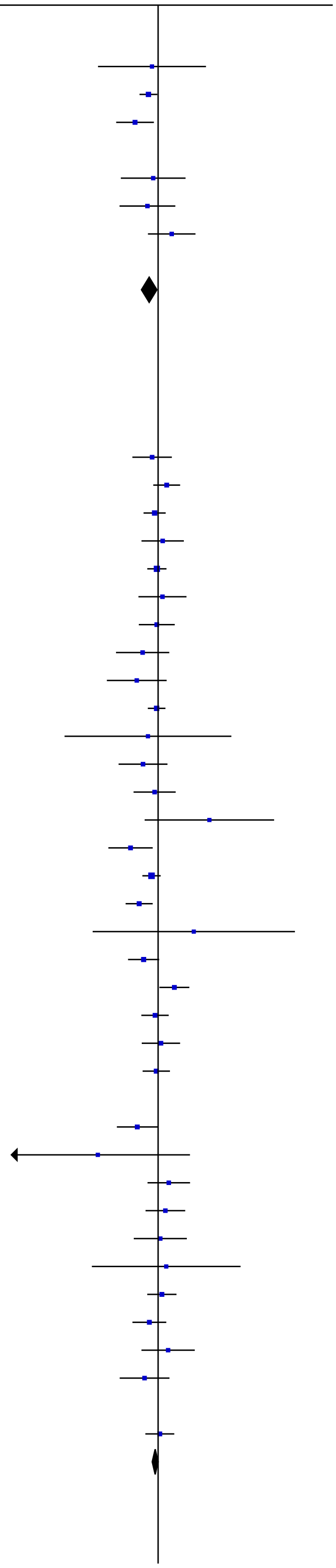
Strus 2018	2	80	4	73	1.3%	0.46 [0.09 , 2.42]
Totsu 2014	2	120	0	102	0.2%	4.26 [0.21 , 87.65]
Wejryd 2019	5	68	5	66	1.5%	0.97 [0.29 , 3.20]
Subtotal (95% CI)		**4656**		**4462**	**83.9%**	**0.79 [0.67 , 0.94]**
Total events:	222		273			
Heterogeneity: Chi² = 30.81, df = 34 (P = 0.62); I² = 0%						
Test for overall effect: Z = 2.73 (P = 0.006)						
3.2.3 Formula only						
Indrio 2017	0	30	0	30		Not estimable
Reuman 1986	1	15	3	15	0.9%	0.33 [0.04 , 2.85]
Stratiki 2007	0	41	3	36	1.1%	0.13 [0.01 , 2.36]
Subtotal (95% CI)		**86**		**81**	**2.0%**	**0.22 [0.04 , 1.21]**
Total events:	1		6			
Heterogeneity: Chi² = 0.28, df = 1 (P = 0.59); I² = 0%						
Test for overall effect: Z = 1.75 (P = 0.08)						
Total (95% CI)		**5230**		**5041**	**100.0%**	**0.76 [0.65 , 0.89]**
Total events:	253		326			
Heterogeneity: Chi² = 37.21, df = 42 (P = 0.68); I² = 0%						
Test for overall effect: Z = 3.46 (P = 0.0005)						
Test for subgroup differences: Chi² = 2.80, df = 2 (P = 0.25), I² = 28.7%						



Analysis 3.3. Comparison 3: Subgroup analysis by type of feeding, Outcome 3: Invasive infection

Study or Subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
3.3.1 Human milk only						
Chrzanowska-Liszewska 2012	2	21	3	26	0.3%	0.83 [0.15 , 4.49]
Roy 2014	31	56	42	56	5.0%	0.74 [0.56 , 0.98]
Samanta 2009	13	91	28	95	3.3%	0.48 [0.27 , 0.88]
Shadkam 2015	0	30	0	30		Not estimable
Shashidhar 2017	6	49	7	49	0.8%	0.86 [0.31 , 2.37]
Tewari 2015	8	123	11	121	1.3%	0.72 [0.30 , 1.72]
Van Niekerk 2014	15	91	10	93	1.2%	1.53 [0.73 , 3.23]
Zeber-Lubecka 2016	0	27	0	28		Not estimable
Subtotal (95% CI)		**488**		**498**	**11.9%**	**0.76 [0.59 , 0.96]**
Total events:	75		101			
Heterogeneity: Chi² = 5.73, df = 5 (P = 0.33); I² = 13%						
Test for overall effect: Z = 2.28 (P = 0.02)						
3.3.2 Mixed- human milk or formula or both						
Al-Hosni 2012	13	50	16	51	1.9%	0.83 [0.45 , 1.54]
Bin-Nun 2005	31	72	24	73	2.8%	1.31 [0.86 , 2.00]
Braga 2011	40	119	42	112	5.2%	0.90 [0.63 , 1.27]
Chandrashekar 2018	15	70	13	70	1.5%	1.15 [0.59 , 2.24]
Costeloe 2015	73	650	77	660	9.1%	0.96 [0.71 , 1.30]
Dani 2002	14	295	12	290	1.4%	1.15 [0.54 , 2.44]
Demirel 2013	20	135	21	136	2.5%	0.96 [0.55 , 1.69]
Dilli 2015	8	100	13	100	1.5%	0.62 [0.27 , 1.42]
Dutta 2015	10	114	6	35	1.1%	0.51 [0.20 , 1.31]
Fernández-Carrocera 2013	42	75	44	75	5.2%	0.95 [0.72 , 1.26]
Fujii 2006	1	11	1	8	0.1%	0.73 [0.05 , 9.97]
Hariharan 2016	9	93	16	103	1.8%	0.62 [0.29 , 1.34]
Hays 2015	25	145	10	52	1.8%	0.90 [0.46 , 1.74]
Hernandez-Enriquez 2016	6	24	1	20	0.1%	5.00 [0.66 , 38.15]
Hikaru 2010	10	108	22	100	2.7%	0.42 [0.21 , 0.84]
Jacobs 2013	72	548	89	551	10.6%	0.81 [0.61 , 1.08]
Kanic 2015	16	40	29	40	3.5%	0.55 [0.36 , 0.84]
Kitajima 1997	1	45	0	46	0.1%	3.07 [0.13 , 73.32]
Lin 2005	22	180	36	187	4.2%	0.63 [0.39 , 1.04]
Lin 2008	40	217	24	217	2.9%	1.67 [1.04 , 2.67]
Manzoni 2006	19	39	22	41	2.6%	0.91 [0.59 , 1.40]
Manzoni 2009	20	238	19	247	2.2%	1.09 [0.60 , 1.99]
Mihatsch 2010	28	91	29	89	3.5%	0.94 [0.61 , 1.45]
Millar 1993	0	10	0	10		Not estimable
Oncel 2014	13	200	25	200	3.0%	0.52 [0.27 , 0.99]
Oshiro 2019	0	17	3	18	0.4%	0.15 [0.01 , 2.72]
Patole 2014	17	77	12	76	1.4%	1.40 [0.72 , 2.73]
Rougé 2009	15	45	13	49	1.5%	1.26 [0.67 , 2.34]
Sadowska-Krawczenko 2012	9	30	7	25	0.9%	1.07 [0.47 , 2.46]
Saengtawesin 2014	2	31	1	20	0.1%	1.29 [0.13 , 13.31]
Sari 2011	29	110	26	111	3.1%	1.13 [0.71 , 1.78]
Serce 2013	19	104	25	104	3.0%	0.76 [0.45 , 1.29]
Strus 2018	12	80	8	73	1.0%	1.37 [0.59 , 3.16]
Totsu 2014	10	120	13	102	1.7%	0.65 [0.30 , 1.43]
Wang 2007	0	22	0	22		Not estimable
Wejryd 2019	25	68	23	66	2.8%	1.05 [0.67 , 1.66]
Subtotal (95% CI)		**4373**		**4179**	**87.2%**	**0.91 [0.83 , 1.00]**
Total events:	686		722			
Heterogeneity: Chi² = 40.61, df = 33 (P = 0.17); I² = 19%						
Test for overall effect: Z = 1.96 (P = 0.05)						

Risk Ratio
M-H, Fixed, 95% CI



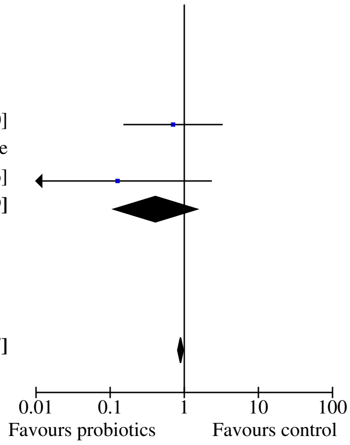
Analysis 3.3. (Continued)

Heterogeneity: Chi² = 40.61, df = 33 (P = 0.17); I² = 19%
Test for overall effect: Z = 1.96 (P = 0.05)

3.3.3 Formula only						
Costalos 2003	3	51	3	36	0.4%	0.71 [0.15 , 3.30]
Indrio 2017	0	30	0	30		Not estimable
Stratiki 2007	0	41	3	36	0.4%	0.13 [0.01 , 2.36]
Subtotal (95% CI)		**122**		**102**	**0.9%**	**0.41 [0.11 , 1.49]**
Total events:	3		6			
Total (95% CI)		**4983**		**4779**	**100.0%**	**0.89 [0.82 , 0.97]**
Total events:	764		829			

3.3.3 Formula only: Heterogeneity: Chi² = 1.10, df = 1 (P = 0.29); I² = 9%
Test for overall effect: Z = 1.36 (P = 0.17)

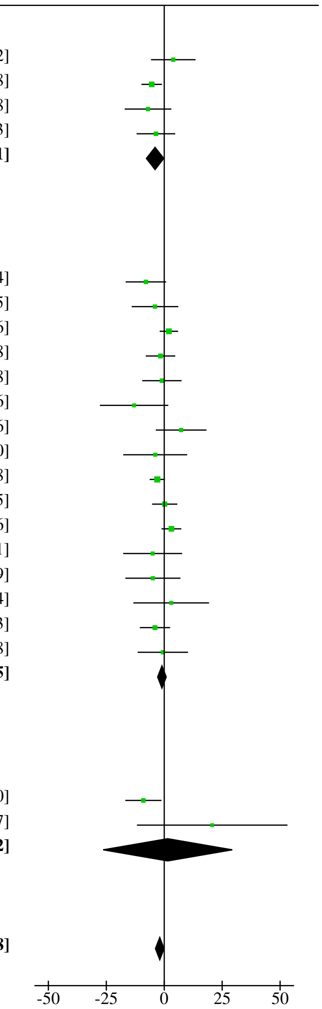
Total: Heterogeneity: Chi² = 50.79, df = 41 (P = 0.14); I² = 19%
Test for overall effect: Z = 2.69 (P = 0.007)
Test for subgroup differences: Chi² = 3.45, df = 2 (P = 0.18), I² = 42.0%



Analysis 3.4. Comparison 3: Subgroup analysis by type of feeding, Outcome 4: Duration of birth hospitalisation (days)

Study or Subgroup	Probiotics Mean	Probiotics SD	Probiotics Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
3.4.1 Human milk only								
Chrzanowska-Liszewska 2012	49.9	18	21	46	15	26	3.1%	3.90 [-5.72 , 13.52]
Roy 2014	25.8	9.2	49	31.2	12.7	48	9.4%	-5.40 [-9.82 , -0.98]
Samanta 2009	17	18	31	24	39	95	2.9%	-7.00 [-17.08 , 3.08]
Shashidhar 2017	27.6	18.5	48	31.2	22.9	48	4.0%	-3.60 [-11.93 , 4.73]
Subtotal (95% CI)			**149**			**217**	**19.4%**	**-3.95 [-7.70 , -0.21]**
Heterogeneity: Tau² = 1.64; Chi² = 3.32, df = 3 (P = 0.34); I² = 10%								
Test for overall effect: Z = 2.07 (P = 0.04)								
3.4.2 Mixed- human milk or formulaor both								
Chandrashekar 2018	15.6	23.6	69	23.5	27.9	66	3.7%	-7.90 [-16.64 , 0.84]
Chowdhury 2016	16	21	52	20	28	44	2.9%	-4.00 [-14.05 , 6.05]
Costeloe 2015	68	37	647	66	36	657	10.6%	2.00 [-1.96 , 5.96]
Dashti 2014	27.2	18.4	69	28.8	19.5	67	6.0%	-1.60 [-7.98 , 4.78]
Demirel 2013	55	33.1	135	56	38	136	3.9%	-1.00 [-9.48 , 7.48]
Dilli 2015	37	38	100	50	65	100	1.5%	-13.00 [-27.76 , 1.76]
Fernández-Carrocera 2013	59.3	35.6	75	52	32.8	75	2.5%	7.30 [-3.66 , 18.26]
Hikaru 2010	91.8	54.1	108	95.7	47.4	100	1.7%	-3.90 [-17.70 , 9.90]
Jacobs 2013	71	28	521	74	26	523	12.5%	-3.00 [-6.28 , 0.28]
Lin 2005	46.7	27.1	180	46.5	26.1	187	7.4%	0.20 [-5.25 , 5.65]
Lin 2008	46.4	24.2	217	43.3	21	217	9.8%	3.10 [-1.16 , 7.36]
Manzoni 2006	30	28	39	35	30	41	1.9%	-5.00 [-17.71 , 7.71]
Rougé 2009	60.7	28.8	45	65.6	30	49	2.2%	-4.90 [-16.79 , 6.99]
Saengtawesin 2014	60	32	31	57	27	20	1.2%	3.00 [-13.34 , 19.34]
Serce 2013	39	24	99	43	23	100	5.8%	-4.00 [-10.53 , 2.53]
Totsu 2014	92.3	44.5	119	92.9	40.2	114	2.5%	-0.60 [-11.48 , 10.28]
Subtotal (95% CI)			**2506**			**2496**	**75.8%**	**-1.00 [-2.84 , 0.85]**
Heterogeneity: Tau² = 1.52; Chi² = 16.89, df = 15 (P = 0.33); I² = 11%								
Test for overall effect: Z = 1.06 (P = 0.29)								
3.4.3 Formula only								
Indrio 2017	13.4	13	30	22.4	17.5	30	4.4%	-9.00 [-16.80 , -1.20]
Reuman 1986	59.4	56.4	15	38.7	30.6	15	0.3%	20.70 [-11.77 , 53.17]
Subtotal (95% CI)			**45**			**45**	**4.7%**	**1.50 [-26.33 , 29.32]**
Heterogeneity: Tau² = 295.88; Chi² = 3.04, df = 1 (P = 0.08); I² = 67%								
Test for overall effect: Z = 0.11 (P = 0.92)								
Total (95% CI)			**2700**			**2758**	**100.0%**	**-1.93 [-3.78 , -0.08]**
Heterogeneity: Tau² = 4.35; Chi² = 28.21, df = 21 (P = 0.13); I² = 26%								
Test for overall effect: Z = 2.05 (P = 0.04)								
Test for subgroup differences: Chi² = 1.98, df = 2 (P = 0.37), I² = 0%								

Mean Difference IV, Random, 95% CI



Comparison 4. Sensitivity analyses: Risk of bias

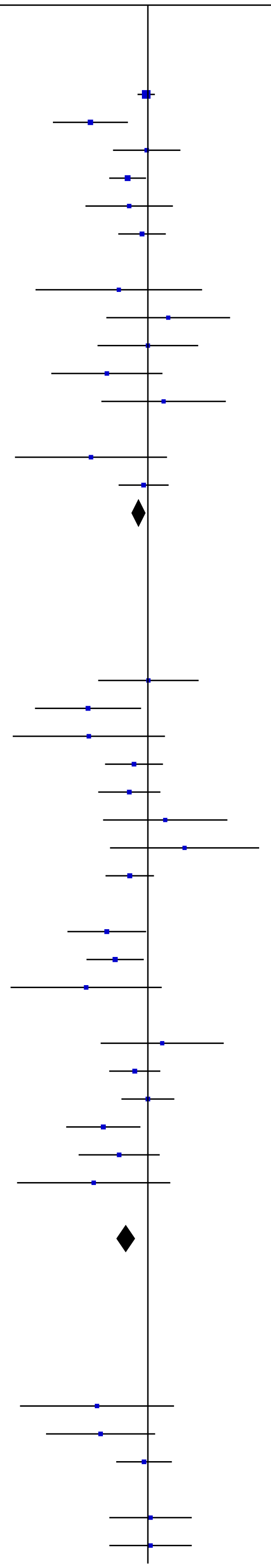
Outcome or subgroup title	No. of studies	No. of partici-pants	Statistical method	Effect size
4.1 Necrotising entero-colitis	54	10800	Risk Ratio (M-H, Fixed, 95% CI)	0.55 [0.46, 0.65]
4.1.1 LOW	16	4597	Risk Ratio (M-H, Fixed, 95% CI)	0.70 [0.55, 0.89]
4.1.2 UNCLEAR	20	3905	Risk Ratio (M-H, Fixed, 95% CI)	0.43 [0.31, 0.59]
4.1.3 HIGH	18	2298	Risk Ratio (M-H, Fixed, 95% CI)	0.41 [0.26, 0.63]
4.2 Mortality	51	10170	Risk Ratio (M-H, Fixed, 95% CI)	0.76 [0.65, 0.89]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
4.2.1 LOW	16	4597	Risk Ratio (M-H, Fixed, 95% CI)	0.86 [0.69, 1.07]
4.2.2 UNCLEAR	19	3818	Risk Ratio (M-H, Fixed, 95% CI)	0.71 [0.54, 0.94]
4.2.3 HIGH	16	1755	Risk Ratio (M-H, Fixed, 95% CI)	0.57 [0.38, 0.85]
4.3 Invasive infection	47	9762	Risk Ratio (M-H, Fixed, 95% CI)	0.89 [0.82, 0.97]
4.3.1 LOW	16	4597	Risk Ratio (M-H, Fixed, 95% CI)	0.90 [0.79, 1.02]
4.3.2 UNCLEAR	18	3700	Risk Ratio (M-H, Fixed, 95% CI)	0.96 [0.84, 1.10]
4.3.3 HIGH	13	1465	Risk Ratio (M-H, Fixed, 95% CI)	0.73 [0.59, 0.90]
4.4 Duration of birth hospitalisation (days)	22	5458	Mean Difference (IV, Random, 95% CI)	-1.93 [-3.78, -0.08]
4.4.1 LOW	6	2786	Mean Difference (IV, Random, 95% CI)	-2.24 [-5.76, 1.29]
4.4.2 UNCLEAR	8	1675	Mean Difference (IV, Random, 95% CI)	-0.99 [-4.07, 2.10]
4.4.3 HIGH	8	997	Mean Difference (IV, Random, 95% CI)	-3.92 [-7.91, 0.07]
4.5 Severe neurodevelopmental impairment	5	1518	Risk Ratio (M-H, Fixed, 95% CI)	1.03 [0.84, 1.26]
4.5.1 LOW	2	913	Risk Ratio (M-H, Fixed, 95% CI)	0.99 [0.76, 1.27]
4.5.2 UNCLEAR	3	605	Risk Ratio (M-H, Fixed, 95% CI)	1.11 [0.79, 1.54]
4.6 Cerebral palsy	5	1512	Risk Ratio (M-H, Fixed, 95% CI)	1.13 [0.74, 1.72]
4.6.1 LOW	2	913	Risk Ratio (M-H, Fixed, 95% CI)	1.14 [0.68, 1.92]
4.6.2 UNCLEAR	3	599	Risk Ratio (M-H, Fixed, 95% CI)	1.09 [0.52, 2.28]
4.7 Visual impairment	4	1356	Risk Ratio (M-H, Fixed, 95% CI)	0.50 [0.14, 1.80]
4.7.1 LOW	2	913	Risk Ratio (M-H, Fixed, 95% CI)	2.91 [0.12, 71.21]
4.7.2 UNCLEAR	2	443	Risk Ratio (M-H, Fixed, 95% CI)	0.31 [0.06, 1.49]
4.8 Hearing impairment	4	1356	Risk Ratio (M-H, Fixed, 95% CI)	0.46 [0.18, 1.17]
4.8.1 LOW	2	913	Risk Ratio (M-H, Fixed, 95% CI)	0.30 [0.09, 0.98]
4.8.2 UNCLEAR	2	443	Risk Ratio (M-H, Fixed, 95% CI)	1.38 [0.23, 8.29]

Analysis 4.1. Comparison 4: Sensitivity analyses: Risk of bias, Outcome 1: Necrotising enterocolitis

Study or Subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
4.1.1 LOW						
Chrzanowska-Liszewska 2012	0	21	0	26		Not estimable
Costeloe 2015	61	650	66	660	19.9%	0.94 [0.67 , 1.31]
Dilli 2015	2	100	18	100	5.5%	0.11 [0.03 , 0.47]
Hays 2015	8	145	3	52	1.3%	0.96 [0.26 , 3.47]
Jacobs 2013	11	548	24	551	7.3%	0.46 [0.23 , 0.93]
Mihatsch 2010	2	91	4	89	1.2%	0.49 [0.09 , 2.60]
Oncel 2014	8	200	10	200	3.0%	0.80 [0.32 , 1.99]
Oshiro 2019	0	17	0	18		Not estimable
Patole 2014	0	77	1	76	0.5%	0.33 [0.01 , 7.95]
Rougé 2009	2	45	1	49	0.3%	2.18 [0.20 , 23.21]
Roy 2014	2	56	2	56	0.6%	1.00 [0.15 , 6.85]
Sadowska-Krawczenko 2012	1	30	4	25	1.3%	0.21 [0.02 , 1.75]
Strus 2018	2	80	1	73	0.3%	1.82 [0.17 , 19.71]
Tewari 2015	0	123	0	121		Not estimable
Van Niekerk 2014	0	91	4	93	1.4%	0.11 [0.01 , 2.08]
Wejryd 2019	7	68	8	66	2.5%	0.85 [0.33 , 2.21]
Subtotal (95% CI)		**2342**		**2255**	**45.0%**	**0.70 [0.55 , 0.89]**
Total events:	106		146			
Heterogeneity: Chi² = 15.98, df = 12 (P = 0.19); I² = 25%						
Test for overall effect: Z = 2.92 (P = 0.004)						
4.1.2 UNCLEAR						
Al-Hosni 2012	2	50	2	51	0.6%	1.02 [0.15 , 6.96]
Bin-Nun 2005	1	72	10	73	3.0%	0.10 [0.01 , 0.77]
Braga 2011	0	119	4	112	1.4%	0.10 [0.01 , 1.92]
Costalos 2003	5	51	6	36	2.1%	0.59 [0.19 , 1.78]
Dani 2002	4	295	8	290	2.4%	0.49 [0.15 , 1.61]
Dashti 2014	2	69	1	67	0.3%	1.94 [0.18 , 20.92]
Dutta 2015	6	114	0	35	0.2%	4.07 [0.23 , 70.49]
Fernández-Carrocera 2013	6	75	12	75	3.6%	0.50 [0.20 , 1.26]
Indrio 2017	0	30	0	30		Not estimable
Lin 2005	2	180	10	187	3.0%	0.21 [0.05 , 0.94]
Lin 2008	4	217	14	217	4.2%	0.29 [0.10 , 0.85]
Manzoni 2009	0	238	5	247	1.6%	0.09 [0.01 , 1.70]
Millar 1993	0	10	0	10		Not estimable
Mohan 2006	2	37	1	32	0.3%	1.73 [0.16 , 18.20]
Sari 2011	6	110	10	111	3.0%	0.61 [0.23 , 1.61]
Serce 2013	7	104	7	104	2.1%	1.00 [0.36 , 2.75]
Shadkam 2015	2	30	11	30	3.3%	0.18 [0.04 , 0.75]
Shashidhar 2017	2	49	6	49	1.8%	0.33 [0.07 , 1.57]
Stratiki 2007	0	41	3	36	1.1%	0.13 [0.01 , 2.36]
Totsu 2014	0	120	0	102		Not estimable
Subtotal (95% CI)		**2011**		**1894**	**34.4%**	**0.43 [0.31 , 0.59]**
Total events:	51		110			
Heterogeneity: Chi² = 17.21, df = 16 (P = 0.37); I² = 7%						
Test for overall effect: Z = 5.07 (P < 0.00001)						
4.1.3 HIGH						
Chandrashekar 2018	0	70	3	70	1.1%	0.14 [0.01 , 2.72]
Chowdhury 2016	1	60	6	59	1.8%	0.16 [0.02 , 1.32]
Demirel 2013	6	135	7	136	2.1%	0.86 [0.30 , 2.50]
Fujii 2006	0	11	0	8		Not estimable
Hariharan 2016	3	93	3	103	0.9%	1.11 [0.23 , 5.35]
Hariharan 2016	3	93	3	103	0.9%	1.11 [0.23 , 5.35]

Risk Ratio
M-H, Fixed, 95% CI



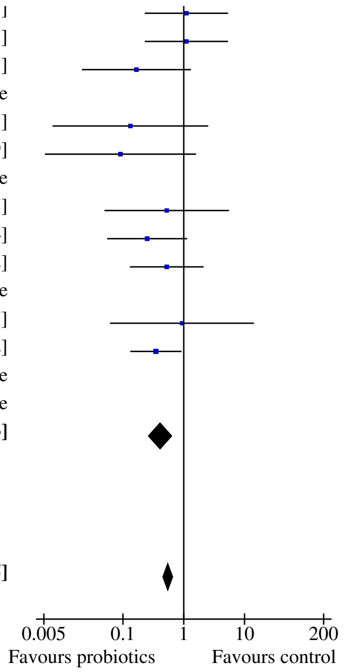
Analysis 4.1. (Continued)

Hariharan 2016	3	93	3	103	0.9%	1.11 [0.23 , 5.35]
Hariharan 2016	3	93	3	103	0.9%	1.11 [0.23 , 5.35]
Hernandez-Enriquez 2016	1	24	5	20	1.7%	0.17 [0.02 , 1.31]
Hikaru 2010	0	108	0	100		Not estimable
Huang 2009	0	95	3	88	1.1%	0.13 [0.01 , 2.53]
Kanic 2015	0	40	5	40	1.7%	0.09 [0.01 , 1.59]
Kitajima 1997	0	45	0	46		Not estimable
Manzoni 2006	1	39	2	41	0.6%	0.53 [0.05 , 5.57]
Rehman 2018	2	73	8	73	2.4%	0.25 [0.05 , 1.14]
Ren 2010	3	80	5	70	1.6%	0.53 [0.13 , 2.12]
Reuman 1986	0	15	0	15		Not estimable
Saengtawesin 2014	1	31	1	29	0.3%	0.94 [0.06 , 14.27]
Samanta 2009	5	91	15	95	4.5%	0.35 [0.13 , 0.92]
Wang 2007	0	22	0	22		Not estimable
Zeber-Lubecka 2016	0	27	0	28		Not estimable
Subtotal (95% CI)		**1152**		**1146**	**20.6%**	**0.41 [0.26 , 0.63]**
Total events:	26		66			

Heterogeneity: Chi² = 9.59, df = 12 (P = 0.65); I² = 0%
Test for overall effect: Z = 4.09 (P < 0.0001)

Total (95% CI)		**5505**		**5295**	**100.0%**	**0.55 [0.46 , 0.65]**
Total events:	183		322			

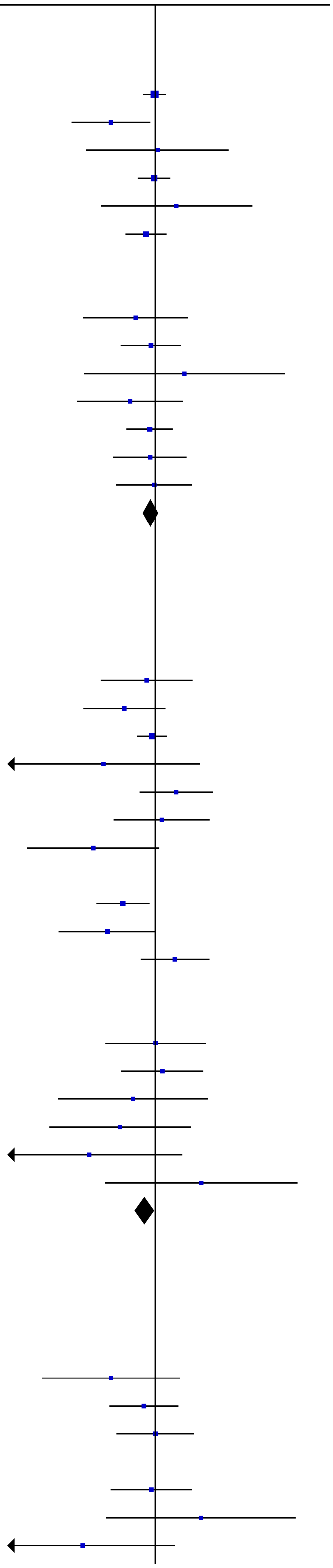
Heterogeneity: Chi² = 49.93, df = 42 (P = 0.19); I² = 16%
Test for overall effect: Z = 6.75 (P < 0.00001)
Test for subgroup differences: Chi² = 7.82, df = 2 (P = 0.02), I² = 74.4%



Analysis 4.2. Comparison 4: Sensitivity analyses: Risk of bias, Outcome 2: Mortality

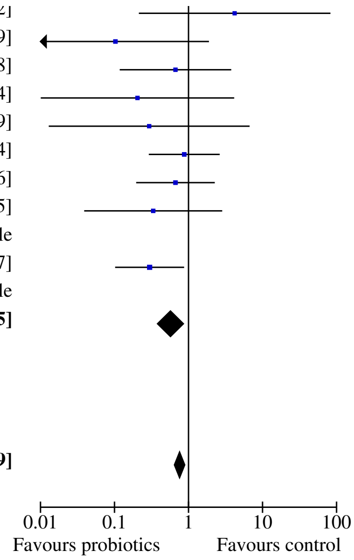
Study or Subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
4.2.1 LOW						
Chrzanowska-Liszewska 2012	0	21	0	26		Not estimable
Costeloe 2015	54	650	56	660	17.0%	0.98 [0.68 , 1.40]
Dilli 2015	3	100	12	100	3.7%	0.25 [0.07 , 0.86]
Hays 2015	3	145	1	52	0.4%	1.08 [0.11 , 10.11]
Jacobs 2013	27	548	28	551	8.5%	0.97 [0.58 , 1.62]
Mihatsch 2010	2	91	1	89	0.3%	1.96 [0.18 , 21.19]
Oncel 2014	15	200	20	200	6.1%	0.75 [0.40 , 1.42]
Oshiro 2019	0	17	0	18		Not estimable
Patole 2014	0	77	0	76		Not estimable
Rougé 2009	2	45	4	49	1.2%	0.54 [0.10 , 2.83]
Roy 2014	7	56	8	56	2.4%	0.88 [0.34 , 2.25]
Sadowska-Krawczenko 2012	1	30	0	25	0.2%	2.52 [0.11 , 59.18]
Strus 2018	2	80	4	73	1.3%	0.46 [0.09 , 2.42]
Tewari 2015	12	123	14	121	4.3%	0.84 [0.41 , 1.75]
Van Niekerk 2014	5	91	6	93	1.8%	0.85 [0.27 , 2.69]
Wejryd 2019	5	68	5	66	1.5%	0.97 [0.29 , 3.20]
Subtotal (95% CI)		**2342**		**2255**	**48.8%**	**0.86 [0.69 , 1.07]**
Total events:	138		159			
Heterogeneity: Chi² = 6.57, df = 12 (P = 0.88); I² = 0%						
Test for overall effect: Z = 1.35 (P = 0.18)						
4.2.2 UNCLEAR						
Al-Hosni 2012	3	50	4	51	1.2%	0.77 [0.18 , 3.25]
Bin-Nun 2005	3	72	8	73	2.4%	0.38 [0.11 , 1.38]
Braga 2011	26	119	27	112	8.5%	0.91 [0.56 , 1.45]
Dani 2002	0	295	2	290	0.8%	0.20 [0.01 , 4.08]
Dashti 2014	8	69	4	67	1.2%	1.94 [0.61 , 6.15]
Dutta 2015	8	114	2	35	0.9%	1.23 [0.27 , 5.52]
Fernández-Carrocera 2013	1	75	7	75	2.1%	0.14 [0.02 , 1.13]
Indrio 2017	0	30	0	30		Not estimable
Lin 2005	7	180	20	187	6.0%	0.36 [0.16 , 0.84]
Lin 2008	2	217	9	217	2.7%	0.22 [0.05 , 1.02]
Manzoni 2009	9	238	5	247	1.5%	1.87 [0.64 , 5.49]
Millar 1993	0	10	0	10		Not estimable
Mohan 2006	0	37	0	32		Not estimable
Sari 2011	3	110	3	111	0.9%	1.01 [0.21 , 4.89]
Serce 2013	5	104	4	104	1.2%	1.25 [0.35 , 4.52]
Shadkam 2015	1	30	2	30	0.6%	0.50 [0.05 , 5.22]
Shashidhar 2017	1	49	3	49	0.9%	0.33 [0.04 , 3.09]
Stratiki 2007	0	41	3	36	1.1%	0.13 [0.01 , 2.36]
Totsu 2014	2	120	0	102	0.2%	4.26 [0.21 , 87.65]
Subtotal (95% CI)		**1960**		**1858**	**32.4%**	**0.71 [0.54 , 0.94]**
Total events:	79		103			
Heterogeneity: Chi² = 20.30, df = 15 (P = 0.16); I² = 26%						
Test for overall effect: Z = 2.38 (P = 0.02)						
4.2.3 HIGH						
Chandrashekar 2018	1	70	4	70	1.2%	0.25 [0.03 , 2.18]
Chowdhury 2016	5	60	7	59	2.2%	0.70 [0.24 , 2.09]
Demirel 2013	5	135	5	136	1.5%	1.01 [0.30 , 3.40]
Fujii 2006	0	11	0	8		Not estimable
Hariharan 2016	4	93	5	103	1.4%	0.89 [0.25 , 3.20]
Hernandez-Enriquez 2016	2	24	0	20	0.2%	4.20 [0.21 , 82.72]
Hikaru 2010	0	108	4	100	1.4%	0.10 [0.01 , 1.89]

Risk Ratio M-H, Fixed, 95% CI



Analysis 4.2. (Continued)

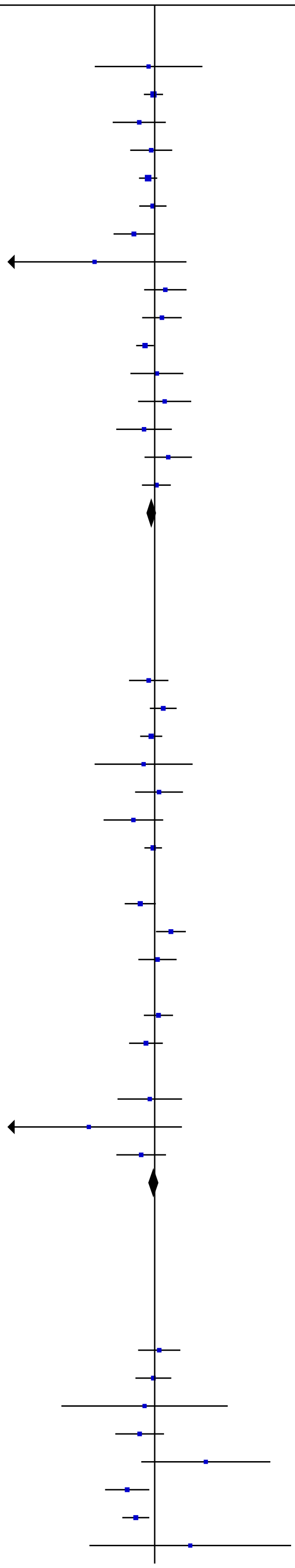
Hernandez-Enriquez 2016	2	24	0	20	0.2%	4.20 [0.21 , 82.72]
Hikaru 2010	0	108	4	100	1.4%	0.10 [0.01 , 1.89]
Kanic 2015	2	40	3	40	0.9%	0.67 [0.12 , 3.78]
Kitajima 1997	0	45	2	46	0.8%	0.20 [0.01 , 4.14]
Li 2019	0	16	1	14	0.5%	0.29 [0.01 , 6.69]
Manzoni 2006	5	39	6	41	1.8%	0.88 [0.29 , 2.64]
Rehman 2018	4	73	6	73	1.8%	0.67 [0.20 , 2.26]
Reuman 1986	1	15	3	15	0.9%	0.33 [0.04 , 2.85]
Saengtawesin 2014	0	31	0	29		Not estimable
Samanta 2009	4	91	14	95	4.2%	0.30 [0.10 , 0.87]
Zeber-Lubecka 2016	0	27	0	28		Not estimable
Subtotal (95% CI)		**878**		**877**	**18.8%**	**0.57 [0.38 , 0.85]**
Total events:	33		60			
Heterogeneity: Chi² = 7.98, df = 12 (P = 0.79); I² = 0%						
Test for overall effect: Z = 2.75 (P = 0.006)						
Total (95% CI)		**5180**		**4990**	**100.0%**	**0.76 [0.65 , 0.89]**
Total events:	250		322			
Heterogeneity: Chi² = 37.21, df = 41 (P = 0.64); I² = 0%						
Test for overall effect: Z = 3.45 (P = 0.0006)						
Test for subgroup differences: Chi² = 3.41, df = 2 (P = 0.18), I² = 41.3%						



Analysis 4.3. Comparison 4: Sensitivity analyses: Risk of bias, Outcome 3: Invasive infection

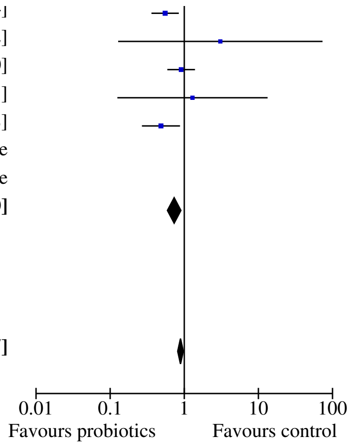
Study or Subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
4.3.1 LOW						
Chrzanowska-Liszewska 2012	2	21	3	26	0.3%	0.83 [0.15 , 4.49]
Costeloe 2015	73	650	77	660	9.1%	0.96 [0.71 , 1.30]
Dilli 2015	8	100	13	100	1.5%	0.62 [0.27 , 1.42]
Hays 2015	25	145	10	52	1.8%	0.90 [0.46 , 1.74]
Jacobs 2013	72	548	89	551	10.6%	0.81 [0.61 , 1.08]
Mihatsch 2010	28	91	29	89	3.5%	0.94 [0.61 , 1.45]
Oncel 2014	13	200	25	200	3.0%	0.52 [0.27 , 0.99]
Oshiro 2019	0	17	3	18	0.4%	0.15 [0.01 , 2.72]
Patole 2014	17	77	12	76	1.4%	1.40 [0.72 , 2.73]
Rougé 2009	15	45	13	49	1.5%	1.26 [0.67 , 2.34]
Roy 2014	31	56	42	56	5.0%	0.74 [0.56 , 0.98]
Sadowska-Krawczenko 2012	9	30	7	25	0.9%	1.07 [0.47 , 2.46]
Strus 2018	12	80	8	73	1.0%	1.37 [0.59 , 3.16]
Tewari 2015	8	123	11	121	1.3%	0.72 [0.30 , 1.72]
Van Niekerk 2014	15	91	10	93	1.2%	1.53 [0.73 , 3.23]
Wejryd 2019	25	68	23	66	2.8%	1.05 [0.67 , 1.66]
Subtotal (95% CI)		**2342**		**2255**	**45.3%**	**0.90 [0.79 , 1.02]**
Total events:	353		375			
Heterogeneity: Chi² = 14.32, df = 15 (P = 0.50); I² = 0%						
Test for overall effect: Z = 1.64 (P = 0.10)						
4.3.2 UNCLEAR						
Al-Hosni 2012	13	50	16	51	1.9%	0.83 [0.45 , 1.54]
Bin-Nun 2005	31	72	24	73	2.8%	1.31 [0.86 , 2.00]
Braga 2011	40	119	42	112	5.2%	0.90 [0.63 , 1.27]
Costalos 2003	3	51	3	36	0.4%	0.71 [0.15 , 3.30]
Dani 2002	14	295	12	290	1.4%	1.15 [0.54 , 2.44]
Dutta 2015	10	114	6	35	1.1%	0.51 [0.20 , 1.31]
Fernández-Carrocera 2013	42	75	44	75	5.2%	0.95 [0.72 , 1.26]
Indrio 2017	0	30	0	30		Not estimable
Lin 2005	22	180	36	187	4.2%	0.63 [0.39 , 1.04]
Lin 2008	40	217	24	217	2.9%	1.67 [1.04 , 2.67]
Manzoni 2009	20	238	19	247	2.2%	1.09 [0.60 , 1.99]
Millar 1993	0	10	0	10		Not estimable
Sari 2011	29	110	26	111	3.1%	1.13 [0.71 , 1.78]
Serce 2013	19	104	25	104	3.0%	0.76 [0.45 , 1.29]
Shadkam 2015	0	30	0	30		Not estimable
Shashidhar 2017	6	49	7	49	0.8%	0.86 [0.31 , 2.37]
Stratiki 2007	0	41	3	36	0.4%	0.13 [0.01 , 2.36]
Totsu 2014	10	120	13	102	1.7%	0.65 [0.30 , 1.43]
Subtotal (95% CI)		**1905**		**1795**	**36.4%**	**0.96 [0.84 , 1.10]**
Total events:	299		300			
Heterogeneity: Chi² = 16.79, df = 14 (P = 0.27); I² = 17%						
Test for overall effect: Z = 0.60 (P = 0.55)						
4.3.3 HIGH						
Chandrashekar 2018	15	70	13	70	1.5%	1.15 [0.59 , 2.24]
Demirel 2013	20	135	21	136	2.5%	0.96 [0.55 , 1.69]
Fujii 2006	1	11	1	8	0.1%	0.73 [0.05 , 9.97]
Hariharan 2016	9	93	16	103	1.8%	0.62 [0.29 , 1.34]
Hernandez-Enriquez 2016	6	24	1	20	0.1%	5.00 [0.66 , 38.15]
Hikaru 2010	10	108	22	100	2.7%	0.42 [0.21 , 0.84]
Kanic 2015	16	40	29	40	3.5%	0.55 [0.36 , 0.84]
Kitajima 1997	1	45	0	46	0.1%	3.07 [0.13 , 73.32]

Risk Ratio M-H, Fixed, 95% CI



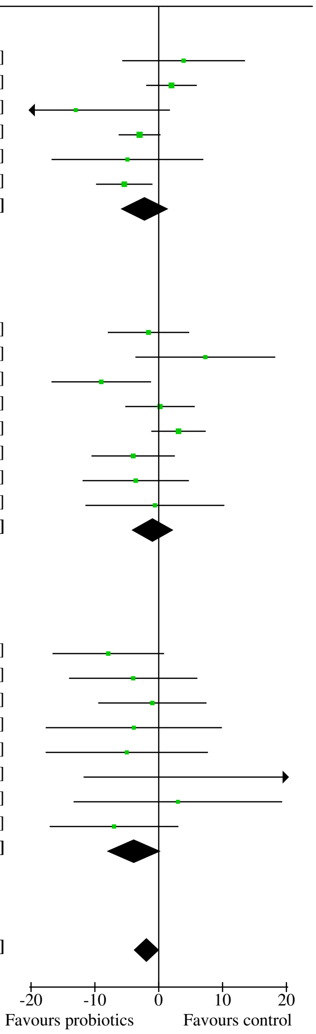
Analysis 4.3. (Continued)

Kanic 2015	16	40	29	40	3.5%	0.55 [0.36 , 0.84]
Kitajima 1997	1	45	0	46	0.1%	3.07 [0.13 , 73.32]
Manzoni 2006	19	39	22	41	2.6%	0.91 [0.59 , 1.40]
Saengtawesin 2014	2	31	1	20	0.1%	1.29 [0.13 , 13.31]
Samanta 2009	13	91	28	95	3.3%	0.48 [0.27 , 0.88]
Wang 2007	0	22	0	22		Not estimable
Zeber-Lubecka 2016	0	27	0	28		Not estimable
Subtotal (95% CI)		**736**		**729**	**18.3%**	**0.73 [0.59 , 0.90]**
Total events:	112		154			
Heterogeneity: Chi² = 14.25, df = 10 (P = 0.16); I² = 30%						
Test for overall effect: Z = 2.96 (P = 0.003)						
Total (95% CI)		**4983**		**4779**	**100.0%**	**0.89 [0.82 , 0.97]**
Total events:	764		829			
Heterogeneity: Chi² = 50.79, df = 41 (P = 0.14); I² = 19%						
Test for overall effect: Z = 2.69 (P = 0.007)						
Test for subgroup differences: Chi² = 4.62, df = 2 (P = 0.10), I² = 56.7%						



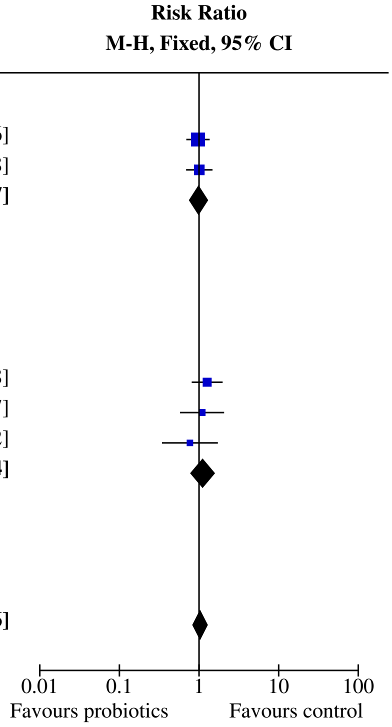
Analysis 4.4. Comparison 4: Sensitivity analyses: Risk of bias, Outcome 4: Duration of birth hospitalisation (days)

Study or Subgroup	Probiotics Mean	Probiotics SD	Probiotics Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
4.4.1 LOW								
Chrzanowska-Liszewska 2012	49.9	18	21	46	15	26	3.1%	3.90 [-5.72 , 13.52]
Costeloe 2015	68	37	647	66	36	657	10.6%	2.00 [-1.96 , 5.96]
Dilli 2015	37	38	100	50	65	100	1.5%	-13.00 [-27.76 , 1.76]
Jacobs 2013	71	28	521	74	26	523	12.5%	-3.00 [-6.28 , 0.28]
Rougé 2009	60.7	28.8	45	65.6	30	49	2.2%	-4.90 [-16.79 , 6.99]
Roy 2014	25.8	9.2	49	31.2	12.7	48	9.4%	-5.40 [-9.82 , -0.98]
Subtotal (95% CI)			**1383**			**1403**	**39.2%**	**-2.24 [-5.76 , 1.29]**
Heterogeneity: Tau² = 8.54; Chi² = 10.35, df = 5 (P = 0.07); I² = 52%								
Test for overall effect: Z = 1.24 (P = 0.21)								
4.4.2 UNCLEAR								
Dashti 2014	27.2	18.4	69	28.8	19.5	67	6.0%	-1.60 [-7.98 , 4.78]
Fernández-Carrocera 2013	59.3	35.6	75	52	32.8	75	2.5%	7.30 [-3.66 , 18.26]
Indrio 2017	13.4	13	30	22.4	17.5	30	4.4%	-9.00 [-16.80 , -1.20]
Lin 2005	46.7	27.1	180	46.5	26.1	187	7.4%	0.20 [-5.25 , 5.65]
Lin 2008	46.4	24.2	217	43.3	21	217	9.8%	3.10 [-1.16 , 7.36]
Serce 2013	39	24	99	43	23	100	5.8%	-4.00 [-10.53 , 2.53]
Shashidhar 2017	27.6	18.5	48	31.2	22.9	48	4.0%	-3.60 [-11.93 , 4.73]
Totsu 2014	92.3	44.5	119	92.9	40.2	114	2.5%	-0.60 [-11.48 , 10.28]
Subtotal (95% CI)			**837**			**838**	**42.3%**	**-0.99 [-4.07 , 2.10]**
Heterogeneity: Tau² = 6.94; Chi² = 11.06, df = 7 (P = 0.14); I² = 37%								
Test for overall effect: Z = 0.63 (P = 0.53)								
4.4.3 HIGH								
Chandrashekar 2018	15.6	23.6	69	23.5	27.9	66	3.7%	-7.90 [-16.64 , 0.84]
Chowdhury 2016	16	21	52	20	28	44	2.9%	-4.00 [-14.05 , 6.05]
Demirel 2013	55	33.1	135	56	38	136	3.9%	-1.00 [-9.48 , 7.48]
Hikaru 2010	91.8	54.1	108	95.7	47.4	100	1.7%	-3.90 [-17.70 , 9.90]
Manzoni 2006	30	28	39	35	30	41	1.9%	-5.00 [-17.71 , 7.71]
Reuman 1986	59.4	56.4	15	38.7	30.6	15	0.3%	20.70 [-11.77 , 53.17]
Saengtawesin 2014	60	32	31	57	27	20	1.2%	3.00 [-13.34 , 19.34]
Samanta 2009	17	18	31	24	39	95	2.9%	-7.00 [-17.08 , 3.08]
Subtotal (95% CI)			**480**			**517**	**18.4%**	**-3.92 [-7.91 , 0.07]**
Heterogeneity: Tau² = 0.00; Chi² = 4.54, df = 7 (P = 0.72); I² = 0%								
Test for overall effect: Z = 1.93 (P = 0.05)								
Total (95% CI)			**2700**			**2758**	**100.0%**	**-1.93 [-3.78 , -0.08]**
Heterogeneity: Tau² = 4.35; Chi² = 28.21, df = 21 (P = 0.13); I² = 26%								
Test for overall effect: Z = 2.05 (P = 0.04)								
Test for subgroup differences: Chi² = 1.30, df = 2 (P = 0.52), I² = 0%								



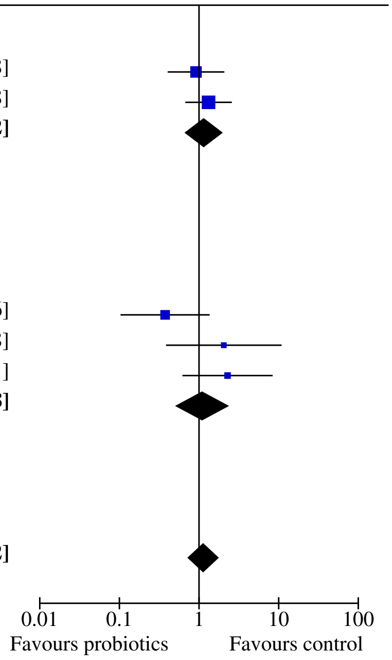
Analysis 4.5. Comparison 4: Sensitivity analyses: Risk of bias, Outcome 5: Severe neurodevelopmental impairment

Study or Subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
4.5.1 LOW						
Jacobs 2013	56	337	56	327	38.6%	0.97 [0.69 , 1.36]
Oncel 2014	37	124	37	125	25.0%	1.01 [0.69 , 1.48]
Subtotal (95% CI)		**461**		**452**	**63.6%**	**0.99 [0.76 , 1.27]**
Total events:	93		93			
Heterogeneity: Chi² = 0.02, df = 1 (P = 0.88); I² = 0%						
Test for overall effect: Z = 0.12 (P = 0.91)						
4.5.2 UNCLEAR						
Lin 2005	37	145	25	124	18.3%	1.27 [0.81 , 1.98]
Sari 2011	16	86	15	88	10.1%	1.09 [0.58 , 2.07]
Totsu 2014	9	80	12	82	8.0%	0.77 [0.34 , 1.72]
Subtotal (95% CI)		**311**		**294**	**36.4%**	**1.11 [0.79 , 1.54]**
Total events:	62		52			
Heterogeneity: Chi² = 1.13, df = 2 (P = 0.57); I² = 0%						
Test for overall effect: Z = 0.60 (P = 0.55)						
Total (95% CI)		**772**		**746**	**100.0%**	**1.03 [0.84 , 1.26]**
Total events:	155		145			
Heterogeneity: Chi² = 1.48, df = 4 (P = 0.83); I² = 0%						
Test for overall effect: Z = 0.29 (P = 0.78)						
Test for subgroup differences: Chi² = 0.30, df = 1 (P = 0.58), I² = 0%						



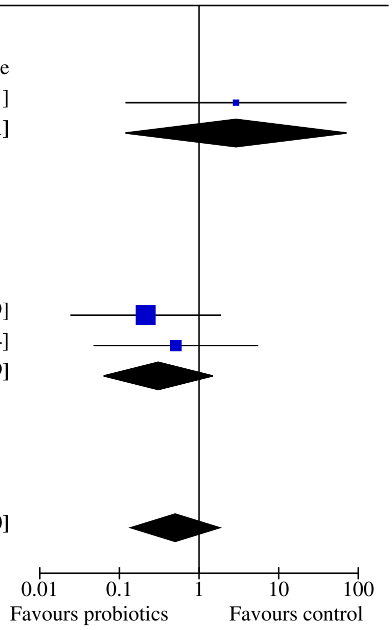
Analysis 4.6. Comparison 4: Sensitivity analyses: Risk of bias, Outcome 6: Cerebral palsy

Study or Subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
4.6.1 LOW						
Oncel 2014	10	124	11	125	28.5%	0.92 [0.40 , 2.08]
Jacobs 2013	19	337	14	327	37.0%	1.32 [0.67 , 2.58]
Subtotal (95% CI)		**461**		**452**	**65.6%**	**1.14 [0.68 , 1.92]**
Total events:	29		25			
Heterogeneity: Chi² = 0.45, df = 1 (P = 0.50); I² = 0%						
Test for overall effect: Z = 0.50 (P = 0.61)						
4.6.2 UNCLEAR						
Totsu 2014	3	78	8	78	20.8%	0.38 [0.10 , 1.36]
Sari 2011	4	86	2	88	5.2%	2.05 [0.38 , 10.88]
Lin 2005	8	145	3	124	8.4%	2.28 [0.62 , 8.41]
Subtotal (95% CI)		**309**		**290**	**34.4%**	**1.09 [0.52 , 2.28]**
Total events:	15		13			
Heterogeneity: Chi² = 4.41, df = 2 (P = 0.11); I² = 55%						
Test for overall effect: Z = 0.23 (P = 0.82)						
Total (95% CI)		**770**		**742**	**100.0%**	**1.13 [0.74 , 1.72]**
Total events:	44		38			
Heterogeneity: Chi² = 4.86, df = 4 (P = 0.30); I² = 18%						
Test for overall effect: Z = 0.55 (P = 0.59)						
Test for subgroup differences: Chi² = 0.01, df = 1 (P = 0.92), I² = 0%						



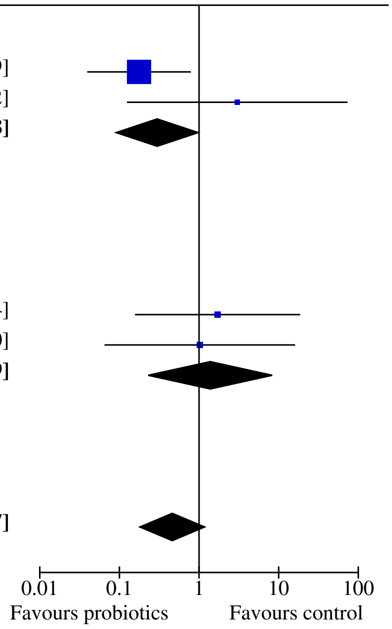
Analysis 4.7. Comparison 4: Sensitivity analyses: Risk of bias, Outcome 7: Visual impairment

Study or Subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
4.7.1 LOW						
Oncel 2014	0	124	0	125		Not estimable
Jacobs 2013	1	337	0	327	7.5%	2.91 [0.12 , 71.21]
Subtotal (95% CI)		**461**		**452**	**7.5%**	**2.91 [0.12 , 71.21]**
Total events:	1		0			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.66 (P = 0.51)						
4.7.2 UNCLEAR						
Lin 2005	1	145	4	124	63.4%	0.21 [0.02 , 1.89]
Sari 2011	1	86	2	88	29.1%	0.51 [0.05 , 5.54]
Subtotal (95% CI)		**231**		**212**	**92.5%**	**0.31 [0.06 , 1.49]**
Total events:	2		6			
Heterogeneity: Chi² = 0.28, df = 1 (P = 0.60); I² = 0%						
Test for overall effect: Z = 1.47 (P = 0.14)						
Total (95% CI)		**692**		**664**	**100.0%**	**0.50 [0.14 , 1.80]**
Total events:	3		6			
Heterogeneity: Chi² = 1.75, df = 2 (P = 0.42); I² = 0%						
Test for overall effect: Z = 1.06 (P = 0.29)						
Test for subgroup differences: Chi² = 1.53, df = 1 (P = 0.22), I² = 34.6%						



Analysis 4.8. Comparison 4: Sensitivity analyses: Risk of bias, Outcome 8: Hearing impairment

Study or Subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
4.8.1 LOW						
Jacobs 2013	2	337	11	327	81.3%	0.18 [0.04 , 0.79]
Oncel 2014	1	124	0	125	3.6%	3.02 [0.12 , 73.52]
Subtotal (95% CI)		**461**		**452**	**84.9%**	**0.30 [0.09 , 0.98]**
Total events:	3		11			
Heterogeneity: Chi² = 2.50, df = 1 (P = 0.11); I² = 60%						
Test for overall effect: Z = 2.00 (P = 0.05)						
4.8.2 UNCLEAR						
Lin 2005	2	145	1	124	7.9%	1.71 [0.16 , 18.64]
Sari 2011	1	86	1	88	7.2%	1.02 [0.07 , 16.10]
Subtotal (95% CI)		**231**		**212**	**15.1%**	**1.38 [0.23 , 8.29]**
Total events:	3		2			
Heterogeneity: Chi² = 0.08, df = 1 (P = 0.78); I² = 0%						
Test for overall effect: Z = 0.35 (P = 0.72)						
Total (95% CI)		**692**		**664**	**100.0%**	**0.46 [0.18 , 1.17]**
Total events:	6		13			
Heterogeneity: Chi² = 4.39, df = 3 (P = 0.22); I² = 32%						
Test for overall effect: Z = 1.62 (P = 0.10)						
Test for subgroup differences: Chi² = 1.96, df = 1 (P = 0.16), I² = 48.9%						



APPENDICES

Appendix 1. Electronic search methodology

Cochrane probiotics search strategies February 2020

Bibliographic databases: Cochrane Central register of Controlled Trials (CENTRAL), CINAHL, Embase, Maternity & Infant Care, MEDLINE

Trial registers: WHO ICTRP & ClinicalTrials.gov

Cochrane Register of Controlled Trials (CENTRAL)

Search date = 18th February 2020; 126 records

#1 MeSH descriptor: [Probiotics] explode all trees

#2 (probiotic*):ti,ab,kw (Word variations have been searched)

#3 MeSH descriptor: [Bifidobacterium] explode all trees

#4 (bifidobacterium*):ti,ab,kw (Word variations have been searched)

#5 MeSH descriptor: [Lactobacillus] explode all trees

#6 (lactobacill*):ti,ab,kw (Word variations have been searched)

#7 MeSH descriptor: [undefined] explode all trees

#8 MeSH descriptor: [Saccharomyces boulardii] this term only

#9 (Saccharomyces):ti,ab,kw (Word variations have been searched)

#10 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9

#11 MeSH descriptor: [Prebiotics] explode all trees

#12 (prebiotic*):ti,ab,kw (Word variations have been searched)

#13 MeSH descriptor: [Oligosaccharides] explode all trees

#14 (oligosaccharide*):ti,ab,kw (Word variations have been searched)

#15 MeSH descriptor: [Inulin] explode all trees

#16 (inulin*):ti,ab,kw (Word variations have been searched)

#17 ((fructooligosaccharide* or fructo-oligosaccharide* or FOS or FOSs or galacto-oligosaccharide* or galactooligosaccharide*)):ti,ab,kw (Word variations have been searched)

#18 MeSH descriptor: [Lactoferrin] explode all trees

#19 (lactoferrin*):ti,ab,kw (Word variations have been searched)

#20 MeSH descriptor: [Lactulose] explode all trees

#21 (lactulose*):ti,ab,kw

#22 #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 or #20 or #21

#23 MeSH descriptor: [Synbiotics] explode all trees

#24 (synbiotic*):ti,ab,kw (Word variations have been searched)

#25 (((probiotic* and prebiotic*) NEAR/4 combin*)):ti,ab,kw (Word variations have been searched)

#26 #23 OR #24 OR #25

#27 #10 AND #22 AND #26

#28 MeSH descriptor: [Infant, Newborn] explode all trees

#29 MeSH descriptor: [Premature Birth] explode all trees

#30 neonat*:ti,ab,kw (Word variations have been searched)

#31 neo-nat*:ti,ab,kw (Word variations have been searched)

#32 newborn or new born* or newly born*:ti,ab,kw (Word variations have been searched)

#33 preterm or preterms or (pre term) or (pre terms):ti,ab,kw (Word variations have been searched)

#34 preemie* or premie or premies:ti,ab,kw (Word variations have been searched)

#35 prematur* near/3 (birth* or born or deliver*):ti,ab,kw (Word variations have been searched)

#36 low near/3 (birthweight* or birth weight*):ti,ab,kw (Word variations have been searched)

#37 lbw or vlbw or elbw:ti,ab,kw (Word variations have been searched)

#38 infan* or baby or babies:ti,ab,kw (Word variations have been searched)

#39 #28 or #29 or #30 or #31 or #32 or #33 or #34 or #35 or #36 or #37 or #38

#40 #27 AND #39

CINAHL Via EBSCO

27 records; 18th February 2020

S35 S31 AND S34 (27)

S34 S32 OR S33 (616,583)

S33 TX ((neonat* or neo nat*)) OR TX ((newborn* or new born* or newly born*)) OR TX ((preterm or preterms or pre term or pre terms)) OR TX ((preemie$ or premie or premies)) OR TX ((prematur* N3 (birth* or born or deliver*))) OR TX ((low N3 (birthweight* or birth weight*))) OR TX ((lbw or vlbw or elbw)) OR TX infan* OR TX ((baby or babies)) (616,583)

S32 (MH "Infant, Newborn+") (126,178)

S31 S22 AND S30 (107)

S30 S28 not S29 (628,752)

S29 (MH animals+ OR MH (animal studies) OR TI (animal model*)) NOT MH (human) (167,644)

S28 S23 OR S24 OR S25 OR S26 OR S27 (657,363)

S27 AB (cluster W3 RCT) (322)

S26 MH placebos OR PT randomized controlled trial OR AB control W5 group OR MH crossover design OR MH comparative studies (401,674)

S25 MH sample size AND AB ((assigned OR allocated OR control)) (3,766)

S24 TI ((randomised OR randomized)) OR AB random* OR TI trial (337,314)

S23 MH Randomized Controlled Trials OR MH double-blind studies OR MH single-blind studies OR MH random assignment OR MH pretest-posttest design OR MH cluster sample (192,625)

S22 S9 AND S18 AND S21 (240)

S21 S19 OR S20 (366)

S20 TI ((probiotic* and prebiotic*) N4 combin*) OR AB ((probiotic* and prebiotic*) N4 combin*) (51)

S19 TI Synbiotic* OR AB Synbiotic* (342)

S18 S10 OR S11 OR S12 OR S13 OR S14 OR S15 OR S16 OR S17 (4,196)

S17 TI Lactoferrin OR AB Lactoferrin (524)

S16 TI fructooligosaccharide* OR AB fructooligosaccharide* OR TI fructo-oligosaccharide* OR AB fructo-oligosaccharide* OR TI galactooligosaccharide* OR AB galactooligosaccharide* OR TI galacto-oligosaccharide* OR AB galacto-oligosaccharide* (363)

S15 TI Inulin OR AB Inulin (515)

S14 TI lactulose* OR AB lactulose* (481)

S13 TI Oligosaccharides OR AB Oligosaccharides (778)

S12 (MH "Oligosaccharides") (932)

S11 TI Prebiotic* OR AB Prebiotic* (1,270)

S10 (MH "Prebiotics") (1,408)

S9 S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 (10,092)

S8 TI Saccharomyces OR AB Saccharomyces (510)

S7 (MH "Saccharomyces") (47)

S6 TI lactobacillus OR AB lactobacillus (2,281)

S5 (MH "Lactobacillus") OR (MH "Lactobacillus Acidophilus") (2,502)

S4 TI bifidobacterium* OR AB bifidobacterium* (875)

S3 (MH "Bifidobacterium") (946)

S2 TI probiotic* OR AB probiotic* (5,016)

S1 MH "Probiotics" (6,611)

Embase Via OVID

Search date 17th February 2020; 5600 records

Database: Embase <1974 to 2020 February 14>

1 Probiotic Agent/ (34490)

2 probiotic$.ti,ab,kw. (31301)

3 exp bifidobacterium/ (12860)

4 bifidobacterium$.ti,ab,kw. (9740)

5 exp lactobacillus/ (43379)

6 lactobacill$.ti,ab,kw. (38688)

7 Saccharomyces/ or Saccharomyces boulardii/ or Saccharomyces cerevisiae/ (98260)

8 Saccharomyces$.ti,ab,kw. (77090)

9 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 (183648)

10 Prebiotic Agent/ (7387)

11 prebiotic$.ti,ab,kw. (9900)

12 exp Oligosaccharide/ (546080)

13 oligosaccharide$.ti,ab,kw. (37361)

14 Galactose oligosaccharide/ (961)

15 (galacto-oligosaccharide$ or galactooligosaccharide$).ti,ab,kw. (1364)

16 Fructose Oligosaccharide/ (2182)

17 (fructooligosaccharide$ or fructo-oligosaccharide$ or FOS or FOSs).ti,ab,kw. (35709)

18 Lactulose/ (8835)

19 lactulose$.ti,ab,kw. (5550)

20 Inulin/ (7321)

21 inulin$.ti,ab,kw. (9557)

22 Lactoferrin/ (10431)

23 lactoferrin$.ti,ab,kw. (9054)

24 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 (617217)

25 Synbiotic Agent/ (1624)

26 synbiotic$.ti,ab,kw. (1737)

27 ((probiotic$ and prebiotic$) adj4 combin$).ti,ab,kw. (411)

28 25 or 26 or 27 (2333)

29 9 or 24 or 28 (778900)

30 Newborn/ (516866)

31 Prematurity/ (99389)

32 (neonat$ or neo nat$).ti,ab. (334397)

33 (newborn$ or new born$ or newly born$).ti,ab. (189575)

34 (preterm or preterms or pre term or pre terms).ti,ab. (102056)

35 (preemie$ or premie or premies).ti,ab. (257)

36 (prematur$ adj3 (birth$ or born or deliver$)).ti,ab. (21105)

37 (low adj3 (birthweight$ or birth weight$)).ti,ab. (42758)

38 (lbw or vlbw or elbw).ti,ab. (11219)

39 infan$.ti,ab. (487240)

40 (baby or babies).ti,ab. (94958)

41 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 (1110575)

42 Randomized controlled trial/ (590055)

43 Controlled clinical study/ (462890)

44 Random$.ti,ab. (1501724)

45 randomization/ (85807)

46 intermethod comparison/ (256520)

47 placebo.ti,ab. (300990)

48 (compare or compared or comparison).ti. (500389)

49 ((evaluated or evaluate or evaluating or assessed or assess) and (compare or compared or comparing or comparison)).ab. (2058845)

50 (open adj label).ti,ab. (76978)

51 ((double or single or doubly or singly) adj (blind or blinded or blindly)).ti,ab. (228154)

52 double blind procedure/ (169466)

53 parallel group$1.ti,ab. (24938)

54 (crossover or cross over).ti,ab. (103058)

55 ((assign$ or match or matched or allocation) adj5 (alternate or group$1 or intervention$1 or patient$1 or subject$1 or participant $1)).ti,ab. (322434)

56 (assigned or allocated).ti,ab. (379281)

57 (controlled adj7 (study or design or trial)).ti,ab. (339741)

58 (volunteer or volunteers).ti,ab. (243065)

59 human experiment/ (484405)

60 trial.ti. (291075)

61 or/42-60 (4900385)

62 (random$ adj sampl$ adj7 ("cross section$" or questionnaire$1 or survey$ or database$1)).ti,ab. not (comparative study/ or controlled study/ or randomi?ed controlled.ti,ab. or randomly assigned.ti,ab.) (7961)

63 Cross-sectional study/ not (randomized controlled trial/ or controlled clinical study/ or controlled study/ or randomi?ed controlled.ti,ab. or control group$1.ti,ab.) (228646)

64 (((case adj control$) and random$) not randomi?ed controlled).ti,ab. (16824)

65 (Systematic review not (trial or study)).ti. (135640)

66 (nonrandom$ not random$).ti,ab. (15874)

67 "Random field$".ti,ab. (2243)

68 (random cluster adj3 sampl$).ti,ab. (1253)

69 (review.ab. and review.pt.) not trial.ti. (777162)

70 "we searched".ab. and (review.ti. or review.pt.) (30687)

71 "update review".ab. (103)

72 (databases adj4 searched).ab. (33664)

73 (rat or rats or mouse or mice or swine or porcine or murine or sheep or lambs or pigs or piglets or rabbit or rabbits or cat or cats or dog or dogs or cattle or bovine or monkey or monkeys or trout or marmoset$1).ti. and animal experiment/ (1045069)

74 Animal experiment/ not (human experiment/ or human/) (2213091)

75 or/62-74 (3395835)

76 61 not 75 (4366247)

77 29 and 41 and 76 (5600)

Maternity & Infant Care Via OVID

Search date 17th February 2020; Records 94

Database: Maternity & Infant Care Database (MIDIRS) <1971 to December 2019>

1 probiotic$.ti,ab,de. (430)

2 bifidobacterium$.ti,ab,de. (153)

3 lactobacill$.ti,ab,de. (306)

4 Saccharomyces$.ti,ab,de. (12)

5 1 or 2 or 3 or 4 (643)

6 prebiotic$.ti,ab,de. (145)

7 oligosaccharide$.ti,ab,de. (139)

8 inulin$.ti,ab,de. (13)

9 (fructooligosaccharide$ or fructo-oligosaccharide$ or FOS or FOSs).ti,ab,de. (39)

10 (galactooligosaccharide$ or galacto-oligosaccharide$).ti,ab,de. (35)

11 lactoferrin$.ti,ab,de. (156)

12 lactulose$.ti,ab,de. (27)

13 6 or 7 or 8 or 9 or 10 or 11 or 12 (413)

14 synbiotic$.ti,ab,de. (27)

15 ((probiotic$ and prebiotic$) adj4 combin$).ti,ab,de. (5)

16 14 or 15 (28)

17 5 or 13 or 16 (932)

18 (neonat$ or neo nat$).ti,ab. (46156)

19 (newborn$ or new born$ or newly born$).ti,ab. (20773)

20 (preterm or preterms or pre term or pre terms).ti,ab. (27396)

21 (preemie$ or premie or premies).ti,ab. (56)

22 (prematur$ adj3 (birth$ or born or deliver$)).ti,ab. (4126)

23 (low adj3 (birthweight$ or birth weight$)).ti,ab. (11086)

24 (lbw or vlbw or elbw).ti,ab. (3170)

25 infan$.ti,ab. (66564)

26 (baby or babies).ti,ab. (29888)

27 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 (123341)

28 17 and 27 (765)

29 limit 28 to randomised controlled trial (94)

MEDLINE Via OVID

Search date 17th February 2020; Records 2054

Database: Ovid MEDLINE(R) ALL <1946 to February 14, 2020>

1 Probiotics/ (16413)

2 probiotic$.ti,ab,kw. (23385)

3 exp bifidobacterium/ (5805)

4 bifidobacterium$.ti,ab,kw. (7563)

5 exp lactobacillus/ (28003)

6 lactobacill$.ti,ab,kw. (34222)

7 Saccharomyces/ or Saccharomyces boulardii/ or Saccharomyces cerevisiae/ (109184)

8 Saccharomyces$.ti,ab,kw. (72585)

9 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 (183166)

10 Prebiotics/ (2477)

11 prebiotic$.ti,ab,kw. (8040)

12 Oligosaccharides/ (24163)

13 oligosaccharide$.ti,ab,kw. (33210)

14 (galactooligosaccharides or galacto-oligosaccharides).ti,ab,kw. (859)

15 (fructooligosaccharide$ or fructo-oligosaccharide$ or FOS or FOSs).ti,ab,kw. (29851)

16 Lactulose/ (2114)

17 lactulose$.ti,ab,kw. (3524)

18 Inulin/ (6862)

19 inulin$.ti,ab,kw. (8603)

20 Lactoferrin/ (5956)

21 lactoferrin$.ti,ab,kw. (7664)

22 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 (100138)

23 Synbiotics/ (525)

24 synbiotic$.ti,ab,kw. (1327)

25 ((probiotic$ and prebiotic$) adj4 combin$).ti,ab,kw. (313)

26 23 or 24 or 25 (1500)

27 9 or 22 or 26 (276802)

28 exp Infant, Newborn/ (599027)

29 Premature Birth/ (13220)

30 (neonat$ or neo nat$).ti,ab. (258480)

31 (newborn$ or new born$ or newly born$).ti,ab. (163361)

32 (preterm or preterms or pre term or pre terms).ti,ab. (72698)

33 (preemie$ or premie or premies).ti,ab. (166)

34 (prematur$ adj3 (birth$ or born or deliver$)).ti,ab. (15366)

35 (low adj3 (birthweight$ or birth weight$)).ti,ab. (33943)

36 (lbw or vlbw or elbw).ti,ab. (8192)

37 infan$.ti,ab. (428676)

38 (baby or babies).ti,ab. (68784)

39 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 (1039559)

40 randomized controlled trial.pt. (500729)

41 controlled clinical trial.pt. (93588)

42 randomized.ab. (470135)

43 placebo.ab. (205251)

44 drug therapy.fs. (2181901)

45 randomly.ab. (327315)

46 trial.ab. (494771)

47 groups.ab. (2009585)

48 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47 (4636061)

49 exp animals/ not humans.sh. (4674306)

50 48 not 49 (4016966)

51 27 and 39 and 50 (2054)

Appendix 2. 'Risk of bias' tool

Sequence generation (checking for possible selection bias). Was the allocation sequence adequately generated?

For each included study, we categorised the method used to generate the allocation sequence as:

- low risk (any truly random process e.g. random number table; computer random number generator);
- high risk (any non-random process e.g. odd or even date of birth; hospital or clinic record number); or
- unclear risk.

Allocation concealment (checking for possible selection bias). Was allocation adequately concealed?

For each included study, we categorised the method used to conceal the allocation sequence as:

- low risk (e.g. telephone or central randomisation; consecutively numbered sealed envelopes);
- high risk (open random allocation; unsealed or non-opaque envelopes, alternation; date of birth); or
- unclear risk.

Blinding of personnel (checking for possible performance bias). Was knowledge of the allocated intervention adequately prevented during the study?

For each included study, we categorised the methods used to blind study participants and personnel from knowledge of which intervention a participant received. Blinding was assessed separately for different outcomes or class of outcomes. We categorised the methods as:

- low risk, high risk or unclear risk for personnel.

Blinding of outcome assessment (checking for possible detection bias). Was knowledge of the allocated intervention adequately prevented at the time of outcome assessment?

For each included study, we categorised the methods used to blind outcome assessment. Blinding was assessed separately for different outcomes or class of outcomes. We categorised the methods as:

- low risk for outcome assessors;
- high risk for outcome assessors; or
- unclear risk for outcome assessors.

Incomplete outcome data (checking for possible attrition bias through withdrawals, dropouts, protocol deviations). Were incomplete outcome data adequately addressed?

For each included study and for each outcome, we described the completeness of data including attrition and exclusions from the analysis. We noted whether attrition and exclusions were reported, the numbers included in the analysis at each stage (compared with the total randomised participants), reasons for attrition or exclusion where reported, and whether missing data were balanced across groups or were related to outcomes. Where sufficient information was reported or supplied by the trial authors, we re-included missing data in the analyses. We categorised the methods as:

- low risk (< 20% missing data);
- high risk (≥ 20% missing data); or
- unclear risk.

Selective reporting bias. Are reports of the study free of suggestion of selective outcome reporting?

For each included study, we described how we investigated the possibility of selective outcome reporting bias and what we found. For studies in which study protocols were published in advance, we compared prespecified outcomes versus outcomes eventually reported in the published results. If the study protocol was not published in advance, we contacted study authors to gain access to the study protocol. We assessed the methods as:

- low risk (where it is clear that all of the study's prespecified outcomes and all expected outcomes of interest to the review have been reported);
- high risk (where not all the study's prespecified outcomes have been reported; one or more reported primary outcomes were not prespecified outcomes of interest and are reported incompletely and so cannot be used; study fails to include results of a key outcome that would have been expected to have been reported); or
- unclear risk.

WHAT'S NEW

Date	Event	Description
4 October 2020	New search has been performed	Inclusion criteria modified to include only very preterm (< 32 weeks' gestation) or very low birth weight infants (< 1500 g) with pre-specified analyses for extremely preterm (< 28 weeks' gestation) or extremely low birth weight (< 1000 g) infants. The literature was searched in February 2020. Thirty-two new published trials were identified.

Date	Event	Description
4 October 2020	New citation required and conclusions have changed	Probiotics may reduce the risk of necrotising enterocolitis, but the certainty of the evidence is "low".

HISTORY

Protocol first published: Issue 4, 2005
Review first published: Issue 1, 2008

Date	Event	Description
1 October 2013	New citation required but conclusions have not changed	Updated search identified eight new trials for inclusion in this review update.
1 October 2013	New search has been performed	This updates Al Faleh 2011
3 November 2010	New search has been performed	This updates the review "Probiotics for prevention of necrotizing enterocolitis in preterm infants" published in the Cochrane Database of Systematic Reviews (Al Faleh 2008). New authorship: Khalid AlFaleh, Jasim Anabrees, Dirk Bassler, Turki Al-Kharfi. Updated search identified seven new trials for inclusion in this review update.
3 November 2010	New citation required and conclusions have changed	With the addition of seven new trials to this update, it brings the total to sixteen eligible trials randomizing 2842 infants. The previous review included nine eligible trials, randomizing 1425 infants.
12 November 2008	Feedback has been incorporated	Feedback incorporated
22 July 2008	Amended	Converted to new review format.

CONTRIBUTIONS OF AUTHORS

SS and SO screened and appraised reports identified in the updated search, and extracted and analysed data.

NM undertook analyses for small-study bias.

WM and MXRR arbitrated inclusion and data extraction disagreements, assessed the certainty of the evidence (GRADE), and drafted the review.

All authors contributed to the final manuscript.

DECLARATIONS OF INTEREST

SS is funded by the UK National Institute of Health Research (NIHR) for the review.

NM: the UK NIHR pays a grant to NM's institution.

MXRR has no interest to declare.

SO: the UK NIHR pays a grant to SO's institution. (SR-PG 13/89/12).

WM: the UK NIHR pays a grant to WM's institution. WM is co-coordinating editor of Cochrane Neonatal.

SOURCES OF SUPPORT

Internal sources

- Centre for Reviews and Dissemination, University of York, UK

  Host department
- Department of Clinical Epidemiology and Biostatistics. Faculty of Medicine. Pontificia Universidad Javeriana, Colombia

  Host department

External sources

- Vermont Oxford Network, USA

  Cochrane Neonatal Reviews are produced with support from Vermont Oxford Network, a worldwide collaboration of health professionals dedicated to providing evidence-based care of the highest quality for newborn infants and their families.
- National Institute of Health Research (NIHR), UK

  This report describes independent research funded by a UK NIHR Cochrane Incentive Award. The views expressed in this publication are those of the review authors and are not necessarily those of the NHS, the NIHR, or the UK Department of Health

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

In the 2020 update:

- new authors updated this review;
- we restricted the population of interest to very preterm and VLBW infants in order to enhance applicability to those infants at high risk of developing NEC and associated complications;
- we added the methodology and plan for 'Summary of findings' tables and GRADE recommendations, which were not included in the original protocol (AlFaleh 2005), or in previous publications of the review (Al Faleh 2008; Al Faleh 2011; Al Faleh 2014);
- we updated the search strategy; and
- we updated the "Risk of Bias" assessments.

INDEX TERMS

Medical Subject Headings (MeSH)

Cross Infection [*prevention & control]; Enterocolitis, Necrotizing [mortality] [*prevention & control]; Infant, Premature; Infant, Very Low Birth Weight; Infusions, Parenteral [methods]; Probiotics [administration & dosage] [*therapeutic use]; Randomized Controlled Trials as Topic

MeSH check words

Humans; Infant, Newborn