Probiotics for prevention of necrotizing enterocolitis in preterm infants (Review)

AlFaleh K, Anabrees J

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[Intervention Review]

Probiotics for prevention of necrotizing enterocolitis in preterm infants

Khalid AlFaleh[1], Jasim Anabrees[2]

[1]Department of Pediatrics (Division of Neonatology), King Saud University, Riyadh, Saudi Arabia. [2]Neonatal Care, Sulaiman Al Habib Medical Group, Riyadh, Saudi Arabia

Contact address: Khalid AlFaleh, Department of Pediatrics (Division of Neonatology), King Saud University, King Khalid University Hospital and College of Medicine, Department of Pediatrics (39), P.O. Box 2925, Riyadh, 11461, Saudi Arabia. kfaleh@ksu.edu.sa. kmfaleh@hotmail.com.

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ABSTRACT

Background

Necrotizing enterocolitis (NEC) and nosocomial sepsis are associated with increased morbidity and mortality in preterm infants. Through prevention of bacterial migration across the mucosa, competitive exclusion of pathogenic bacteria, and enhancing the immune responses of the host, prophylactic enteral probiotics (live microbial supplements) may play a role in reducing NEC and the associated morbidity.

Objectives

To compare the efficacy and safety of prophylactic enteral probiotics administration versus placebo or no treatment in the prevention of severe NEC or sepsis, or both, in preterm infants.

Search methods

For this update, searches were made of MEDLINE (1966 to October 2013), EMBASE (1980 to October 2013), the Cochrane Central Register of Controlled Trials (CENTRAL) in *The Cochrane Library* (2013, Issue 10), and abstracts of annual meetings of the Society for Pediatric Research (1995 to 2013).

Selection criteria

Only randomized or quasi-randomized controlled trials that enrolled preterm infants < 37 weeks gestational age or < 2500 g birth weight, or both, were considered. Trials were included if they involved enteral administration of any live microbial supplement (probiotics) and measured at least one prespecified clinical outcome.

Data collection and analysis

Standard methods of The Cochrane Collaboration and its Neonatal Group were used to assess the methodologic quality of the trials and for data collection and analysis.

Main results

Twenty-four eligible trials were included. Included trials were highly variable with regard to enrolment criteria (that is birth weight and gestational age), baseline risk of NEC in the control groups, timing, dose, formulation of the probiotics, and feeding regimens. In a meta-analysis of trial data, enteral probiotics supplementation significantly reduced the incidence of severe NEC (stage II or more) (typical relative risk (RR) 0.43, 95% confidence interval (CI) 0.33 to 0.56; 20 studies, 5529 infants) and mortality (typical RR 0.65, 95% CI 0.52 to 0.81; 17 studies, 5112 infants). There was no evidence of significant reduction of nosocomial sepsis (typical RR 0.91, 95% CI 0.80 to 1.03; 19 studies, 5338 infants). The included trials reported no systemic infection with the supplemental probiotics organism. Probiotics preparations containing either lactobacillus alone or in combination with bifidobacterium were found to be effective.

Authors' conclusions

Enteral supplementation of probiotics prevents severe NEC and all cause mortality in preterm infants. Our updated review of available evidence strongly supports a change in practice. Head to head comparative studies are required to assess the most effective preparations, timing, and length of therapy to be utilized.

PLAIN LANGUAGE SUMMARY

Probiotics for prevention of necrotizing enterocolitis in preterm infants

Necrotizing enterocolitis (NEC) is a serious disease that affects the bowel of premature infants in the first few weeks of life. Although the cause of NEC is not entirely known, milk feeding and bacterial growth play a role. Probiotics (dietary supplements containing potentially beneficial bacteria or yeast) have been used to prevent NEC. Our review of studies found that the use of probiotics reduces the occurrence of NEC and death in premature infants born weighing less than 1500 grams. There is insufficient data with regard to the benefits and potential adverse effects in the most at risk infants weighing less than 1000 grams at birth.

BACKGROUND

Description of the condition

Necrotizing enterocolitis (NEC) is the most common serious acquired disease of the gastrointestinal tract in preterm infants (Lee 2003). It is characterized by bowel wall necrosis, of various length and depth. Bowel perforation occurs in one third of the affected infants (Kafetzis 2003). Although 5% to 25% of cases occur in term infants, it is primarily a disease of preterm infants with the majority of cases occurring in very low birth weight infants (infants with birth weight < 1500 g) (Kosloske 1994). The incidence of NEC varies across countries and neonatal centers. It has been reported to affect up to 10% of very low birth weight infants (VLBW) (Kosloske 1994). In a recent report of the Vermont Oxford Network for VLBW infants the incidence of NEC has risen slightly between 2000 to 2009 (Horbar 2012). NEC is categorized into three different stages, with clinical symptoms varying from feeding intolerance to severe cardiovascular compromise, coagulopathy, and peritonitis with or without pneumoperitoneum (Bell 1978).

The pathogenesis of NEC remains incompletely understood. NEC most likely represents a complex interaction of factors causing mucosal injury (Neu 1996). It is speculated that NEC occurs with the coincidence of two of the three pathologic events of intestinal ischemia, colonization of the intestine by pathologic bacteria, and excess protein substrate in the intestinal lumen (Kosloske 1984; La Gamma 1994). Bacterial colonization is necessary for the development of NEC (Kosloske 1990; Musemeche 1986). When compared to term infants, VLBW infants at risk of NEC have abnormal fecal colonization, demonstrate a paucity of normal enteric bacterial species, and have delayed onset of bacterial colonization (Gewolb 1999; Goldmann 1978). Nosocomial infection is also a frequent complication in VLBW infants. Data from the National Institute of Child Health and Human Development (NICHD) Network demonstrated that as many as 25% of these infants have at least one or more positive blood cultures, and 5% have positive cerebrospinal fluid cultures over the course of their hospitalization (Stoll 1996). Late onset sepsis is associated with an increased risk

of death, neonatal morbidity, and prolonged hospitalization (Stoll 2002a; Stoll 2002b).

Description of the intervention

Probiotic bacteria are live microbial supplements that colonize the gastrointestinal tract and potentially provide benefit to the host (Millar 2003). The most frequently used probiotics are lactobacillus and bifidobacterium. There is increasing interest in the potential health benefits of proactive colonization of the gastrointestinal tract of preterm infants (Millar 2003).

How the intervention might work

Potential mechanisms by which probiotics may protect high risk infants from developing NEC or sepsis, or both, include an increased barrier to migration bacteria and their products across the mucosa (Mattar 2001; Orrhage 1999), competitive exclusion of potential pathogens (Reid 2001), modification of host response to microbial products (Duffy 2000), augmentation of immunoglobulin A (IGA) mucosal responses, enhancement of enteral nutrition that inhibits the growth of pathogens, and up-regulation of immune responses (Link-Amster 1994).

Why it is important to do this review

VLBW infants with NEC have a mortality rate of up to 20% (Caplan 2001; Holman 1997). Approximately 27% to 63% of affected infants require surgical intervention (Lee 2003). Strictures, primarily in the colon, occur in more than one third of affected infants (Ricketts 1994). An increased rate of total parenteral nutrition (TPN) related complications and extended hospitalization have been reported (Bisquera 2002). Recent data from the NICHD Network suggest an increase in neurodevelopmental impairment rates among infants with NEC and sepsis (Stoll 2004). There is a theoretical risk of bacteremia secondary to enterally administered probiotics strains, though few data support this concern. Bacillus species administered as probiotics were reported to be associated with invasive disease in target populations (Richard 1988).

OBJECTIVES

The primary objective was to compare the efficacy and safety of prophylactic enteral probiotics administration versus placebo or no treatment in the prevention of severe (stage II or more) NEC or sepsis, or both, in preterm infants.

The secondary objective was to conduct a subgroup analysis to investigate the effect of probiotics:

- in very low birth weight ((VLBW) (birth weight < 1500 g) and extremely low birth weight (ELBW) infants (birth weight < 1000 g);
- according to species, time of initiation, and the duration of probiotics administrations.

METHODS

Criteria for considering studies for this review

Types of studies

Only randomized and quasi-randomized controlled trials were included.

Types of participants

Preterm infants < 37 weeks and birth weight < 2500 g, or both.

Types of interventions

Enteral administration of any live microbial supplement (probiotics) at any dose for more than seven days compared to placebo or no treatment.

Types of outcome measures

Primary outcomes

- Severe NEC (stage II or more) as per Bell's criteria (Bell 1978; Walsh 1986), diagnosed prior to discharge
- Nosocomial sepsis, defined as positive blood or cerebrospinal fluid cultures taken beyond five days of age
- All cause mortality

Secondary outcomes

- Any NEC (according Bell's criteria)
- The composite of nosocomial sepsis or NEC or death
- Systemic infection with the supplemented organism
- Duration of total parenteral nutrition (days)
- Time to establish full enteral feeds (days)
- Duration of hospitalization (days)
- Weight gain (any measurement scale)
- Neurodevelopmental impairment i.e. rates of cerebral palsy, cognitive delay, deafness, blindness, or their composite, reported at 18 months corrected age or later.

Search methods for identification of studies

Electronic searches

Our search was updated from October 2010 to October 2013. We used the standard search strategy for the Cochrane Neonatal Review Group. Randomized and quasi-randomized controlled trials that compared enteral probiotics to placebo or no treatment in premature infants were identified from Ovid MEDLINE, National Library of Medicine (1966 to October 2013) using the following subject headings (MeSH) and text word terms: "neonate(s), newborn(s), infant(s), probiotics, lactobacillus, bifidobacterium, saccharomyces and publication type 'controlled trial'. We restricted our search to English literature. Other databases were searched including: Cochrane Central Register of Controlled Trials (CENTRAL) in *The Cochrane Library* (2013, Issue 10) and EMBASE (1980 to October 2013). Review authors performed the electronic database search independently.

Searching other resources

A manual search was performed of the abstract books published from the Society of Pediatric Research (SPR) and the European Society of Pediatric Research (ESPR) for the period from 1998 to 2013. Additional citations were sought using the references in articles retrieved from the searches. Subject experts were contacted to identify unpublished and ongoing studies. Authors of published trials were contacted to clarify or provide additional information. The review authors independently screened the candidate articles to check their eligibility for inclusion in the review.

We also searched clinical trials registries for ongoing or recently completed trials (clinicaltrials.gov; controlled-trials.com; and who.int/ictrp).

Data collection and analysis

The standard methods of the Cochrane Neonatal Review Group were employed in creating this update.

Selection of studies

Retrieved articles were independently assessed for eligibility by two review authors. Discrepancies were resolved by discussion and consensus.

Data extraction and management

Data were abstracted independently by two review authors. Discrepancies were resolved by discussion and consensus. Where data were incomplete, the primary investigator was contacted for further information and clarification.

Assessment of risk of bias in included studies

Standard methods of The Cochrane Collaboration and the Neonatal Review Group were used to assess the methodological quality (to meet the validity criteria) of the trials. For each trial, information was sought regarding the method of randomization, and the blinding and reporting of all outcomes of all the infants enrolled in the trial. Each criterion was assessed as yes, no, can't tell. Two review authors separately assessed each study. Any disagreement was resolved by discussion. This information was added to the table Characteristics of included studies. In addition, for the updates in 2010 and 2013, the following issues were evaluated and entered into the risk of bias table.

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

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

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

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

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

- adequate (e.g. telephone or central randomization; consecutively numbered sealed opaque envelopes);
- inadequate (open random allocation; unsealed or non-opaque envelopes, alternation; date of birth);
- unclear.

(3) Blinding (checking for possible performance bias). Was knowledge of the allocated intervention adequately prevented during the study? At study entry? At the time of outcome assessment?

For each included study, we categorized 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 classes of outcomes. We categorized the methods as:

- adequate, inadequate or unclear for participants;
- adequate, inadequate or unclear for personnel;
- adequate, inadequate or unclear for outcome assessors.

(4) 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 randomized 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 categorized the methods as:

- adequate (< 20% missing data);
- inadequate (≥ 20% missing data);
- unclear.

(5) 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. We assessed the methods as:

- adequate (where it is clear that all of the study's pre-specified outcomes and all expected outcomes of interest to the review have been reported);
- inadequate (where not all the study's pre-specified outcomes have been reported; one or more reported primary outcomes were not pre-specified outcomes of interest 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);
- unclear.

(6) Other sources of bias. Was the study apparently free of other problems that could put it at a high risk of bias?
For each included study, we described any important concerns we had about other possible sources of bias (for example, whether there was a potential source of bias related to the specific study design or whether the trial was stopped early due to some data-dependent process). We assessed whether each study was free of other problems that could put it at risk of bias as:

- yes;
- no;
- unclear.

If needed, we planned to explore the impact of the level of bias through undertaking sensitivity analyses.

Measures of treatment effect

For dichotomous outcomes, relative risk (RR), risk difference (RD), and the number needed to treat to benefit (NNTB) and the associated confidence intervals (CIs) were calculated. For continuous outcomes, treatment effect was expressed as mean difference (MD) and its calculated standard deviation (SD). When median, range, and sample size were reported, the mean and SD were estimated using established methods (Hozo 2005).

Assessment of heterogeneity

Heterogeneity was defined as a significant test of heterogeneity ($P < 0.1$) and differences in the treatment effects across studies. Tests for between-study heterogeneity (including the I^2 statistic) were applied. If noticed, possible sources of heterogeneity were examined, including differences in the type or dose of probiotics used, the population under study (VLBW versus ELBW infants), and the quality of the study.

Data synthesis

Review Manager 5.2 software was used for statistical analysis. For estimates of typical RR and RD we used the Mantel-Haenszel method. For measured quantities we used the inverse variance method. All meta-analyses were done using the fixed-effect model.

Subgroup analysis and investigation of heterogeneity

The secondary objective was to conduct a subgroup analysis to investigate the effect of the probiotics in and for the following.

- VLBW infants.
- ELBW infants.
- Different species of probiotics.
- Different times of initiation of probiotics.
- Different durations of probiotics administration.

Sensitivity analysis

A sensitivity analysis was carried out to assess the effect of trials methodological quality on the results of the meta-analysis. Studies were considered to be of high quality if allocation was concealed and adequately described.

RESULTS

Description of studies

See the tables Characteristics of included studies; Characteristics of excluded studies.
Our updated search in October 2013 yielded eight additional studies meeting our inclusion criteria (Al-Hosni 2012; Braga 2011; Demirel 2013; Fernández-Carrocera 2013; Mihatsch 2010; ProPrems 2013; Rojas 2012; Romeo 2011a). Therefore, a total of 24 randomized trials were included in our updated review. Excluded studies and reasons for exclusion are outlined in Characteristics of excluded studies. The details of six identified ongoing studies are provided in Characteristics of ongoing studies.

Participants

Twenty-four included studies reported outcomes on 2761 infants treated with probiotics and 2768 control infants.
[ed note: please check the math. These numbers discussed here are only the infants enrolled in the studies that report on NEC. The total numbers must be greater]

While all studies enrolled infants < 37 weeks or with birth weight < 2500 g, or both, the entry criteria varied between studies. Al-Hosni 2012; Bin-Nun 2005; Braga 2011; Fernández-Carrocera 2013; Kitajima 1997; Li 2004; Lin 2005; Lin 2008; Manzoni 2006; Manzoni 2009; Reuman 1986; and Rojas 2012 enrolled infants based on birth weight criteria. On the other hand, Costalos 2003; Mihatsch 2010; Millar 1993; Mohan 2006; and Stratiki 2007 enrolled infants based on their gestational age. Dani 2002; Demirel 2013; Romeo 2011a; Rougé 2009; Samanta 2009; and Sari 2010 utilized both criteria to enroll infants. Only Al-Hosni 2012 limited enrolment to ELBW infants.

Intervention

The included studies randomized infants to different preparations, times of initiation and duration of therapy of probiotics.

While Dani 2002; Manzoni 2006; Manzoni 2009; Millar 1993; Reuman 1986; Rojas 2012; Romeo 2011a; and Sari 2010 administered *Lactobacillus* species to the intervention groups, Kitajima 1997; Li 2004; Mihatsch 2010; Mohan 2006; and Stratiki 2007 utilized the *Bifidobacterium* species; Costalos 2003 and Demirel 2013 utilized *Saccharomyces boulardii*, and Al-Hosni 2012; Bin-Nun 2005; Braga 2011; Fernández-Carrocera 2013; Lin 2005; Lin 2008; ProPrems 2013; Rougé 2009; and Samanta 2009 used a mixture of species of probiotics.

The time of initiation was different among the included studies. Probiotics were administered either during the first 24 hours of life in Kitajima 1997; Li 2004; and Reuman 1986, on the second day in Braga 2011, at less than 48 hours of age in Rojas 2012, on the third day of life in Manzoni 2009, in the first 72 h in Romeo 2011a, at the time of the first feed in Al-Hosni 2012; Dani 2002; Fernández-Carrocera 2013; Lin 2005; Lin 2008; Mihatsch 2010; Millar 1993; Rougé 2009; Samanta 2009; and Sari 2010, when an infant was receiving at least 1 mL of milk four hourly in ProPrems 2013, or during the first week when enteral feeds were tolerated in Costalos 2003; Manzoni 2006; and Mohan 2006.

The duration of probiotics administration varied from two weeks in Reuman 1986, four to six weeks in Costalos 2003; Kitajima 1997; Lin 2008; and Manzoni 2009, until discharge in Al-Hosni 2012; Dani 2002; Fernández-Carrocera 2013; Li 2004; Lin 2005; Manzoni 2006; Mihatsch 2010; Rojas 2012; Rougé 2009; Samanta 2009; and Sari 2010, at discharge if it happened before the 30th day in Braga 2011, until discharge from hospital or 40 weeks postmenstrual age (term corrected age) in ProPrems 2013, or six weeks or until they were discharged from the neonatal intensive care unit (NICU) in Romeo 2011a.

Outcomes

The major outcomes reported in THE included studies were severe stage II-III NEC (Al-Hosni 2012; Bin-Nun 2005; Braga 2011; Costalos 2003; Dani 2002; Fernández-Carrocera 2013; Kitajima 1997; Lin 2005; Lin 2008; Manzoni 2006; Manzoni 2009; Mihatsch 2010; Mohan 2006; Rojas 2012; Rougé 2009; Samanta 2009; Sari 2010; Stratiki 2007), all cause mortality (Al-Hosni 2012; Bin-Nun 2005; Braga 2011; Dani 2002; Fernández-Carrocera 2013; Kitajima 1997; Lin 2005; Lin 2008; Manzoni 2006; Manzoni 2009; Mihatsch 2010; Reuman 1986; Rojas 2012; Rougé 2009; Samanta 2009), and any culture proven sepsis (Al-Hosni 2012; Bin-Nun 2005; Braga 2011; Costalos 2003; Dani 2002; Kitajima 1997; Lin 2005; Lin 2008; Manzoni 2006; Manzoni 2009; Mihatsch 2010; Millar 1993; Rojas 2012; Rougé 2009; Samanta 2009; Sari 2010; Stratiki 2007). Weight gain was reported in five studies (Al-Hosni 2012; Costalos 2003; Millar 1993; Reuman 1986; Sari 2010) using different measurement scales. Only one study reported data on apnea and long term neurosensory outcomes (Kitajima 1997).

Risk of bias in included studies

Details of THE included studies are presented in the table Characteristics of included studies. The methodologic details of the studies were extracted from the published data and by contacting the primary authors.

- Al-Hosni 2012: this was a multicenter study. All premature infants with birth weight 501 to 1000 g, appropriate for gestational age, and less than or equal to 14 days of age at the time of feeding were randomized to receive either probiotics consisting of *Lactobacillus rhamnosus GG* (LGG) (Culturelle, Amerifit Brand, Cromwell, CT, USA) at 500 million colony forming units (CFU) and *Bifidobacterium infantis* (Align, Procter and Gamble, Cincinnati, OH, USA) at 500 million CFU suspended in 0.5 mL of infant's milk or to receive unsupplemented milk added to their daily feeding. Probiotic supplementation was added to the first enteral feeding and continued once daily with feedings thereafter until discharge or until 34 weeks postmenstrual age. The milk type was not known. Information regarding allocation concealment was not specified, the intervention and outcome assessment were blinded.

- Bin-Nun 2005: this was a single centre study. Infants less than 1500 g were randomized to receive either probiotics mixture (*Lactobacillus bifidus*, *streptococcus thermophillus*, and *bifidobactrium infantis*) or placebo. Expressed mother's milk, when available, or Similac Special Care formula was used. Information regarding allocation concealment was not specified, the intervention was masked, and blinding of outcome assessment was not specified. Of note, this trial was 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, which suggests a change in the a priori specified criteria and multiple looks at the trials results.

- Braga 2011: this was a single center, prospective, double-blind, randomized controlled study. Infants with weights 750 to

1500 g were randomized to receive either 3 mL of pasteurized human milk once a day or *Lactobacillus casei* and *Bifidobacterium breve* (Yakult - LB) diluted with 3 mL of pasteurized human milk once a day on the second to the 30th day of life, or at discharge if it happened before the 30th day. All enrolled infants received human (expressed breast or donor) milk. Information regarding allocation concealment was adequate. Intervention and outcome assessment were masked. Of note, this study was terminated by the External Study Committee for a clear benefit in one of the probiotic groups after enrolment of 231 infants.

- Costalos 2003: this was a single center study. Infants were randomized to receive either enteral probiotics (*Saccharomyces boulardii*) added to preterm formula or the same formula with maltodextrins. All enrolled infants received formula milk. Allocation concealment was apparently adequate. Intervention and outcome assessment were masked. All infants were accounted for in the final results. There was a discrepancy with regard to the infants enrolled in the groups (51 in the treatment group and 36 in the control). The author presented no explanation of whether this discrepancy was a result of imbalance in the randomization process or losses to follow-up.

- Dani 2002: this was a multicenter study. Infants were randomized to receive either enteral probiotics (*Lactobacillus GG*) or placebo. Allocation was adequately concealed. The intervention was masked. Milk type was not known. All enrolled infants were accounted for and outcome measurement was blinded.

- Demirel 2013: this was a single center study. Infants were randomized to receive either enteral probiotics, 250 mg (5 billion CFU) *Saccharomyces boulardii* (N = 135) added to breast milk or formula, or the control group (N = 136) that were fed as usual, without *S. boulardii* supplementation. Allocation concealment was apparently adequate. Intervention and outcome assessment were masked. All infants were accounted for in the final results.

- Fernández-Carrocera 2013: this was a single center study. Infants (N = 150) were randomly assigned to the study group (N = 75) that received their regular feeds and a daily multispecies probiotic feeding supplement of 1 g/d diluted in 3 mL of expressed mother's milk, when available, or a premature infant formula or to the control group (N = 75) that received their regular feeds from their mother's own milk, when available, with nothing added or a premature infant formula. Allocation concealment was apparently adequate. Intervention and outcome assessment were masked.

- Kitajima 1997: this was a single center study; 91 infants were randomized to receive enteral probiotics (*Bifidobacterium breve*) or to the control group. All enrolled infants received expressed breast milk and premature formula. It was unclear whether allocation was concealed, the intervention blinded, or the outcome assessment was blinded. Not all enrolled infants were accounted for in the final results (six infants were excluded for various reasons).

- Li 2004: this was a single center study. Infants were randomized in to three groups to receive either enteral probiotics (*Bifidobacterium breve*) (group A, B) or control (group C). All enrolled infants received breast or artificial milk. Allocation concealment was not described. It was unclear whether the intervention or outcome assessment were blinded and whether all infants were included in the final results.

- Lin 2005: this was a single centre study; infants less than 1500 g were randomized to either probiotics (Infloran® - *L acidophilus* and *B infantis*) or to a control group. All enrolled infants received maternal or banked breast milk. Allocation was adequately concealed. The intervention was masked (except for investigators and breast milk team). All enrolled infants were accounted for. Outcomes measurement was blinded.

- Lin 2008: this was a multicenter trial. Infants less than 1500g were randomized to either probiotics (n = 217) given *Bifidobacterium bifidum* and *Lactobacillus acidophilus*, added to breast milk or mixed feeding (breast milk and formula), twice daily for six weeks or to control (n = 217) fed with breast milk or mixed feeding. Allocation was adequately concealed. The intervention was masked. All enrolled infants were accounted for. Outcomes measurement was blinded.

- Manzoni 2006: this was a single centre study. Infants less than 1500 g were randomized to either probiotics (Dicoflor, *Lactobacillus casei*) or to a control group, all receiving human milk. All enrolled infants received only human (maternal or pooled donors') milk. Although the authors utilized computer generated randomization, allocation concealment was not described. The intervention was masked for the human bank and microbiology workers, however it was unclear whether the care givers were masked or not. All enrolled infants were accounted for. Blinding of outcomes measurement was reported.

- Manzoni 2009: this was a multicenter study. Infants less than 1500 g and younger than three days were randomized to either bovine lactoferrin (BLF) (100 mg/d) (LF100; Dicofarm SpA, Rome, Italy) alone or BLF plus LGG (6×10^9 CFU/d) (Dicoflor 60; Dicofarm SpA); the control group received placebo (2 mL of a 5% glucose solution). Treatment lasted six weeks (for birth weight 1000 g) or four weeks (birth weight 1001 to 1500 g) unless neonates were discharged earlier. Drug administration began on the third day of life with one daily dose; all doses including placebo were diluted in prepared milk so as to maintain blinding. Enrolled infants received any combination of expressed breast milk, donor breast milk, and preterm formula. Allocation was adequately concealed. The i ntervention was masked. All enrolled infants were accounted for. Outcomes measurement was blinded.

- Mihatsch 2010: this was a single center study. VLBW infants less than 30 weeks were randomized to either receive *B. lactis* BB12 suspension or placebo given in addition to human milk, fortified human milk, or preterm formula. BB12 was provided as lyophilized powder mixed with a standard preterm infant human milk fortifier. Human milk fortifier powder only (Nestlé FM 85) was used as the placebo. In infants < 1500 g, 1 g of powder was dissolved in 10 ml of sterile water once a day. In infants ≥ 1500 g, 2 g of powder was dissolved in 20 mL of sterile water once a day. The control group received the identical volume of placebo suspension. All enrolled infants received maternal breast or formula milk. Allocation was adequately concealed. The intervention was masked. All enrolled infants were accounted for. Outcomes measurement was blinded.

- Millar 1993: this was a single center study. Twenty infants were randomized to receive either enteral probiotics (*Lactobacillus GG*) or control. The infants received expressed breast milk or preterm formula, or both. The intervention was masked. All enrolled infants were accounted for. It was unclear whether the outcome assessment was blinded or not.

- Mohan 2006: this was a single center study. Infants less than 37 weeks were randomized to the probiotic (n = 37) and placebo (n = 32) groups. The formula-based placebo (Nestlé FM 2000B) and verum (Nestlé FM 2000A) preparations were supplied by Nestlé, Konolfingen, Switzerland. The verum contained 2 x 10^9 cells of *Bifidobacterium lactis* Bb12 per g of powder. The administration of the study preparation started on the first day after birth and continued for 21 days. The study ended at the 35th day after birth or when the infant was discharged from the hospital, if earlier. Allocation concealment was not described. The intervention was double masked; however it was unclear whether the outcomes assessment was masked or not. All enrolled infants were accounted for. Of note, clinical data obtained through contact with the corresponding author were different from those recently published by Deshpande 2010.

- ProPrems 2013: this was a multicenter study. The data in our systematic review were unpublished and extracted from the Society of Pediatric Research meeting 2013 proceedings and an oral presentation by the primary author. Infants were randomized to receive either a probiotic (n = 548) combination of *B. infantis*, *Streptococcus thermophilus* and *B. lactis* (ABC Dophilus Probiotic Powder for Infants®, Solgar, USA) with 1 x 10^9 total organisms per 1.5 g or maltodextrin powder as the placebo (n = 551). All enrolled infants received breast or formula milk. Randomization was adequate but allocation concealment was not clear. The intervention was double blinded. All enrolled infants were accounted for and outcome assessment was blinded.

- Reuman 1986: this was a single center study. Three groups of infants were randomized to receive either enteral probiotics (*Lactobacillus*) or control. All enrolled infants received formula milk. Randomization and allocation concealment were clearly inadequate. The intervention was double masked. All infants enrolled were accounted for and outcome assessment was blinded.

- Rojas 2012: this was a multicenter study. Infants were randomized to receive either probiotics, five drops of an oil-based suspension containing 10^8 CFU of *L. reuteri* DSM 17938 (BioGaia AB, Stockholm, Sweden) once a day, or placebo in an equal number of drops from an identical vial containing only the oil base. Enrolled infants received any combination of maternal breast milk and preterm formula. Randomization and allocation concealment were adequate. The intervention was double masked. All enrolled infants were accounted for and outcome assessment was blinded.

- Romeo 2011a and Romeo 2011b (the same study): this was a single center study. Infants were randomized to either: Group I (n = 83; 12 with a birth weight < 1500 g, 71 ≥ 1500 g) that received supplementation with *L. reuteri* American Type Culture Collection (ATCC) 55730, 5 drops daily; Group II (n = 83; 28 < 1500 g, 55 ≥ 1500 g) that received supplementation with *L. rhamnosus* ATCC 53103 1 capsule daily; or Group III that included infants with no probiotics (control) (n = 83; 16 < 1500 g, 67 ≥ 1500 g). Patients received supplementation from the first 72 h after hospitalization for six weeks or until they were discharged from the NICU. All enrolled infants received breast or formula milk. Allocation concealment and blinding of intervention and outcome assessment were not documented. All enrolled infants were accounted for.

- Rougé 2009: this trial was conducted in two centers. Infants less than 1500 g and gestational age < 32 weeks were randomized to either the probiotic group (n = 45; 10^8 lyophilized cells per unit of the probiotics *L. rhamnosus* GG (Valio, Ltd) and *B. longum* BB536 (Morinaga Milk Industry Co, Ltd, Tokyo, Japan) and maltodextrin beginning on the day when enteral feeding started until discharge) or the placebo group (n = 49; 4 daily capsules of a supplement containing maltodextrin alone). Infants were fed human (own mother's expressed milk or bank milk) or preterm formula, or both. Allocation was adequately concealed. The intervention was masked. All enrolled infants were accounted for. Outcomes measurement was blinded.

- Samanta 2009: this was a single center study. Infants < 32 weeks and < 1500 g started feed enterally and those that survived beyond 48 h of life were randomized to receive a probiotic mixture (*Bifidobacteria infantis*,*Bifidobacteria bifidum*,*Bifidobacteria longum*, and*Lactobacillus acidophilus*, each 2.5 billion CFU) with expressed breast milk twice daily till discharge, the dosage being 125 g/kg, or breast milk only (control). The infants were fed only breast milk. Allocation concealment and blinding of intervention and outcome assessment were not adequately described. All enrolled infants were accounted for.

- Sari 2010: this was a single center study. Infants < 33 weeks and < 1500 g who survived to start enteral feeding were randomized into two groups. Infants in the study group received *L. sporogenes* with a dose of 350 x 10^6 CFU added to breast milk or formula once a day, starting with the first feed, until discharge. All enrolled infants received breast milk or mixed feeding (breast milk and formula). Infants in the control group received no supplementation. Allocation concealment, blinding of the intervention and outcome assessment were adequately described. All enrolled infants were accounted for.

- Stratiki 2007: this was a single center study. Infants (81 infants) with gestational ages between 27 and 37 weeks, stable state, and formula fed were randomized to group A given a BL supplemented preterm formula (Prenan Nestlé BLSPF) at a concentration of 2 x 10^7 CFU/g of milk powder or group B (control), which received exactly the same formula but without the addition of BL. All enrolled infants received only formula milk. Allocation concealment was not described. The intervention and outcome assessment were blinded and all infants were included in the final results.

Effects of interventions

Probiotics versus control (Comparison 1)

Primary outcomes

Severe necrotizing enterocolitis (stage II to III) (Outcome 1.1)

Twenty studies reported on severe stage II to III NEC (Al-Hosni 2012; Bin-Nun 2005; Braga 2011; Costalos 2003; Dani 2002; Demirel 2013; Fernández-Carrocera 2013; Kitajima 1997; Lin 2005; Lin 2008; Manzoni 2006; Manzoni 2009; Mihatsch 2010; Mohan 2006; ProPrems 2013; Rojas 2012; Rougé 2009; Samanta 2009; Sari 2010; and Stratiki 2007). The administration of prophylactic probiotics significantly reduced the incidence of severe stage II to III NEC (typical RR 0.43, 95% CI 0.33 to 0.56, NNTB 30).

Culture proven sepsis (Outcome 1.2)

Any sepsis (Outcome 1.2.1)

Ninteen studies reported on any culture proven sepsis (Al-Hosni 2012; Bin-Nun 2005; Braga 2011; Costalos 2003; Dani 2002; Demirel 2013; Kitajima 1997; Lin 2005; Lin 2008; Manzoni 2006; Manzoni 2009; Mihatsch 2010; Millar 1993; ProPrems 2013; Rojas 2012; Rougé 2009; Samanta 2009; Sari 2010; Stratiki 2007). Although there was a positive trend, probiotics didn't significantly alter the rate of culture proven sepsis in the pooled effect (typical RR 0.91, 95% CI 0.80 to 1.03).

Any bacterial sepsis (Outcome 1.2.2)

Only Al-Hosni 2012 reported on any bacterial sepsis; no significant difference was observed (typical RR 0.70, 95% CI 0.36 to 1.36).

Any fungal sepsis (Outcome 1.2.3)

Only Al-Hosni 2012 reported on any fungal sepsis with no significant difference among the groups (typical RR 5.10, 95% CI 0.25 to 103.6).

Mortality (Outcome 1.3)

Seventeen studies reported on mortality (Al-Hosni 2012; Bin-Nun 2005; Braga 2011; Dani 2002; Demirel 2013; Fernández-Carrocera 2013; Kitajima 1997; Lin 2005; Lin 2008; Manzoni 2006; Manzoni 2009; Mihatsch 2010; ProPrems 2013; Reuman 1986; Rojas 2012; Rougé 2009; Samanta 2009). Mortality was significantly lowered in the probiotics group (typical RR 0.65, 95% CI 0.52 to 0.81, NNTB 41). Seven studies (Bin-Nun 2005; Dani 2002; Kitajima 1997; Lin 2008; Mihatsch 2010; ProPrems 2013; Sari 2010) reported NEC related mortality. A similar positive effect was observed (typical RR 0.39, 95% 0.18 to 0.82).

Secondary outcomes

Parenteral nutrition duration (days) (Outcome 1.4)

Six studies reported this outcome (Dani 2002; Demirel 2013; Fernández-Carrocera 2013; Lin 2005; ProPrems 2013; Romeo 2011a; Romeo 2011b). Probiotics administration didn't decrease the total days of parenteral nutrition (typical weighted mean difference (WMD) -0.25, 95% CI -0.52 to 0.03).

Hospitalization duration (days) (Outcome 1.5)

Ten studies reported this outcome (Demirel 2013; Fernández-Carrocera 2013; Lin 2005; Lin 2008; ProPrems 2013; Reuman 1986; Rojas 2012; Romeo 2011a; Romeo 2011b; Rougé 2009; Samanta 2009). Probiotics administration significantly shortened hospitalization days compared to control (typical WMD -3.71, 95% CI -4.32 to -3.11).

Weight gain (Outcome 1.6)

Five studies (Al-Hosni 2012; Reuman 1986; Millar 1993; Costalos 2003; Sari 2010) reported weight gain results. No significant statistical difference in weight gain was observed among the study groups. Due to the use of different scales, that is g/week, g/day and g/kg/day, these results were not pooled.

Time to full enteral feeds (Outcome 1.7)

Eight studies (Braga 2011; Demirel 2013; Fernández-Carrocera 2013; Manzoni 2009; Mihatsch 2010; ProPrems 2013; Samanta 2009; Sari 2010) reported time to full enteral feeds. Pooled data of the studies showed a significant reduction in time to reach full enteral feeds (typical WMD -1.32, 95% CI -1.48 to -1.17).

The composite of death or severe NEC or sepsis (Outcome 1.8)

Only one study reported this outcome (Lin 2005). Probiotics significantly reduced the incidence of this composite endpoint (typical RR 0.54, 95% CI 0.37 to 0.79).

Long term outcomes (Outcome 1.9)

Kitajima 1997 reported mental retardation and cerebral palsy at six years. No significant statistical difference was observed among the study groups.

Systemic infection with the supplemented organism

None of the included studies reported any systemic infection caused by the supplemented probiotics organisms.

Subgroup comparisons

Very low birth weight infants (VLBW) (Comparison 2)

Seventeen trials reported on severe stage II to III NEC (Al-Hosni 2012; Bin-Nun 2005; Braga 2011; Dani 2002; Demirel 2013; Fernández-Carrocera 2013; Kitajima 1997; Lin 2005; Lin 2008; Manzoni 2006; Manzoni 2009; Mihatsch 2010; ProPrems 2013; Rojas 2012; Rougé 2009; Samanta 2009; Sari 2010) including VLBW infants only (< 1500 g at birth). The administration of prophylactic probiotics significantly reduced the incidence of severe stage II to III NEC in VLBW infants (typical RR 0.41, 95% CI 0.31 to 0.56) with no significant effect on culture proven sepsis (typical RR 0.92, 95% CI 0.81 to 1.04). Probiotics significantly reduced mortality (typical RR 0.63, 95% CI 0.50 to 0.81) and NEC related mortality (typical RR 0.38, 95% CI 0.18 to 0.82).

Extremely low birth weight infants (ELBW) (Comparison 3)

Al-Hosni 2012 and ProPrems 2013 were the only trials that limited their inclusion to ELBW infants. The administration of prophylactic probiotics did not reduce the incidence of severe stage II to III NEC (typical RR 0.76, 95% CI 0.37 to 1.58), sepsis (typical RR 0.82, 95% CI 0.63 to 1.06), or mortality (typical RR 0.94, 95% CI 0.58 to 1.53). However, the number of included ELBW infants was too small to detect a small meaningful clinical difference in this subgroup of infants.

Effect of different species of probiotics (Comparison 4)

Severe NEC - species of probiotics (Outcome 4.1)

Both the administration of *Lactobacillus* species (five trials) and a mixture of probiotics (nine trials) significantly reduced the incidence of severe stage II to III NEC (RR 0.45, 95% CI 0.27 to 0.75; RR 0.37, 95% CI 0.25 to 0.54 respectively). Four trials utilized *bifidobactirium* species alone, the pooled effect of included trials showed a lack of significant reduction of severe NEC stage II to III (RR 0.48, 95% CI 0.16 to 1.47). Two trials utilized *Saccharomyces boulardii* alone, the pooled effect of the included trials showed a lack of significant reduction of severe NEC stage II to III (RR 0.72, 95% CI 0.34 to 1.55).

Culture proven sepsis - species of probiotics (Outcome 4.2)

The administration of *Lactobacillus* species alone (five trials), *bifidobactirium* species alone (three trials), *Saccharomyces boulardii* alone (two trials), or a mixture of probiotics (nine trials) did not reduce the incidence of culture proven sepsis (RR 0.91, 95% CI 0.71 to 1.16; RR 0.88, 95% CI 0.58 to 1.34; RR 0.92, 95% CI 0.54 to 1.57; RR 0.91, 95% CI 0.78 to 1.06 respectively).

Mortality - species of probiotics (Outcome 4.3)

The administration of a mixture of probiotics (nine trials) significantly reduced the incidence of mortality (RR 0.62, 95% CI 0.47 to 0.81). The administration of *Lactobacillus* species alone (four trials), *bifidobactirium* species alone (two trials), or *Saccharomyces boulardii* alone (one trial) did not reduce mortality (RR 0.72, 95% CI 0.47 to 1.10; RR 0.71, 95% CI 0.14 to 3.6; RR 1.01, 95% CI 0.30 to 3.4 respectively).

Effect of different time of initiation of probiotics (Comparison 5)

Severe NEC - time of initiation (Outcome 5.1)

Probiotics were initiated at different times in the included studies. Nine studies started probiotics administration at the time of the first feed, with a typical RR of 0.44 (95% Cl 0.30 to 0.65). Most included studies initiated prophylaxis within the first week of life, therefore a significant overlap of time of initiation is observed among the included trials.

Culture proven sepsis - time of initiation (Outcome 5.2)

Nine studies started probiotics administration at the time of the first feed, with a typical RR of 0.96 (95% Cl 0.81 to 1.14).

Mortality - time of initiation (Outcome 5.3)

Nine studies started probiotics administration at the time of the first feed, with a typical RR of 0.41 (95% Cl 0.26 to 0.63).

Effect of different duration of probiotics administration (Comparison 6)

Severe NEC - the duration of probiotics administration (Outcome 6.1)

The included trials administered probiotics for either four to six weeks duration or till discharge. Both administration durations produced positive significant effects in reduction of severe stage II to III NEC.

Culture proven sepsis - the duration of probiotics administration (Outcome 6.2)

Included trials administered probiotics for either four to six weeks duration or till discharge. Both administration durations produced no significant effects in terms of reduction of culture proven sepsis.

Mortality - the duration of probiotics administration (Outcome 6.3)

Included trials administered probiotics for either four to six weeks duration or till discharge. Trials that administered probiotics for more than six weeks duration or till discharge showed significant effects in reducing mortality, with a typical RR of 0.65 (95% Cl 0.49 to 0.87).

High quality studies (Comparison 7)

Our results were not altered when a sensitivity analysis including only high quality studies was performed (typical RR for severe stage II or III NEC 0.41, 95% CI 0.29 to 0.58).

DISCUSSION

Our updated review summarizes the evidence on probiotics efficacy in preterm infants. Twenty-four randomized trials and more than 5000 preterm infants are included. Since the publication of our first review, we note a tremendous increase in published studies, reviews, and editorials addressing the efficacy and safety of probiotics utilization in the preterm host. Probiotics are one of the most studied interventions in neonatal medicine.

Our update with more robust data shows that enteral administration of probiotics reduces the incidence of severe NEC, mortality, and NEC related mortality. In addition, the administration of probiotic organisms resulted in a shorter time to full feeds. Our data shows a trend toward a benefit in reduction of sepsis, however this didn't reach statistical significance. Although only two studies limited their inclusion criteria to ELBW infants, included studies had a large number of ELBW infants to assure sceptics of the value of this intervention in a high risk population. Based on the available evidence for probiotics efficacy and safety in preterm infants, the number of infants enrolled, the narrow confidence intervals, and the probiotics safety profile, a change in practice is warranted at this stage. More studies to address the optimal preparation, dosing, and duration of therapy are still needed in head to head comparative studies rather than placebo controlled trials.

Eleven of our included trials were classified as high quality trials based on adequacy of allocation concealment procedures and blinding of the intervention. Although all included trials evaluated probiotics use in preterm infants, the trials were highly variable with regard to enrolment criteria (that is birth weight and gestational age), baseline risk of NEC in the control groups, timing, dose, formulation of probiotic used, and feeding regimens.

Case reports of systemic infections caused by probiotic organisms are found in the biomedical literature. None of our included studies reported this adverse effect. The use of probiotics was described as safe and well tolerated. Our update provides more robust safety data of probiotics utilization in the preterm host.

This review utilized a very thorough and comprehensive search strategy. All attempts were made to minimize potential publication bias. Only randomized or quasi-randomized controlled trials were included. To minimize the reviewer bias, all steps of this review were conducted independently by the review authors. The validity of our review's results is potentially compromised as the included trials utilized different preparations and dosing regimens of the intervention under study; and data on the highest risk population (ELBW infants) could not be retrieved.

The issue of whether it is time to change practice and adopt the use of probiotics as a standard of care in preterm infants has been widely discussed in the medical literature over the last few years. While some advocate a change in practice based on significant reduction in severe NEC and all cause mortality (Tarnow-Mordi

2010), others suggest to wait until precise data on efficacy and safety in ELBW infants are available, in addition to the determination of the most effective preparation and dosing to be utilized (Soll 2010). The evidence on probiotics efficacy and safety is substantial compared to other innovative interventions in neonatal medicine such as surfactant, hypothermia, and room air resuscitation (Janvier 2013). We believe that based on the available evidence and in comparison to other effective interventions in neonatal medicine, a change in practice is warranted. Recently, experts and scientific bodies have started to endorse probiotics utilization in the management of preterm infants (Downard 2012; Janvier 2013).

Probiotics are not licensed by regulatory authorities in many countries including the United States, and hence the wide availability of these products to the public, ethical questions and concerns could be raised in the adoption of this intervention or in the conduct of more placebo controlled trials. We believe that parents' choice to give or withhold probiotics in the management of preterm infants should be respected. Consent forms of planned or ongoing randomized trials should describe the positive effects of probiotics on severe NEC and mortality and the lack of significant side effects prior to enrolling infants in such trials. Enrolment into a randomized trial should not be a condition to receive probiotics in the institutions undertaking these trials (Janvier 2013).

AUTHORS' CONCLUSIONS

Implications for practice

Enteral supplementation of probiotics prevents severe NEC and all cause mortality in preterm infants. Our review strongly supports a change in practice and adoption of probiotics prophylaxis in the management of preterm infants.

Implications for research

More studies are needed to investigate the most effective formulation and dose to be utilized. Parents of preterm infants should be informed of current evidence if further placebo controlled randomized trials are to be conducted.

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* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

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

Al-Hosni 2012

Methods	Multicenter randomized controlled double blinded study
Participants	101 infants 501-1000 g, appropriate for gestational age, and ≤ 14 days of age at the time of feeding initiation Exclusion: Major congenital anomalies, and have known PS before study Demographic data: Probiotics Group N=50, Gestational age (weeks) 25.7 (1.4), birth weight 778 (138) Placebo Group N=51, Gestational age (weeks) 25.7 (1.4), birth weight 779 (126)
Interventions	Probiotic group was given supplement consisting of *Lactobacillus rhamnosus GG* (LGG) (Culturelle, Amerifit Brand, Cromwell, CT, USA) 500 million colony forming units (CFU) and Bifidobacterium infantis (Align, Procter and Gamble, Cincinnati, OH, USA) 500 million CFU suspended in 0.5 mL of infant's milk. Probiotic supplementation was added to the first enteral feeding and continued once daily with feedings thereafter until discharge or until 34 weeks postmenstrual age. The control group received unsupplemented milk added to their daily feeding Milk type was not known
Outcomes	Primary outcome: Weight <10th percentile at 34 weeks Secondary outcomes: Average volume of feeding, Growth velocity, Average daily weight gain, Antimicrobial days, Antibacterial days, Antifungal days, NEC, IVH, ROP, and CLD
Notes	

Risk of bias

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

Bin-Nun 2005

Methods	Single centre randomized study Method of generating randomization sequence: not described Blinding of randomization: not described Blinding of intervention: yes Blinding of outcome measurement: yes Completeness of follow-up: not specified
Participants	145 infants less than 1500 g at birth Demographic data: Probiotics Group N=72, Gestational age (weeks) 29.2 (2.6), birth weight 1152 (262) Placebo Group N=73, Gestational age (weeks) 29.3 (4.3), birth weight 1111 (278)
Interventions	Probiotics group (N=72) received mixture of *Lactobacillus bifidus, streptococcus thermophillus,* and*bifidobactrium infantis* added to 3 ml of expressed breast milk or premature formula enteral feeds Control group (N=73) received 3 ml of expressed milk or premature formula with no supplements added
Outcomes	Stage 2 or 3 NEC Mortality NEC or mortality Sepsis Days to full feeds Days till TPN stopped
Notes	Israel Period of study: Sept 2001-Sept 2004 Published: Journal of Pediatrics 2005 Source of Funding: ABC Dophilus

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Method of generating randomization sequence: not described
Allocation concealment (selection bias)	Unclear risk	Blinding of randomization: not described
Blinding (performance bias and detection bias) All outcomes	Low risk	Blinding of intervention: yes Blinding of outcome measurement: yes
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Completeness of follow-up: not specified
Selective reporting (reporting bias)	Low risk	All clinically important outcomes are described

Braga 2011

Methods	A prospective, double blind, randomized controlled trial
Participants	231 Infants with weight 750-1500 g Demographic data: Probiotics Group N=119, Gestational age (weeks) 29.5 (2.5), birth weight 1194.7 (206. 3) Placebo Group N=112, Gestational age (weeks) 29.2 (2.6), birth weight 1151.4 (224.9)
Interventions	The participants randomised into two groups of 231 infants: Control group: 3 mL of pasteurized human milk once a day Intervention group: *Lactobacillus casei* and*Bifidobacterium breve* (Yakult - LB) diluted with 3 mL of pasteurized human milk once a day on the second day to the 30th day of life, or at discharge if it happens before the 30th day All enrolled infants received human (expressed breast milk or donor) milk
Outcomes	Primary: Necrotising enterocolitis classified as higher or equal to 2 according to Bell's criteria Secondary: The pathogenic bacteria in the faeces, duration of birth weight recovery, Time to full enteral feeds, and hospital stay
Notes	Brazil ISRCTN67165178 Supported by Conselho Nacional de Desenvolvimento Cient ´ fico e Tecnolo´ gico (grant number 473704/2006-4) and research grants (to PIC de Lira and M de Carvalho Lima) External Study Committee observed a major benefit in one of the groups and recommended that the study be interrupted; at this time there were a total of 231 participants

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	
Allocation concealment (selection bias)	Low risk	
Blinding (performance bias and detection bias) All outcomes	Low risk	
Incomplete outcome data (attrition bias) All outcomes	Low risk	
Selective reporting (reporting bias)	Low risk	
Other bias	Low risk	

Costalos 2003

Methods	Single center randomized double blind study Method of generating randomization sequence: Cards in sealed envelopes Allocation concealment: Possibly adequate Blinding of intervention: Yes Blinding of outcome measurement: Not described Complete follow-up: Yes
Participants	87 infants, gestational age 28-32 weeks Exclusion criteria: Major anomalies, receiving antibiotics or anti-fungals, receiving breast milk Demographic data: Probiotics Group N=51, Gestational age (weeks) 31.1 (2.5), birth weight 1651 (470) Placebo Group N=36, Gestational age (weeks) 31.8 (2.7), birth weight 1644 (348)
Interventions	Probiotics group (N=51) received preterm formula containing approximately 15 nmol/dL polyamines with added Saccharomyces boulardii 50mg/kg every 12 hours during the first week of life when enteral feed are tolerated for 30 days Placebo group (N=36) received same formula with maltodextrins All enrolled infants received formula milk
Outcomes	NEC Weight gain Abdominal distension Vomiting Gastric retention Stool characteristics Sepsis
Notes	Greece Period of study: not specified Published: 2003 Source of Funding: Unclear

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Method of generating randomization sequence: Cards in sealed envelopes
Allocation concealment (selection bias)	Low risk	Allocation concealment: Possibly adequate
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Blinding of intervention: Yes Blinding of outcome measurement: Not described
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete follow-up: Yes

Dani 2002

Methods	Multicenter randomized double blind study (12 centers) Method of generating randomization sequence: not described Allocation concealment: Clearly adequate Blinding of intervention: Yes Blinding of outcome measurement: Yes Complete follow-up: Yes
Participants	585 infants, < 33 weeks gestation or <1500 g birth weight enrolled Exclusion criteria: Congenital malformation and death within two weeks of birth Demographic data: Probiotics Group N=295, gestational age (weeks) 30.8 (2.4), birth weight 1325 (361) Placebo Group N=290, gestational age (weeks) 30.7 (2.3), birth weight 1345 (384) Milk type was not known
Interventions	Probiotics group (N=295) received standard milk with *Lactobacillus GG* (Dicoflor®, Dicofarm, Rome, Italy) with an added dose of 6×109 colony forming units (cfu) once a day until discharge, starting with first feed Placebo group (N=290) received standard milk with placebo which was an indistinguishable dried powder of maltodextrins
Outcomes	Severe NEC Incidence of PDA Duration of parenteral nutrition Urinary tract infection Bacterial sepsis (culture proven) Stage 2 and 3 NEC Single course of antibiotics treatment NEC related mortality
Notes	Italy Period of study: not specified in paper Published: 2002 Source of Funding: not specified in paper

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Not described
Allocation concealment (selection bias)	Low risk	Clearly adequate
Blinding (performance bias and detection bias) All outcomes	Low risk	Blinding of Intervention: Yes Blinding of outcome measurement: Yes

Dani 2002 (*Continued*)

Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete Follow-up: Yes
Selective reporting (reporting bias)	Low risk	
Other bias	Low risk	

Demirel 2013

Methods	Double blind placebo controlled randomized trial
Participants	271 infants Inclusion criteria: Infants with gestational age ≤32 weeks and birth weight ≤1500 g who survived to start enteral feeding were enrolled in the study Exclusion criteria: major congenital anomalies and lack of parental consent Demographic data: Probiotics group N=135, gestational age (weeks) 29.4 (2.3), birth weight 1164 (261) Placebo group N=136, gestational age (weeks) 29.2 (2.5), birth weight 1131 (284) All enrolled infants received breast milk or formula
Interventions	The infants in the study group were given 250 mg (5 billion CFU) *S. boulardii* added to breast milk or formula once a day, starting with the first feed, until they were discharged. The infants in the control group were fed as usual, without supplementation. The supplementation did not change the physical appearance of the milk or formula Feeding commenced within 48 h of birth when the infant had stable vital signs, active bowel sounds without abdominal distension, and no bile or blood from the nasogastric tube
Outcomes	Primary Outcome: NEC stage ≥2 and death Secondary Outcomes: clinical or culture-proven sepsis, feeding difficulties, and days required to reach full enteral feeding
Notes	NCT01315821

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Randomisation was simple and unadjusted and was performed using sequential numbers generated at the computer centre of the NICU
Allocation concealment (selection bias)	Low risk	The allocations were sealed in opaque, sequentially numbered envelopes

Demirel 2013 (*Continued*)

Blinding (performance bias and detection bias) All outcomes	Low risk	The supplements were prepared by personnel on the breast milk team following the instructions in the sealed envelope. These individuals were the only personnel who were aware of the group assignments, and they were not involved in the care of the infants
Incomplete outcome data (attrition bias) All outcomes	Low risk	
Selective reporting (reporting bias)	Low risk	
Other bias	Low risk	

Fernández-Carrocera 2013

Methods	A randomized, double blind clinical trial
Participants	150 infants <1500 g birth weight enrolled Demographic data: Probiotics Group N=75, gestational age (weeks) 31.2 (26-35.4), birth weight 1090 (580-1495) Placebo Group N=75, gestational age (weeks) 31 (27-36), birth weight 1170 (540-1492) Exclusion criteria: Preterm newborns with a low Apgar score (<6 at 5 min), gastrointestinal malformations, genetic syndromes, asphyxia and IA-IB NEC stages were excluded
Interventions	Infants were randomly assigned to: The study group received their regular feeds and a daily multi species probiotic feeding supplement of 1 g/d diluted in 3 ml of expressed mother's milk when available or a premature infant formula The control group received their regular feeds from their mother's own milk when available with nothing added, or a premature infant formula
Outcomes	Primary outcome: the occurrence of NEC Secondary outcomes: sepsis, apnea, anaemia, patent ductus arteriosus, and death
Notes	

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	
Allocation concealment (selection bias)	Low risk	

Fernández-Carrocera 2013 (*Continued*)

Blinding (performance bias and detection bias) All outcomes	Low risk	
Incomplete outcome data (attrition bias) All outcomes	Low risk	
Selective reporting (reporting bias)	Low risk	
Other bias	Low risk	

Kitajima 1997

Methods	Single center randomized study Method of generating randomization sequence: Not described Allocation concealment: Not described Blinding of intervention: Not described Blinding of outcome measurement: Not described Complete follow-up: No (6 patients dropped)
Participants	91 infants, birth weight <1500 g enrolled Exclusion criteria: Major anomalies, severe asphyxia, severe IUGR Demographic data: Probiotics Group N=45, gestational age (weeks) 28.3 (2.3), birth weight 1026 (24) Placebo Group N=46, gestational age (weeks) 28.2 (2.1), birth weight 1026 (205)
Interventions	Probiotics group (N=45) received 1 ml supplement of *Bifidobacterium breve* with distilled water 0.5×109 of live *B. breve* within the 1st 24 hrs of life once per day for 28 days Control group (N=46) received distilled water All enrolled infants received expressed breast milk and premature formula
Outcomes	Colonization rate Mean aspired air volume Vomiting times/week Apnoea times/week Weight gain Mental retardation and cerebral palsy outcome at 6 years
Notes	Japan Period of study: May 1990-April 1991 Published: 1997 Source of funding: Unclear

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**

Kitajima 1997 (*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	Unclear risk	Blinding of Intervention: Not described Blinding of outcome measurement: Not described
Incomplete outcome data (attrition bias) All outcomes	High risk	Complete Follow-up: No (6 patients dropped)
Selective reporting (reporting bias)	High risk	Important patient oriented outcomes are not included

Li 2004

Methods	Single center randomized study
Participants	30 infants, of low birth weight. Exclusion criteria: Major anomalies, chromosomal anomalies, intrauterine infection Demographic data: Probiotics Group A N=10, gestational age (weeks) 33.8 (2.9), birth weight 1523 (490) Probiotics Group B N=10, gestational age (weeks) 33.8 (3.2), birth weight 1354 (280) Control (C) Group N=10, gestational age (weeks) 32.4 (3.1), birth weight 1480 (237)
Interventions	Probiotics group (N=10) received through gastric tube Bifidobacterium breve twice a day with feeds till discharge. Group A within several hours of birth, while group B after the 1st 24 hrs Control group (N=10) received no supplement Breast and artificial milk was utilized for feeding
Outcomes	Colonization rate Sepsis
Notes	Japan Period of study: Jan 2000- Aug 2002 Published: 2004 Source of funding: Morinaja Milk industry and Meiji Dairies

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Unclear

Li 2004 (*Continued*)

Allocation concealment (selection bias)	Unclear risk	Not described
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Blinding of intervention: Not described Blinding of outcome measurement: Not described
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Complete follow-up: Unclear
Selective reporting (reporting bias)	High risk	Important patient oriented outcomes are not included

Lin 2005

Methods	Single centre randomized study Method of generating randomization sequence: Random-number table sequence. Allocation concealment: Clearly adequate Blinding of intervention: Yes, only investigators and breast milk team were unblinded. Blinding of outcome measurement: Yes Completeness of follow up: Yes
Participants	367 infants less than 1500 g at birth, survived beyond 7 days of life, and started on enteral feed were enrolled Demographic data: Probiotics Group N=180, gestational age (weeks) 28.5(2.5), birth weight 1104 (242) Placebo Group N=187, gestational age (weeks) 28.2 (2.5), birth weight 1071 (243)
Interventions	Probiotics group (N=180) received Infloran® (*L. acidophilus* and *B. infantis*) obtained from the American Type Culture Collection in 1973, 125 mg/kg/dose twice daily with breast milk until discharge. All enrolled infants received maternal or banked breast milk Control group (N=187) received breast milk without any addition (no placebo)
Outcomes	Death Stage 2 or 3 NEC Sepsis (culture proven) Composite outcomes of death + NEC, sepsis + NEC, death + NEC + sepsis Duration of parenteral nutrition Hospitalization days
Notes	Taiwan Period of study: July 1999- December 2003 Published: 2005 Source of funding: supported by research department of China medical university hospital

Risk of bias

Bias	Authors' judgement	Support for judgement

Lin 2005 (*Continued*)

Random sequence generation (selection bias)	Low risk	Method of generating randomization sequence: Random number table sequence
Allocation concealment (selection bias)	Low risk	Allocation concealment: Clearly adequate
Blinding (performance bias and detection bias) All outcomes	Low risk	Blinding of intervention: Yes, only investigators and breast milk team were unblinded Blinding of outcome measurement: Yes
Incomplete outcome data (attrition bias) All outcomes	Low risk	Completeness of follow up: Yes
Selective reporting (reporting bias)	Low risk	
Other bias	Low risk	

Lin 2008

Methods	Multicenter trial Method of generating randomization sequence: Sequential numbers generated at the computer center Allocation concealment: Adequate Blinding of intervention: Yes Blinding of outcome measurement: Yes Completeness of follow up: Yes
Participants	Very low birth weight infants (birth weight ≤1500 g) Demographic data: The study group N=217, birth weight 1028.9 (246) The control Group N=217, birth weight 1077 (214.4)
Interventions	Infants in the study group were given *Bifidobacterium bifidum* and*Lactobacillus acidophilus*, added to breast milk or mixed feeding (breast milk and formula), twice daily for 6 weeks Infants in the control group were fed with breast milk or mixed feeding
Outcomes	Death or severe NEC NEC, ≥ stage 2 Death not attributable to NEC Death attributable to NEC Sepsis CLD PVL IVH, ≥ grade 3
Notes	7 NICUs in Taiwan Period of study: January 2005 - May 2007 Published: 2008

Lin 2008 (*Continued*)

	Sources of support: National Science Council of Taiwan	
Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Method of generating randomization sequence: Sequential numbers generated at the computer center
Allocation concealment (selection bias)	Low risk	Allocation concealment: Adequate
Blinding (performance bias and detection bias) All outcomes	Low risk	Blinding of intervention: Yes Blinding of outcome measurement: Yes
Incomplete outcome data (attrition bias) All outcomes	Low risk	Completeness of follow-up: Yes
Selective reporting (reporting bias)	Low risk	
Other bias	Low risk	

Manzoni 2006

Methods	Single center randomized study Method of generating randomization sequence: Computer generated randomization Allocation concealment: Unclear Blinding of intervention: Can't tell Blinding of outcome measurement: Can't tell Completeness of follow up: Yes
Participants	80 infants less than 1500 g at birth, survived beyond 3 days of life, and started on human or donor milk enteral feed were enrolled Demographic data: Probiotics Group N=39, gestational age (weeks) 29.6 (5), birth weight 1212 (290) Placebo Group N=41, gestational age (weeks) 41 (4), birth weight 1174 (340)
Interventions	Probiotics group (N=39) received LGG (Diclofor 60; Dicofarm spa); single dose (1/2 packet of Diclofor 60) daily mixed with human or donor milk till end of the sixth week or discharge Control group (N=41) received human or donor milk without any addition (no placebo)
Outcomes	Fungal colonization rates Stage 2 or 4 NEC Death Sepsis (culture proven) Time to full feeds

Manzoni 2006 (*Continued*)

Notes	Italy Period of study: 12 months Published: 2006 Sources of support: non reported

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Method of generating randomization sequence: computer generated randomization
Allocation concealment (selection bias)	Unclear risk	Allocation concealment: Unclear
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Blinding of intervention: Can't tell Blinding of outcome measurement: Can't tell
Incomplete outcome data (attrition bias) All outcomes	Low risk	Completeness of follow up: Yes
Selective reporting (reporting bias)	Low risk	

Manzoni 2009

Methods	Multicenter trial Method of generating randomization sequence: using ralloc.ado version 3.2.5 in Stata 9.2 (Stata-Corp, College Station, Texas) Allocation concealment: Yes Blinding of intervention: Yes Blinding of outcome measurement: Yes Completeness of follow up: Yes
Participants	VLBW neonates younger than 3 days Demographic data: Probiotics Group N=151, gestational age (weeks) 29.8 (23-35), birth weight 1138 (550-1500) Control Group N=153, gestational age (weeks) 29.5 (23-39), birth weight 1109 (437-1500)
Interventions	Infants received either BLF (Bovine Lactoferrin) (100mg/d) (LF100; Dicofarm SpA, Rome, Italy) alone or BLF plus LGG ($6x10^9$ colony-forming units/d) (Dicoflor60; Dicofarm SpA); the control group received placebo (2 mL of a 5% glucose solution). Treatment lasted 6 (birth weight 1000 g) or 4 (birth weight 1001-1500 g) weeks, unless neonates were discharged earlier. Drug administration began on the third day of life with 1 daily dose; all doses including placebo were diluted in prepared milk so as to maintain blinding

	Enrolled infants received any combination of expressed breast milk, donor breast milk, and preterm formula
Outcomes	First episode of late-onset sepsis Incidence of gram-positive/gram-negative bacterial and fungal sepsis Mortality prior to discharge Incidence of urinary tract infections, fungal colonization, progression from fungal colonization to invasive fungal infection Severe NEC Threshold ROP Severe (grade 3-4) IVH BPD Alteration of liver function Adverse effects or intolerance
Notes	11 Italian tertiary NICU Period of study: October 1, 2007, and July 31, 2008 Published: 2009 Source of Funding: Dicofarm SpA

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Method of generating randomization sequence:using ralloc.ado version 3.2.5 in Stata 9.2 (Stata-Corp, College Station, Texas)
Allocation concealment (selection bias)	Low risk	Allocation concealment: Yes
Blinding (performance bias and detection bias) All outcomes	Low risk	Blinding of intervention: Yes Blinding of outcome measurement: Yes
Incomplete outcome data (attrition bias) All outcomes	Low risk	Completeness of follow-up: Yes
Selective reporting (reporting bias)	Low risk	
Other bias	Low risk	

Mihatsch 2010

Methods	A randomized controlled trial
Participants	183 VLBW infants <30 weeks of gestation Demographic data: Probiotics Group N=91, gestational age (weeks) 26.6 (1.8), birth weight 856 (251) Control Group N=89, gestational age (weeks) 26.7 (1.7), birth weight 871 (287) Exclusion criteria were major congenital malformations and anomalies which might interfere with nourishing
Interventions	B. lactis BB12 suspension or placebo was given in addition to human milk, fortified human milk or preterm formula. BB12 was provided as lyophilized powder mixed with a standard preterm infant human milk fortifier. Human milk fortifier powder only (FM85; Nestlé) was used as placebo. In infants <1,500 g, 1 g of powder was dissolved once a day in 10 ml of sterile water. In infants ≥1,500 g, 2 g of powder were dissolved once a day in 20 ml of sterile water The control group received the identical volume of placebo suspension All enrolled infants received maternal breast or formula milk
Outcomes	Primary outcome was the 'incidence density' of nosocomial infections from day 7 after initiation of milk feeding until the 42nd day of life Secondary outcomes was the incidence of necrotizing enterocolitis (NEC; ≥ stage 2)
Notes	Division of Neonatology (Children's Hospital, University of Ulm, Germany) The study was supported by Nestlé AG, Frankfurt, Germany.

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	sealed envelopes, computer-generated, blocked randomization lists, block size of four
Allocation concealment (selection bias)	Low risk	The two indistinguishable powders were provided as blinded coded 10 gram sachets
Blinding (performance bias and detection bias) All outcomes	Low risk	
Incomplete outcome data (attrition bias) All outcomes	Low risk	
Selective reporting (reporting bias)	Low risk	
Other bias	Low risk	

Millar 1993

Methods	Single center randomized blinded study Method of generating randomization sequence: Not described Allocation concealment: Not described Blinding of Intervention: Yes Blinding of outcome measurement: Unclear Complete follow-up: Yes
Participants	20 infants, < 33 weeks gestation enrolled Demographic data: Probiotics Group N=10, gestational age (weeks) 30.5(26-33), birth weight 1445 (800-2560) Placebo Group N=10, gestational age (weeks) 30.0 (24-33), birth weight 1500 (830-2150)
Interventions	Probiotics group received milk feeds with *Lactobacillus GG* 10^8 (cfu) twice a day for 14 days, starting with first feed Placebo group received unsupplemented milk. Enrolled infants received any combination of expressed breast milk, formula, and preterm formula
Outcomes	Weight gain Sepsis clinical or lab proven Antibiotics treatment Oxygen and ventilatory requirements Hospital stay Perineal candidal infection Duration of hospital stay
Notes	UK Period of study: Sept 1991-Jan 1992 Published: 1993 Source of 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	Blinding of intervention: Yes Blinding of outcome measurement: Unclear
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete follow-up: Yes

Millar 1993 (*Continued*)

Selective reporting (reporting bias)	High risk	Important patient oriented outcomes are not included

Mohan 2006

Methods	A double blind, placebo controlled, randomized trial Method of generating randomization sequence: Randoma software version 4.3 Allocation concealment: Not described Blinding of intervention: Yes Blinding of outcome measurement: Unclear Complete follow-up: Yes
Participants	Gestational age of less than 37 weeks No demographic data were provided
Interventions	69 preterm infants The probiotic and placebo groups contained 37 and 32 preterm infants, respectively The verum contained 2 $x10^9$ cells of *Bifidobacterium lactis* Bb12 per gram of powder. The concentration of Bb12 in 1 ml solution of verum in water was 4 $x10^8$. The verum group received 1.6 $x10^9$ cells on day 1 to 3 and 4.8 $x10^9$ cells from day 4 onward. Started on the first day after birth and continued for 21 days. The study ended at the 35th day after birth or when the infant was discharged from the hospital, if earlier The formula-based placebo (Nestlé FM 2000B) and verum (Nestlé FM 2000A) preparations were supplied by Nestlé, Konolfingen, Switzerland
Outcomes	No clinical outcomes were presented in the published data NEC and sepsis data were collected by contacting the corresponding author
Notes	The Ernst von Bergmann hospital, Potsdam, Germany Period of study: August 2003 - June 2005 Published: 2006 Source of funding: Not reported

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Method of generating randomization sequence: Randoma software version 4.3
Allocation concealment (selection bias)	Unclear risk	Allocation concealment: Not described
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Blinding of intervention: Yes Blinding of outcome measurement: Unclear
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete follow-up: Yes

Mohan 2006 (*Continued*)

Selective reporting (reporting bias)	High risk	Important patient oriented outcomes are not included

ProPrems 2013

Methods	A prospective multicenter, double blinded, placebo controlled, randomized trial
Participants	Infants, born <32 completed weeks' gestation and weighing <1500g, were eligible for enrolment within 72 hours of birth Infants were excluded if they had major congenital or chromosomal anomalies, if death was considered likely within 72 hours of birth, or if the mother was taking non-dietary probiotic supplements
Interventions	The intervention was the probiotic combination *B. infantis*, *Streptococcus thermophilus* and *B. lactis* (ABC Dophilus Probiotic Powder for Infants®, Solgar, USA) with 1 x 10^9 total organisms per 1.5 g, in a maltodextrin base powder. The placebo was maltodextrin powder. The intervention was only administered when an infant was receiving at least 1mL of milk 4 hourly. The daily dose was two 1mL spoons, equivalent to 1.5g of study powder, reconstituted with 3mL breast milk or formula. When an infant received <3mL milk per feed, one 1mL spoon of powder was mixed with 1·5mL milk and given twice daily. The dose was the same irrespective of the infant's current weight or postnatal age and was administered daily by gastric tube or mouth, until discharge from hospital or term corrected age All enrolled infants received breast or formula milk
Outcomes	The primary outcome was the incidence of at least one episode of definite late-onset sepsis before 40 weeks' postmenstrual age or discharge home, whichever occurred first Secondary outcomes were the incidence of definite or clinical sepsis, the composite outcome of definite or clinical late-onset sepsis, the number of courses and duration of antibiotic treatment, the incidence of definite sepsis with a probiotic species, mortality, the incidence of NEC, duration of primary hospitalization and intravenous nutrition, time to enteral feeds of 120 mL/kg/day for ≥3 days, breast milk feeding rates, days to regain birth weight, weight at 28 days of age and at discharge, PDA treated, IVH grade 3 or 4 or cystic PVL, ROP ≥ grade 3, oxygen treatment and/ or respiratory support
Notes	ProPrems trial was conducted in Australia (n = 8) and New Zealand (n = 2) ACTRN12607000144415 Included data in this review are unpublished

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	

ProPrems 2013 (*Continued*)

Allocation concealment (selection bias)	Low risk	The schedule was provided to the pharmacist at RWH who made up individual bottles for each randomized infant, coded by sequential study number
Blinding (performance bias and detection bias) All outcomes	Low risk	
Incomplete outcome data (attrition bias) All outcomes	Low risk	
Selective reporting (reporting bias)	Low risk	
Other bias	Low risk	

Reuman 1986

Methods	Randomized double blind study Method of generating randomization sequence: random number charts and the last digit of patient's chart number, the next matched infants is assigned to the opposite group Allocation concealment: clearly inadequate Blinding of intervention: Yes Blinding of outcome measurement: Yes Complete follow-up: Yes
Participants	45 infants, <2000 gm at birth weight who survived beyond first 24 hrs and are younger than 72 hrs Demographic data: Probiotics Group N=15, gestational age (weeks) 30.6 (2.7), birth weight 1366 (302) Placebo Group N=15, gestational age (weeks) 30.5 (2.8), birth weight 1377 (344) Untreated group N=15, gestational age(weeks) 30.7 (2.9), birth weight 1329 (337)
Interventions	Probiotics group received at least 1 mL of formula containing *lactobacillus*. $5x10^{10}$ organisms/mL preparation diluted 100 times in infants formula Placebo group received 1 mL of formula with no added *lactobacillus* Both groups started within 72 hrs of birth The untreated group received nothing per mouth for 2 weeks All enrolled infants received formula milk
Outcomes	Death Colonization rates Hospitalization duration Daily weight gain Hospital acquired infection

Reuman 1986 (*Continued*)

Notes	US Period of study: not specified in paper Published: 1986 Source of Funding: not specified in paper

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	High risk	Method of generating randomization sequence: random number charts and the last digit of patient's chart number, the next matched infants is assigned to the opposite group
Allocation concealment (selection bias)	High risk	Allocation concealment: Clearly inadequate
Blinding (performance bias and detection bias) All outcomes	Low risk	Blinding of intervention: Yes Blinding of outcome measurement: Yes
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete follow-up: Yes
Selective reporting (reporting bias)	High risk	

Rojas 2012

Methods	Multicenter, double blinded, randomized, placebo controlled trial
Participants	Inclusion criteria: admission to the NICU, birth weight $\leq$2000 g, hemodynamically stable, and $\leq$48 hours of age Infants with evidence or suspicion of congenital intestinal obstruction or perforation, gastroschisis, large omphalocele, congenital diaphragmatic hernia, major congenital heart defects, or anticipated transfer to a NICU not participating in the study were excluded
Interventions	Infants in the probiotic group received 5 drops of an oil-based suspension containing 10^8 colony-forming units of *L. reuteri* DSM 17938 (BioGaia AB, Stockholm, Sweden) once a day For infants in the placebo group, an equal number of drops from an identical vial containing only the oil base were administered Enrolled infants received any combination of maternal breast milk and/or preterm formula

Rojas 2012 (*Continued*)

Outcomes	The primary outcome was death or NI Secondary outcomes included nosocomial pneumonia, NEC, feeding intolerance, and duration of hospitalization
Notes	Colombia

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer generated balanced block randomization scheme
Allocation concealment (selection bias)	Low risk	Sealed, sequentially numbered, opaque envelopes, color-coded for strata, available in each NICU pharmacy
Blinding (performance bias and detection bias) All outcomes	Low risk	
Incomplete outcome data (attrition bias) All outcomes	Low risk	
Selective reporting (reporting bias)	Low risk	
Other bias	Low risk	

Romeo 2011a

Methods	Prospective randomized trial (Romeo11a and Romeo 2011b are the same trial)
Participants	249 preterms with a birth weight <2500 g and a gestational age <37 weeks All the infants were outborn. Inclusion criteria were admission to the NICU, a stable oral feeding within 72 h of birth and an informed parental consent; exclusion criteria were the presence of major congenital malformation or antenatal and perinatal risk factors for sepsis
Interventions	The newborns were randomized into three groups: Group I (n=83; 12 with a birth weight <1500 g, 71 ≥1500 g) received supplementation with *L. reuteri* American Type Culture Collection (ATCC) 55730 5 drops daily Group II (n=83; 28 <1500 g, 55 ≥1500 g) received supplementation with *L. rhamnosus* ATCC 53103 1 capsule daily Group III included newborns with no probiotics (control; n=83; 16 <1500 g, 67 ≥1500 g). Patients received supplementation from the first 72 h after hospitalization for 6 weeks or until they were discharged from the NICU All enrolled infants received breast or formula milk

Romeo 2011a (*Continued*)

Outcomes	The primary outcome was to evaluate the incidence of enteric fungal colonization The secondary outcomes were days of parenteral nutrition, days of antibiotic treatment, days of hospitalization, etc
Notes	NICU of the Policlinico University of Catania, Italy

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Random number table
Allocation concealment (selection bias)	Unclear risk	Not mentioned
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Not mentioned
Incomplete outcome data (attrition bias) All outcomes	Low risk	
Selective reporting (reporting bias)	Low risk	
Other bias	Low risk	

Romeo 2011b

Methods	Prospective randomized trial (Romeo11a and Romeo 2011b are the same trial)
Participants	249 preterms with a birth weight <2500 g and a gestational age <37 weeks All the infants were outborn. Inclusion criteria were admission to the NICU, a stable oral feeding within 72 h of birth and an informed parental consent; exclusion criteria were the presence of major congenital malformation or antenatal and perinatal risk factors for sepsis
Interventions	The newborns were randomized into three groups: Group I (n=83; 12 with a birth weight <1500 g, 71 ≥1500 g) received supplementation with *L. reuteri* American Type Culture Collection (ATCC) 55730 5 drops daily Group II (n=83; 28 <1500 g, 55≥1500 g) received supplementation with *L. rhamnosus* ATCC 53103 1 capsule daily Group III included newborns with no probiotics (control; n=83; 16 <1500 g, 67 ≥1500 g). Patients received supplementation from the first 72 h after hospitalization for 6 weeks or until they were discharged from the NICU All enrolled infants received breast or formula milk

Romeo 2011b (*Continued*)

Outcomes	The primary outcome was to evaluate the incidence of enteric fungal colonization The secondary outcomes were days of parenteral nutrition, days of antibiotic treatment, days of hospitalization, etc
Notes	NICU of the Policlinico University of Catania, Italy

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Random number table
Allocation concealment (selection bias)	Unclear risk	Not mentioned
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Not mentioned
Incomplete outcome data (attrition bias) All outcomes	Low risk	
Selective reporting (reporting bias)	Low risk	
Other bias	Low risk	

Rougé 2009

Methods	Two centers
Participants	Gestational age, <32 wk, a birth weight, <1500 g Demographic data: Probiotics Group N=45, gestational age (weeks) 28.1 (1.9), birth weight 1115 (251) Placebo Group N=49, gestational age (weeks) 28.1 (1.8), birth weight 1057 (260)
Interventions	Placebo group (N 49) receive 4 daily capsules of a supplement containing maltodextrin alone Probiotic group (N 45) 10^8 lyophilized cells per unit of the probiotics *L. rhamnosus* GG (Valio, Ltd) and B. longum BB536 (Morinaga Milk Industry Co, Ltd, Tokyo, Japan) and maltodextrin beginning on the day when enteral feeding started until discharge Infants were fed human (own mother's expressed milk or bank milk) and/or preterm formula
Outcomes	The percentage of infants receiving more than 50% of their nutritional needs via enteral feeding on the 14th day of life Nutrition on day 14 (more than 50% of calories received enterally and total calories delivered enterally) Nosocomial infections

Rougé 2009 (*Continued*)

	Sepsis with positive blood culture Duration of antibiotic use Necrotizing enterocolitis Duration of ventilatory support Duration of CPAP Duration of oxygen therapy Systemic postnatal corticoid treatment Duration of hospital stay Death
Notes	France Period of study: Aprill 2005 - January 2007 Published: 2009 Source of Funding:from the Programme Hospitalier de Recherche Clinique of the French Ministry of Health and the Délégation à la Recherche Clinique, CHU de Nantes

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Method of generating randomization sequence: In-house software (Nantes University Hospital, Nantes, France)
Allocation concealment (selection bias)	Unclear risk	Not mentioned
Blinding (performance bias and detection bias) All outcomes	Low risk	Blinding of intervention: Yes Blinding of outcome measurement: Yes
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete follow-up: Yes
Selective reporting (reporting bias)	Low risk	
Other bias	Low risk	

Samanta 2009

Methods	Prospective randomized double blind controlled trial
Participants	Gestational age <32 weeks and VLBW infants (<1500 g) started feed enterally and survived beyond 48 h of life Demographic data: Probiotics Group N=91, gestational age 30.12 (weeks) (1.63), birth weight 1172 (143) Control Group N=95, gestational age 30.14 (weeks) (1.59), birth weight 1210 (143)
Interventions	The probiotic group received a probiotic mixture (*Bifidobacteria infantis, Bifidobacteria bifidum, Bifidobacteria longum* and*Lactobacillus acidophilus*, each 2.5 billion CFU) with

Samanta 2009 (*Continued*)

	expressed breast milk twice daily, the dosage being 125 g kg^{-1} till discharge. The control group was fed with breast milk only Infants were fed breast milk only
Outcomes	Feed tolerance in terms of days required to reach full enteral feeding Length of hospital stay NEC Sepsis Death due to NEC or sepsis
Notes	Neonatal Care Unit of Medical College and Hospital, Kolkata, India Period of study: October 2007 - March 2008 Published: 2009 Source of Funding: not specified in paper

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Can't tell
Allocation concealment (selection bias)	Unclear risk	Can't tell
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Blinding of intervention: Can't tell Blinding of outcome measurement: Can't tell
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete follow-up: Yes

Sari 2010

Methods	Single center
Participants	Gestational age <33 weeks or birth weight <1500 g Demographic data: Probiotics Group N=110, gestational age 29.5 (weeks) (2.4), birth weight 1231 (262) Control Group N=111, gestational age 29.7 (weeks) (2.4), birth weight 1278 (282)
Interventions	VLBW infants who survived to start enteral feeding were randomized The study group were given L. sporogenes with a dose of 350.000.000 colony forming units added to breast milk or formula once a day starting with first feed until discharge. The control group were fed without L. sporogenes supplementation All enrolled infants received breast milk or mix feeding (breast milk and formula)

Sari 2010 (*Continued*)

Outcomes	Death or severe NEC NEC (stage 2, 3, ≥ 2) Death (attributable to NEC, not attributable to NEC) Total parental nutrition Intraventricular hemorrhage, grade 3-4, Sepsis (culture proven, gram negative, gram positive, fungus) NICU stay Feeding (amount, full feeding, intolerance) Weight gain
Notes	Turkey Period of study: October 2008 and June 2009 Published: Unpublished Source of Funding: not specified in paper

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Method of generating randomization sequence: Sequential numbers generated at the computer center of the NICU
Allocation concealment (selection bias)	Unclear risk	Allocation concealment: Can't tell
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Blinding of intervention: Can't tell Blinding of outcome measurement: Yes
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete follow-up: Yes
Selective reporting (reporting bias)	Low risk	

Stratiki 2007

Methods	Single center
Participants	Gestational age between 27 and 37 weeks, stable state, formula fed Demographic data: Probiotics Group N=41, gestational age 31 weeks (27-37), birth weight 1500 (900-1780) Control Group N=34, gestational age 30.5 weeks (26-37), birth weight 1500 (700-1900)
Interventions	81 infants Group A (study group) was given a BL supplemented preterm formula - Prenan Nestlé - (BLSPF) at a concentration of 2×107 CFU/g of milk powder Group B (control) received exactly the same formula but without the addition of BL

	All enrolled infants received only formula milk
Outcomes	Intestinal permeability Somatic growth Tolerance Sepsis Necrotizing enterocolitis
Notes	Greece Period of study: January 2004 - December 2005 Published: 2007 Source of Funding: not specified in paper (Nestlé Company, Vevey provide the *B. lactis* supplemented milk formula)

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Method of generating randomization sequence: Can't tell
Allocation concealment (selection bias)	Unclear risk	Allocation concealment: Can't tell
Blinding (performance bias and detection bias) All outcomes	Low risk	Blinding of intervention: Yes Blinding of outcome measurement: Yes
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete follow-up: Yes
Selective reporting (reporting bias)	High risk	Important patient oriented clinical outcomes are not included

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

Study	Reason for exclusion
Agarwal 2003	No clinical outcomes were presented (Agarwal 2003)
Awad 2010	Data included full term infants (Awad 2010)
Di 2010	Article in Chinese, reference retrieved by manual search of included studies references in Wang 2012
Havranek 2013	A substudy of multicenter trial by Al-Hosni 2012
Huang 2009	Article in Chinese, reference retrieved by manual search of included studies references in Wang 2012

(*Continued*)

Ke 2008	Article in Chinese, reference retrieved by manual search of included studies references in Wang 2012
Ren 2010	Article in Chinese, reference retrieved by manual search of included studies references in Wang 2012
Stansbridge 1993	No clinical outcomes were presented (Stansbridge 1993)
Uhlemann 1999	Data included full term infants (Uhlemann 1999)

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

Cooper

Trial name or title	Necrotizing Enterocolitis and *B. Lactis* in Premature Babies
Methods	Multi-centre double-blind placebo-controlled randomized trial
Participants	Inclusion Criteria: Weight between 800-1500 g, Tolerating enteral feeding within 48 hours, Having obtained his/her parents or legal representative informed consent Exclusion Criteria: Chromosomal abnormality, Hydrops featalis, Congenital malformation of the gastrointestinal tract, Congenital heart defects or other major congenital abnormalities likely to affect feeding and/or feeding tolerance, or Currently participating in another clinical trial
Interventions	One capsule containing probiotics per day added to milk
Outcomes	Primary Outcome: NEC onset Secondary Outcome: Antibiotic administration and stool microbiology
Starting date	November 2009
Contact information	Peter A. Cooper peter.cooper@wits.ac.za
Notes	Sponsor by Nestlé South Africa NCT00977912 Ongoing

Costeloe

Trial name or title	The administration of probiotic to premature babies to prevent infection, severe intestinal complication (i.e. necrotising enterocolitis) and death
Methods	Multicenter double-blind placebo-controlled randomized trial

Costeloe (*Continued*)

Participants	1. Both males and females, born before 31 completed weeks of gestation, i.e. up to and including 30 weeks + 6 days by the best estimate of Expected Date of Delivery 2. Less than 48 hours old 3. With written informed parental consent 4. Babies already on antibiotics for suspected or proven infection are eligible for recruitment to the study
Interventions	*Bifidobacterium breve* strain BBG (B breve BBG) The placebo is corn starch alone Both products are manufactured in identical foil sachets each containing 1 gram of product The intervention will be given once daily starting as soon as possible after randomization and continuing until 36 completed weeks of post-menstrual age (36 weeks + 0 days) or death or discharge from hospital if sooner 1,300 babies will be recruited over 30 months
Outcomes	Primary: 1. Any baby with an episode of blood stream infection, with any organism other than a skin commensal 2. Necrotising enterocolitis, Bell stage II or III 3. Death before discharge Secondary: 1. Number of babies with the composite outcome of any or a combination of the 3 primary outcomes Outcomes 2 to 7 are for samples taken more than 72 hours after birth and before death or discharge home: 2. Number of babies with any positive blood culture with an organism recognized as a skin commensal e.g. CoNS or Corynebacteria 3. Number of babies with blood cultures taken 4. Number of blood cultures taken per baby 5. Number of babies with episodes of blood stream infection with organisms other than skin commensals by organism 6. Number of babies with isolates of organisms other than skin commensals from a normally sterile site other than blood 7. Number of babies with a positive culture of B breve BBG from any normally sterile site 8. Total duration of days of antibiotics and/or anti-fungals administered per baby after 72 hours and until death or discharge 9. The number of babies colonized with the administered probiotic strain 10. Stool flora 11. Age at achieving full enteral nutrition (defined as 150 ml/kg/day for 1 day) 12. Change of weight Z score from birth to 36 weeks post-menstrual age or discharge from hospital if sooner 13. Broncho-pulmonary dysplasia 14. Hydrocephalus and/or intraparenchymal cysts confirmed by cerebral ultrasound scan performed during the baby's in-patient stay 15. Worst stage of retinopathy of prematurity in either eye at discharge or death 16. Length of stay in intensive, high dependency and special care (British Association of Perinatal Medicine (BAPM) 2001: definitions)
Starting date	01/12/2009
Contact information	Prof Kate Costeloe Barts and the London School of Medicine and Dentistry Neonatal Unit Homerton University Hospital

Costeloe (*Continued*)

	Homerton Row
Notes	UK ISRCTN05511098 Completed

Kusuda

Trial name or title	Effect of *Bifidobacterium Bifidum* Supplementation on Morbidity of Very Low Birth Weight Infants
Methods	Double-blind placebo-controlled randomized trial
Participants	Inclusion Criteria: Infants with birth weight less than 1500g Exclusion Criteria: Sever bacteremia, Congenital anomaly, Not suitable for the trial defined by an attending neonatologist
Interventions	*B. bifidum* (OLB6378) supplementation with approximately 2.5*10 to 9th bacteria per day
Outcomes	Primary Outcome:Postnatal day when enteral feeding exceeded at 100ml/kg/day Secondary Outcome:standard deviation scores of bodyweight and head circumference Necrotizing enterocolitis or sepsis Intestinal flora
Starting date	January 2010
Contact information	Satoshi Kusuda Tokyo Women's Medical University
Notes	NCT01375309 Completed

Moral

Trial name or title	Effects of *Lactobacillus Reuteri* in Premature Infants
Methods	Multicenter double-blind placebo-controlled randomized trial
Participants	Inclusion criteria: • Preterm newborns admitted to the neonatal intensive care units with a birth weight 700-1500 g and who survive more than 3 days Exclusion criteria: • Chromosomal anomalies. • Major congenital anomalies (complex cardiac anomalies, congenital hydrocephalus, renal dysplasia) • Congenital (e.g. jejunal atresia) and acquired (e.g. GI perforation) gastrointestinal pathology precluding oral feed and/or requiring major surgical or medical intervention • Parental refusal • Prior enrolment into a conflicting clinical trial

Moral (*Continued*)

Interventions	*L. reuteri DSM* will be given at a dose of $1x10^8$ colony forming units (CFU)/day
Outcomes	Primary Outcome: Time to reach full feeds Secondary Outcome: Intestinal colonization and Intestinal immunological response
Starting date	July 2010
Contact information	Teresa del Moral University of Miami
Notes	NCT01181791 Chile

Oncel

Trial name or title	*Lactobacillus Reuteri* for Prevention of Necrotizing Enterocolitis in Very Low-birth Weight Infants
Methods	Randomised placebo controlled trial
Participants	Inclusion Criteria: Very low birth weight infants < 1500 g, Gestational age < 32 weeks Exclusion Criteria: Genetic anomalies, Short bowel syndrome, Not willing to participate, Allergy to *L. reuteri* components
Interventions	*L. reuteri* 100 million CFU/day for 3 months
Outcomes	Primary Outcome: NEC in VLBW infants Secondary Outcomes:Culture proved sepsis, Weight gain, and Length of hospital stay
Starting date	February 2012
Contact information	Mehmet Yekta Oncel Zekai Tahir Burak Maternity Teaching Hospital, Neonatology Unit
Notes	NCT01531179 Completed

Punnahitananda

Trial name or title	Effect of Oral Probiotic Supplementation on The Rate of Hospital Acquired Infection and Necrotizing Enterocolitis in PretermVery Low Birth Weight Infants
Methods	Randomised placebo controlled trial
Participants	VLBW preterm infants (Gestational age < 35 weeks , BW < 1500 g) admitted to the NICU who survived the first 3 days of life Exclusion Criteria: Infants with chromosome abnormality or severe congenital defects, especially gastroin-

Punnahitananda (*Continued*)

	testinal anomalies and infants with unstable hemodynamic status
Interventions	Daily enteral probiotic supplementation (live *Lactobacillus acidophilus* and*Bifidobacterium infantis*) at a dose of 2.5 x 10^8 CFU of each strain once a day for at least 28 days or until discharge The control group received daily placebo
Outcomes	Primary Outcome:incidence of nosocomial infections Secondary Outcome:incidence of NEC, feeding tolerance, time to full enteral feeding
Starting date	January 2005
Contact information	Santi Punnahitananda, Faculty of Medicine Chulalongkorn University Thailand
Notes	Study First Received: April 19, 2011 ISRCTN 39142169 Completed

DATA AND ANALYSES

Comparison 1. Probiotics versus control (all infants)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Severe necrotising enterocolitis (stage II-III)	20	5529	Risk Ratio (M-H, Fixed, 95% CI)	0.43 [0.33, 0.56]
2 Culture proven sepsis	19		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.1 Any sepsis	19	5338	Risk Ratio (M-H, Fixed, 95% CI)	0.91 [0.80, 1.03]
2.2 Any Bacterial sepsis	1	101	Risk Ratio (M-H, Fixed, 95% CI)	0.70 [0.36, 1.36]
2.3 Any Fungal sepsis	1	101	Risk Ratio (M-H, Fixed, 95% CI)	5.10 [0.25, 103.60]
3 Mortality	18		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
3.1 All causes of neonatal mortality	17	5112	Risk Ratio (M-H, Fixed, 95% CI)	0.65 [0.52, 0.81]
3.2 NEC related mortality	7	2755	Risk Ratio (M-H, Fixed, 95% CI)	0.39 [0.18, 0.82]
4 Parenteral nutrition duration (days)	7	2804	Mean Difference (IV, Fixed, 95% CI)	-0.25 [-0.52, 0.03]
5 Hospitalization duration (days)	11	3713	Mean Difference (IV, Fixed, 95% CI)	-3.71 [-4.32, -3.11]
6 Weight gain	5		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
6.1 g/week	1	87	Mean Difference (IV, Fixed, 95% CI)	7.20 [-0.06, 14.46]
6.2 g/day	2	131	Mean Difference (IV, Fixed, 95% CI)	2.14 [0.01, 4.27]
6.3 g/kg/day	2	241	Mean Difference (IV, Fixed, 95% CI)	0.28 [-0.93, 1.49]
7 Time to full enteral feeds	8	2657	Mean Difference (IV, Fixed, 95% CI)	-1.32 [-1.48, -1.17]
8 Death or severe NEC or sepsis	1	367	Risk Ratio (M-H, Fixed, 95% CI)	0.54 [0.37, 0.79]
9 Long-term outcomes	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
9.1 Mental retardation and Cerebral palsy	1	85	Risk Ratio (M-H, Fixed, 95% CI)	1.02 [0.15, 6.94]

Comparison 2. Probiotics versus control (infants < 1500 g)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Severe necrotising enterocolitis (stage II-III)	17	4914	Risk Ratio (M-H, Fixed, 95% CI)	0.41 [0.31, 0.56]
2 Culture proven sepsis	16		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.1 Any sepsis	16	5154	Risk Ratio (M-H, Fixed, 95% CI)	0.92 [0.81, 1.04]
2.2 Any Bacterial sepsis	1	101	Risk Ratio (M-H, Fixed, 95% CI)	0.70 [0.36, 1.36]
2.3 Any Fungal sepsis	1	101	Risk Ratio (M-H, Fixed, 95% CI)	5.10 [0.25, 103.60]
3 Mortality	17		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
3.1 All causes of neonatal mortality	17	5303	Risk Ratio (M-H, Fixed, 95% CI)	0.66 [0.53, 0.82]
3.2 NEC related mortality	7	2755	Risk Ratio (M-H, Fixed, 95% CI)	0.39 [0.18, 0.82]

Comparison 3. Probiotics versus control (infants < 1000 g)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Severe necrotising enterocolitis (stage II-III)	2	575	Risk Ratio (M-H, Fixed, 95% CI)	0.76 [0.37, 1.58]
2 Culture proven sepsis	2	1200	Risk Ratio (M-H, Fixed, 95% CI)	0.82 [0.63, 1.06]
3 Mortality	2	1199	Risk Ratio (M-H, Fixed, 95% CI)	0.94 [0.58, 1.53]

Comparison 4. Probiotics versus control (species of probiotic)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Severe NEC- Species of probiotics	20		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.1 Lactobacillus	5	1955	Risk Ratio (M-H, Fixed, 95% CI)	0.45 [0.27, 0.75]
1.2 Bifidobacterium	4	409	Risk Ratio (M-H, Fixed, 95% CI)	0.48 [0.16, 1.47]
1.3 Saccharomyces boulardii	2	357	Risk Ratio (M-H, Fixed, 95% CI)	0.72 [0.34, 1.55]
1.4 A mixture of two to three species of probiotics	9	2807	Risk Ratio (M-H, Fixed, 95% CI)	0.37 [0.25, 0.54]
2 Culture proven sepsis	19		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.1 Lactobacillus	5	1955	Risk Ratio (M-H, Fixed, 95% CI)	0.91 [0.71, 1.16]
2.2 Bifidobacterium	3	348	Risk Ratio (M-H, Fixed, 95% CI)	0.88 [0.58, 1.34]
2.3 Saccharomyces boulardii	2	358	Risk Ratio (M-H, Fixed, 95% CI)	0.92 [0.54, 1.57]
2.4 A mixture of two to three species of probiotics	9	2677	Risk Ratio (M-H, Fixed, 95% CI)	0.91 [0.78, 1.06]
3 Mortality	16		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
3.1 Lactobacillus	4	1734	Risk Ratio (M-H, Fixed, 95% CI)	0.72 [0.47, 1.10]
3.2 Bifidobacterium	2	271	Risk Ratio (M-H, Fixed, 95% CI)	0.71 [0.14, 3.60]
3.3 Saccharomyces boulardii	1	271	Risk Ratio (M-H, Fixed, 95% CI)	1.01 [0.30, 3.40]
3.4 A mixture of two to three species of probiotics	9	2806	Risk Ratio (M-H, Fixed, 95% CI)	0.62 [0.47, 0.81]

Comparison 5. Probiotics versus control (time of initiation)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Severe NEC- Time of initiation	16		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.1 Less than 48 hours of age	3	1072	Risk Ratio (M-H, Fixed, 95% CI)	0.49 [0.23, 1.05]
1.2 More than 48 hours of age	1	319	Risk Ratio (M-H, Fixed, 95% CI)	0.05 [0.00, 0.90]
1.3 At the time of the first feed	9	2318	Risk Ratio (M-H, Fixed, 95% CI)	0.44 [0.30, 0.65]

1.4 During the first week when enteral feeds were tolerated	3	236	Risk Ratio (M-H, Fixed, 95% CI)	0.64 [0.26, 1.55]
2 Culture proven sepsis	16	4017	Risk Ratio (M-H, Fixed, 95% CI)	0.91 [0.79, 1.05]
2.1 Less than 48 hours of age	3	1072	Risk Ratio (M-H, Fixed, 95% CI)	1.06 [0.79, 1.44]
2.2 More than 48 hours of age	1	319	Risk Ratio (M-H, Fixed, 95% CI)	0.27 [0.12, 0.60]
2.3 At the time of the first feed	10	2459	Risk Ratio (M-H, Fixed, 95% CI)	0.96 [0.81, 1.14]
2.4 During the first week when enteral feeds were tolerated	2	167	Risk Ratio (M-H, Fixed, 95% CI)	0.88 [0.58, 1.34]
3 Mortality	14	3838	Risk Ratio (M-H, Fixed, 95% CI)	0.61 [0.47, 0.79]
3.1 Less than 48 hours of age	3	1072	Risk Ratio (M-H, Fixed, 95% CI)	0.82 [0.58, 1.17]
3.2 More than 48 hours of age	1	319	Risk Ratio (M-H, Fixed, 95% CI)	0.56 [0.21, 1.45]
3.3 At the time of the first feed	9	2367	Risk Ratio (M-H, Fixed, 95% CI)	0.41 [0.26, 0.63]
3.4 During the first week when enteral feeds were tolerated	1	80	Risk Ratio (M-H, Fixed, 95% CI)	0.88 [0.29, 2.64]

Comparison 6. Probiotics versus control (duration of probiotics administration)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Severe NEC- The duration of probiotics administration	16		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.1 Four to six weeks	5	1162	Risk Ratio (M-H, Fixed, 95% CI)	0.26 [0.13, 0.52]
1.2 More than six weeks or until discharged from NICU	11	2985	Risk Ratio (M-H, Fixed, 95% CI)	0.53 [0.37, 0.75]
2 Culture proven sepsis	14	3247	Risk Ratio (M-H, Fixed, 95% CI)	0.88 [0.77, 1.02]
2.1 Four to six weeks	5	1162	Risk Ratio (M-H, Fixed, 95% CI)	0.91 [0.71, 1.18]
2.2 More than six weeks or until discharged from NICU	9	2085	Risk Ratio (M-H, Fixed, 95% CI)	0.87 [0.73, 1.04]
3 Mortality	14		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
3.1 Four to six weeks	4	1075	Risk Ratio (M-H, Fixed, 95% CI)	0.67 [0.45, 1.00]
3.2 More than six weeks or until discharged from NICU	10	3591	Risk Ratio (M-H, Fixed, 95% CI)	0.65 [0.49, 0.87]

Comparison 7. Probiotics versus control (high quality studies)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Severe necrotising enterocolitis (stage II-III)	11	4473	Risk Ratio (M-H, Fixed, 95% CI)	0.43 [0.31, 0.59]
2 Culture proven sepsis	10		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.1 Any sepsis	10	4323	Risk Ratio (M-H, Fixed, 95% CI)	0.89 [0.77, 1.04]
3 Mortality	10		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only

3.1 All causes of neonatal mortality	10	4386	Risk Ratio (M-H, Fixed, 95% CI)	0.71 [0.55, 0.91]
3.2 NEC related mortality	4	2298	Risk Ratio (M-H, Fixed, 95% CI)	0.47 [0.20, 1.09]

Analysis 1.1. Comparison 1 Probiotics versus control (all infants), Outcome 1 Severe necrotising enterocolitis (stage II-III).

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 1 Probiotics versus control (all infants)

Outcome: 1 Severe necrotising enterocolitis (stage II-III)

Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Al-Hosni 2012	2/50	2/51		1.02 [0.15, 6.96]
Bin-Nun 2005	1/72	10/73		0.10 [0.01, 0.77]
Braga 2011	0/119	4/112		0.10 [0.01, 1.92]
Costalos 2003	5/51	6/36		0.59 [0.19, 1.78]
Dani 2002	4/295	8/290		0.49 [0.15, 1.61]
Demirel 2013	6/135	7/136		0.86 [0.30, 2.50]
Fern ndez-Carrocera 2013	6/75	12/75		0.50 [0.20, 1.26]
Kitajima 1997	0/45	0/46		0.0 [0.0, 0.0]
Lin 2005	2/180	10/187		0.21 [0.05, 0.94]
Lin 2008	4/217	14/217		0.29 [0.10, 0.85]
Manzoni 2006	1/39	3/41		0.35 [0.04, 3.23]
Manzoni 2009	0/151	10/168		0.05 [0.00, 0.90]
Mihatsch 2010	2/91	4/89		0.49 [0.09, 2.60]
Mohan 2006	2/37	1/32		1.73 [0.16, 18.20]
ProPrems 2013	11/548	24/551		0.46 [0.23, 0.93]
Rojas 2012	9/372	15/378		0.61 [0.27, 1.38]
Roug˙x00e9˙ 2009	2/45	1/49		2.18 [0.20, 23.21]
Samanta 2009	5/91	15/95		0.35 [0.13, 0.92]
Sari 2010	6/110	10/111		0.61 [0.23, 1.61]
Stratiki 2007	0/38	3/31		0.12 [0.01, 2.19]
Total (95% CI)	**2761**	**2768**		**0.43 [0.33, 0.56]**
Total events: 68 (Probiotics), 159 (Control)				

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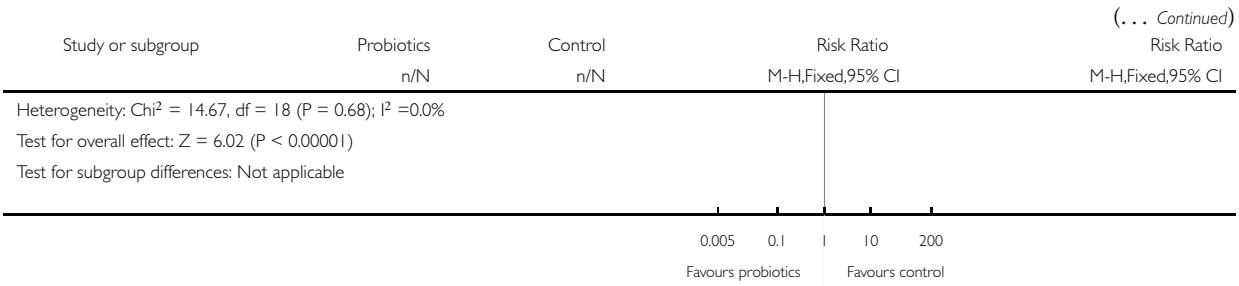
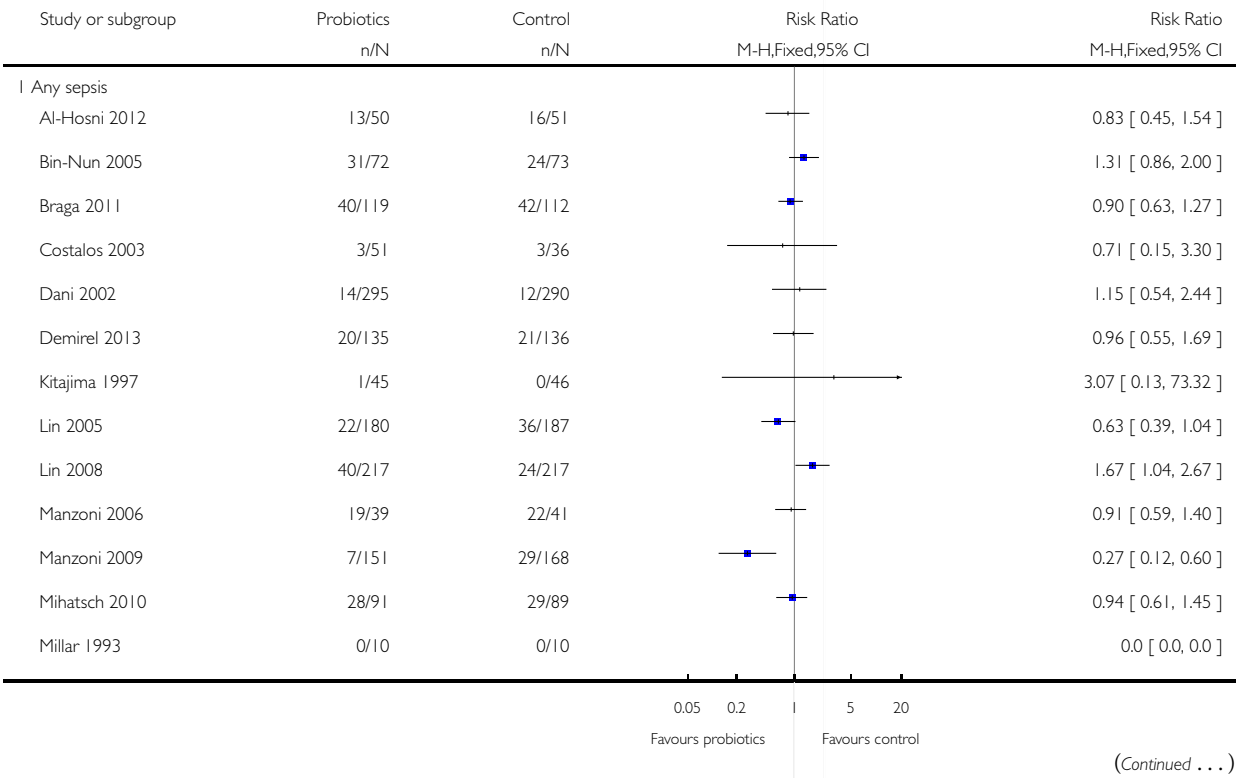


Analysis 1.2. Comparison 1 Probiotics versus control (all infants), Outcome 2 Culture proven sepsis.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 1 Probiotics versus control (all infants)

Outcome: 2 Culture proven sepsis



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Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
ProPrems 2013	72/548	89/551		0.81 [0.61, 1.08]
Rojas 2012	24/372	17/378		1.43 [0.78, 2.63]
Roug˙x00e9˙ 2009	15/45	13/49		1.26 [0.67, 2.34]
Samanta 2009	13/91	28/95		0.48 [0.27, 0.88]
Sari 2010	29/110	26/111		1.13 [0.71, 1.78]
Stratiki 2007	0/41	3/36		0.13 [0.01, 2.36]
Subtotal (95% CI)	**2662**	**2676**		**0.91 [0.80, 1.03]**
Total events: 391 (Probiotics), 434 (Control)				
Heterogeneity: Chi^2 = 32.28, df = 17 (P = 0.01); I^2 =47%				
Test for overall effect: Z = 1.56 (P = 0.12)				
2 Any Bacterial sepsis				
Al-Hosni 2012	11/50	16/51		0.70 [0.36, 1.36]
Subtotal (95% CI)	**50**	**51**		**0.70 [0.36, 1.36]**
Total events: 11 (Probiotics), 16 (Control)				
Heterogeneity: not applicable				
Test for overall effect: Z = 1.05 (P = 0.29)				
3 Any Fungal sepsis				
Al-Hosni 2012	2/50	0/51		5.10 [0.25, 103.60]
Subtotal (95% CI)	**50**	**51**		**5.10 [0.25, 103.60]**
Total events: 2 (Probiotics), 0 (Control)				
Heterogeneity: not applicable				
Test for overall effect: Z = 1.06 (P = 0.29)				
Test for subgroup differences: Chi^2 = 1.84, df = 2 (P = 0.40), I^2 =0.0%				

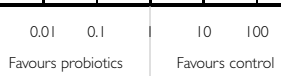
Analysis 1.3. Comparison 1 Probiotics versus control (all infants), Outcome 3 Mortality.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 1 Probiotics versus control (all infants)

Outcome: 3 Mortality

Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 All causes of neonatal mortality					
Al-Hosni 2012	3/50	4/50		2.2 %	0.75 [0.18, 3.18]
Bin-Nun 2005	3/72	8/73		4.4 %	0.38 [0.11, 1.38]
Braga 2011	26/119	27/112		15.5 %	0.91 [0.56, 1.45]
Dani 2002	0/295	2/290		1.4 %	0.20 [0.01, 4.08]
Demirel 2013	5/135	5/136		2.8 %	1.01 [0.30, 3.40]
Fern ndez-Carrocera 2013	1/75	7/75		3.9 %	0.14 [0.02, 1.13]
Kitajima 1997	0/45	2/46		1.4 %	0.20 [0.01, 4.14]
Lin 2005	7/180	20/187		10.9 %	0.36 [0.16, 0.84]
Lin 2008	2/217	9/217		5.0 %	0.22 [0.05, 1.02]
Manzoni 2006	5/39	6/41		3.3 %	0.88 [0.29, 2.64]
Manzoni 2009	6/151	12/168		6.3 %	0.56 [0.21, 1.45]
Mihatsch 2010	2/91	1/89		0.6 %	1.96 [0.18, 21.19]
ProPrems 2013	27/548	28/551		15.5 %	0.97 [0.58, 1.62]
Reuman 1986	1/15	3/15		1.7 %	0.33 [0.04, 2.85]
Rojas 2012	22/372	28/378		15.4 %	0.80 [0.47, 1.37]
Roug˙x00e9˙ 2009	2/45	4/49		2.1 %	0.54 [0.10, 2.83]
Samanta 2009	4/91	14/95		7.6 %	0.30 [0.10, 0.87]
Subtotal (95% CI)	**2540**	**2572**	◆	**100.0 %**	**0.65 [0.52, 0.81]**
Total events: 116 (Probiotics), 180 (Control)					
Heterogeneity: Chi^2 = 16.58, df = 16 (P = 0.41); I^2 =4%					
Test for overall effect: Z = 3.77 (P = 0.00017)					
2 NEC related mortality					
Bin-Nun 2005	0/72	3/73		14.2 %	0.14 [0.01, 2.75]
Dani 2002	0/295	2/290		10.3 %	0.20 [0.01, 4.08]
Kitajima 1997	0/45	2/46		10.1 %	0.20 [0.01, 4.14]
Lin 2008	2/217	3/217		12.3 %	0.67 [0.11, 3.95]



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Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Mihatsch 2010	1/91	0/89		2.1 %	2.93 [0.12, 71.10]
ProPrems 2013	4/548	11/551		44.9 %	0.37 [0.12, 1.14]
Sari 2010	0/110	1/111		6.1 %	0.34 [0.01, 8.17]
Subtotal (95% CI)	**1378**	**1377**		**100.0 %**	**0.39 [0.18, 0.82]**
Total events: 7 (Probiotics), 22 (Control)					
Heterogeneity: Chi² = 2.72, df = 6 (P = 0.84); I² =0.0%					
Test for overall effect: Z = 2.48 (P = 0.013)					

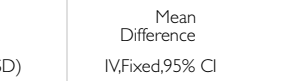
Analysis 1.4. Comparison 1 Probiotics versus control (all infants), Outcome 4 Parenteral nutrition duration (days).

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 1 Probiotics versus control (all infants)

Outcome: 4 Parenteral nutrition duration (days)

Study or subgroup	Probiotics N	Mean(SD)	Control N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Weight	Mean Difference IV,Fixed,95% CI
Dani 2002	295	12.8 (13.9)	290	14.7 (18.7)		1.0 %	-1.90 [-4.57, 0.77]
Demirel 2013	135	8.38 (1.27)	136	8.47 (3.41)		20.0 %	-0.09 [-0.70, 0.52]
Fern ndez-Carrocera 2013	75	18.75 (12.1)	75	23.25 (20.6)		0.3 %	-4.50 [-9.91, 0.91]
Lin 2005	180	14.7 (5.7)	187	13.9 (5)		6.2 %	0.80 [-0.30, 1.90]
ProPrems 2013	548	12.25 (2.6)	551	12.5 (2.9)		70.8 %	-0.25 [-0.58, 0.08]
Romeo 2011a	83	6.8 (5.4)	83	13.5 (11.6)		1.0 %	-6.70 [-9.45, -3.95]
Romeo 2011b	83	13 (10.5)	83	13.5 (11.6)		0.7 %	-0.50 [-3.87, 2.87]
Total (95% CI)	**1399**		**1405**			**100.0 %**	**-0.25 [-0.52, 0.03]**
Heterogeneity: Chi² = 28.72, df = 6 (P = 0.00007); I² =79%							
Test for overall effect: Z = 1.76 (P = 0.078)							
Test for subgroup differences: Not applicable							



Analysis 1.5. Comparison 1 Probiotics versus control (all infants), Outcome 5 Hospitalization duration (days).

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 1 Probiotics versus control (all infants)

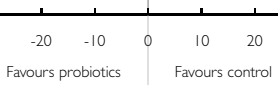
Outcome: 5 Hospitalization duration (days)

Study or subgroup	Probiotics N	Mean(SD)	Control N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Weight	Mean Difference IV,Fixed,95% CI
Demirel 2013	135	55 (33.12)	136	56 (38.02)		0.5 %	-1.00 [-9.49, 7.49]
Fern ndez-Carrocera 2013	75	59.25 (35.6)	75	52 (32.8)		0.3 %	7.25 [-3.71, 18.21]
Lin 2005	180	46.7 (27.1)	187	46.5 (26.1)		1.2 %	0.20 [-5.25, 5.65]
Lin 2008	217	46.4 (24.2)	217	43.3 (21)		2.0 %	3.10 [-1.16, 7.36]
ProPrems 2013	548	72 (10.98)	551	74.75 (10.1)		23.5 %	-2.75 [-4.00, -1.50]
Reuman 1986	15	59.4 (56.4)	15	38.7 (30.6)		0.0 %	20.70 [-11.77, 53.17]
Rojas 2012	372	21 (6.4)	378	22.25 (7.9)		34.6 %	-1.25 [-2.28, -0.22]
Romeo 2011a	83	17.8 (7.9)	83	31.3 (16.3)		2.4 %	-13.50 [-17.40, -9.60]
Romeo 2011b	83	26.9 (15.7)	83	31.3 (16.3)		1.5 %	-4.40 [-9.27, 0.47]
Roug˙x00e9˙ 2009	45	60.7 (28.8)	49	65.6 (30)		0.3 %	-4.90 [-16.79, 6.99]
Samanta 2009	91	17.17 (3.23)	95	24.07 (4)		33.6 %	-6.90 [-7.94, -5.86]
Total (95% CI)	**1844**		**1869**			**100.0 %**	**-3.71 [-4.32, -3.11]**

Heterogeneity: Chi^2 = 102.76, df = 10 (P<0.00001); I^2 =90%

Test for overall effect: Z = 12.04 (P < 0.00001)

Test for subgroup differences: Not applicable

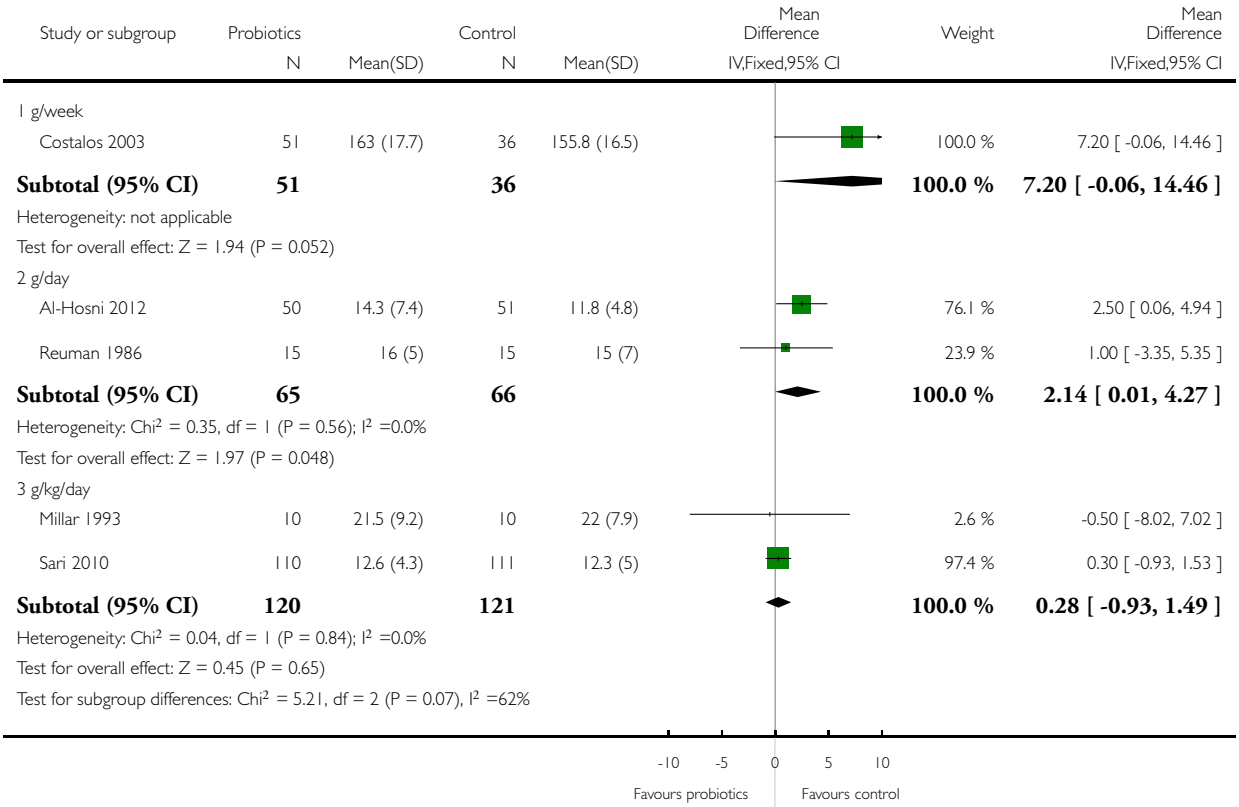


Analysis 1.6. Comparison 1 Probiotics versus control (all infants), Outcome 6 Weight gain.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 1 Probiotics versus control (all infants)

Outcome: 6 Weight gain



Analysis 1.7. Comparison 1 Probiotics versus control (all infants), Outcome 7 Time to full enteral feeds.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 1 Probiotics versus control (all infants)

Outcome: 7 Time to full enteral feeds

Study or subgroup	Probiotics N	Mean(SD)	Control N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Weight	Mean Difference IV,Fixed,95% CI
Braga 2011	119	15.2 (5.2)	112	17.4 (5.7)		1.2 %	-2.20 [-3.61, -0.79]
Demirel 2013	135	11.74 (0.46)	136	13.24 (1.23)		48.6 %	-1.50 [-1.72, -1.28]
Fern ndez-Carrocera 2013	75	23 (16.3)	75	17.25 (11.3)		0.1 %	5.75 [1.26, 10.24]
Manzoni 2009	151	13.4 (5.1)	168	14.8 (4.7)		2.0 %	-1.40 [-2.48, -0.32]
Mihatsch 2010	91	17.9 (6.8)	89	18 (7.4)		0.5 %	-0.10 [-2.18, 1.98]
ProPrems 2013	548	12.25 (2.02)	551	12.75 (2.05)		40.9 %	-0.50 [-0.74, -0.26]
Samanta 2009	91	13.76 (2.28)	95	19.2 (2.02)		6.2 %	-5.44 [-6.06, -4.82]
Sari 2010	110	17.3 (8.7)	111	18.3 (9.8)		0.4 %	-1.00 [-3.44, 1.44]
Total (95% CI)	**1320**		**1337**			**100.0 %**	**-1.32 [-1.48, -1.17]**

Heterogeneity: Chi^2 = 229.21, df = 7 (P<0.00001); I^2 =97%

Test for overall effect: Z = 16.83 (P < 0.00001)

Test for subgroup differences: Not applicable

Analysis 1.8. Comparison 1 Probiotics versus control (all infants), Outcome 8 Death or severe NEC or sepsis.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 1 Probiotics versus control (all infants)

Outcome: 8 Death or severe NEC or sepsis

Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Lin 2005	31/180	60/187		100.0 %	0.54 [0.37, 0.79]
Total (95% CI)	**180**	**187**		**100.0 %**	**0.54 [0.37, 0.79]**
Total events: 31 (Probiotics), 60 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 3.19 (P = 0.0014)					
Test for subgroup differences: Not applicable					

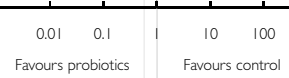
Analysis 1.9. Comparison 1 Probiotics versus control (all infants), Outcome 9 Long-term outcomes.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 1 Probiotics versus control (all infants)

Outcome: 9 Long-term outcomes

Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Mental retardation and Cerebral palsy					
Kitajima 1997	2/42	2/43		100.0 %	1.02 [0.15, 6.94]
Subtotal (95% CI)	**42**	**43**		**100.0 %**	**1.02 [0.15, 6.94]**
Total events: 2 (Probiotics), 2 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.02 (P = 0.98)					
Test for subgroup differences: Not applicable					



Analysis 2.1. Comparison 2 Probiotics versus control (infants < 1500 g), Outcome 1 Severe necrotising enterocolitis (stage II-III).

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 2 Probiotics versus control (infants < 1500 g)

Outcome: 1 Severe necrotising enterocolitis (stage II-III)

Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Al-Hosni 2012	2/50	2/51		1.02 [0.15, 6.96]
Bin-Nun 2005	1/72	10/73		0.10 [0.01, 0.77]
Braga 2011	0/119	4/112		0.10 [0.01, 1.92]
Dani 2002	4/295	8/290		0.49 [0.15, 1.61]
Demirel 2013	6/135	7/136		0.86 [0.30, 2.50]
Fern ndez-Carrocera 2013	6/75	12/75		0.50 [0.20, 1.26]
Kitajima 1997	0/45	0/46		0.0 [0.0, 0.0]
Lin 2005	2/180	10/187		0.21 [0.05, 0.94]
Lin 2008	4/217	14/217		0.29 [0.10, 0.85]
Manzoni 2006	1/39	3/41		0.35 [0.04, 3.23]
Manzoni 2009	0/151	10/168		0.05 [0.00, 0.90]
Mihatsch 2010	2/91	4/89		0.49 [0.09, 2.60]
ProPrems 2013	11/548	24/551		0.46 [0.23, 0.93]
Rojas 2012	6/176	10/184		0.63 [0.23, 1.69]
Roug˙x00e9˙ 2009	2/45	1/49		2.18 [0.20, 23.21]
Samanta 2009	5/91	15/95		0.35 [0.13, 0.92]
Sari 2010	6/110	10/111		0.61 [0.23, 1.61]
Total (95% CI)	**2439**	**2475**		**0.41 [0.31, 0.56]**

Total events: 58 (Probiotics), 144 (Control)

Heterogeneity: $Chi^2 = 12.32$, df = 15 (P = 0.65); $I^2 = 0.0\%$

Test for overall effect: Z = 5.83 (P < 0.00001)

Test for subgroup differences: Not applicable

Analysis 2.2. Comparison 2 Probiotics versus control (infants < 1500 g), Outcome 2 Culture proven sepsis.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 2 Probiotics versus control (infants < 1500 g)

Outcome: 2 Culture proven sepsis

Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Any sepsis					
Al-Hosni 2012	13/50	16/51		3.7 %	0.83 [0.45, 1.54]
Bin-Nun 2005	31/72	24/73		5.6 %	1.31 [0.86, 2.00]
Braga 2011	40/119	42/112		10.2 %	0.90 [0.63, 1.27]
Dani 2002	14/295	12/290		2.8 %	1.15 [0.54, 2.44]
Demirel 2013	20/135	21/136		4.9 %	0.96 [0.55, 1.69]
Kitajima 1997	1/45	0/46		0.1 %	3.07 [0.13, 73.32]
Lin 2005	22/180	36/187		8.3 %	0.63 [0.39, 1.04]
Lin 2008	40/217	24/217		5.6 %	1.67 [1.04, 2.67]
Manzoni 2006	19/39	22/41		5.0 %	0.91 [0.59, 1.40]
Manzoni 2009	7/151	29/168		6.5 %	0.27 [0.12, 0.60]
Mihatsch 2010	28/91	29/89		6.9 %	0.94 [0.61, 1.45]
ProPrems 2013	72/548	89/551		20.9 %	0.81 [0.61, 1.08]
Rojas 2012	24/372	17/378		4.0 %	1.43 [0.78, 2.63]
Roug˙x00e9˙ 2009	15/45	13/49		2.9 %	1.26 [0.67, 2.34]
Samanta 2009	13/91	28/95		6.4 %	0.48 [0.27, 0.88]
Sari 2010	29/110	26/111		6.1 %	1.13 [0.71, 1.78]
Subtotal (95% CI)	**2560**	**2594**		**100.0 %**	**0.92 [0.81, 1.04]**
Total events: 388 (Probiotics), 428 (Control)					
Heterogeneity: Chi^2 = 30.32, df = 15 (P = 0.01); I^2 =51%					
Test for overall effect: Z = 1.40 (P = 0.16)					
2 Any Bacterial sepsis					
Al-Hosni 2012	11/50	16/51		100.0 %	0.70 [0.36, 1.36]
Subtotal (95% CI)	**50**	**51**		**100.0 %**	**0.70 [0.36, 1.36]**
Total events: 11 (Probiotics), 16 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.05 (P = 0.29)					
3 Any Fungal sepsis					
Al-Hosni 2012	2/50	0/51		100.0 %	5.10 [0.25, 103.60]

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Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Subtotal (95% CI)	**50**	**51**		**100.0 %**	**5.10 [0.25, 103.60]**
Total events: 2 (Probiotics), 0 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.06 (P = 0.29)					
Test for subgroup differences: Chi² = 1.87, df = 2 (P = 0.39), I² =0.0%					

Analysis 2.3. Comparison 2 Probiotics versus control (infants < 1500 g), Outcome 3 Mortality.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 2 Probiotics versus control (infants < 1500 g)

Outcome: 3 Mortality

Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 All causes of neonatal mortality					
Al-Hosni 2012	3/50	4/50		2.2 %	0.75 [0.18, 3.18]
Bin-Nun 2005	3/72	8/73		4.4 %	0.38 [0.11, 1.38]
Braga 2011	26/119	27/112		15.4 %	0.91 [0.56, 1.45]
Dani 2002	0/295	2/290		1.4 %	0.20 [0.01, 4.08]
Demirel 2013	5/135	5/136		2.8 %	1.01 [0.30, 3.40]
Fern ndez-Carrocera 2013	1/75	7/75		3.9 %	0.14 [0.02, 1.13]
Kitajima 1997	0/45	2/46		1.4 %	0.20 [0.01, 4.14]
Lin 2005	7/180	20/187		10.9 %	0.36 [0.16, 0.84]
Lin 2008	2/217	9/217		5.0 %	0.22 [0.05, 1.02]
Manzoni 2006	5/39	6/41		3.2 %	0.88 [0.29, 2.64]
Manzoni 2009	6/151	12/168		6.3 %	0.56 [0.21, 1.45]
Mihatsch 2010	2/91	1/89		0.6 %	1.96 [0.18, 21.19]

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Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
ProPrems 2013	27/548	28/551		15.4 %	0.97 [0.58, 1.62]
Rojas 2012	22/372	28/378		15.4 %	0.80 [0.47, 1.37]
Roug˙x00e9˙ 2009	2/45	4/49		2.1 %	0.54 [0.10, 2.83]
Samanta 2009	4/91	14/95		7.6 %	0.30 [0.10, 0.87]
Sari 2010	3/110	4/111		2.2 %	0.76 [0.17, 3.30]
Subtotal (95% CI)	**2635**	**2668**		**100.0 %**	**0.66 [0.53, 0.82]**
Total events: 118 (Probiotics), 181 (Control)					
Heterogeneity: Chi^2 = 16.13, df = 16 (P = 0.44); I^2 =1%					
Test for overall effect: Z = 3.68 (P = 0.00023)					
2 NEC related mortality					
Bin-Nun 2005	0/72	3/73		14.2 %	0.14 [0.01, 2.75]
Dani 2002	0/295	2/290		10.3 %	0.20 [0.01, 4.08]
Kitajima 1997	0/45	2/46		10.1 %	0.20 [0.01, 4.14]
Lin 2008	2/217	3/217		12.3 %	0.67 [0.11, 3.95]
Mihatsch 2010	1/91	0/89		2.1 %	2.93 [0.12, 71.10]
ProPrems 2013	4/548	11/551		44.9 %	0.37 [0.12, 1.14]
Sari 2010	0/110	1/111		6.1 %	0.34 [0.01, 8.17]
Subtotal (95% CI)	**1378**	**1377**		**100.0 %**	**0.39 [0.18, 0.82]**
Total events: 7 (Probiotics), 22 (Control)					
Heterogeneity: Chi^2 = 2.72, df = 6 (P = 0.84); I^2 =0.0%					
Test for overall effect: Z = 2.48 (P = 0.013)					
Test for subgroup differences: Chi^2 = 1.77, df = 1 (P = 0.18), I^2 =44%					

Analysis 3.1. Comparison 3 Probiotics versus control (infants < 1000 g), Outcome 1 Severe necrotising enterocolitis (stage II-III).

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 3 Probiotics versus control (infants < 1000 g)

Outcome: 1 Severe necrotising enterocolitis (stage II-III)

Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Al-Hosni 2012	2/50	2/51		12.5 %	1.02 [0.15, 6.96]
ProPrems 2013	10/235	14/239		87.5 %	0.73 [0.33, 1.60]
Total (95% CI)	**285**	**290**		**100.0 %**	**0.76 [0.37, 1.58]**

Total events: 12 (Probiotics), 16 (Control)

Heterogeneity: $Chi^2 = 0.10$, df = 1 (P = 0.75); $I^2 = 0.0\%$

Test for overall effect: Z = 0.73 (P = 0.47)

Test for subgroup differences: Not applicable

Analysis 3.2. Comparison 3 Probiotics versus control (infants < 1000 g), Outcome 2 Culture proven sepsis.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 3 Probiotics versus control (infants < 1000 g)

Outcome: 2 Culture proven sepsis

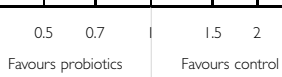
Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Al-Hosni 2012	13/50	16/51		15.1 %	0.83 [0.45, 1.54]
ProPrems 2013	72/548	89/551		84.9 %	0.81 [0.61, 1.08]
Total (95% CI)	**598**	**602**		**100.0 %**	**0.82 [0.63, 1.06]**

Total events: 85 (Probiotics), 105 (Control)

Heterogeneity: $Chi^2 = 0.00$, df = 1 (P = 0.96); $I^2 = 0.0\%$

Test for overall effect: Z = 1.53 (P = 0.13)

Test for subgroup differences: Not applicable



Analysis 3.3. Comparison 3 Probiotics versus control (infants < 1000 g), Outcome 3 Mortality.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 3 Probiotics versus control (infants < 1000 g)

Outcome: 3 Mortality

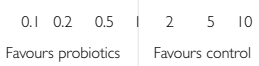
Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Al-Hosni 2012	3/50	4/50		12.5 %	0.75 [0.18, 3.18]
ProPrems 2013	27/548	28/551		87.5 %	0.97 [0.58, 1.62]
Total (95% CI)	**598**	**601**		**100.0 %**	**0.94 [0.58, 1.53]**

Total events: 30 (Probiotics), 32 (Control)

Heterogeneity: $Chi^2 = 0.11$, df = 1 (P = 0.74); $I^2 = 0.0\%$

Test for overall effect: Z = 0.24 (P = 0.81)

Test for subgroup differences: Not applicable

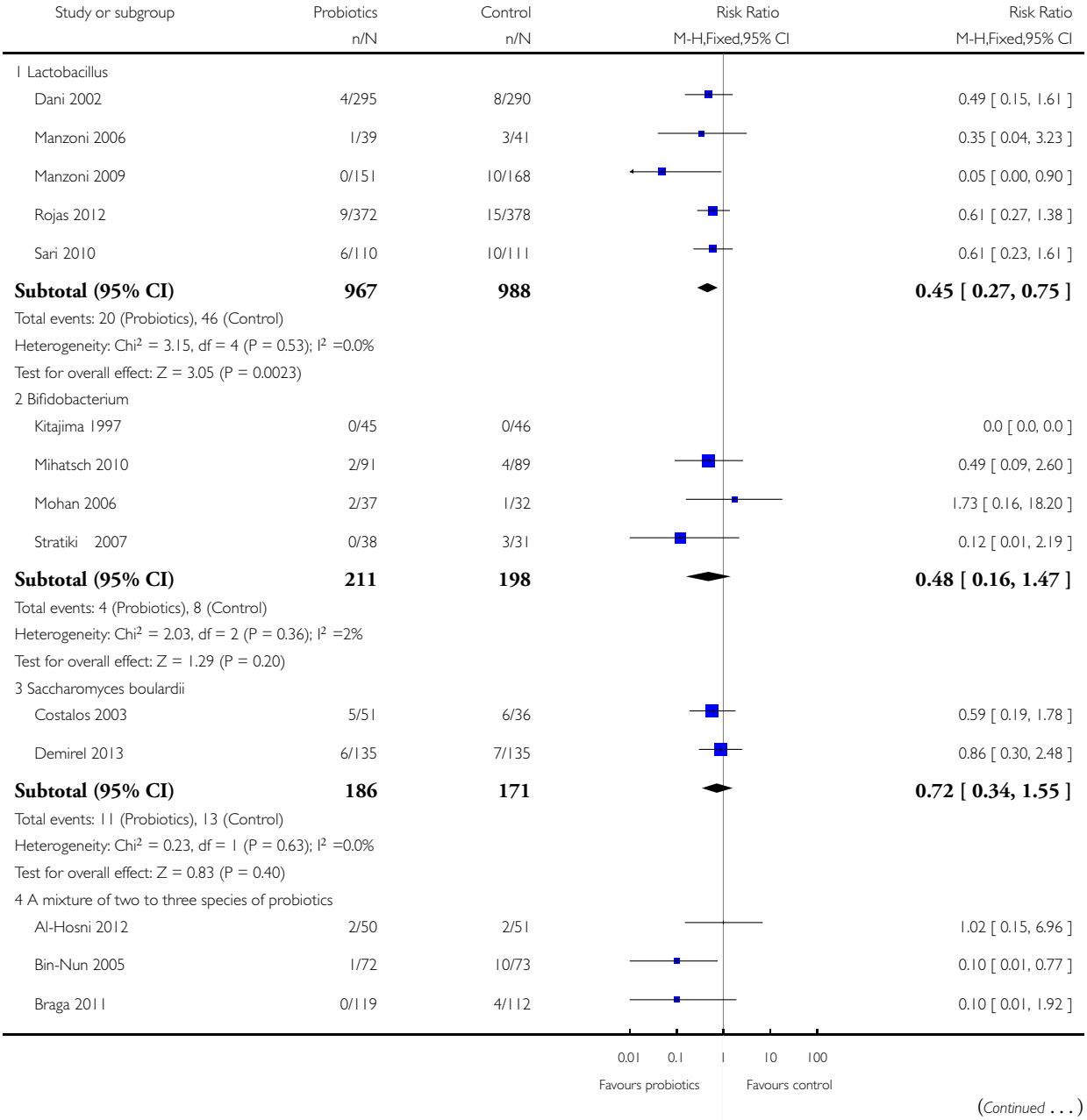


Analysis 4.1. Comparison 4 Probiotics versus control (species of probiotic), Outcome 1 Severe NEC- Species of probiotics.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 4 Probiotics versus control (species of probiotic)

Outcome: 1 Severe NEC- Species of probiotics



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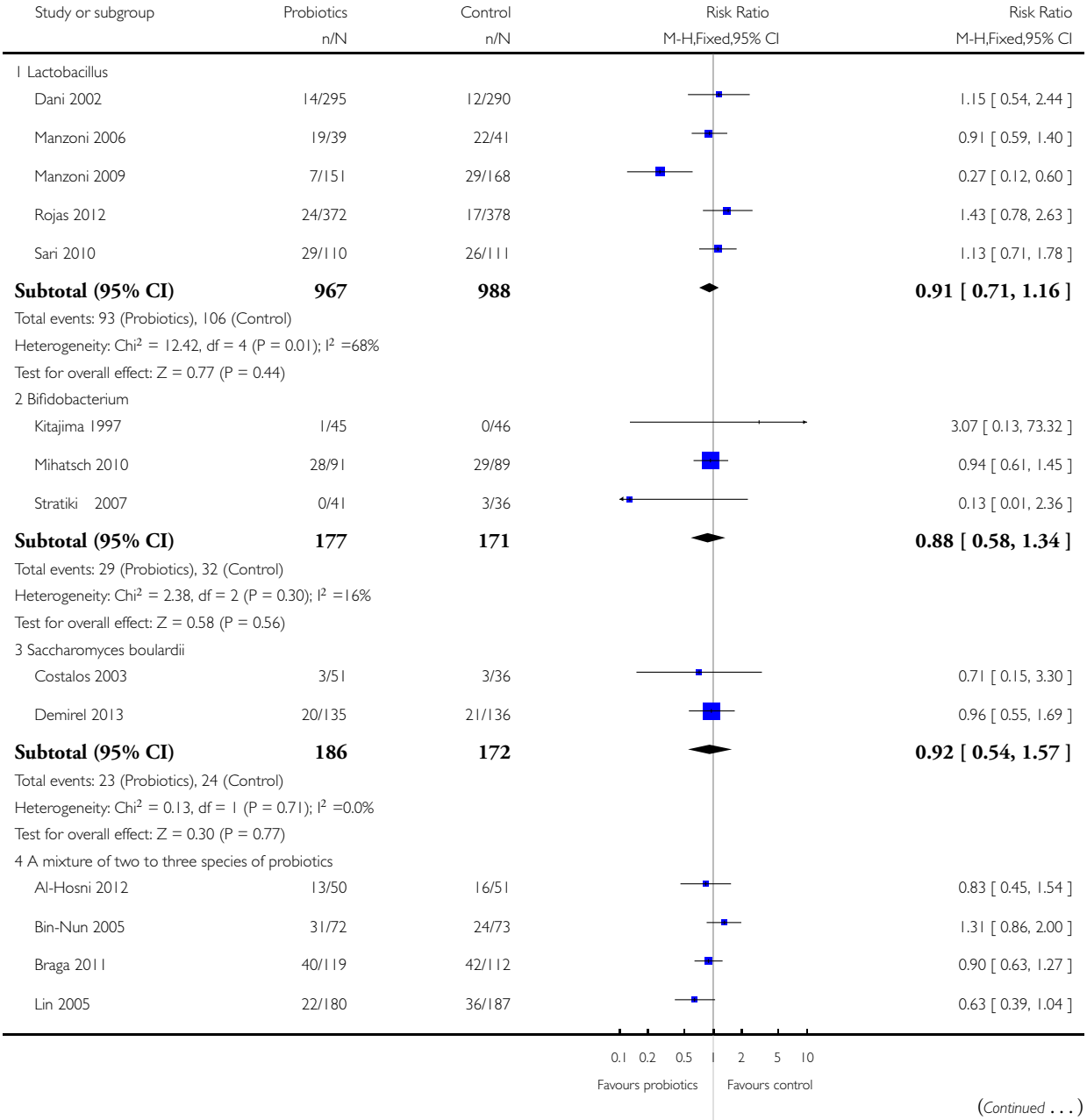
Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Fern ndez-Carrocera 2013	6/75	12/75		0.50 [0.20, 1.26]
Lin 2005	2/180	10/187		0.21 [0.05, 0.94]
Lin 2008	4/217	14/217		0.29 [0.10, 0.85]
ProPrems 2013	11/548	24/551		0.46 [0.23, 0.93]
Roug˙x00e9˙ 2009	2/45	1/49		2.18 [0.20, 23.21]
Samanta 2009	5/91	15/95		0.35 [0.13, 0.92]
Subtotal (95% CI)	**1397**	**1410**		**0.37 [0.25, 0.54]**
Total events: 33 (Probiotics), 92 (Control)				
Heterogeneity: Chi^2 = 7.11, df = 8 (P = 0.52); I^2 =0.0%				
Test for overall effect: Z = 5.09 (P < 0.00001)				
Test for subgroup differences: Chi^2 = 2.47, df = 3 (P = 0.48), I^2 =0.0%				

Analysis 4.2. Comparison 4 Probiotics versus control (species of probiotic), Outcome 2 Culture proven sepsis.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 4 Probiotics versus control (species of probiotic)

Outcome: 2 Culture proven sepsis



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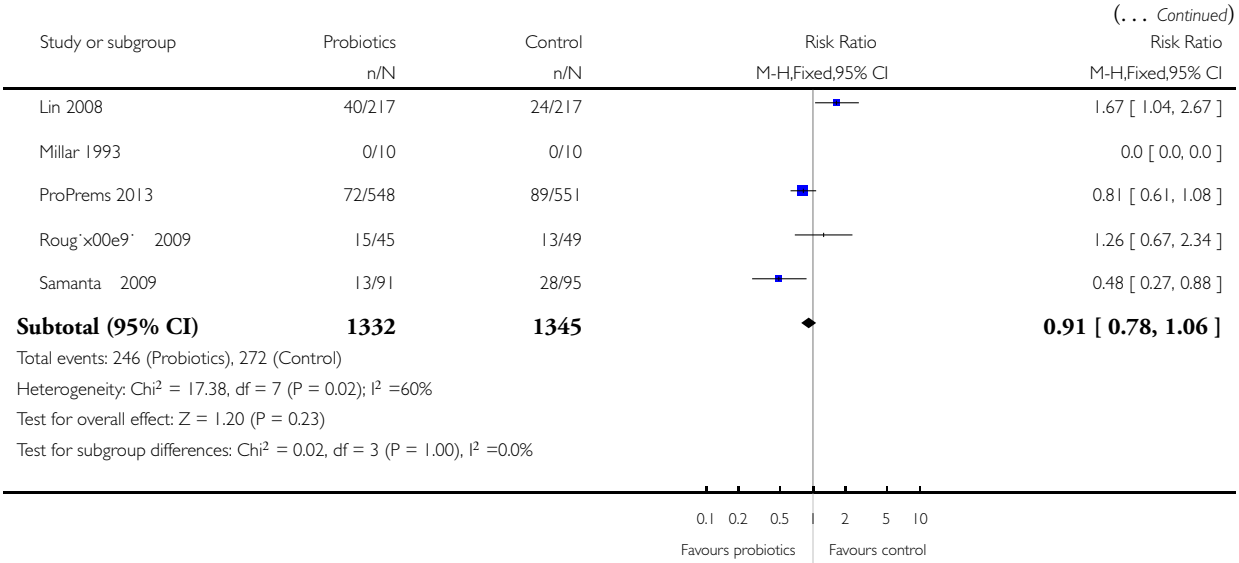
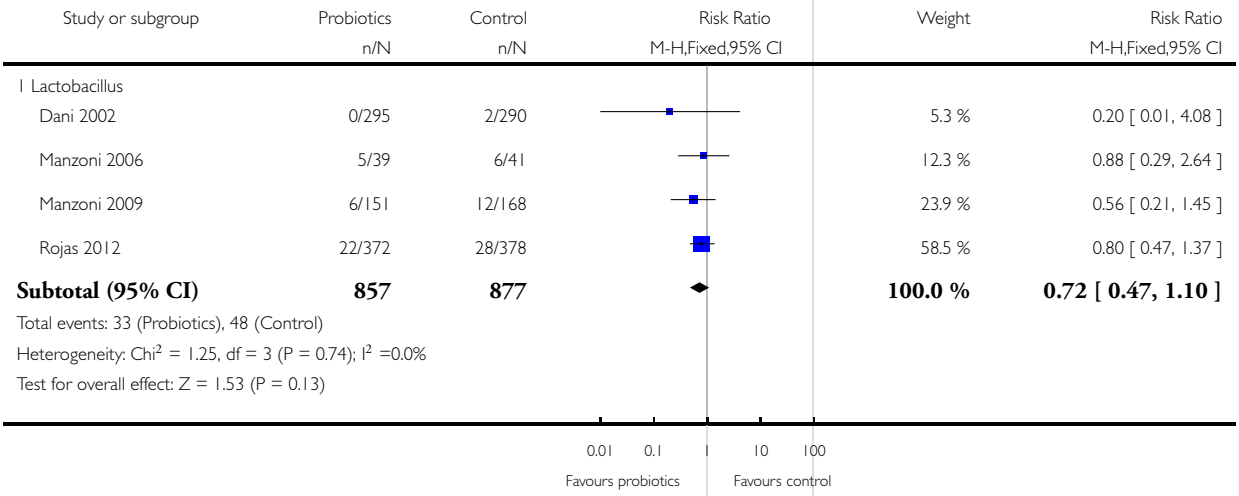


Analysis 4.3. Comparison 4 Probiotics versus control (species of probiotic), Outcome 3 Mortality.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 4 Probiotics versus control (species of probiotic)

Outcome: 3 Mortality



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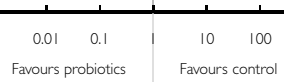
Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
2 Bifidobacterium					
Kitajima 1997	0/45	2/46		71.0 %	0.20 [0.01, 4.14]
Mihatsch 2010	2/91	1/89		29.0 %	1.96 [0.18, 21.19]
Subtotal (95% CI)	**136**	**135**		**100.0 %**	**0.71 [0.14, 3.60]**
Total events: 2 (Probiotics), 3 (Control)					
Heterogeneity: Chi^2 = 1.35, df = 1 (P = 0.24); I^2 =26%					
Test for overall effect: Z = 0.41 (P = 0.68)					
3 Saccharomyces boulardii					
Demirel 2013	5/135	5/136		100.0 %	1.01 [0.30, 3.40]
Subtotal (95% CI)	**135**	**136**		**100.0 %**	**1.01 [0.30, 3.40]**
Total events: 5 (Probiotics), 5 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.01 (P = 0.99)					
4 A mixture of two to three species of probiotics					
Al-Hosni 2012	3/50	4/50		3.3 %	0.75 [0.18, 3.18]
Bin-Nun 2005	3/72	8/73		6.6 %	0.38 [0.11, 1.38]
Braga 2011	26/119	27/112		23.0 %	0.91 [0.56, 1.45]
Fern ndez-Carrocera 2013	1/75	7/75		5.8 %	0.14 [0.02, 1.13]
Lin 2005	7/180	20/187		16.2 %	0.36 [0.16, 0.84]
Lin 2008	2/217	9/217		7.4 %	0.22 [0.05, 1.02]
ProPrems 2013	27/548	28/551		23.1 %	0.97 [0.58, 1.62]
Roug˙x00e9˙ 2009	2/45	4/49		3.2 %	0.54 [0.10, 2.83]
Samanta 2009	4/91	14/95		11.3 %	0.30 [0.10, 0.87]
Subtotal (95% CI)	**1397**	**1409**		**100.0 %**	**0.62 [0.47, 0.81]**
Total events: 75 (Probiotics), 121 (Control)					
Heterogeneity: Chi^2 = 13.08, df = 8 (P = 0.11); I^2 =39%					
Test for overall effect: Z = 3.45 (P = 0.00057)					
Test for subgroup differences: Chi^2 = 0.85, df = 3 (P = 0.84), I^2 =0.0%					



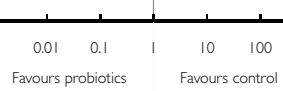
Analysis 5.1. Comparison 5 Probiotics versus control (time of initiation), Outcome 1 Severe NEC- Time of initiation.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 5 Probiotics versus control (time of initiation)

Outcome: 1 Severe NEC- Time of initiation

Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
1 Less than 48 hours of age				
Braga 2011	0/119	4/112		0.10 [0.01, 1.92]
Kitajima 1997	0/45	0/46		0.0 [0.0, 0.0]
Rojas 2012	9/372	15/378		0.61 [0.27, 1.38]
Subtotal (95% CI)	**536**	**536**		**0.49 [0.23, 1.05]**
Total events: 9 (Probiotics), 19 (Control)				
Heterogeneity: Chi^2 = 1.36, df = 1 (P = 0.24); I^2 =26%				
Test for overall effect: Z = 1.83 (P = 0.067)				
2 More than 48 hours of age				
Manzoni 2009	0/151	10/168		0.05 [0.00, 0.90]
Subtotal (95% CI)	**151**	**168**		**0.05 [0.00, 0.90]**
Total events: 0 (Probiotics), 10 (Control)				
Heterogeneity: not applicable				
Test for overall effect: Z = 2.04 (P = 0.042)				
3 At the time of the first feed				
Al-Hosni 2012	2/50	2/51		1.02 [0.15, 6.96]
Dani 2002	4/295	8/290		0.49 [0.15, 1.61]
Fern ndez-Carrocera 2013	6/75	12/75		0.50 [0.20, 1.26]
Lin 2005	2/180	10/187		0.21 [0.05, 0.94]
Lin 2008	4/217	14/217		0.29 [0.10, 0.85]
Mihatsch 2010	2/91	4/89		0.49 [0.09, 2.60]
Roug˙x00e9˙ 2009	2/45	1/49		2.18 [0.20, 23.21]
Samanta 2009	5/91	15/95		0.35 [0.13, 0.92]
Sari 2010	6/110	10/111		0.61 [0.23, 1.61]
Subtotal (95% CI)	**1154**	**1164**		**0.44 [0.30, 0.65]**
Total events: 33 (Probiotics), 76 (Control)				
Heterogeneity: Chi^2 = 4.80, df = 8 (P = 0.78); I^2 =0.0%				
Test for overall effect: Z = 4.05 (P = 0.000051)				
4 During the first week when enteral feeds were tolerated				
Costalos 2003	5/51	6/36		0.59 [0.19, 1.78]



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Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Manzoni 2006	1/39	3/41		0.35 [0.04, 3.23]
Mohan 2006	2/37	1/32		1.73 [0.16, 18.20]
Subtotal (95% CI)	**127**	**109**		**0.64 [0.26, 1.55]**
Total events: 8 (Probiotics), 10 (Control)				
Heterogeneity: Chi^2 = 0.99, df = 2 (P = 0.61); I^2 =0.0%				
Test for overall effect: Z = 0.99 (P = 0.32)				
Test for subgroup differences: Chi^2 = 2.83, df = 3 (P = 0.42), I^2 =0.0%				

Analysis 5.2. Comparison 5 Probiotics versus control (time of initiation), Outcome 2 Culture proven sepsis.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 5 Probiotics versus control (time of initiation)

Outcome: 2 Culture proven sepsis

Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
1 Less than 48 hours of age				
Braga 2011	40/119	42/112		0.90 [0.63, 1.27]
Kitajima 1997	1/45	0/46		3.07 [0.13, 73.32]
Rojas 2012	24/372	17/378		1.43 [0.78, 2.63]
Subtotal (95% CI)	**536**	**536**		**1.06 [0.79, 1.44]**
Total events: 65 (Probiotics), 59 (Control)				
Heterogeneity: Chi^2 = 2.30, df = 2 (P = 0.32); I^2 =13%				
Test for overall effect: Z = 0.40 (P = 0.69)				
2 More than 48 hours of age				
Manzoni 2009	7/151	29/168		0.27 [0.12, 0.60]
Subtotal (95% CI)	**151**	**168**		**0.27 [0.12, 0.60]**
Total events: 7 (Probiotics), 29 (Control)				
Heterogeneity: not applicable				

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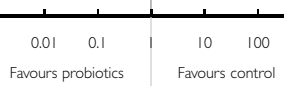
Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Test for overall effect: Z = 3.24 (P = 0.0012)				
3 At the time of the first feed				
Al-Hosni 2012	13/50	16/51		0.83 [0.45, 1.54]
Dani 2002	14/295	12/290		1.15 [0.54, 2.44]
Demirel 2013	20/135	21/136		0.96 [0.55, 1.69]
Lin 2005	22/180	36/187		0.63 [0.39, 1.04]
Lin 2008	40/217	24/217		1.67 [1.04, 2.67]
Mihatsch 2010	28/91	29/89		0.94 [0.61, 1.45]
Millar 1993	0/10	0/10		0.0 [0.0, 0.0]
Roug`x00e9` 2009	15/45	13/49		1.26 [0.67, 2.34]
Samanta 2009	13/91	28/95		0.48 [0.27, 0.88]
Sari 2010	29/110	26/111		1.13 [0.71, 1.78]
Subtotal (95% CI)	**1224**	**1235**		**0.96 [0.81, 1.14]**
Total events: 194 (Probiotics), 205 (Control)				
Heterogeneity: Chi^2 = 14.78, df = 8 (P = 0.06); I^2 =46%				
Test for overall effect: Z = 0.45 (P = 0.65)				
4 During the first week when enteral feeds were tolerated				
Costalos 2003	3/51	3/36		0.71 [0.15, 3.30]
Manzoni 2006	19/39	22/41		0.91 [0.59, 1.40]
Subtotal (95% CI)	**90**	**77**		**0.88 [0.58, 1.34]**
Total events: 22 (Probiotics), 25 (Control)				
Heterogeneity: Chi^2 = 0.10, df = 1 (P = 0.75); I^2 =0.0%				
Test for overall effect: Z = 0.60 (P = 0.55)				
Total (95% CI)	**2001**	**2016**		**0.91 [0.79, 1.05]**
Total events: 288 (Probiotics), 318 (Control)				
Heterogeneity: Chi^2 = 27.03, df = 14 (P = 0.02); I^2 =48%				
Test for overall effect: Z = 1.25 (P = 0.21)				
Test for subgroup differences: Chi^2 = 10.31, df = 3 (P = 0.02), I^2 =71%				

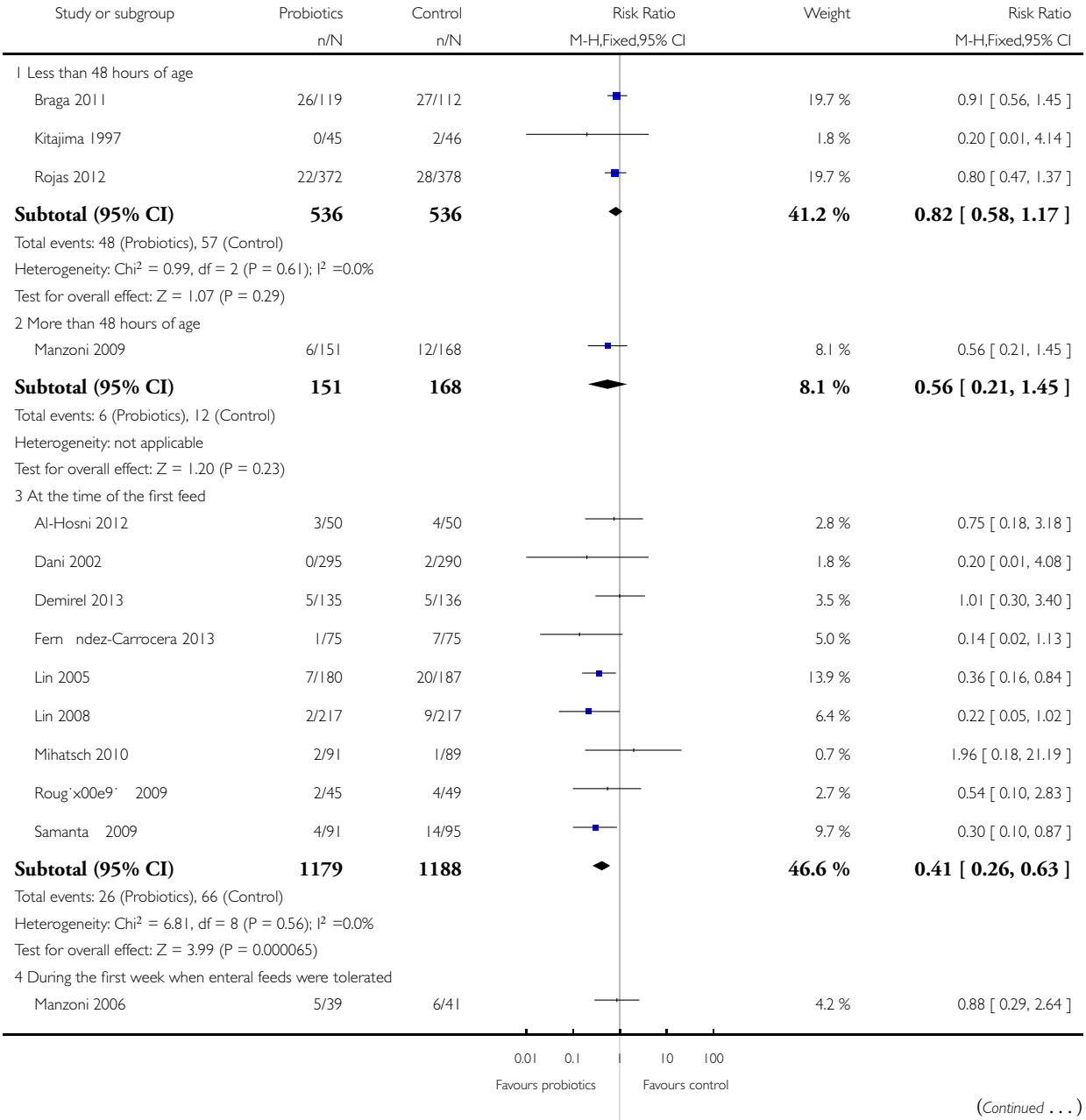


Analysis 5.3. Comparison 5 Probiotics versus control (time of initiation), Outcome 3 Mortality.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 5 Probiotics versus control (time of initiation)

Outcome: 3 Mortality



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Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Subtotal (95% CI)	**39**	**41**		**4.2 %**	**0.88 [0.29, 2.64]**
Total events: 5 (Probiotics), 6 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.24 (P = 0.81)					
Total (95% CI)	**1905**	**1933**		**100.0 %**	**0.61 [0.47, 0.79]**
Total events: 85 (Probiotics), 141 (Control)					
Heterogeneity: Chi^2 = 13.56, df = 13 (P = 0.41); I^2 =4%					
Test for overall effect: Z = 3.78 (P = 0.00015)					
Test for subgroup differences: Chi^2 = 6.38, df = 3 (P = 0.09), I^2 =53%					

Analysis 6.1. Comparison 6 Probiotics versus control (duration of probiotics administration), Outcome 1 Severe NEC- The duration of probiotics administration.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 6 Probiotics versus control (duration of probiotics administration)

Outcome: 1 Severe NEC- The duration of probiotics administration

Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
1 Four to six weeks				
Braga 2011	0/119	4/112		0.10 [0.01, 1.92]
Costalos 2003	5/51	6/36		0.59 [0.19, 1.78]
Kitajima 1997	0/45	0/46		0.0 [0.0, 0.0]
Lin 2008	4/217	14/217		0.29 [0.10, 0.85]
Manzoni 2009	0/151	10/168		0.05 [0.00, 0.90]
Subtotal (95% CI)	**583**	**579**		**0.26 [0.13, 0.52]**
Total events: 9 (Probiotics), 34 (Control)				
Heterogeneity: Chi^2 = 3.75, df = 3 (P = 0.29); I^2 =20%				
Test for overall effect: Z = 3.76 (P = 0.00017)				
2 More than six weeks or until discharged from NICU				

(*Continued* . . .)

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Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Al-Hosni 2012	2/50	2/51		1.02 [0.15, 6.96]
Dani 2002	4/295	8/290		0.49 [0.15, 1.61]
Demirel 2013	6/135	7/136		0.86 [0.30, 2.50]
Fern ndez-Carrocera 2013	6/75	12/75		0.50 [0.20, 1.26]
Lin 2005	2/180	10/187		0.21 [0.05, 0.94]
Manzoni 2006	1/39	3/41		0.35 [0.04, 3.23]
Mihatsch 2010	2/91	4/89		0.49 [0.09, 2.60]
Rojas 2012	9/372	15/378		0.61 [0.27, 1.38]
Roug˙x00e9˙ 2009	2/45	1/49		2.18 [0.20, 23.21]
Samanta 2009	5/91	15/95		0.35 [0.13, 0.92]
Sari 2010	6/110	10/111		0.61 [0.23, 1.61]
Subtotal (95% CI)	**1483**	**1502**		**0.53 [0.37, 0.75]**

Total events: 45 (Probiotics), 87 (Control)

Heterogeneity: Chi^2 = 5.20, df = 10 (P = 0.88); I^2 =0.0%

Test for overall effect: Z = 3.60 (P = 0.00032)

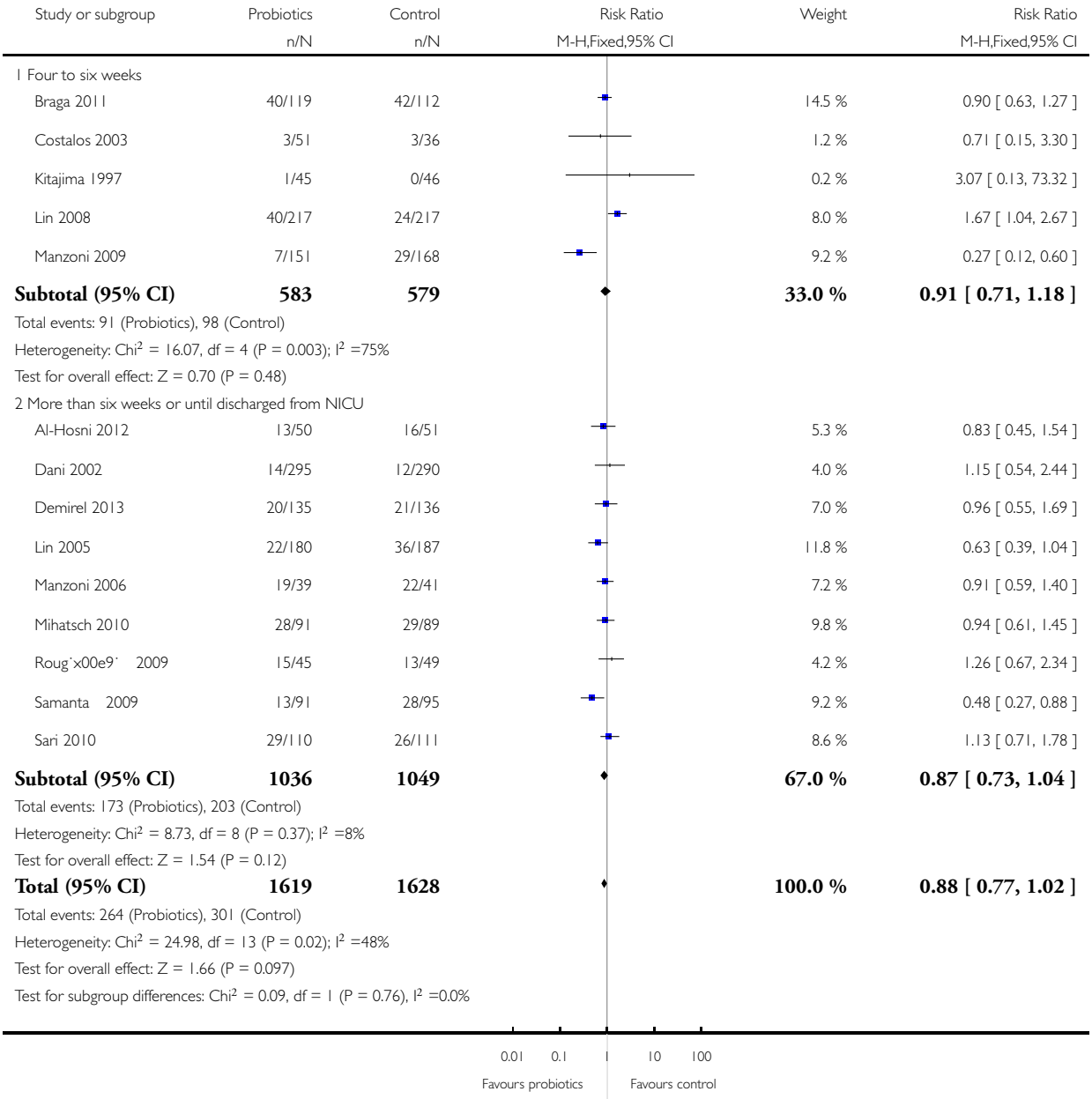
Test for subgroup differences: Chi^2 = 3.15, df = 1 (P = 0.08), I^2 =68%

Analysis 6.2. Comparison 6 Probiotics versus control (duration of probiotics administration), Outcome 2 Culture proven sepsis.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 6 Probiotics versus control (duration of probiotics administration)

Outcome: 2 Culture proven sepsis

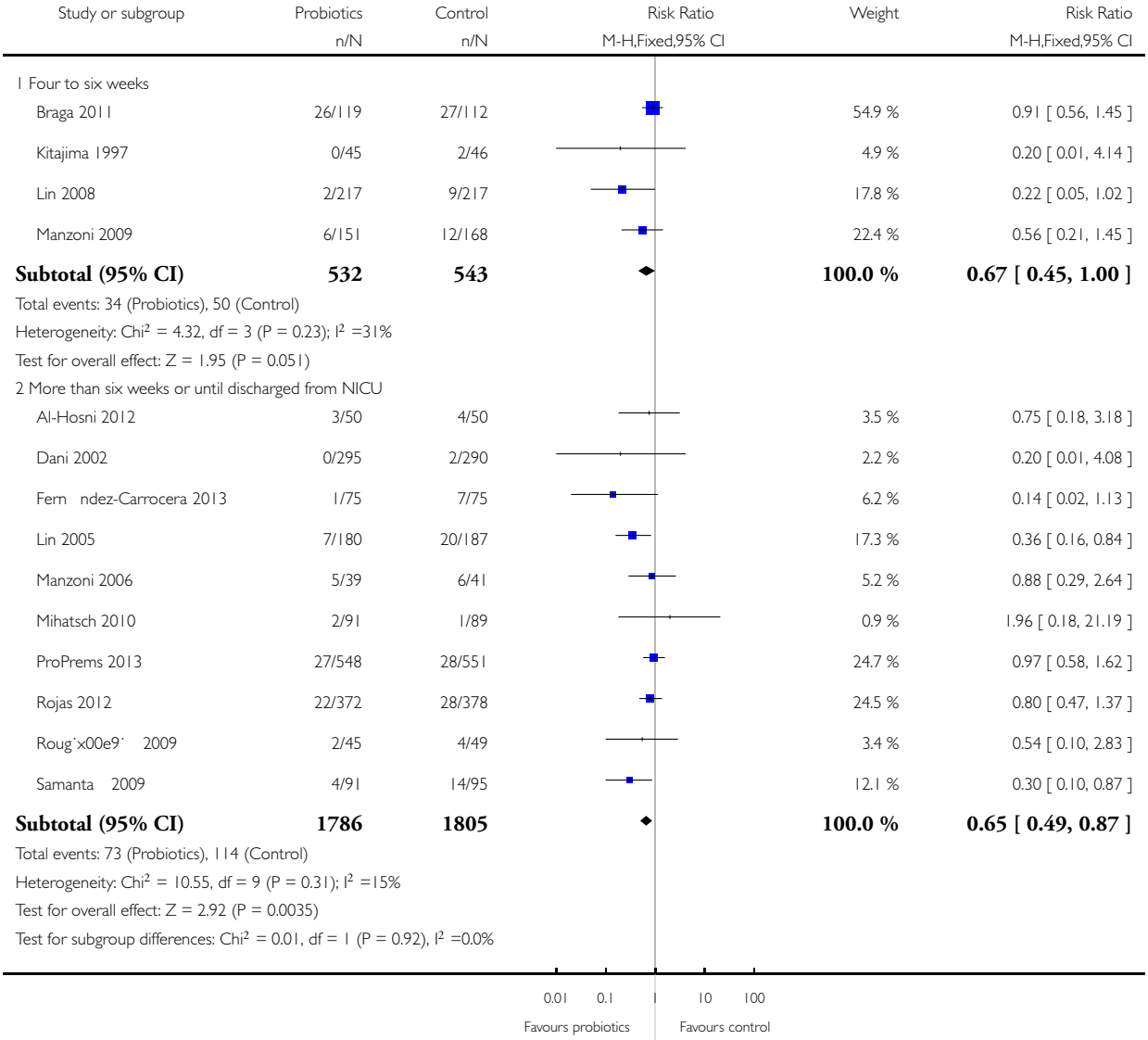


Analysis 6.3. Comparison 6 Probiotics versus control (duration of probiotics administration), Outcome 3 Mortality.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 6 Probiotics versus control (duration of probiotics administration)

Outcome: 3 Mortality



Analysis 7.1. Comparison 7 Probiotics versus control (high quality studies), Outcome 1 Severe necrotising enterocolitis (stage II-III).

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 7 Probiotics versus control (high quality studies)

Outcome: 1 Severe necrotising enterocolitis (stage II-III)

Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Braga 2011	0/119	4/112		4.0 %	0.10 [0.01, 1.92]
Costalos 2003	5/51	6/36		6.1 %	0.59 [0.19, 1.78]
Dani 2002	4/295	8/290		7.0 %	0.49 [0.15, 1.61]
Demirel 2013	6/135	7/136		6.0 %	0.86 [0.30, 2.50]
Fern ndez-Carrocera 2013	6/75	12/75		10.4 %	0.50 [0.20, 1.26]
Lin 2005	2/180	10/187		8.5 %	0.21 [0.05, 0.94]
Lin 2008	4/217	14/217		12.1 %	0.29 [0.10, 0.85]
Manzoni 2009	0/151	10/168		8.6 %	0.05 [0.00, 0.90]
Mihatsch 2010	2/91	4/89		3.5 %	0.49 [0.09, 2.60]
ProPrems 2013	11/548	24/551		20.8 %	0.46 [0.23, 0.93]
Rojas 2012	9/372	15/378		12.9 %	0.61 [0.27, 1.38]
Total (95% CI)	**2234**	**2239**		**100.0 %**	**0.43 [0.31, 0.59]**

Total events: 49 (Probiotics), 114 (Control)

Heterogeneity: $Chi^2 = 7.36$, df = 10 (P = 0.69); $I^2 = 0.0\%$

Test for overall effect: Z = 5.10 (P < 0.00001)

Test for subgroup differences: Not applicable

Analysis 7.2. Comparison 7 Probiotics versus control (high quality studies), Outcome 2 Culture proven sepsis.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 7 Probiotics versus control (high quality studies)

Outcome: 2 Culture proven sepsis

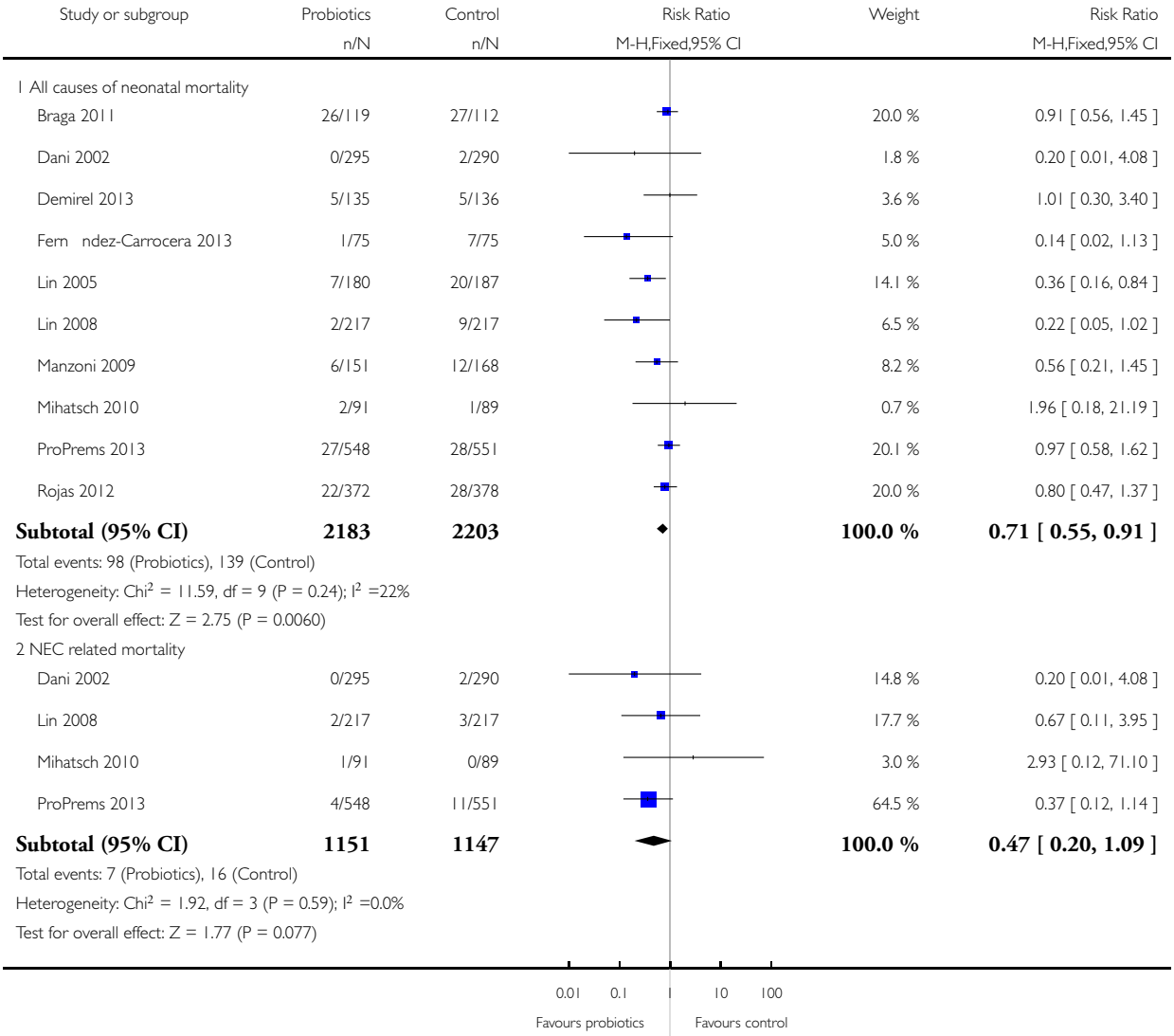
Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Any sepsis					
Braga 2011	40/119	42/112		14.4 %	0.90 [0.63, 1.27]
Costalos 2003	3/51	3/36		1.2 %	0.71 [0.15, 3.30]
Dani 2002	14/295	12/290		4.0 %	1.15 [0.54, 2.44]
Demirel 2013	20/135	21/136		6.9 %	0.96 [0.55, 1.69]
Lin 2005	22/180	36/187		11.7 %	0.63 [0.39, 1.04]
Lin 2008	40/217	24/217		8.0 %	1.67 [1.04, 2.67]
Manzoni 2009	7/151	29/168		9.1 %	0.27 [0.12, 0.60]
Mihatsch 2010	28/91	29/89		9.7 %	0.94 [0.61, 1.45]
ProPrems 2013	72/548	89/551		29.4 %	0.81 [0.61, 1.08]
Rojas 2012	24/372	17/378		5.6 %	1.43 [0.78, 2.63]
Subtotal (95% CI)	**2159**	**2164**		**100.0 %**	**0.89 [0.77, 1.04]**
Total events: 270 (Probiotics), 302 (Control)					
Heterogeneity: Chi^2 = 20.82, df = 9 (P = 0.01); I^2 =57%					
Test for overall effect: Z = 1.49 (P = 0.14)					
Test for subgroup differences: Not applicable					

Analysis 7.3. Comparison 7 Probiotics versus control (high quality studies), Outcome 3 Mortality.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 7 Probiotics versus control (high quality studies)

Outcome: 3 Mortality



FEEDBACK

Davies, 9 May 2008

Summary

I read with interest the review by AlFaleh and Bassler. It was a well conducted systematic review that revealed that the use of probiotics in preterm infants significantly reduces the incidence of NEC and death in preterm infants. I am not sure why the authors have concluded that probiotics should only be used for preterm infants with a birth weight greater than 1000 grams. If we assume that the data on birth weight from individual studies are normally distributed, we can surmise from the mean birth weight and standard deviations that approximately 25% of babies included in the studies that contribute to the two main meta-analyses (for the outcomes of severe NEC and mortality) had a birth weight of less than 1000 grams. Only about 3% or less had a birth weight of greater than 1500 grams. The authors conclusions imply that the use of probiotics is supported for infants who are preterm (born at < 37 weeks gestational age) and who had a birth weight of > 1500 grams (less than ~3% of the study population), but is not supported for infants who had a birth weight of <1000 grams (~25% of the study population). The results of the review and its meta-analysis are highly significant, both statistically and clinically. They should be applicable to the population of infants that contributed to the pooled data, i.e., preterm babies who were (almost all) <1500 grams at birth.

The authors should provide justification for their recommendation that extremely low birth weight infants should not be given this intervention that provides a 57% reduction in the risk of death. Also, if further large randomized controlled trial[s] are done they must include assessment of long-term

neurodevelopmental outcomes, not just important intermediate neonatal outcomes.

Reply

We first would like to thank you for your thoughtful comments on our recently published systematic review. Your question/comment was a one that we have thought of and discussed quite extensively prior to the publication of the review.

Although we agree that the efficacy of the probiotics in prevention of NEC or mortality holds true for the ELBW infant, we could not ensure the safety of this new intervention in a highly vulnerable group with the number of infants enrolled; especially with few cases of probiotics species sepsis reported in the literature.

Contributors

Khalid M Al-Faleh, July 2008

WHAT'S NEW

Last assessed as up-to-date: 1 October 2013.

Date	Event	Description
1 October 2013	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 2011).

(*Continued*)

1 October 2013	New citation required but conclusions have not changed	Updated search identified eight new trials for inclusion in this review update

HISTORY

Protocol first published: Issue 4, 2005

Review first published: Issue 1, 2008

Date	Event	Description
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
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
12 November 2008	Feedback has been incorporated	Feedback incorporated
22 July 2008	Amended	Converted to new review format.

CONTRIBUTIONS OF AUTHORS

KA and JA updated the review.

DECLARATIONS OF INTEREST

None

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Internal sources

- McMaster University Medical Center, Canada.

External sources

- Eunice Kennedy Shriver National Institute of Child Health and Human Development National Institutes of Health, Department of Health and Human Services, USA.

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DIFFERENCES BETWEEN PROTOCOL AND REVIEW

None

INDEX TERMS

Medical Subject Headings (MeSH)

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

MeSH check words

Humans