Prevention of necrotizing enterocolitis with probiotics: a systematic review and meta-analysis

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Context Necrotizing enterocolitis (NEC) is the most frequent gastrointestinal emergency in neonates. The microbiome of the preterm gut may regulate the integrity of the intestinal mucosa. Probiotics may positively contribute to mucosal integrity, potentially reducing the risk of NEC in neonates.

Objective To perform an updated systematic review and meta-analysis on the efficacy and safety of probiotics for the prevention of NEC in premature infants.

Data Sources Structured searches were performed in: Medline, Embase, and the Cochrane Central Register of Controlled Trials (all via Ovid, from 2013 to January 2015). Clinical trial registries and electronically available conference materials were also searched. An updated search was conducted June 3, 2016.

Study Selection Randomized trials including infants less than 37 weeks gestational age or less than 2500 g on probiotic vs. standard therapy.

Data Extraction Data extraction of the newly-identified trials with a double check of the previously-identified trials was performed using a standardized data collection tool.

Results Thirteen additional trials (n = 5033) were found. The incidence of severe NEC (RR 0.53 [95% CI 0.42 to 0.66]) and all-cause mortality (RR 0.79[95% CI 0.68 to 0.93]) were reduced. No difference was shown in culture-proven sepsis RR 0.88 [95% CI 0.77 to 1.00].

Limitations Heterogeneity of organisms and dosing regimens studied prevent a species-specific treatment recommendation from being made.

Conclusions Preterm infants benefit from probiotics to prevent severe NEC and death.
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Abstract

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Introduction

Rationale

Necrotizing enterocolitis (NEC) is a gastrointestinal (GI) syndrome characterized by transmural inflammation and necrosis of the large or small bowel and subsequent translocation of gas-forming organisms into the intestinal wall.\textsuperscript{1,2} Primarily seen in infants, the incidence of NEC is inversely correlated with gestational age (GA) and birth weight.\textsuperscript{3,4} The overall incidence of NEC in all infants $\leq$ 33 weeks GA in a survey of Canadian neonatal intensive care units (NICUs) was 5%, and 7% for infants less than 1500 g birth weight in 2013.\textsuperscript{5} The consequences of NEC are potentially devastating — 20% to 40% of patients require surgical intervention and mortality ranges from 15% to 30%.\textsuperscript{6,7} Survivors of NEC risk significant morbidity including short gut syndrome, strictures, and neurodevelopmental impairment.\textsuperscript{5,6}

The signs and symptoms of NEC were classified by Bell in 1978 and gave rise to modified criteria for diagnosis of NEC in 1996 by Neu.\textsuperscript{7} The modified Bell’s criteria describe the systemic clinical signs of NEC, the important GI signs (which can help differentiate NEC from sepsis), and the radiologic features.

The immature GI tract of preterm infants is particularly susceptible to mucosal injury from a variety of factors. Intestinal and immunological deficiencies associated with prematurity, enteral feeding, microbial overgrowth, and circulatory instability have all been implicated in the pathogenesis of NEC.\textsuperscript{8}

Recent research has focused on microbial overgrowth in the GI tract of premature infants, with an overabundance of pathogenic organisms and lack of microbial diversity being key discoveries. These observations imply that a disturbance in the microbiome, and not a single
pathogen, may be a causative factor of NEC. The lower prevalence of protective *Lactobacillus* or *Bifidobacterium* species in preterm infants compared to term infants make probiotics a potential intervention for the prevention of NEC.

Previous systematic reviews

At the time of our search, there were two recent systematic reviews and meta-analyses on this topic. The Cochrane review on this topic is thorough, but it was last updated in October 2013. The Yang review included many of the same studies but included additional studies as a result of a Chinese trial database search. Since the publication of these two systematic reviews, more large randomized clinical trials have been published.

Objective

The objective of this systematic review was to assess the efficacy and safety of probiotics for the prevention of NEC in premature infants. We planned to update the previous systematic reviews using similar eligibility criteria.

Methods

Protocol/registration

The systematic review methods and analysis plans were undertaken according to published guidelines by PRISMA.

Eligibility criteria

Studies: All randomized clinical trials were considered for inclusion. No language restrictions were applied.

Participants: Infants of less than 37 weeks gestation or weighing less than 2500 g at birth.

Interventions: Probiotics in any species and any dose, or prebiotic/probiotic combinations (synbiotics) of any species and any dose.
Comparators: Probiotic products with different species than the intervention group (i.e. RCTs comparing one species to another head-to-head), placebo, or standard therapy.

Outcomes: The primary outcome of the review was the incidence of severe NEC (Bell’s Stage 2 or greater). Secondary outcomes included all-cause mortality, all-cause sepsis, culture-proven sepsis, bacterial sepsis, fungal sepsis, length of stay in hospital, time to achieve full feeds, duration of parenteral nutrition, and weight gain.

Outcome definitions

1. Sepsis was accepted as defined by the authors of the individual trials.

2. Culture-proven sepsis was accepted as defined by the authors but needed to include a positive culture (blood, urine, or cerebrospinal fluid) to qualify.

3. Length of stay in hospital and length of stay in NICU were considered equivalent. Many studies discharged infants home directly from the NICU.

4. We considered the outcome “age at which full enteral feeding was reached” to be the same as “time to reach full feeds”. We considered the “age at which parenteral nutrition stopped” to be the same as the outcome of “duration of parenteral nutrition”.

5. We subgrouped trials in duration of therapy categories based on the durations reported in the results section of each paper, not the planned duration. We only placed a trial in a specific subgroup if the duration category encompassed the median and the entire interquartile range (IQR) reported in the study paper.

Information Sources & Search

Pre-existing trials

Randomized clinical trials included in the previous systematic reviews\(^{11,12}\) (hereafter referred to as the “old trials”) were included in this review. Chinese language studies were
translated to complete the data extraction.\textsuperscript{13–15,18,19} The studies by Romeo\textsuperscript{20} and Underwood\textsuperscript{21} were divided into two separate trials due to multiple arms.

**Updated Search**

Trials published after completion of the two previous systematic reviews (hereafter referred to as the “new trials”) were identified by searches of Medline, Embase, and the Cochrane Central Register of Controlled Trials. The search was developed and conducted by one of the authors. See Supplementary materials Appendix A for the detailed search strategies for the three databases used in this review. Limits were applied to obtain trials from 2013 onwards. No language restrictions were applied.

Updated searches were conducted January 19, 2015. Clinical trial registries were searched on January 14, 2015. Abstracts and conference proceedings were searched on January 15, 2015. On June 3, 2016 another full update of our search strategy was conducted.

We searched for ongoing, unpublished, and terminated trials using the National Library of Medicine and National Institutes of Health clinical trials database and the World Health Organization International Clinical Trials Registry Platform.\textsuperscript{22,23} Other sources included electronically available conference materials (2014) from the Society of Pediatric Research (SPR) and the European Society of Pediatric Research (ESPR).\textsuperscript{24,25}

**Study selection**

After de-duplication, two reviewers independently screened titles and abstracts for inclusion using a standardized screening tool. Full text screening was completed independently in duplicate by two authors using a full-text screening tool. Cohen’s Kappa was used to assess agreement between the two reviewers on the selection of full-text articles for inclusion.\textsuperscript{26}
A standardized data collection form was developed \textit{a priori} and two authors independently extracted the relevant outcomes and validity criteria from the new trials. The data pertaining to the old trials, including risk of bias assessment, was extracted by one author. Disagreements were resolved by consensus and a third party was consulted if necessary. Author contact was attempted for outcome data in the included trials which was missing or unclear. The complete list of the data extracted from the included trials is included in Supplementary materials, Appendix B.

Study outcome data published in duplicate was included once, but all versions of the publication were utilized for maximal data extraction. In the event of inconsistency between multiple reports of one study, the peer-reviewed publication was used as the primary data set.

Two authors independently assessed the risk of bias for each of the new included studies using the criteria outlined in the \textit{Cochrane Handbook for Systematic Reviews of Interventions}.\textsuperscript{27} A summary table and a graph for risk of bias were created using Review Manager (RevMan) software.\textsuperscript{28} The risk of bias assessment from the studies included in the previous systematic reviews\textsuperscript{11,12} were double-checked for accuracy by a single author.

When possible, the results were synthesized using RevMan 5.3.\textsuperscript{28} A random effects model\textsuperscript{27} was chosen to account for the clinical and statistical heterogeneity expected when including different species and regimens of probiotics, different neonatal ages and weights, different feeding regimens (breast milk, formula, combination feeding, and parenteral nutrition supplementation as needed), as well as the varied countries conducting RCTs in this area. Relative risks (RRs) with 95\% confidence intervals (CI) were used for dichotomous variables.
and mean differences (MDs) with 95% CIs for continuous variables. If the continuous variables
in the studies were measured in different scales, we calculated the standardized mean difference
(SMD).

Analysis was done on an intention-to-treat (ITT) basis.\textsuperscript{27} If patients discontinued the
intervention after randomization, they were still counted in our analysis for outcomes (such as
mortality) where this was possible. Author contact was attempted to clarify any missing outcome
data.

If trials had two intervention arms, both of which contained a probiotic, both probiotic
arms were included and the number of patients in the comparator arm divided by the number of
active arms to prevent double counting. If the trial had two or more intervention arms and only
one of them contained a probiotic, the data from the corresponding non-probiotic arm was used
as the comparator.\textsuperscript{27} In trials where patients received a co-intervention, the co-intervention had to
be present in both the active and control arms to be included.

The $I^2$ statistic was used to quantify statistical heterogeneity (the percentage of total
variation across studies due to heterogeneity). Statistical heterogeneity as measured by $I^2$ was
described as “small” (\(\leq 25\%\)), “moderate” (between 26\% and 49\%) and “large” (\(\geq 50\%\)).\textsuperscript{29}
Forest plots were visually inspected for possible sources of heterogeneity.

\textit{Additional Analysis}

Subgroup analysis was planned \textit{a priori} for the following subgroups: infant weight
(exremely-low birth weight (ELBW) [less than 1000 g] and very low birth weight (VLBW) [less
than 1500 g]), timing of probiotic initiation, duration of probiotic therapy, sepsis types (including
“any sepsis”), and use of breast milk vs. formula for feeding.
Results

Study Selection

The previously published systematic reviews included a total of 37 unique randomized clinical trials.\textsuperscript{13–15,19–21,30–52} Electronic database searches (including the 2016 search update) yielded 475 citations, conference searching yielded 115 citations, and clinical trials database searching yielded 35 citations. After de-duplication, 412 citations remained for title and abstract screening (see Figure 1 for the detailed flow diagram of study selection). Cohen’s Kappa was 0.723 (good agreement) between the two reviewers for selection of new full-text trials for inclusion.\textsuperscript{53} The study by Manzoni 2014\textsuperscript{54} was included in our review as it was a randomized extension of a previously published trial.\textsuperscript{42} The ProPrems study was added to the previous review as unpublished data, but is now published and was included in our review.\textsuperscript{46} The updated search in 2016 resulted in a follow up to the Oncel trial\textsuperscript{55} (Akar\textsuperscript{56}), and new trials by Costeloe\textsuperscript{57} (previously on our ongoing trials list), Dilli\textsuperscript{58}, Dutta\textsuperscript{59}, Sinha\textsuperscript{60} and Tewari\textsuperscript{61}. Three trials included in previous reviews were excluded from our review as they were determined to be non-randomized.\textsuperscript{62–64} The overall updated search added a total of 13 randomized controlled trials (two trials split due to multiple arms) with over 5000 new evaluable patients to previous systematic reviews, bringing the total to 42 included trials.\textsuperscript{18,54,55,57–61,65–68}

Study Characteristics

When verifying the outcome data included in the previous reviews, a number of methodological flaws and errors of data synthesis were noted.\textsuperscript{11,12} A decision was made to re-extract the data from the “old trials” instead of re-entering the data from the previously published reviews (see Supplementary materials, Appendix C).

See Table 1 for characteristics of included studies. All studies were conducted in preterm infants admitted to the NICU. Twenty-four studies limited birth weight to 1500 g or less. Weight
was not part of the inclusion criteria in nine studies.\textsuperscript{13,18,33,44,45,52,57,59,61} Gestational age was not part of the inclusion criteria in five studies but all of these studies had birth weight inclusion criteria for preterm infants less than 1500 g.\textsuperscript{32,37,39,41,54} One trial did not specify gestational age but enrolled babies 1500-2500 g.\textsuperscript{60} Five trials were translated from Chinese for use in the review.\textsuperscript{13–15,18,38}

Type of feeding was variable across the included trials. Nine trials included infants exclusively fed breastmilk.\textsuperscript{39,41,49,50,60,61,66,69} One trial had infants fed exclusively preterm formula.\textsuperscript{52} The trials published in Chinese did not consistently specify this information on translation.\textsuperscript{13–15,19,38} Costeloe had 46\% of infants exclusively fed breastmilk, but the rest of the infants had a combination of feeding types.\textsuperscript{57} Overall, the number of trials were split evenly between multiple species and single species probiotics (22 trials each). The Sari trial (\textit{Bacillus coagulans} formerly known as \textit{Lactobacillus sporogenes})\textsuperscript{51} and the Tewari trial (\textit{Bacillus clausii})\textsuperscript{61} used single species that were not used in any other trial. Sinha used a multi-organism product containing eight species.\textsuperscript{60} All studies used a variety of organisms and dose regimens. Comparators were matching placebo, standard therapy, or prebiotics (two trials).\textsuperscript{54,58} There were no trials comparing one probiotic preparation with another, but two trials had multiple arms with different probiotics.\textsuperscript{20,21} One trial used varying durations of probiotics and doses but fit within the range of doses and duration of therapy seen with all included trials, so the three treatment arms were combined into one.\textsuperscript{59}

Timing of probiotic initiation was variable. Twenty-one trials started probiotics with the first feed, six trials started within 48 hours of birth, one within 72 hours, four within the first week, and in twelve trials therapy started at the “more than 48 hours” time point.
Duration of probiotic therapy ranged from 7 days to 6 weeks. One trial did not specify a duration of therapy. Most studies were classified in the “28 days or more” subgroup for the purposes of analysis by extraction of the actual duration of therapy (when provided) in trials that specified duration as “until discharge”.

**Outcomes**

**Risk of Bias within Studies**

See Supplemental Figure 1 for the risk of bias assessment for all included trials. All included trials were randomized (five were judged to have uncertainty around the method of randomization). All of these trials were previously included in the AlFaleh review.

Seven trials had a degree of selective reporting one of the trials being from the updated search. Of the translated trials, randomization was clearly stated, but uncertainty remains about blinding status, allocation concealment, and selective reporting.

**Synthesis of Results**

Two of the “old trials” did not contribute any outcome data to the meta-analysis and were excluded. Data used for the Mohan trial in the previous review appears to be based on personal communication with the authors and could not be corroborated with the published trial. Li did not report on any usable outcomes.

**All infants**

The primary outcome, severe NEC, was significantly reduced in infants who received probiotics compared to placebo with 38 trials (10,520 patients) reporting on this outcome — RR 0.53 [95% CI 0.42 to 0.66] - see Figure 2. The incidence of culture-proven sepsis was not different between the probiotics and control — RR 0.88 [95% CI 0.77 to 1.00] in 31 trials comprising 8707 patients, see Figure 3. The incidence of all-cause mortality was significantly reduced in infants receiving probiotics in 29 trials (9507 patients) — RR 0.79 [95% CI 0.68 to
Other statistically significant findings included shorter duration of hospitalization, increased weight gain (g/day), and reduced time to reach full enteral feeds, all in favor of using probiotics (Table 2).

There was a moderate to large degree of heterogeneity in the results for culture-proven sepsis, duration of hospitalization, duration of parenteral nutrition, and time to achieve full feeds.

**VLBW Infants**

The incidence of severe NEC was significantly reduced in VLBW infants who received probiotics compared to placebo including 25 trials (6587 patients) — RR 0.47 [95% CI 0.36 to 0.61] (Figure 5). The incidence of all-cause mortality was significantly reduced in VLBW infants who received probiotics compared to infants who received placebo in 24 trials (6736 patients) with RR 0.74 [95% CI 0.61 to 0.90]. Compared to VLBW infants who received placebo, those who received probiotics had a significantly reduced duration of parenteral nutrition (Table 2).

There was significant heterogeneity in the outcomes of duration of hospitalization, and time to full feeds.

**ELBW infants**

Eight trials reported outcome data on this weight group. The only trial to enroll infants solely in this weight group was Al-Hosni. ELBW infants were a pre-specified subgroup in the Jacobs trial. In the remaining six trials, outcome data for ELBW infants was presented as a post-hoc subgroup analysis. ELBW infants who received probiotics had a significantly shorter duration of hospitalization and reached full enteral feeding sooner compared to infants who received placebo, see Table 2.
No statistically significant differences were demonstrated for the incidence of NEC (Figure 6), mortality, culture-proven sepsis, any bacterial sepsis and any fungal sepsis. There was significant heterogeneity in the outcomes of culture-proven sepsis and mortality. Other outcomes were only reported in a small number of patients and trials.

Initiation of Probiotics

Severe NEC was significantly reduced in trials where patients were started on probiotics at more than 48 hours of age — RR 0.36 [95% CI 0.24 to 0.53] or in those trials where probiotics were started at the time of the first feed — RR 0.55 [95% CI 0.41 to 0.75] (Supplemental Figure 2). The incidence of culture-proven sepsis was significantly reduced in the 11 trials in which therapy was started at more than 48 hours of age — RR 0.65 [95% CI 0.51 to 0.82]. A reduction in the incidence of mortality was significant in trials when probiotics were started with the first feed — RR 0.68 [95% CI 0.51 to 0.90].

Duration of Probiotics

Subgroups with probiotic duration of at least 14 days or until discharge were statistically significant for a reduced incidence of severe NEC (Supplemental Figure 3). The largest amount of data was in the 28 days or more category, with 28 trials contributing outcome data.

Species of Probiotics

Outcomes were compared according to the various probiotic species included in the trials. Incidence of severe NEC was significantly reduced in infants receiving a Lactobacillus species (8 trials) — RR 0.61 [95% CI 0.40 to 0.95], Bifidobacterium species (6 trials) — RR 0.37 [95% CI 0.14 to 0.97], or multispecies (two or more) supplement (18 trials) — RR 0.41 [95% CI 0.29 to 0.56]. Incidence of NEC was not significantly different from control in infants receiving only a Saccharomyces boulardii supplement (2 trials) — RR 0.72 [95% CI 0.33 to
1.54]. Incidence of culture-proven sepsis was not significantly different from control in infants receiving any probiotic species. Incidence of mortality was significantly reduced only in infants receiving a multispecies supplement (15 trials) — RR 0.66 [95% CI 0.5 to 0.87].

**Breast milk vs. formula feeding**

Comparison of the rates of severe NEC between infants fed using breast milk alone and those fed formula alone was not possible due to the lack of studies containing infants fed only formula.

**Discussion**

This review was done in accordance with current guidelines and strict attention to best practice of systematic reviews and meta-analysis. It has added randomized data from over 5000 infants to the previous meta-analyses. Based on high-quality evidence, the use of probiotics in preterm infants reduces the incidence of severe NEC. The effect size has changed slightly in comparison to the Cochrane review but the precision of the result remains the same, despite the additional patients. This may be related to the wide range of probiotic species and regimens included in the analysis and use of the more conservative random effects model for meta-analysis. There was no statistical heterogeneity in the primary outcome, despite the inclusion of diverse probiotic regimens and species. No other intervention to prevent NEC has demonstrated this effect size.

This review showed a decrease in all-cause mortality with probiotics, which confirms the findings of previous reviews and re-affirms the important benefit of this therapy.

The concern about bacterial translocation beyond the preterm infant gut should be reflected in the outcome of culture-proven sepsis and/or all-cause mortality. This review found
no increased risk of culture-proven sepsis. No sepsis due to probiotic species was reported among the included trials.

A statistically significant reduction of three days was shown in duration of hospitalization. The clinical significance of this reduction is unclear given a mean length of stay in Canadian NICUs of 63.2 days in 2013.\textsuperscript{73}

The reduction in the duration of parenteral nutrition and time to full enteral feeds is of importance for this patient population, as prolonged parenteral nutrition may be associated with increased hospital stay, mortality, and morbidity.\textsuperscript{74} Recently published evidence-based guidelines echo the need and benefits of achieving full feeds in an efficient manner.\textsuperscript{75}

In the ELBW infants, the lack of benefit on severe NEC, culture-proven sepsis or mortality outcomes was consistent with the previous reviews (despite the addition of four new randomized trials almost doubling the number of infants studied). The direction and magnitude of the point estimates for the effect of probiotics on the incidence of severe NEC and all-cause mortality are consistent with those of the “all infant” sample.

The incidence of NEC and mortality outcomes had little to no heterogeneity which gives substantial confidence in those results. The substantial heterogeneity in sepsis, duration of hospitalization and duration of parenteral nutrition outcomes would suggest caution in interpreting the results.
Timing of probiotic initiation is a clinically important question which was not resolved in the previous reviews. In this review, subgroups for timing mirrored those in the Alfaleh review. The time of initiation of probiotics seemed to have a variable influence on the main three outcomes of severe NEC, culture-proven sepsis, and mortality. When probiotics were started very early (48 hours of age or less) there was no difference in any of the outcomes. There were few trials placed in this category, and therefore the outcomes may lack power to detect a statistical difference. Many trials described initiating probiotic supplementation at the time of first feeding. Without access to individual patient level data, it is unclear how many of the infants categorized into this group could also be included in the 48 hours of age or less category. Consequently, we cannot definitively state that probiotic supplementation should be withheld until at least 48 hours of age or until feeding. Starting probiotics with the initiation of feeds did reduce the incidence of both NEC and mortality and does have some practical advantages in terms of drug administration which make it an opportune time to initiate probiotic prophylaxis. There was a lack of effect on mortality when probiotic supplementation was started after 48 hours of age. We can find no explanation for this, especially since the benefit on NEC remained when therapy was started after 48 hours.

Determining the appropriate duration of therapy is equally important as the timing of initiation. Clinically it seems prudent to continue therapy for as long as there is risk for NEC. A minimum of two weeks of probiotic therapy continued for as long as the patient is judged to be at risk (up to six weeks) can be recommended, since trials in these subgroups showed a lower incidence of NEC.
Feeding infants with human milk compared to formula has been previously shown to have a protective effect on the incidence of NEC.\textsuperscript{76,77} This review found only one trial in which infants were fed exclusively formula (most other trials included a combination of feeding types), precluding definitive conclusions based on feeding method. The majority of infants were fed a combination of human milk and formula reflecting clinical practice. Future trials may consider having a pre-defined subgroup of breastfed vs. formula fed infants to definitively answer this question.

A post hoc subgroup analysis to examine if the effects on severe NEC were consistent based on the underlying background incidence of NEC across the included trials (grouped by less than 5%, 5-7% and more than 7%\textsuperscript{5}) was undertaken. Most of the trials were in the low baseline incidence subgroup (18 trials, 4905 patients). The primary outcome remained significant across all groups and reinforces that no matter the institution’s incidence of NEC, infants had the same reduction in severe NEC.

In many countries, probiotics are not regulated as drugs and products are not subject to the same rigorous quality assurance standards.\textsuperscript{78} Stability and/or species testing was confirmed in nine of the included trials.\textsuperscript{21,30,36,43,44,65,68,79} Hospitals either did their own testing or requested the information from the manufacturer of the probiotic being studied. Institutions are encouraged to conduct their own quality assessment or request quality certificates from the manufacturer of the product being used.\textsuperscript{80,81}

\textit{Limitations}

The limitations to this systematic review were as follows:

1. Three of the Chinese language trials\textsuperscript{16,82,83} included in the older review\textsuperscript{12} could not be obtained in full text and were not included in this review.
2. No unpublished data was requested from any of the manufacturers of probiotic products assessed in this review.

3. Only one trial in the previous review addressed long term neurodevelopmental outcomes, but this information could not be confirmed.\textsuperscript{37} Akar 2016\textsuperscript{56} and the abstract from one of the ProPrems conference presentations\textsuperscript{84} also reports on neurodevelopmental outcomes. If the Kitijama\textsuperscript{37} and ProPrems results were available these could be combined for a summary effect estimate in a future review.

\textit{Remaining Uncertainties}

The outcome of fungal sepsis showed a definite benefit with no heterogeneity (Table 2).

Some of the included studies employed antifungal prophylaxis (either systemic or topical) in their infants as per their normal NICU practice. This choice is not the routine practice at all institutions and is not standard practice.\textsuperscript{85,86} The impact of these studies with background antifungal therapy was not explored in sensitivity analyses but could be considered in future reviews for its impact on the outcome of fungal sepsis.

Which probiotic product to use remains uncertain, since the total body of evidence comprises a heterogeneous group of probiotics (individual species and combination products, and regimens). In the previous review, only the \textit{Lactobacillus} and multispecies supplements were shown to be effective for this outcome. We would recommend a regulatory body-approved product and that quality assessment be requested from the manufacturer to validate the purity of product. The evidence of benefit was clear for \textit{Lactobacillus} or \textit{Bifidobacterium} species and multiple species products so any of these would be reasonable choices.

\textbf{Conclusions}
For infants born at less than 37 weeks gestation or less than 2500 g birth weight there is clear benefit from the use of probiotics to prevent severe NEC and all-cause mortality, with no increase in culture-proven sepsis. We would recommend using probiotics in premature infants with these characteristics. The evidence for babies of birth weight less than 1000 grams is less clear and we cannot make as strong a recommendation in this class of infants.

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Table 1: Characteristics of Included Studies
Figure 1: PRISMA Flow Diagram
Figure 2: Severe (≥ Stage 2 Bell’s Criteria) NEC - all infants
Figure 3: Culture-proven sepsis - all infants
Figure 4: Mortality - all infants
Table 2: Additional important findings
Figure 5: Severe NEC - VLBW (Less than 1500 g) infants
Figure 6: Severe NEC - ELBW (Less than 1000 g) infants

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Table 1 (on next page)

Characteristics of included trials
| Identifier   | Inclusion Criteria | Gestational Age | Birth weight | Other inclusion criteria | Number randomized in each group | Probiotic Species (Brand names)                                                                 | Total Dose (cfu/day) | Initiation | Duration | Feeding (B, PF, F, Mixed) |
|--------------|-------------------|-----------------|--------------|--------------------------|-------------------------------|---------------------------------------------------------------------------------|---------------------|------------|----------|--------------------------|
| Al-Hosni 2012 | "preterm"         | 30              | 501-1000 g   | 14 days of age or less at the time of initiation of feeds | Probiotic: 50 Control: 51     | Lactobacillus rhamnosus GG LGG – 0.5 billion (Culturelle®)                        | 1 billion           | At the time of first feeding | 28 days or more | Not stated |
| Bin-Nun 2005  | "preterm"         |                 | Less than 1500 g | None                     | Probiotic: 72 Control: 73     | Lactobacillus casei – 0.002 to 2 billion (ABC Dophilus®)                        | 0.035 to 3.5 billion | 48 hours or less |          | Mixed |
| Braga 2011    | None              | 750-1499 g      | Born locally and admitted to NICU | Probiotic: 119 Control: 112 | Lactobacillus casei – 0.002 to 2 billion (ABC Dophilus®)                        | 0.035 to 3.5 billion | 48 hours or less |          | Mixed |
| Costalos 2003 | 28-32 weeks       | None            | None         | Probiotic: 51 Control: 36 | Saccharomyces boulardii        | 2 billion                       | At the time of first feeding | 28 days or more |          | Mixed |
| Costeloe 2016 | 23 weeks up to 30 weeks and 6 days | None          | None         | Probiotic: 650 Control: 660 | Lactobacillus casei – 0.002 to 2 billion (ABC Dophilus®)                        | 0.067 – 6.7 billion | 48 hours or less | 28 days or more | Mixed/B (46%) |
| Dani 2002     | Less than 33 weeks | Less than 1500 g | None         | Probiotic: 295 Control: 290 | Lactobacillus rhamnosus GG (Dicoflor®)                                       | 6 billion           | At the time of first feeding | 28 days or more | Mixed |
| Demiril 2013  | Less than 32 weeks | 1500 g or less  | Survival to start enteral feeding | Probiotic: 135 Control: 136 | Lactobacillus boulardii (Reflor®)                                              | 5 billion           | At the time of first feeding | 28 days or more | Mixed |
| Dilli 2015    | Less than 32 weeks | Less than 1500 g | 7 days of age or less at the time of initiation of feeds | Probiotic: 100 Synbiotic: 100 Prebiotic: 100 Control: 100 | B. lactis 5 billion B. lactis 5 billion + inulin Inulin 900 mg (Maflor®)         | 5 billion           | More than 48 hours | 28 days or more | Mixed |
| Dutta 2015    | 27 – 33 weeks     |                 | Aged less than 96 hrs, likely to remain in hospital or reside within 30 km for 28 days, tolerating 15 mL/kg/d of milk feeds | Probiotic: 100 Control: 100 | B. lactis 5 billion B. lactis 5 billion + inulin Inulin 900 mg (Maflor®)         | 5 billion           | More than 48 hours | 28 days or more | Mixed |
| Fernández-Carrasco 2013 | "preterm" | Less than 1500 g | None         | Probiotic: 75 Control: 75 | Lactobacillus acidophilus - 1 billion Lactobacillus rhamnosus - 0.44 billion | 2.65 billion | At the time of first feeding | 28 days or more | Mixed |
| STUDY | WEEKS | BIRTHWEIGHT | BOC | PROBIOTIC | DOSAGE | PROTOCOL | OUTCOME |
|-------|-------|-------------|-----|-----------|--------|----------|---------|
| Hua 2014 | Less than 37 weeks | None | Anticipated to start enteral feeding within 72 hrs. Anticipated length of stay at least 7 days. | Probiotic: L casei - 1 billion Lactobacillus plantarum - 0.176 billion B infantis - 0.0276 billion S thermophillus - 0.0066 billion (Lactipan®) | Biﬁdobacterium longum Lactobacillus bulgaricus S thermophilus (Golden Bifid®) | 3 billion | At the time of first feeding | 14 to 27 days | Mixed |
| Huang 2009 | 28-32 weeks | Less than 1500 g | None | Probiotic: Bifidobacterium adolescentis | 0.05 billion | More than 48 hrs | Up to 13 days | Unknown |
| Jacobs 2013 (ProPrems) | Less than 32 weeks | Less than 1500 g | Enrolled within 72 hours of birth. | Probiotic: B bifidus - 0.35 billion L acidophilus - 1 billion (Bifico®) | Biﬁdobacterium adolescentis | 1 billion | More than 48 hrs | 28 days or more | Mixed |
| Ke 2008 | Less than 37 weeks | None | None | Probiotic: Enterococcus faecalis - 1 billion B longum - 1 billion L acidophilus - 1 billion (Bifico®) | Biﬁdobacterium adolescentis | 3 billion | More than 48 hrs | Until Discharge | Unknown |
| Kitajima 1997 | None | Less than 1500 g | None | Probiotic: B breve YIT4010 (Yakult® Honsya Co. Ltd., Tokyo, Japan) | Biﬁdobacterium adolescentis | 0.5 billion | At the time of first feeding | 28 days or more | Mixed |
| Li 2004 | 27.8-37.6 weeks | 780-2250 g | Slated as low birth weight infants | Probiotic: B breve | Biﬁdobacterium adolescentis | 0.32 billion | 48 hours or less | Until Discharge | Unknown |
| Lin 2005 | None | Less than 1500 g | None | Probiotic: L acidophilus - 1 billion/250 mg cap B infantis - 1 billion/250 mg cap | Biﬁdobacterium adolescentis | 1 billion/kg | At the time of first feeding | 28 days or more | B |
| Lin 2008 | Less than 34 weeks | Less than 1500 g | None | Probiotic: L acidophilus - 1 billion/250 mg cap B bifidum - 1 billion/250 mg cap | Biﬁdobacterium adolescentis | 1 billion/kg | At the time of first feeding | Until Discharge | Mixed |
| Manzoni 2006 | None | Less than 1500 g | Less than 3 days of age, started oral feeding with human milk, no baseline fungal colonization at enrollment, no other antifungal prophylaxis, oral feeding was stable and was tolerated by neonate | Probiotic: L rhamnosus GG (Dicoflor®) | Biﬁdobacterium adolescentis | 6 billion | More than 48 hrs | 28 days or more | B |
| Manzoni 2014 | None | Less than 1500 g | Less than 48 hours of age | Synbiotic: L rhamnosus GG 6 billion + Bovine Lactoferrin 100 mg (Dicoflor®) Bovine Lactoferrin 100 mg (Dicofarm®) | Biﬁdobacterium adolescentis | 6 billion | More than 48 hrs | 28 days or more | Mixed |
| Mihatsch 2010 | Less than 30 weeks | Less than 1500 g | None | Probiotic: B bifidus - 0.35 billion L acidophilus - 1 billion (Nestlé®) | Biﬁdobacterium adolescentis | 12 billion/kg | At the time of first feeding | 28 days or more | Mixed |
| Study Year | Duration | Gestation | Birth Weight | Probiotics | Control | Description |
|------------|----------|-----------|--------------|------------|---------|-------------|
| Millar 1993 | 33 weeks or less | None | None | Probiotic: 10 (L. rhamnosus GG) | Control: 10 | 0.2 billion at the time of first feeding |
| Mohan 2006 | Less than 37 weeks | None | None | Probiotic: 37 (B. lactis Bb12) | Control: 32 | 4.8 billion 48 hours or less |
| Oncel 2014 | 32 weeks or less | 1500 g or less | None | Probiotic: 200 (L. reuteri DSM 17938) | Control: 200 | 0.1 billion at the time of first feeding |
| Patole 2014 | Less than 33 weeks | Less than 1500 g | Ready to commence or on enteral feeds for <12 hours | Probiotic: 77 (B. infantis) | Control: 70 | 3 billion at the time of first feeding |
| Ren 2010 | 28-33 weeks | 1000-1800 g | None | Probiotic: 80 (L. acidophilus) | Control: 70 | 0.016 billion at the time of first feeding |
| Reuman 1986 | *preterm* | Less than 2000 g | Greater than 24 hrs, but less than 72 hrs old | Probiotic: 15 (L. acidophilus) | Control: 15 | 0.018 billion within 72 hrs |
| Rojas 2012 | *preterm* | 2000 g or less | None | Probiotic: 372 (L. reuteri DSM 17938) | Control: 378 | 0.1 billion 48 hours or less |
| Romeo 2011 | Less than 37 weeks | Less than 2500 g | Age < 2wks feeds within 72 hrs | Probiotic (L. reuteri): 83 | Control: 83 | 0.1 billion L. reuteri or 6 billion L. rhamnosus more than 48 hrs |
| Rougé 2009 | Less than 32 weeks | Less than 1500 g | Postnatal age <= 2 week, the absence of any disease other than those linked to prematurity and the start of enteral feeding | Probiotic: 43 (L. rhamnosus) | Placebo: 49 | 0.8 billion at the time of first feeding |
| Roy 2014 | Less than 37 weeks | Less than 2500 g | Stable oral feeding within 72 h of birth, adequate renal and liver function, a postnatal age < 2 week | Probiotic: 56 (L. acidophilus) | Control: 56 | 1.25 billion more than 48 hrs |
| Samanta 2009 | Less than 32 weeks | Less than 1500 g | Started feed enterally and survived beyond 48 h of life | Probiotic: 91 (B. infantis) | Control: 95 | 20 billion more than 48 hrs |
| Sari 2011 | Less than 33 weeks | Less than 1500 g | Who survived to feed enterally | Probiotic: 110 (Bacillus coagulans) | Control: 111 | 0.35 billion at the time of first feeding |
| Study | Weeks or Less | Birth Weight | Feeding Method | Probiotic | Control | Probiotic Description | Control Description | Duration | Start of Therapy | Notes |
|-------|---------------|--------------|----------------|------------|---------|------------------------|--------------------|----------|-----------------|-------|
| Serce 2013<sup>27</sup> | 32 weeks or less | 1500 g or less | Survival to feed enterally | Probiotic: 104 | Control: 104 | S boulardii (Reflor®) | 1 billion | At the time of first feeding | 28 days or more | Mixed |
| Sinha 2015<sup>30</sup> | None | 1500-2500 g | Residing within 20-25 km of hospital and not planning to shift residences for at least the next 2 months | Probiotic: 668 | Control: 672 | VSL#3®: Streptococcus thermophilus, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus paracasei and Lactobacillus delbrueckii spp bulgaricus. | 10 billion | Within the first week | 28 days or more | B |
| Stratiki 2007<sup>32</sup> | 27 to 37 weeks | None | formula fed | Probiotic: 41 | Control: 34 | B lactis (Prenan Nestlé®) | 0.2 billion/kg | 48 hours or less | Not stated | PF |
| Tewari 2015<sup>31</sup> | 27-30 weeks + 6 days and 31-33 weeks + 6 days | None | None | Probiotic:123 | Control:121 | Bacillus clausii 2 billion (Enterogermina®) | 6 billion | More than 48 hrs | 28 days or more | B |
| Underwood 2009<sup>24</sup> | Less than 35 weeks | 750 to 2000 g | Younger than 7 days old | Probiotic: (Culturelle): 30 | Probiotic: (ProBioPlus): 31 | L rhamnosus GG - 10 billion/cap (ProBioPlus DDS) B infantis - 10 billion/cap B bifidum - 10 billion/cap B longum - 10 billion/cap L acidophilus - 10 billion/cap (Culturelle®) | 0.5 billion Culturelle or 2 billion ProBioPlus | Within the First Week | 28 days or more | Mixed |
| Van Niekerk 2014<sup>48,50</sup> | Less than 34 weeks | 500 to 1250 g | HIV exposed and unexposed born to HIV positive or negative mothers who agreed to breastfeed | Probiotic: 91 | Control: 29 | L rhamnosus GG - 0.35 billion B infantis - 0.35 billion (Pro-B2®) | 0.7 billion | At the time of first feeding | 28 days or more | B |
| Yang 2011<sup>19</sup> | Less than 37 weeks | <1500 to >2500 g | 2 week length of stay and admitted within 24 hours | Probiotic: 31 | Control: 31 | L longum - 0.005 billion L acidophilus - 0.005 billion E faecalis - 0.005 billion | 0.03 billion | At the time of first feeding | Up to 13 days | Unknown |

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2. Initiation of probiotic therapy was categorized to fit the defined subgroups for data analysis.
3. Duration of probiotic therapy was categorized to fit the defined subgroups for data analysis.
4. Handled as two trials (4 arms).
5. Randomized extension of the 2009 publication.
6. Handled as two trials to account for the 3 arms in the trial.
7. Included two randomized clinical studies, one of HIV-exposed and one of HIV-unexposed preterm infants which were analyzed as two trials.
8. B: breastfeeding only, PF: preterm formula, F: formula, Mixed: mixed feeding types
Table 2 (on next page)

Table 2 - Additional Important Findings
### Table 2 - Additional important findings

| Outcome                                      | Number of studies / participants | Effect size | 95% CI          | I² (%) |
|----------------------------------------------|----------------------------------|-------------|-----------------|--------|
| **All Infants**                              |                                  |             |                 |        |
| Bacterial sepsis                             | 9 / 2212                         | RR 0.86     | 0.62 to 1.18    | 52     |
| Fungal sepsis                                | 12 / 3756                        | RR 0.67     | 0.43 to 1.06    | 10     |
| Duration of hospitalization (days)           | 16 / 4915                        | MD -3.2     | -5.5 to -0.9    | 84     |
| Weight gain (g/day)                          | 3 / 314                          | MD +1.7     | 1.0 to 2.3      | 0      |
| Time to achieve full feeds (days)            | 17 / 4448                        | MD -1.2     | -2.2 to -0.1    | 93     |
| **VLBW infants**                             |                                  |             |                 |        |
| Culture-proven sepsis                        | 24 / 6616                        | RR 0.93     | 0.82 to 1.05    | 15     |
| Duration parenteral nutrition (days)         | 4 / 1210                         | MD -1.2     | -2.3 to -0.02   | 0      |
| **ELBW infants**                             |                                  |             |                 |        |
| Culture-proven sepsis                        | 6 / 1703                         | RR 0.95     | 0.72 to 1.26    | 41     |
| Mortality                                    | 4 / 1122                         | RR 0.92     | 0.046 to 1.83   | 47     |
| Duration of hospitalization (days)           | 2 / 218                          | MD -6.4     | -12.6 to -0.1   | 0      |
| Time to achieve full feeds (days)            | 2 / 218                          | MD -1.8     | -2.9 to -0.7    | 0      |

MD: mean difference, RR: risk ratio, CI: confidence interval, NEC: necrotizing enterocolitis, VLBW: very low birth weight (<1500 g), ELBW: extremely low birth weight (<1000 g)
Figure 1 (on next page)

PRISMA Flow Diagram
Clinical trials registries (n = 35)
Conference abstracts (n = 115)

Electronic databases (Embase, Medline, Central) (n = 475)

Previous systematic reviews (n = 51)

Records screened after deduplication:
- Trial registries (n = 28)
- Conference abstracts (n = 115)
- Electronic databases (n = 233)
- Previous systematic reviews (n = 36)
  (n = 412)

Records excluded after title and abstract screening:
- Trial registries (n = 25)
- Electronic databases (n = 195)
  (n = 220)

Full-text articles assessed for eligibility:
- Trial registries (n = 3)
- Conference abstracts (n = 115)
- Electronic databases (n = 38)
- Previous systematic reviews (n = 36)
  (n = 192)

Records excluded after full-text screening:
- Additional records of already included trials (electronic databases n = 5, conference abstracts n = 1)
- Conference abstracts (n = 112)
- Electronic databases (n = 22)
- Previous systematic reviews (n = 6)
  (n = 146)

Studies included in qualitative synthesis
- Trial registries (n = 3)
- Conference abstracts (n = 2)
- Electronic databases (n = 11)
- Previous systematic reviews (n = 30)
  (n = 46)

Records excluded:
- Trial registries (n = 3 - ongoing trials)
- Conference abstracts (n = 2 - not available in full-text)
- Previous systematic reviews (n = 2 - no data added)
  (n = 7)

Studies included in quantitative synthesis (meta-analysis)
- Electronic databases (n = 13)
- Previous systematic reviews (n = 29)
  (n = 42)

Records added; trial split due to multiple arms:
- Electronic databases (n = 2)
- Previous systematic reviews (n = 1)
**Figure 2** (on next page)

Forest plot showing the effect of probiotics on severe NEC in all infants
| Study or Subgroup       | Probiotics Events | Control Events | Total | Weight | Risk Ratio M-H, Random, 95% CI | Risk Ratio M-H, Random, 95% CI |
|------------------------|-------------------|----------------|-------|--------|-------------------------------|-------------------------------|
| Al-Hodhi 2012          | 2                 | 50             | 51    | 1.3%   | 1.02 [0.15, 8.98]             |                               |
| Bin-Nun 2006           | 1                 | 72             | 73    | 1.2%   | 0.19 [0.01, 0.77]             |                               |
| Braga 2011             | 0                 | 119            | 112   | 0.8%   | 0.10 [0.01, 1.02]             |                               |
| Costalos 2003          | 5                 | 61             | 66    | 3.8%   | 0.59 [0.34, 0.99]             |                               |
| Costeloe 2015          | 51                | 650            | 681   | 18.1%  | 0.37 [0.27, 0.50]             |                               |
| Dami 2002              | 4                 | 295            | 299   | 3.2%   | 0.49 [0.19, 1.51]             |                               |
| Dammacco 2013          | 8                 | 135            | 143   | 3.9%   | 0.80 [0.39, 1.65]             |                               |
| Dalli 2015a            | 2                 | 100            | 102   | 2.2%   | 0.11 [0.03, 0.47]             |                               |
| Dalli 2015b            | 4                 | 100            | 104   | 3.8%   | 0.33 [0.11, 1.00]             |                               |
| Dutta 2015             | 0                 | 114            | 114   | 0.0%   | 4.67 [0.23, 70.49]            |                               |
| Fernandez-Cancino 2013 | 6                 | 76             | 82    | 4.9%   | 0.59 [0.20, 1.30]             |                               |
| Hus 2014               | 0                 | 118            | 118   | 0.5%   | 0.23 [0.01, 4.78]             |                               |
| Huang 2009             | 0                 | 66             | 66    | 0.9%   | 0.13 [0.01, 2.53]             |                               |
| Ks 2008                | 7                 | 438            | 445   | 5.9%   | 0.30 [0.13, 0.69]             |                               |
| Katajima 1997          | 0                 | 46             | 46    |        | Not estimable                 |                               |
| Lin 2005               | 2                 | 110            | 112   | 2.1%   | 0.21 [0.05, 0.94]             |                               |
| Lin 2008               | 4                 | 222            | 226   | 3.7%   | 0.29 [0.10, 0.89]             |                               |
| Manzoni 2006           | 1                 | 39             | 40    | 1.0%   | 0.35 [0.04, 3.23]             |                               |
| Manzoni 2014           | 0                 | 236            | 236   | 0.7%   | 0.09 [0.01, 1.70]             |                               |
| Minakata 2010          | 2                 | 86             | 88    | 1.7%   | 0.49 [0.09, 2.60]             |                               |
| Orend 2014             | 8                 | 200            | 208   | 5.0%   | 0.39 [0.22, 0.70]             |                               |
| Palis 2014             | 0                 | 79             | 79    | 0.0%   | 0.34 [0.01, 1.69]             |                               |
| ProFprems 2013         | 11                | 946            | 957   | 7.5%   | 0.46 [0.23, 0.93]             |                               |
| Rieh 2010              | 3                 | 60             | 63    | 2.4%   | 0.53 [0.13, 2.12]             |                               |
| Reunan 1998            | 0                 | 15             | 15    |        | Not estimable                 |                               |
| Ruc 2012               | 0                 | 372            | 372   | 8.0%   | 0.61 [0.27, 1.38]             |                               |
| Roug 2009              | 2                 | 48             | 50    | 0.9%   | 2.18 [0.20, 23.21]            |                               |
| Rou 2014               | 2                 | 56             | 58    | 1.3%   | 1.05 [0.15, 8.95]             |                               |
| Samaret 2009           | 5                 | 91             | 96    | 4.5%   | 0.35 [0.13, 0.92]             |                               |
| Satt 2011              | 6                 | 121            | 127   | 4.4%   | 0.60 [0.23, 1.50]             |                               |
| Sia 2013               | 7                 | 122            | 129   | 4.2%   | 1.03 [0.36, 2.77]             |                               |
| Staal 2007             | 0                 | 41             | 41    | 0.0%   | 0.12 [0.01, 2.23]             |                               |
| Tawar 2015             | 0                 | 128            | 128   |        | Not estimable                 |                               |
| Underwood 2009a        | 1                 | 30             | 31    | 0.7%   | 0.50 [0.03, 7.45]             |                               |
| Underwood 2009b multi  | 1                 | 31             | 32    | 0.9%   | 1.44 [0.66, 3.12]             |                               |
| Van Niekerk 2014a (-IV-exposed) | 0       | 37             | 37    | 0.5%   | 0.20 [0.01, 4.03]             |                               |
| Van Niekerk 2014b (-IV-unexposed) | 0   | 54             | 54    | 0.5%   | 0.21 [0.01, 4.22]             |                               |
| Yang 2011              | 2                 | 51             | 53    | 1.9%   | 0.67 [0.12, 3.72]             |                               |

Total (95% CI): 5304 5216 100.0% 0.53 [0.42, 0.66]

Total events: 170 311

Heterogeneity: Tau² = 0.04; Chi² = 98.08, df = 34 (P = 0.00001); P = 11%

Test for overall effect: Z = 5.91 (P < 0.00001)
Figure 3 (on next page)

Forest plot showing the effect of probiotics on culture-proven sepsis in all infants
| Study or Subgroup       | Probiotics Events | Probiotics Total | Control Events | Control Total | Weight | Risk Ratio M-H, Random, 95% CI |
|------------------------|-------------------|-----------------|----------------|--------------|--------|--------------------------------|
| Al-Heidi 2012          | 13                | 50              | 18             | 51           | 2.3%   | 0.93 [0.45, 1.94]             |
| Bin-Nun 2005           | 31                | 72              | 24             | 73           | 5.5%   | 1.31 [0.88, 2.00]             |
| Costalos 2003          | 3                 | 51              | 3              | 36           | 0.7%   | 0.71 [0.15, 3.30]             |
| Costalos 2018          | 73                | 600             | 77             | 580          | 7.9%   | 0.69 [0.71, 1.30]             |
| Dani 2002              | 14                | 295             | 12             | 290          | 2.4%   | 1.15 [0.54, 2.41]             |
| Demir 2013             | 20                | 135             | 21             | 116          | 3.9%   | 0.95 [0.59, 1.69]             |
| Dill 2015a             | 8                 | 100             | 13             | 100          | 2.1%   | 0.82 [0.27, 2.94]             |
| Dill 2015b             | 8                 | 100             | 10             | 100          | 1.9%   | 0.80 [0.33, 1.94]             |
| Duffa 2015             | 10                | 114             | 8              | 35           | 1.7%   | 0.51 [0.20, 1.31]             |
| Fernandez-Carreno 2013 | 42                | 78              | 44             | 75           | 8.2%   | 0.96 [0.72, 1.28]             |
| Hu 2014                | 2                 | 119             | 0              | 119          | 0.7%   | 0.29 [0.08, 0.94]             |
| Lin 2005               | 22                | 100             | 28             | 108          | 4.9%   | 0.63 [0.39, 1.04]             |
| Lin 2008               | 40                | 222             | 24             | 221          | 4.9%   | 1.68 [1.04, 2.69]             |
| Manzoni 2006           | 13                | 36              | 22             | 44           | 5.4%   | 0.91 [0.59, 1.40]             |
| Manzoni 2009           | 7                 | 101             | 9              | 100          | 1.3%   | 0.79 [0.33, 2.03]             |
| Milan 1992             | 0                 | 10              | 0              | 10           | Not estimable                   |
| Oncel 2014             | 13                | 200             | 25             | 200          | 3.1%   | 0.52 [0.27, 0.99]             |
| Patino 2014            | 17                | 79              | 12             | 80           | 2.9%   | 1.43 [0.73, 2.80]             |
| ProFrem 2013           | 72                | 548             | 89             | 551          | 7.9%   | 0.81 [0.61, 1.08]             |
| Rojas 2012             | 24                | 372             | 17             | 378          | 3.4%   | 1.43 [0.78, 2.63]             |
| Romeo 2011a            | 1                 | 63              | 5              | 62           | 0.4%   | 0.19 [0.01, 0.94]             |
| Romeo 2011b            | 2                 | 83              | 4              | 82           | 0.3%   | 0.25 [0.09, 0.69]             |
| Roupe 2008             | 15                | 45              | 13             | 45           | 3.3%   | 1.26 [0.67, 2.34]             |
| Roy 2014               | 31                | 56              | 42             | 64           | 0.1%   | 0.74 [0.59, 0.99]             |
| Samardzic 2009         | 13                | 98              | 28             | 95           | 3.5%   | 0.49 [0.27, 0.88]             |
| Sari 2011              | 28                | 121             | 26             | 121          | 4.0%   | 1.12 [0.70, 1.78]             |
| Serc 2013              | 19                | 122             | 25             | 122          | 4.0%   | 0.78 [0.44, 1.31]             |
| Strakos 2007           | 0                 | 41              | 3              | 41           | 0.2%   | 0.12 [0.01, 2.23]             |
| Tetani 2015            | 0                 | 123             | 11             | 121          | 1.9%   | 0.72 [0.30, 1.72]             |
| Van Niekerk 2014a      | 2                 | 37              | 4              | 37           | 0.3%   | 0.59 [0.30, 1.10]             |
| Van Niekerk 2014b      | 5                 | 64              | 4              | 65           | 1.0%   | 1.30 [0.87, 2.00]             |

Total (events) | 4418 | 4289 | 100.0% | 0.88 [0.77, 1.00] |
Figure 4 (on next page)

Forest plot showing the effect of probiotics on all-cause mortality in all infants
Figure 5 (on next page)

Forest plot showing the effect of probiotics in VLBW infants
### Table

| Study or Subgroup | Prebiotics Events | Control Events | Total | Weight | Risk Ratio M-H, Random, 95% CI | Risk Ratio M-H, Random, 95% CI |
|-------------------|-------------------|----------------|-------|--------|-------------------------------|-------------------------------|
| Al-Nassri 2012    | 2                 | 50             | 51    | 1.9%   | 1.02 [0.15, 8.98]             |                               |
| Bin-Nun 2005      | 1                 | 72             | 73    | 1.7%   | 0.19 [0.01, 0.77]             |                               |
| Braga 2011        | 0                 | 118            | 118   | 0.8%   | 0.10 [0.01, 0.92]             |                               |
| Dani 2002         | 4                 | 285            | 290   | 4.9%   | 0.49 [0.13, 1.61]             |                               |
| Demirel 2013      | 6                 | 135            | 141   | 9.1%   | 0.99 [0.30, 2.50]             |                               |
| Dhill 2013a       | 2                 | 100            | 102   | 3.4%   | 0.11 [0.03, 0.47]             |                               |
| Dhill 2015a       | 4                 | 100            | 104   | 5.7%   | 0.33 [0.11, 1.00]             |                               |
| Fernandez-Camacho 2013 | 6             | 76             | 82    | 8.0%   | 0.50 [0.20, 1.28]             |                               |
| Kajijima 1997     | 0                 | 45             | 45    |        |                               |                               |
| Lin 2005          | 2                 | 180            | 182   | 3.1%   | 0.21 [0.05, 0.94]             |                               |
| Lin 2008          | 4                 | 217            | 221   | 5.0%   | 0.29 [0.12, 0.70]             |                               |
| Manzoni 2006      | 1                 | 30             | 31    | 1.4%   | 0.35 [0.04, 3.23]             |                               |
| Manzoni 2014      | 0                 | 238            | 238   | 0.3%   | 0.03 [0.01, 1.78]             |                               |
| Mihatsch 2010     | 2                 | 81             | 83    | 2.5%   | 0.49 [0.09, 2.49]             |                               |
| Onora 2014        | 8                 | 200            | 208   | 8.4%   | 0.80 [0.32, 2.20]             |                               |
| Pastore 2014      | 0                 | 79             | 79    | 0.7%   | 0.34 [0.01, 0.98]             |                               |
| Proferias 2013    | 11                | 946            | 957   | 13.9%  | 0.45 [0.23, 0.89]             |                               |
| Rozas 2012        | 8                 | 176            | 184   | 7.0%   | 0.83 [0.23, 3.09]             |                               |
| Rouge 2009        | 2                 | 45             | 47    | 1.2%   | 2.18 [0.20, 23.2]             |                               |
| Sansores 2009     | 5                 | 91             | 96    | 7.5%   | 0.35 [0.13, 0.92]             |                               |
| Sant 2011         | 6                 | 110            | 116   | 7.2%   | 0.81 [0.23, 2.91]             |                               |
| Sarve 2013        | 7                 | 122            | 129   | 6.7%   | 1.00 [0.30, 2.77]             |                               |
| Tawani 2015       | 0                 | 61             | 61    |        |                               |                               |
| Van Nielkerk 2014a (HIV-exposed) | 0          | 37             | 37    | 0.3%   | 0.20 [0.01, 4.03]             |                               |
| Van Nielkerk 2014b (HIV-unexposed) | 0         | 64             | 64    | 0.3%   | 0.21 [0.01, 4.22]             |                               |
| **Total (95% CI)** | **3279**          | **3308**       | **10.0%** | **0.47 [0.36, 0.61]** | **[.]** | **[.]** |

**Total events:** 7981

**Heterogeneity:** Tau² = 0.00, Chi² = 19.68, df = 22 (P = 0.60), P = 0.0%

**Test for overall effect:** Z = 5.87 (P < 0.0001)
Figure 6 (on next page)

Forest plot showing the effect of probiotics in ELBW infants
Manuscript to be reviewed

| Study or Subgroup   | Events | Total | Events | Total | Weight | Risk Ratio M.H, Random, 95% CI |
|---------------------|--------|-------|--------|-------|--------|-------------------------------|
| Al-Hassan 2012      | 2      | 50    | 2      | 51    | 2.3%   | 1.02 [0.15, 6.98]             |
| Costelloe 2016      | 60     | 317   | 52     | 327   | 88.8%  | 0.97 [0.88, 1.36]             |
| Lin 2008             | 4      | 102   | 7      | 79    | 6.1%   | 0.44 [0.13, 1.45]             |
| Onoel 2014           | 5      | 93    | 9      | 103   | 7.7%   | 0.62 [0.21, 1.77]             |
| ProPrems 2013        | 10     | 235   | 14     | 230   | 13.6%  | 0.73 [0.33, 1.66]             |
| Roy 2014             | 1      | 11    | 1      | 11    | 1.2%   | 1.00 [0.07, 14.05]            |
| **Total (95% CI)**   | 808    | 810   | 100.0% | 0.86 [0.64, 1.16] |

Total events: 72, 88

Heterogeneity: Tau² = 0.00; Chi² = 2.27, df = 5 (P = 0.81), I² = 0%

Test for overall effect: Z = 1.00 (P = 0.32)