Probiotics for prevention of necrotizing enterocolitis in preterm infants: systematic review and meta-analysis

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Abstract

Necrotizing enterocolitis (NEC) affects predominantly preterm infants, who have specific risk factors leading to intestinal dysbiosis. Manipulations of gut microbiota through probiotics have the potential to prevent NEC. The aim of this systematic review and meta-analysis was to evaluate the effect of probiotics for NEC prevention in preterm infants, with a focus on specific strains, microbiological strength of currently available studies, and high-risk populations.

PubMed and the Cochrane Library were searched for trials published within 4th February 2015. Randomized-controlled trials reporting on NEC and involving preterm infants who were given probiotics in the first month of life were included in the systematic review.

Twenty-six studies were suitable for inclusion in the meta-analysis. Data about study design, population, intervention and outcome were extracted and summarized independently by two observers. Study quality and quality of evidence were also evaluated.

Fixed-effects models were used and random-effects models where significant heterogeneity was present. Subgroup analyses were performed to explore sources of heterogeneity among studies. Results were expressed as risk ratio (RR) with 95% confidence interval (CI).

The main outcome was incidence of NEC stage ≥2 according to Bell’s criteria. Probiotics prevented NEC in preterm infants (RR 0.47 [95% CI 0.36–0.60], p < 0.00001). Strain-specific sub-meta-analyses showed a significant effect for Bifidobacteria (RR 0.24 [95% CI 0.10–0.54], p = 0.00006) and for probiotic mixtures (RR 0.39 [95% CI 0.27–0.56], p < 0.00001). Probiotics prevented NEC in very-low-birth-weight infants (RR 0.48 [95% CI 0.37–0.62], p < 0.00001); there were insufficient data for extremely-low-birth-weight infants. The majority of studies presented severe or moderate microbiological flaws.

Probiotics had an overall preventive effect on NEC in preterm infants. However, there are still insufficient data on the specific probiotic strain to be used and on the effect of probiotics in high-risk populations such as extremely-low-birth-weight infants, before a widespread use of these products can be recommended.

Keywords: Probiotics, Newborn, Necrotizing enterocolitis, Meta-analysis

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Background

Necrotizing enterocolitis (NEC), which is one of the most devastating neonatal diseases, has become a priority for research [1]. Despite great advances in neonatal care, the morbidity, mortality, and health-care costs directly related to the disease are substantial: during hospital stay, the economic burden of NEC in the United States has been estimated as high as several billions USD per year, which is approximately 20 % of the costs for Neonatal Intensive Care Units in the country; furthermore, this estimate is likely to be much higher when the costs of long-term care of survivors are taken into account [2].

NEC is a multifactorial disease: prematurity is a well-recognized risk factor, and approximately 90% of the infants who develop NEC are born preterm [3]. This is probably due to specific comorbidities of prematurity, such as immunodeficiency, use of broad-spectrum antimicrobials, delayed enteral feeding, and low availability of human milk.

Recently, research has focused on the role of gut microbiota and its manipulations, such as the use of probiotics, on disease and health status. Probiotics are live microorganisms which, when ingested in adequate amounts, confer a health-benefit to the host through an interaction with gut microbiota [4]. The intestinal microbiota undergoes dynamic changes during childhood. Gut colonization in preterm infants occurs differently than in healthy term newborns [5], and preterm infants frequently have delayed and aberrant acquisition of the “normal” digestive flora. Recent studies performed in preterm foetuses and infants demonstrated that amniotic fluid and meconium are not sterile, suggesting an intraterine origin of gut microbiota [6, 7]; after birth, the preterm infant’s immature intestine is exposed to an unique environment and to several iatrogenic manipulations, including the use of broad-spectrum antibiotics. The subsequent intestinal dysbiosis is recognized as a risk factor for NEC: actually, it has been shown that preterm infants with NEC have reduced bacterial gut diversity and different bacterial strains compared to healthy controls [8]. In this perspective, provision of probiotics to preterm infants has the potential to “normalize” the abnormal colonization pattern, thus preventing the occurrence of the disease [9].

The use of probiotics for the prevention of NEC in preterm infants has been extensively investigated in many randomized-controlled trials, whose results have been summarized in several systematic-reviews and meta-analyses [10, 11]. The authors of these meta-analyses, which show that probiotics reduce NEC and mortality in preterm infants, strongly encourage a change in practice, promoting a widespread use of probiotics in this population [11], and also claim that withholding probiotics from high-risk neonates would be almost unethical [10]. However, the position of the American Academy of Paediatrics is more cautious, highlighting the need for more studies to address unanswered questions on the amount and specificity of which probiotic or mixture of probiotics should be used [12]. In addition, a recent systematic review, which analyzed the level of evidence of randomized-controlled trials on probiotics in preterm infants, concluded that there is still insufficient evidence to recommend routine probiotics use, but also that present data are encouraging and justify further research on specific probiotic products [13].

Actually, the beneficial effects of probiotics appear to be strain-specific, and pooling data from studies using different strains can result in misleading conclusions [14]. Furthermore, currently available studies often lack specificity in reporting correct identification of probiotic strain [15], dosage regimen and duration, and gut colonization, which are all fundamental to assess the ability of a probiotic to confer a health benefit to the host [16].

The aim of this meta-analysis is thus to evaluate in detail the effect of probiotics for the prevention of NEC in preterm infants, with a focus on specific strains, on microbiological strength of currently available studies, and on high-risk populations.

Methods

Literature search

The study protocol was designed jointly by the members of the Task Force on Probiotics of the Italian Society of Neonatology.

A systematic review of published studies reporting the use of probiotics for the prevention of NEC in preterm infants was performed, following PRISMA guidelines [17].

Criteria for inclusion in the meta-analysis were the following: randomized and quasi-randomized controlled trials involving preterm infants (gestational age <37 weeks) and reporting on NEC (any stage, according to modified Bell staging criteria [18, 19]); enteral administration of any probiotic starting within one month of age, compared to placebo or no treatment. Being the search strategy focused specifically on NEC, data on different outcomes, such as sepsis or mortality, which were reported in the studies retrieved by the literature search, were not evaluated by meta-analysis.

A search was conducted in PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) for studies published before 4th February 2015, using the search string reported in Fig. 1. This string was built up combining all the terms related to NEC and probiotics, using PubMed MeSH terms and free-text words and their combinations through the most proper Boolean operators, in order to be as comprehensive as possible. Similar criteria were used for searching the Cochrane Library. The review was limited to studies written in English and involving human subjects.
The search was conducted by AA and LC: relevant studies were identified from the abstract, and reference lists of papers retrieved were searched for additional studies. “Snowballing” technique was also used [20].

Data extraction and meta-analysis
Study details, including study population, characteristics of the intervention, use of placebo, and outcome, were assessed independently by AA and LC, and checked by DG. Study quality was evaluated independently using the risk of bias tool as proposed by the Cochrane collaboration (Chapter 8 of the Cochrane Handbook of Systematic Reviews) [21]. In addition, an assessment of the body of evidence using the GRADE working group approach was used in order to grade the quality of evidence. The evaluation was carried out following the Chapter 12 of the Cochrane Handbook [21] and classifying the evidence as high,
| Author, year | Study details | Study population | Intervention | Type of milk | Placebo |
|-------------|---------------|------------------|--------------|--------------|---------|
| Al-Hosni, 2012 [33] | P DB R C Multic. | Preterm infants with BW 501–1000 g, appropriate for gestational age, and ≤ 14 days of age at time of feeding initiation | *Lactobacillus rhamnosus* GG LGG *Bifidobacterium infantis* D: 0.5 × 10⁹ CFU each probiotic, OD S: first enteral feeding E: discharge or until 34 w postmenstrual age | Non specified | Extra milk |
| Bin-Nun, 2005 [40] | P B R C | Preterm infants with BW < 1500 g, who began enteral feeding on a weekday | *Bifidobacterium infantis* *Streptococcus thermophilus* *Bifidobacterium bifidus* D: 0.35 × 10⁹ CFU each probiotic, OD S: start of enteral feeding E: 36 w postconceptual age | OMM, PFM | HM or FM |
| Braga, 2011 [35] | P DB R C | Inborn infants with BW 750–1499 g | *Lactobacillus casei* *Bifidobacterium breve* D: 3.5 × 10⁷ CFU to 3.5 × 10⁹ CFU OD S: day 2 E: day 30, NEC diagnosis, discharge, death, whichever occurred first | HM | Extra HM |
| Costalos, 2003 [49] | P R C | GA 28–32 w No major GI problem Not receiving antibiotics Not receiving breast milk | *Saccharomyces boulardii* D: 1 × 10⁹ CFU BD S: non-specified | PFM | MDX |
| Dani, 2002 [42] | P DB R C Multic. | Infants with GA < 33 w or BW < 1500 g | *Lactobacillus rhamnosus* GG D: 6 × 10⁶ CFU OD S: first feed E: discharge | OMM, DM or FM | MDX |
| Demirél, 2013 [28] | P B R | Preterm infants with GA ≤ 32 w and BW ≤ 1500 g, who survived to feed enterally | *Saccharomyces boulardii* D: 5 × 10⁶ CFU OD S: first feed | HM, FM | None |
| Study | Authors | Study Population | Intervention | Outcomes |
|-------|---------|------------------|--------------|----------|
| Dilli, 2015 [44] | P | Preterm infants with GA <32 weeks and BW <1500 g, born at or transferred to the NICU within the first week of life and fed enterally before inclusion | Bifidobacterium infantis | E: discharge |
| | DB | | Lactis | HM, FM |
| | R | | D: $5 \times 10^6$ CFU | MDX powder |
| | C | | S: beyond d7 after birth | |
| | Multic. | | E: death or discharge (max 8 weeks) | |
| Fernández-Carrocerca, 2013 [32] | P | Preterm infants with BW <1500 g | Lactobacillus acidophilus | OMM, PFM |
| | DB | | Lactobacillus rhamnosus | None |
| | | | 4.4 x 10^8 CFU/g | |
| | R | | Lactobacillus casei | |
| | C | | 1 x 10^9 CFU/g | |
| | Multic. | | Bifidobacterium infantis | |
| | | | 2.76 x 10^7 CFU/g | |
| | | | Streptococcus thermophilus | |
| | | | 6.6 x 10^5 CFU/g | |
| | | | Total D: 1 g powder OD | |
| | | | S: start of enteral feeding | |
| | | | E: non-specified | |
| Jacobs, 2013 [26] | P | Preterm infants with GA <32 w and BW <1500 g | Bifidobacterium infantis BB-02 | HM, FM |
| | DB | | 300 CFU x 10^6 | MDX powder |
| | R | | Streptococcus thermophilus Th-4 | |
| | C | | 350 CFU x 10^6 | |
| | Multic. | | Bifidobacterium lactis BB-12 | |
| | | | 350 CFU x 10^6 | |
| | | | Total D: $1 \times 10^9$ CFU x 1.5 g maltodextrin powder OD | |
| | | | S: enteral feed $\geq$ 1 ml every 4 h | |
| | | | E: discharge or term corrected age | |
| Kitajima, 1997 [52] | P | Preterm infants with BW <1500 g | Bifidobacterium breve YIT4010 | OMM, FM after full enteral feeding |
| | R | | Distilled water had been reached | Distilled water |
| | C | | D: $0.5 \times 10^6$ CFU OD | |
| | | | S: within 24 h of life | |
| | | | Duration of probiotic supplementation: 28 days | |
| Lin, 2005 | P | Infants with BW <1500 g, who started to feed enterally and survived beyond day 7 | Lactobacillus acidophilus | OMM, DM |
| | M | | Bifidobacterium infantis | None |
| | R | | D: $\geq 10^6$ CFU each probiotic (=125 mg/kg), BD | |
| | C | | S: start of enteral feeding | |
| | | | E: discharge | |
| Lin, 2008 | P | Preterm infants with GA <34 w and BW $\leq$ 1500 g, who survived to feed enterally | Lactobacillus acidophilus NCDO 1746 | HM, FM |
| | B | | Bifidobacterium bifidum NCDO 1453 | None |
| | R | | D: $1 \times 10^6$ CFU each probiotic (=125 mg/kg) | BD |
Table 1: Studies included in the systematic review and meta-analysis (Continued)

| Study | Design | Population | Intervention | Duration | Outcome |
|-------|--------|------------|--------------|----------|---------|
| Manzoni, 2006 [37] | Multic. | Infants with BW < 1500 g, ≥ 3 days of life, who started enteral feeding with HM | *Lactobacillus rhamnosus LGG* | 6 × 10^9 CFU/day | None |
| | | | | | |
| | | | | | |
| Milhatsch, 2010 [43] | | Preterm infants with GA < 30 w and BW ≤ 1500 g | *Bifidobacterium lactis BB12* | 2 × 10^9 CFU/kg 6 times a day | Indistinguishable powder |
| | | | | | |
| | | | | | |
| Mohan, 2006 [53] | | Preterm infants (GA < 37 w) | *Bifidobacterium lactis BB12* | 1 × 10^8 CFU OD | Not stated |
| | | | | | |
| | | | | | |
| Oncel, 2013 [25] | | Preterm infants with GA ≤ 32 w and BW ≤ 1500 g, who survived to feed enterally | *Lactobacillus reuteri DSM 17938* | 1 × 10^8 CFU OD | Oil base |
| | | | | | |
| | | | | | |
| Patole, 2014 [45] | | Preterm infants with GA < 33 w and BW < 1500 g | *Bifidobacterium breve M16-V* | 3 × 10^9 CFU OD (1.5 × 10^9 CFU OD for newborns ≤ 27 w until they reached 50 ml/kg/day enteral feeds) | Dextrin |
| | | | | | |
| | | | | | |
| Rojas, 2012 [30] | | Preterm infants with BW ≤ 2000 g, hemodynamically stable, ≤ 48 h of age (regardless start of enteral feeding) | *Lactobacillus reuteri DSM 17938* | 1 × 10^8 CFU OD | Oil base |
| | | | | | |
| | | | | | |
| Rougé, 2009 [50] | Multic. | Preterm infants with GA < 32 w and BW < 1500 g, ≤ 2 w of age, without any disease other than those linked to prematurity, who started enteral feeding before inclusion | *Bifidobacterium longum BB536* | 1 × 10^8 CFU/day | MDX |
| | | | | | |
| | | | | | |
| Study Reference       | Study Design | Population Description                                                                 | Probiotics Used | Dose                                                                 | Start | End     |
|-----------------------|--------------|----------------------------------------------------------------------------------------|-----------------|----------------------------------------------------------------------|-------|---------|
| Roy, 2014 [58]        | P            | Preterm infants (GA < 37w) and BW < 2500 g, with stable enteral feeding within 72 h of birth | Lactobacillus acidophilus 1.25 × 10^9 CFU × 1 g, B. longum 0.125 × 10^9 CFU × 1 g, B. bifidum 0.125 × 10^9 CFU × 1 g, B. lactis 1 × 10^9 CFU × 1 g | Half a 1 g sachet | From 72 h of life | After 6 w or at discharge |
| Saengtawesin, 2014 [48]| P            | Preterm infants with GA ≤ 34 w and BW ≤ 1500 g                                          | Lactobacillus acidophilus 1 × 10^9 CFU, Bifidobacterium | Half a 1 g sachet | Start of feeding | 6 w of age or discharge |
| Samanta, 2009         | P            | Preterm infants with GA < 32 w and BW < 1500 g, who started enteral feeding and survived beyond 48 h of age | Bifidobacterium infantis, Bifidobacterium bifidum, Bifidobacterium longum, Lactobacillus acidophilus | 2.5 × 10^9 CFU each probiotic, BD | Start of enteral feeding | Discharge |
| Sari, 2011 [34]       | P            | Preterm infants with GA < 32 w or BW < 1500 g, who survived to feed enterally            | Lactobacillus sporogenes 0.35 × 10^9 CFU OD | First feed | Discharge | None |
| Serce, 2013 [27]      | P            | Preterm infants with GA ≤ 32 w and BW ≤ 1500 g, who survived to feed enterally           | Saccharomyces boulardii 0.5 × 10^9 CFU/kg BD | Non-specified | Non-specified | Distilled water |
| Stratiki, 2007 [39]   | P            | Preterm infants with GA 27–32 w, formula-fed, without major congenital anomalies         | Bifidobacterium lactis 2 × 10^7 CFU/g of milk powder | Start of enteral feeding | Non-specified | None |
Table 1  Studies included in the systematic review and meta-analysis (Continued)

| Study                | Intervention                                      | Comparator | Outcome |
|----------------------|---------------------------------------------------|------------|---------|
| Totsu, 2014 [46]    | P Infants with BW < 1500 g Bifidobacterium bifidum | HM, FM     | Dextrin |
|                      | DB D: 2.5 x 10^9 CFU, divided in two doses        |            |         |
|                      | CLR S: within 48 h after birth                    |            |         |
|                      | C E: body weight 2000 g                           |            |         |
|                      | Multic.                                           |            |         |

P prospective, DB double-blinded, R randomized, C controlled, Multic multicentric, B blinded, M masked, Bic bicentric, BW birth weight, GA gestational age, NEC necrotizing enterocolitis, HM human milk, CFU colony forming unit, OD once daily, BD twice daily, OMM own mother’s milk, PFM preterm formula, DM donor milk, FM formula, MDX maltodextrin
moderate, low and very low (as suggested by the GRADE Working Group) [22].

The association between probiotic use and NEC was evaluated by meta-analyses, conducted by AA and DG, using the RevMan software (version 5.3.5; downloaded from the Cochrane website: http://tech.cochrane.org/revman/download). Risk ratio (RR) was calculated using the Mantel-Haenszel method, and reported with 95% confidence interval (CI).

The following sub-meta-analyses were also performed, in order to evaluate the effect of probiotics:

- in specific subgroups of patients (very-low-birth-weight [VLBW] infants);
- in surgical NEC;
- according to NEC incidence in different populations: the incidence of NEC stage ≥2 in the control population was used as a reference, because only a minority of studies reported NEC incidence in the general population. Studies were arbitrarily divided into three groups defined as “low-risk” (NEC incidence <5 %), “medium-risk” (incidence 5–10 %), and “high-risk” (incidence >10 %);
- according to probiotic strain: studies were divided according to the specific probiotic strain used, and were considered as suitable for inclusion in the sub-meta-analyses when the same probiotic strain was used in at least two studies. Studies which used a probiotic mixture were considered together.

Microbiological quality of all the studies was evaluated by MLC and LM. Studies were defined as having severe, moderate or minor microbiological flaws according to the evaluation of proper strain identification and microbiological assessment. Specifically, the lack of proper strain identification was considered as a severe flaw; the lack of microbiological assessment regarding the probiotic persistence in stools was considered as a moderate flaw, whereas a low flaw was defined when the presence of the probiotic in stools was evaluated by indirect approaches such as the quantification of its species belonging.

A fixed-effect model was used for the analyses. Heterogeneity was measured using the I² test. If significant heterogeneity was present (p < 0.05 from the χ² test), a random-effects model was used [23]. The random-effects model was also used when heterogeneity was not significant but the number of studies was ≤5, because the test for heterogeneity is known to have low power when the number of studies is small [24].

Forest plots were used to illustrate results from meta-analyses, and funnel plots to investigate bias.

The online version of GraphPad Quickcalcs software was used to calculate number needed to treat (NNT).

Results

Literature search

Three-hundred-sixty-eight papers were identified through the literature search (310 through PubMed and 58 through the Cochrane Library). Thirty-eight studies met the inclusion criteria: 22 were identified through the PubMed search [25–48] and 16 through the Cochrane Library search [25, 26, 28–32, 34–37, 40–43, 45]. Ten additional papers were identified from the reference lists of included studies [49–58]. Of these 47 studies, 16 were excluded, as they were duplicates retrieved both by PubMed and Cochrane Library search. Six additional studies were excluded after examining the full-texts: one study reported maternal probiotic supplementation during pregnancy [29], one cohort was reported twice [31], one study included both term and preterm infants [36], two studies did not report NEC data [55, 56], and in one study randomization was not declared [54].

Twenty-six studies were suitable for inclusion in the meta-analysis [10, 25–28, 30, 32–35, 37–42, 44, 46, 48–53, 58]. A description of included studies is provided in Table 1; excluded studies are described in Table 2.

All the studies reported NEC data in a form suitable for meta-analysis, except one [53], for which data included in a previous Cochrane review were used [59].

### Table 2: Studies excluded from the systematic review and meta-analysis

| Author, year | Study summary | Reason for exclusion |
|--------------|---------------|----------------------|
| Awad, 2010   | Living vs. killed *Lactobacillus acidophilus* vs. placebo given to neonates admitted to the study NICU | Term and preterm infants included |
| Benor, 2014  | *Lactobacillus acidophilus* and *Bifidobacteria lactis* vs. placebo given to mothers of VLBW infants | Maternal probiotic supplementation |
| Li, 2004     | *Bifidobacterium breve* given to LBW infants | Randomization not declared |
| Millar, 1993 | *Lactobacillus GG* given to preterm infants with GA < 33 w | No NEC data |
| Reuman, 1986 | Formula containing lactobacilli vs. placebo given to preterm infants | No NEC data |
| Sari, 2012   | *Lactobacillus sporogenes* given to preterm infants with GA < 32 w or BW < 1500 g, who survived to feed enterally | Duplicate population (Sari, 2011 [34]) |

NICU: neonatal intensive care unit, VLBW: very low birth weight, LBW: low birth weight, GA: gestational age, NEC: necrotizing enterocolitis, BW: birth weight
| Author, year          | Previous NEC rate | Number of subjects | NEC in probiotic group | NEC in control group |
|----------------------|-------------------|--------------------|------------------------|----------------------|
| Al-Hosni, 2012 [33]  | Not stated        | 50 probiotic       | 3/50 any stage         | 4/51 any stage       |
|                      |                   | 51 control         | 1/50 stage 1           | 2/51 stage 1         |
|                      |                   |                    | 0/50 stage 2           | 0/51 stage 2         |
|                      |                   | 2/50 stage 3       | 2/51 stage 3           |                      |
| Bin-Nun, 2005 [40]   | 15 %              | 72 probiotic       | 3/72 any stage         | 12/73 any stage      |
|                      |                   | 73 control         | 1/72 stage ≥2          | 10/73 stage ≥2       |
|                      |                   |                    | 1/72 stage 2           | 7/73 stage 2         |
|                      |                   |                    | 0/72 stage 3           | 3/73 stage 3         |
| Braga, 2011 [35]     | 10 %              | 119 probiotic      | 0/119 stage ≥2         | 4/112 stage ≥2       |
|                      |                   | 112 placebo        |                        |                      |
| Costalos, 2003 [49]  | Not stated        | 51 probiotic       | 5/51 any stage         | 6/36 any stage       |
|                      |                   | 36 placebo         |                        |                      |
| Dani, 2002 [42]      | Not stated        | 295 probiotic      | 4/295 stage ≥2         | 8/290 stage ≥2       |
|                      |                   | 290 placebo        |                        |                      |
| Demirel, 2013 [28]   | 32 %              | 135 probiotic      | 6/135 stage ≥2         | 7/136 stage ≥2       |
|                      |                   | 136 control        |                        |                      |
| Dilli, 2015 [44]     | Not stated        | 100 probiotic      | 2/100 stage ≥2         | 18/100 stage ≥2      |
|                      |                   | 100 placebo        |                        |                      |
| Fernández-Carrocera, | 20 %              | 75 probiotic       | 6/75 stage ≥2          | 12/75 stage ≥2       |
| 2013 [32]            |                   | 75 placebo         |                        |                      |
| Jacobs, 2013 [26]    | Not stated        | 548 probiotic      | 11/548 stage ≥2        | 24/551 stage ≥2      |
|                      |                   | 551 placebo        |                        |                      |
| Kitajima, 1997 [52]  | Not stated        | 45 probiotic       | 0/45 any stage         | 0/46 any stage       |
|                      |                   | 46 placebo         |                        |                      |
| Lin, 2005 [41]       | Approx. 23 % (NEC | 180 probiotic      | 2/180 stage ≥2         | 10/187 stage ≥2      |
|                      | or death)         | 187 control        | 2/180 stage 2          | 4/187 stage 2        |
|                      |                   |                    | 0/180 stage 3          | 6/187 stage 3        |
| Lin, 2008 [37]       | Approx.           | 217 placebo        | 4/217 any stage        | 14/217 any stage     |
|                      |                   | 217 control        | 2/217 stage 2          | 9/217 stage 2        |
|                      |                   |                    | 2/217 stage 3          | 5/217 stage 3        |
| Manzoni, 2006 [37]   | Not stated        | 39 probiotic       | 1/39 any stage         | 3/41 any stage       |
|                      |                   | 41 control         | 1/39 stage 2           | 2/41 stage 2         |
|                      |                   |                    | 0/39 stage 3           | 1/41 stage 3         |
| Mihatsch, 2010 [43]  | Not stated        | 84 probiotic       | 2/84 stage ≥2          | 4/82 stage ≥2        |
|                      |                   | 82 placebo         |                        |                      |
| Mohan, 2006 [53]     | Not stated        | 21 probiotic       | 2/37 stage ≥2          | 1/32 stage ≥2        |
|                      |                   | 17 placebo         | Unpublished data, taken from Alfaleh 2011 [58] | Unpublished data, taken from Alfaleh 2011 [58] |
| Oncel, 2013 [25]     | 15 %              | 200 probiotic      | 8/200 stage ≥2         | 10/200 stage ≥2      |
|                      |                   | 200 placebo        |                        |                      |
| Patole, 2014 [45]    | Not stated        | 74 probiotic       | 0/74 stage ≥2          | 1/66 stage ≥2        |
|                      |                   | 66 placebo         |                        |                      |
| Rojas, 2012 [30]     | Not stated        | 372 probiotic      | NEC stage ≥2           | NEC stage ≥2         |
|                      |                   | 378 placebo        | ≤1500 g               | ≤1500 g              |
For each study, NEC rate in the probiotic and in the placebo/control group is reported in Table 3. For the purpose of the meta-analysis, data on NEC stage ≥ 2 were used.

**Probiotics and NEC stage ≥2**

Data from 6605 infants (3324 in the probiotic group and 3281 in the control group) were analyzed. Fewer infants in the probiotic group developed NEC stage ≥ 2 compared to infants in the control group (88 [2.65 %] vs. 188 [5.73 %], respectively). The RR was significantly lower in infants treated with probiotics (0.47 [95 % CI 0.36–0.60], p < 0.00001; fixed-effect analysis). NNT was 33 (95 % CI 24.7–47.2), which means that 33 infants needed to be treated with probiotics in order to prevent one more case of NEC stage ≥ 2. Heterogeneity among trials was absent (I² = 0 %, p = 0.63; Fig. 2a). The funnel plot did not show any clear asymmetry (Fig. 2b).

**VLBW infants**

Twenty-two studies [25–28, 30, 32–35, 37, 38, 40–42, 44–46, 48, 50–52] reported data from 5912 VLBW infants, 2959 in the probiotic and 2953 in the control group. NEC stage ≥ 2 occurred less frequently in the probiotic group than in controls (82 [2.77 %] infants vs. 174 [5.89 %], respectively), with a RR of 0.48 ([95 % CI 0.37–0.62], p < 0.00001; fixed-effect analysis; I² = 0 %, p = 0.56). NNT was 33 (95 % CI 24.1–47.9).

**Table 3 Incidence of necrotizing enterocolitis in infants treated with probiotics and in controls (Continued)**

| Study | Randomization | Sample | Probiotic NEC | Control NEC |
|-------|---------------|--------|---------------|-------------|
| Rough, 2009 [50] | Not stated | 45 | 6/176 | 10/184 |
| Rough, 2009 [50] | Not stated | 49 | 2/45 | 1/49 |
| Roy, 2014 [58] | Not stated | 56 | 2/56 | 2/56 |
| Saengtawesin, 2014 [48] | Not stated | 31 | 1/31 | 1/29 |
| Samanta, 2009 | Not stated | 91 | 5/91 | 15/95 |
| Sari, 2011 [34] | Approx. 32 % (death or NEC) | 110 | 6/110 | 10/111 |
| Serce, 2013 [27] | 17 % | 104 | 7/104 | 7/104 |
| Stratiki, 2007 [39] | Not stated | 41 | 0/41 | 3/34 |
| Totsu, 2014 [46] | Not stated | 153 | 0/153 | 0/130 |

**Surgical NEC**

Only 6 studies [33, 34, 37, 40, 41, 51] reported separate data for surgical NEC (NEC stage 3), which occurred in 6/668 (0.90 %) infants in the probiotic group and in 20/680 (2.94 %) infants in the control group. The RR for NEC stage 3 was significantly lower in the probiotic group (0.35 [95 % CI 0.16–0.81], p = 0.01; fixed-effect analysis; I² = 0 %, p = 0.69). NNT was 49 (95 % CI 28.6–170.8).

**NEC incidence**

NEC incidence in controls was <5 % in 13 studies (Fig. 3a) [26, 30, 33, 35, 42, 43, 45, 46, 48, 50, 52, 53, 58], between 5 and 10 % in 8 studies (Fig. 3b) [25, 27, 28, 34, 37, 39, 41, 51], and >10 % in 5 studies (Fig. 3c) [32, 38, 40, 44, 49].

The RR for NEC stage ≥ 2 was significantly lower in the probiotic group compared to the control group in all the three populations (RR 0.52 [95 % CI 0.35–0.78], p = 0.001; RR 0.54 [95 % CI 0.36–0.80], p = 0.002; RR 0.33 [95 % CI 0.17–0.62], p = 0.0006, respectively). Heterogeneity was non-significant in all the three sub-analyses.

**Probiotic strain**

*Lactobacillus GG* was used in 2 studies [42, 51] and *Lactobacillus reuteri* in 2 other studies [25, 30]: the effect of these probiotics in reducing NEC was not significant, either for *Lactobacillus GG* and *Lactobacillus*...
\textit{reuteri} (RR 0.50 [95% CI 0.17–1.44], \( p = 0.20 \) [Fig. 4a], and RR 0.69 [95% CI 0.38–1.26], \( p = 0.23 \) [Fig. 4b]). One study used \textit{Lactobacillus sporogenes} [34].

The results of all the studies including \textit{Lactobacilli} were pooled, except for the study by Sari et al. [34]: \textit{Lactobacillus sporogenes} is a species which has not an international recognition, shows characteristics of both \textit{genera Lactobacillus} and \textit{Bacillus}, and its strains should be better classified as \textit{Bacillus coagulans} [60]. Thus, when the results of studies using \textit{Lactobacillus GG} and \textit{reuteri} were pooled, no significant reduction in the RR for NEC in the probiotic group was observed (0.62 [95% CI 0.37–1.05], \( p = 0.07 \), Fig. 4c).

Four studies used \textit{Bifidobacterium lactis} [39, 43, 44, 53], 2 studies \textit{Bifidobacterium breve} [45, 52] and 1 study \textit{Bifidobacterium bifidum} [46]. The use of
Bifidobacterium lactis resulted in a significant reduction in the RR for NEC (0.23 [95 % CI 0.10–0.55], \( p = 0.0008 \), Fig. 5a). No effect of Bifidobacterium breve in reducing NEC was documented (RR 0.30 [95 % CI 0.01–7.19], \( p = 0.46 \), Fig. 5b); the only study reporting the use of Bifidobacterium bifidum did not report any case on NEC. When the results of studies using Bifidobacteria were pooled, a significant reduction in the RR for NEC in the probiotic group was observed (0.24 [95 % CI 0.10–0.54], \( p = 0.0006 \), Fig. 5c).

Saccharomyces boulardii was used in 3 studies [27, 28, 49]: no significant effect of this probiotic was documented (RR 0.81 [95 % CI 0.44–1.49], \( p = 0.50 \); random effects analysis).

The pooled analysis of the 11 studies [26, 32, 33, 35, 37, 38, 40, 41, 48, 50, 58] in which a probiotic mixture was used showed an overall and significant benefit of these products in reducing NEC (RR 0.39 [95 % CI 0.27–0.56], \( p < 0.00001 \), Fig. 6).

Study quality
Evaluation of the quality of the studies included in the meta-analysis according to the risk of bias tool as proposed by the Cochrane Collaboration is showed in Table 4, which also shows the level of evidence evaluated following the recommendations of the GRADE Working Group.

Microbiological quality
Microbiological quality of included studies is described in Table 5. Eight studies were evaluated as having severe microbiological flaws [27, 32, 34, 35, 38, 40, 41, 49], meaning that they did not report a proper probiotic strain identification. Thirteen studies [25, 26, 28, 30, 33, 37, 42–44, 46, 48, 51, 58] were evaluated as having moderate microbiological flaws, because none of them evaluated the probiotic persistence in stools. There were only five studies [39, 45, 50, 52, 53] with minor microbiological flaws.
Discussion

The results of this systematic review and meta-analysis show an overall benefit of probiotic supplementation for the prevention of NEC in preterm infants. These results are strengthened by the absence of significant statistical heterogeneity among studies and by the low-risk of publication bias documented by the funnel plot.

However, despite the overall benefit, it is remarkable that the 26 studies included in the meta-analysis were extremely heterogeneous in terms of probiotic strain, dosage, duration of intervention, and target population. Furthermore, only few studies documented an effective colonization of the infants’ gut with the probiotic strain. Thus, the proposal made by the authors of the recent Cochrane review of a “change in practice” in the use of probiotics in preterm infants [11] might require further investigation.

Currently available literature does not provide any definite conclusion on which probiotic strain should be used, and which group of preterm infants would benefit most from a probiotic intervention. It is important to note that the effect of a live-microorganism used as a probiotic is strictly strain-specific [61]. In this paper we aimed to perform strain-specific sub-meta-analyses but our efforts were weakened by the fact that in very few studies the same probiotic strain was used. For this reason, we were unable to draw definite conclusions on which single-strain of probiotics would be more effective in reducing NEC. When studies using single strains were pooled according to the probiotic genus, no significant effect was documented for *Lactobacilli* and *Saccharomyces*. This is partially in contrast with the recent Cochrane review on probiotics and NEC [11], which showed a beneficial effect of *Lactobacilli*: this discrepancy appears to be due mainly to differences in the studies included in the two sub-meta-analyses. Actually, the present meta-analysis included the study by Oncel et al. [25], which was on-going when the Cochrane review was published, but excluded the study by Manzoni et al. [57], where probiotics were used in addition to lactoferrin, and the study by Sari et al. [34], which used a probiotic product which is not properly a *Lactobacillus* [60].

The analysis of studies using *Bifidobacteria* showed a significant effect of *Bifidobacterium breve* in reducing NEC. This is also in contrast with the results of the Cochrane review; however, the discrepancy is explained by the inclusion in the present meta-analysis of the recent study by Dilli et al. [44], which appears to drive the beneficial effect documented for *Bifidobacteria*. Similarly to the Cochrane review [11], the analysis of studies in which more than one strain was used documented a strong and significant effect of these products in the prevention of NEC. No definite conclusion can be drawn from these results, even if it could be suggested that further research should be focused on mixed rather than on single-strain products; a potential rationale for this
approach could be that a mix of strains might be more effective in providing an ecological barrier than a single strain.

The evidence that probiotics are effective in reducing NEC in VLBW infants does not necessarily apply also to extremely LBW infants (ELBWs), who are the highest-risk population. Only three studies [26, 33, 58] reported the rate of NEC in ELBWs: in two of these studies [33, 58], the same number of ELBWs in the probiotic and control group developed NEC [33], while in the ProPrems trial NEC incidence was slightly lower in the probiotic group [26]. Given the relatively small number of ELBWs and the inconclusive results, no specific recommendation can be drawn from the analysis of these two studies. Similarly, no study reported separate data for intrauterine-growth-restricted (IUGR) infants, and thus
no recommendation can be made either for this high-risk population.

In the analysis of trials evaluating a specific intervention, it is pivotal to understand whether the results of these trials are generalizable or applicable only in specific clinical settings. According to our data, the common belief that probiotics are more effective in populations with a high rate of NEC [62] can be called into question: actually, when studies were divided according to NEC incidence in the control population, NEC reduction was striking and significant also when NEC rate in controls was extremely low. NEC rate in controls can be considered as a proxy for the quality of neonatal care: in this perspective, it is interesting to note that, in contrast with previous data, probiotics appear to confer a preventive benefit also in high quality-of-care settings. NEC rate in control populations was used for the analysis, rather than the baseline NEC rate stated by the authors and used in several studies for sample size calculation: this approach was considered more appropriate, because baseline NEC rate was not provided in many studies and, when provided, there was often a discrepancy with NEC rate detected in controls.

The analysis of included studies according to their microbiological quality points out that clinical studies aiming at evaluate the preventive effect of probiotics on NEC often lack an adequate microbiological assessment and this represents a major limitation of these studies. Actually, it is well known that the correct identification of a probiotic at species level corresponds to evaluate its safety, whereas the identification at strain level is extremely relevant as probiotic beneficial properties are strain-specific. Furthermore, the evaluation of probiotic colonisation, even if temporary,

### Table 4 Evaluation of the quality of the studies included in the meta-analysis according to the risk of bias tool as proposed by the Cochrane collaboration and evaluation of the level of evidence according to the GRADE approach

| Study                          | Random sequence generation | Allocation concealment | Blinding | Incomplete outcome data | Selective outcome reporting | Other sources of bias              | Levels of quality of evidence in the grade approach |
|--------------------------------|---------------------------|------------------------|----------|-------------------------|----------------------------|---------------------------------|--------------------------------------------------|
| Al-Hosni, 2012 [33]            | UNCLEAR                   | UNCLEAR                | LOW      | UNCLEAR                 | UNCLEAR                   | UNCLEAR                        | LOW                                             |
| Bin-Nun, 2005 [40]             | UNCLEAR                   | UNCLEAR                | HIGH     | UNCLEAR                 | UNCLEAR                   | UNCLEAR                        | VERYLOW                                         |
| Braga, 2011 [35]               | LOW                       | LOW                    | LOW      | LOW                     | UNCLEAR                   | LOW                             | HIGH                                            |
| Costalos, 2003 [49]            | LOW                       | LOW                    | LOW      | LOW                     | LOW                       | LOW                             | HIGH                                            |
| Dani, 2002 [42]                | UNCLEAR                   | LOW                    | LOW      | LOW                     | UNCLEAR                   | UNCLEAR                        | MODERATE                                        |
| Demirel, 2013 [28]             | LOW                       | LOW                    | UNCLEAR  | UNCLEAR                 | UNCLEAR                   | UNCLEAR                        | MODERATE                                        |
| Dilli, 2015 [44]               | LOW                       | LOW                    | LOW      | UNCLEAR                 | UNCLEAR                   | UNCLEAR                        | MODERATE                                        |
| Fernández-Carrocera, 2013 [32]| LOW                       | LOW                    | LOW      | UNCLEAR                 | UNCLEAR                   | LOW                             | HIGH                                            |
| Jacobs, 2013 [26]              | LOW                       | UNCLEAR                | LOW      | UNCLEAR                 | UNCLEAR                   | UNCLEAR                        | LOW                                             |
| Kitajima, 1997 [52]            | LOW                       | UNCLEAR                | LOW      | LOW                     | UNCLEAR                   | UNCLEAR                        | MODERATE                                        |
| Lin, 2005 [41]                 | LOW                       | LOW                    | LOW      | LOW                     | UNCLEAR                   | LOW                             | HIGH                                            |
| Lin, 2008 [37]                 | LOW                       | LOW                    | LOW      | LOW                     | UNCLEAR                   | LOW                             | HIGH                                            |
| Manzoni, 2006 [37]             | LOW                       | LOW                    | LOW      | NEC                     | UNCLEAR                   | UNCLEAR                        | LOW MODERATE                                    |
| Mihatsch, 2010 [43]            | LOW                       | UNCLEAR                | LOW      | UNCLEAR                 | LOW                       | UNCLEAR                        | LOW MODERATE                                    |
| Mohan, 2006 [53]               | UNCLEAR                   | LOW                    | LOW      | UNCLEAR                 | LOW                       | UNCLEAR                        | LOW                                             |
| Oncel, 2013 [25]               | LOW                       | LOW                    | LOW      | UNCLEAR                 | LOW                       | LOW                             | MODERATE                                        |
| Patole, 2014 [45]              | LOW                       | LOW                    | LOW      | UNCLEAR                 | LOW                       | LOW                             | HIGH                                            |
| Rojas, 2012 [30]               | LOW                       | LOW                    | LOW      | UNCLEAR                 | LOW                       | LOW                             | HIGH                                            |
| Rougé, 2009 [50]               | LOW                       | UNCLEAR                | LOW      | UNCLEAR                 | HIGH                      | LOW                             | LOW                                             |
| Roy, 2014 [58]                 | LOW                       | UNCLEAR                | LOW      | LOW                     | UNCLEAR                   | UNCLEAR                        | MODERATE                                        |
| Saengtawesin, 2014 [48]        | HIGH                      | HIGH                   | HIGH     | UNCLEAR                 | UNCLEAR                   | UNCLEAR                        | LOW                                             |
| Samanta, 2009                  | LOW                       | LOW                    | LOW      | UNCLEAR                 | UNCLEAR                   | UNCLEAR                        | MODERATE                                        |
| Sari, 2011 [34]                | LOW                       | LOW                    | LOW      | UNCLEAR                 | UNCLEAR                   | UNCLEAR                        | MODERATE                                        |
| Serge, 2013 [27]               | LOW                       | LOW                    | LOW      | UNCLEAR                 | UNCLEAR                   | UNCLEAR                        | MODERATE                                        |
| Stratiki, 2007 [39]            | UNCLEAR                   | UNCLEAR                | LOW      | UNCLEAR                 | UNCLEAR                   | UNCLEAR                        | LOW                                             |
| Totsu, 2014 [46]               | LOW                       | LOW                    | LOW      | UNCLEAR                 | UNCLEAR                   | UNCLEAR                        | MODERATE                                        |
Table 5 Evaluation of the included studies according to their microbiological quality

| Author, year | Probiotic strain | Strain identification | Microbiological assessment | Microbiological flaw |
|--------------|------------------|-----------------------|---------------------------|---------------------|
| Al-Hosni, 2012 [33] | Lactobacillus rhamnosus LGG | LGG identified at the strain level | No assessment | Moderate |
|                | Bifidobacterium infantis | B. infantis identified via the web site of the producer: Bifantis (Bifidobacterium infantis 35624) | | |
| Bin-Nun, 2005 [40] | Bifidobacterium infantis | Strains not identified at the strain level | No assessment | Severe |
|                | Streptococcus thermophilus | | | |
|                | Bifidobacterium bifidus | | | |
| Braga, 2011 [35] | Lactobacillus casei | Strains not identified clearly | No assessment | Severe |
|                | Bifidobacterium breve | | | |
| Costalos, 2003 [49] | Saccharomyces boulardii | Strain not identified at the strain level | S. boulardii not characterized in stools. | Severe |
|                | | Gut flora assessed by plate count | | |
| Dani, 2002 [42] | Lactobacillus rhamnosus GG | Strain identified | No assessment | Moderate |
| Demirel, 2013 [28] | Saccharomyces cerevisiae | Strain identified | No assessment | Moderate |
| Dilli, 2015 [44] | B. lactis | Strain non identified at the strain level but probably Bb12 | No assessment | Moderate |
| Fernández-Carrocera, 2013 [32] | Lactobacillus acidophilus | Strains not identified at the strain level | No assessment | Severe |
|                | Lactobacillus casei | | | |
|                | Lactobacillus plantarum | | | |
|                | Bifidobacterium bifidus | | | |
| Jacobs, 2013 [26] | Bifidobacterium infantis | Strains identified at the strain level | No assessment | Moderate |
|                | Streptococcus thermophilus | | | |
|                | Bifidobacterium lactis | | | |
| Kitajima, 1997 [52] | Bifidobacterium breve | Strain identified | Assessment by a strain-specific monoclonal antibody conjugated with colloidal gold particle | Minor |
| Lin, 2005 [41] | Lactobacillus acidophilus | Strains not identified at the strain level | No assessment | Severe |
|                | Bifidobacterium infantis | | | |
| Lin, 2008 [37] | Lactobacillus acidophilus | Strain identified | No assessment | Moderate |
|                | Bifidobacterium bifidum | | | |
| Manzoni, 2006 [51] | Lactobacillus rhamnosus LGG | Strain identified | No assessment | Moderate |
| Mihatsch, 2010 [43] | Bifidobacterium lactis | Strain identified | No assessment | Moderate |
| Mohan, 2006 [53] | Bifidobacterium lactis | Strain identified | Species-specific (not strain-specific) assessment | Minor |
| Oncel, 2013 [25] | Lactobacillus reuteri | Strain identified | No assessment | Moderate |
| Patole, 2014 [45] | Bifidobacterium breve | Strain identified | Microbiological assessment by PCR | Minor |
| Rojas, 2012 [30] | Lactobacillus reuteri | Strain identified | No assessment | Moderate |
| Rougé, 2009 [50] | Bifidobacterium longum | Strain identified | Microbiological assessment by PCR | Minor |
| Roy, 2014 [58] | Lactobacillus acidophilus, B. longum, B. bifidum, and B. lactis | Strains not identified at the strain level/identification of the commercial product | No assessment | Moderate |
| Saengtawesin, 2014 [48] | Lactobacillus acidophilus and Bifidobacterium bifidum | | No assessment | Moderate |
is important to correlate the probiotic presence to the beneficial effects.

The development of gut microbiota in preterm infants is known to be influenced by several factors, including gestational age, mode of delivery, diet, and antibiotic exposure [63]. All these factors are likely to be significant confounders in the relationship between probiotics and NEC; actually, it is well documented that infants fed maternal or donor breast milk have a lower risk of NEC compared to formula-fed infants [64], and that caesarean delivery is associated with a disruption in gut microbiota [65]. Quite surprisingly, however, in published studies data are not analyzed taking these confounders into account [66]. Given the definite protective role of human milk feeding and the symbiotic properties of human milk, it would be fundamental to understand whether the use of probiotics should be encouraged also in human-milk fed infants, or if this intervention should be directed towards exclusively formula-fed infants.

The studies included in the meta-analysis did not report any short-term adverse effect of probiotic supplementation (i.e., bloodstream infection with the probiotic strain). Growing evidence suggests the influence of gut microbiota on long-term health and disease, including both type 1 and type 2 diabetes mellitus, atherosclerosis, asthma, colon cancer, and inflammatory bowel disease [67]. However, at present little is known on the long-term outcome possibly related to the alteration of gut flora in preterm infants, which is the result of the supplementation with exogenous strains.

The choice to investigate a single outcome might be viewed as a limitation of the study: however, this choice was deliberate, as the literature search strategy was focused exclusively on NEC. Any speculation on different outcomes such as sepsis or mortality would have been inevitably misleading, because it would have been impossible to be sure to have identified all the studies reporting on those outcomes.

Conclusions
Meta-analyses give a valuable contribution in guiding researchers to focus future clinical studies on specific unanswered questions. The results of the present meta-analysis confirm that research on probiotics and NEC is on the right track, but also suggest that there are several unanswered questions which should be addressed before radically changing clinical practice. Our data highlight the need for further, well-designed studies aimed at clarifying the specific effect of probiotics in high-risk populations (i.e., ELBWs, IUGRs) and at addressing the choice of the most effective probiotic product, at the proper dose and duration of supplementation. For this reason, we encourage, for future studies, the publication of study protocols detailing study population and characteristics of the intervention, in order to narrow probiotic research to the most promising strains or combination of strains and to the most vulnerable populations, thus allowing a confirmative individual patient data analysis.

Competing interests
None of the authors has any conflict of interest to declare in connection with this paper.

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

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References
1. Neu J, Walker WA. Necrotizing enterocolitis. N Engl J Med. 2011;364:255–64.
2. Bisquera JA, Cooper TR, Berseth CL. Impact of necrotizing enterocolitis on length of stay and hospital charges in very low birth weight infants. Pediatrics. 2002;109:423–8.
3. Gephart S, McGrath J, Effren J, Halpern M. Necrotizing enterocolitis risk: state of the science. Adv Neonatal Care. 2012;12:77–89.
4. Sanders ME, Guerrier F, Guerrier R, Holt PR, Quigley EMM, Sartor RB, et al. An update on the use and investigation of probiotics in health and disease. Gut. 2013;62:387–96.
5. Martin CR, Walker WA. Probiotics: role in pathophysiology and prevention in necrotizing enterocolitis. Semin Perinatol. 2008;32:127–37.
6. DiGilio DB, Romero R, Amogn P, Husakow JP, Bia EM, Gotsch F, et al. Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: a molecular and culture-based investigation. PLoS One. 2008;3:e3056.
7. Mohviladze M, Neu J, Shuster J, Theriaque D, Li N, Mai V. Intestinal microbial ecology in premature infants assessed with non-culture-based techniques. J Pediatr. 2010;156:20–20.
8. Wang Y, Hoening JD, Malin KL, Qamar S, Petrof EO, Sun J, et al. 16S rRNA gene-based analysis of fecal microbiota from preterm infants with and without necrotizing enterocolitis. ISME J. 2009;3:944–54.
9. Murguía-Peniche T, Mihatsch WA, Zegarra J, Supaparnachant S, Ding Z-Y, Neu J. Intestinal mucosal defense system, Part 2: Probiotics and prebiotics. J Pediatr. 2013;162(3 Suppl:S64–71.
10. Deshpande G, Rao S, Patole S, Bulsara M. Updated meta-analysis of probiotics for preventing necrotizing enterocolitis in preterm neonates. Pediatrics. 2010;125:921–30.
11. Affale K, Anabrees J, Basiller D. Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev. 2014;4:CD005496. doi:10.1002/14651858.CD005496.pub4.
12. Thomas DW, Greer FR. Probiotics and prebiotics in pediatrics. Pediatrics. 2010;126:2137–13.
13. Mihatsch WA, Braegger CP, Deici T, Kolacek S, Lanzinger H, Mayer B, et al. Critical systematic review of the level of evidence for routine use of probiotics for reduction of mortality and prevention of necrotizing enterocolitis and sepsis in preterm infants. Clin Nutr. 2012;31:6–15.
14. Szajewska H. Probiotics and prebiotics in preterm infants: where are we? Where are we going? Early Hum Dev. 2010;86 Suppl 1:81.
15. Hill C, Guerrier F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014;11:506–14.
16. FAQ, WHO. Probiotics in food. Health and nutritional properties and guidelines for evaluation. 2006.
17. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009;6:e1000097.
18. Bell MJ, Ternberg JL, Feigin RD, King JP, Marshall R, Barton L, et al. Neonatal necrotizing enterocolitis: therapeutic decisions based upon clinical staging criteria. Ann Surg. 1978;178:171–7.
19. Walsh MC, Thompson SG. Detecting and describing heterogeneity in meta-analysis. Stat Med. 1998;17:841–56.
20. Greenhalgh T, Peacock R. Effectiveness and efficiency of search methods in systematic reviews of interventions. [http://handbook.cochrane.org/].
21. Cochrane handbook for systematic reviews of interventions. [http://handbook.cochrane.org/].
22. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ. 2008;336:924–6.
23. Higgins JPT, Green S, editors. Cochrane handbook for systematic reviews of interventions. Version 5.10 (March 2011). 2011.
24. Hardy RJ, Thompson SG. Detecting and describing heterogeneity in meta-analysis. Stat Med. 1998;17:841–56.
43. Mihattsch WA, Vosbeck S, Eilkmanns B, Hoegel J, Pohlmdt F. Effect of Bifidobacterium lactis on the incidence of nosocomial infections in very-low-birth-weight infants: a randomized controlled trial. Neonatology. 2010;98:156–63.

44. Dill D, Aydin B, Ferhat M, Ozyazici E, Beken S, Zenciroglu A, et al. The ProPre-Save study: effects of probiotics and prebiotics alone or combined on necrotizing enterocolitis in very low birth weight infants. J Pediatr. 2015. In press.

45. Patole S, Keil AD, Chang A, Nathan E, Doherty D, Simmer K, et al. Effect of Bifidobacterium breve M-16 V supplementation on fecal bifidobacteria in preterm neonates—a randomised double blind placebo controlled trial. PLoS One. 2014;9:e89511.

46. Totsu S, Yamasaki T, Terahara M, Uchiyama A, Kusuda S. Bifidobacterium, and enteral feeding in preterm infants: Cluster-randomized trial. Pediatr Int. 2014;56:714–9.

47. Sehgal A, Mak W, Dunn M, Kelly E, Whyte H, McCrindle B, et al. Haemodynamic changes after delivery room surfactant administration to very low birth weight infants. Arch Dis Child Fetal Neonatal Ed. 2010;95:F345–51.

48. Saengtawesin V, T Ashton William R. Randomisation of oral probiotics supplementation in the prevention of necrotizing enterocolitis among very low birth weight preterm infants. J Med Assoc Thai. 2014;97:S20–5.

49. Costalos C, Skoutelis A, Gounaris A, Vassilopoulou S, Triandafilidou A, Lecchi R, et al. Enteral feeding of premature infants with Saccharomyces boulardii. Early Hum Dev. 2003;74:89–96.

50. Rougé C, Pilouquet H, Burtel M-J, Berger B, Cheah C, Peciaris L, et al. Oral supplementation with probiotics in very-low-birth-weight preterm infants: a randomized, double-blind, placebo-controlled trial. Am J Clin Nutr. 2009;89:1828–35.

51. Manzoni P, Mostert M, Leonessa ML, Priolo C, Farina D, Monetti C, et al. Oral supplementation with Lactobacillus casei subsp. rhamnosus prevents enteric colonization by Candida species in preterm neonates: a randomized study. Clin Infect Dis. 2006;42:1735–42.

52. Kitajima H, Sumida Y, Tanaka R, Yuki N, Takayama H, Fujimura M. Early administration of Bifidobacterium breve to preterm infants: randomised controlled trial. Arch Dis Child Fetal Neonatal Ed. 1997;76:F101–7.

53. Mohan R, Koebnick C, Schildt J, Schmidt S, Mueller M, Possner M, et al. Effects of Bifidobacterium lactis Bb12 supplementation on intestinal microbiota of preterm infants: a double-blind, placebo-controlled, randomized study. J Clin Microbiol. 2006;44:4025–31.

54. Li Y, Shimizu T, Hosaka A, Kaneko N, Ohtsuka Y, Yamashiro Y. Effects of bifidobacterium breve supplementation on intestinal flora of low birth weight infants. Pediatr Int. 2004;46:509–15.

55. Mihatsch WA, Vosbeck S, Eilkmanns B, Hoegel J, Pohlndt F. Effect of Bifidobacterium lactis on the incidence of nosocomial infections in very-low-birth-weight infants: a randomized controlled trial. Neonatology. 2010;98:156–63.

56. Rougé C, Pilouquet H, Burtel M-J, Berger B, Cheah C, Peciaris L, et al. Oral supplementation with probiotics in very-low-birth-weight preterm infants: a randomized, double-blind, placebo-controlled trial. Am J Clin Nutr. 2009;89:1828–35.

57. Roy A, Chaudhuri J, Sarkar D, Ghosh P, Chakraborty S. Role of enteric supplementation of Probiotics on late-onset sepsis by Candida species in preterm low-birth-weight neonates. JAMA. 2009;302:1421–8.

58. Villar MR, Vågberg C, Smith R, Walker V, Hall MA. Enteral feeding of premature infants with Lactobacillus GG. Arch Dis Child. 1993;68:483–7.

59. Rouman PO, Duckworth D, Smith KL, Kagan R, Bucciarelli RL, Ayoub EM. Lack of effect of Lactobacillus on gastrointestinal bacterial colonization in premature infants. Pediatr Infect Dis. 1986;5:663–8.

60. Alfaaleh K, Abarees J, Badler D. Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev. 2011;3:CD005496. doi:10.1002/14651858.CD005496.pub3.

61. Diogo L, De Vecchi E. Should Lactobacillus sporogenes and Bacillus coagulans have a future? J Chemother. 2009;21:371–7.

62. Guarnieri F, Malgalda J-R. Gut flora in health and disease. Lancet. 2003;361:512–9.

63. Luedtke SA, Yang JT, Wild HE. Probiotics and necrotizing enterocolitis: finding the missing pieces of the probiotic puzzle. J Pediatr Pharmacol Ther. 2012;17:308–28.

64. Berrington JE, Stewart CJ, Emeleton ND, Cummings SP. Gut microbiota in preterm infants: assessment and relevance to health and disease. Arch Dis Child Fetal Neonatal Ed. 2013;98:F286–90.

65. Patole S, Keil AD, Chang A, Nathan E, Doherty D, Simmer K, et al. Effect of Bifidobacterium breve M-16 V supplementation on fecal bifidobacteria in preterm neonates—a randomised double blind placebo controlled trial. PLoS One. 2014;9:e89511.