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# Updated Meta-analysis of Probiotics for Preventing Necrotizing Enterocolitis in Preterm Neonates

Girish Deshpande, Shripada Rao, Sanjay Patole and Max Bulsara

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# Updated Meta-analysis of Probiotics for Preventing Necrotizing Enterocolitis in Preterm Neonates

**AUTHORS:** Girish Deshpande, FRACP,[a,b] Shripada Rao, MD,[a,b] Sanjay Patole, DrPH,[a,c] and Max Bulsara, PhD[d]

*[a]Department of Neonatal Paediatrics, King Edward Memorial Hospital for Women, Perth, Western Australia, Australia; [b]Department of Neonatal Paediatrics, Princess Margaret Hospital for Children, Perth, Western Australia, Australia; [c]School of Women's and Infant's Health, University of Western Australia, Perth, Western Australia, Australia; and [d]Department of Biostatistics, University of Notre Dame, Perth, Western Australia, Australia*

**KEY WORDS**

neonates, necrotizing enterocolitis, preterm, probiotics, systematic review

**ABBREVIATIONS**

NEC—necrotizing enterocolitis
LOS—late-onset sepsis
VLBW—very low birth weight
RCT—randomized, controlled trial
CNRG—Cochrane Neonatal Review Group
TFF—time to full feeds
TSA—trial sequential analysis
RR—relative risk
CI—confidence interval
NNT—numbers needed to treat
TPN—total parenteral nutrition
NDI—neurodevelopmental impairment
CONS—coagulase-negative *Staphylococcus*
ELBW—extremely low birth weight

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Dr Deshpande participated in the literature search, selection of trials, quality assessment of trials, contacting the authors of the trials for additional information, performing analysis, and writing the manuscript; Dr Rao did independent literature search, identified trials for inclusion and exclusion, assessed the methodologic quality of included trials, and extracted and also verified the data entered by Dr Deshpande in the RevMan software; Prof Bulsara was responsible for conducting the trial sequential analysis and interpreting its results and also contributed to the revised manuscript; and Dr Patole was responsible for the concept, design, interpretation of analysis, and writing of the final version of the manuscript that was seen and approved by all authors. All authors had full access to all of the data (including statistical reports and tables) in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Address correspondence to Sanjay Patole, DrPH, Department of Neonatal Paediatrics, King Edward Memorial Hospital for Women, 374 Bagot Rd, Subiaco, Perth, Western Australia 6008, Australia. E-mail: sanjay.patole@health.wa.gov.au

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**WHAT'S KNOWN ON THIS SUBJECT:** Previous systematic reviews of RCTs indicated significantly lower risk for all-cause mortality and definite NEC and shorter time to full feeds after probiotic supplementation in preterm (<34 weeks' gestation) VLBW (birth weight <1500 g) neonates.

**WHAT THIS STUDY ADDS:** The results of this conclusive updated meta-analysis confirm the benefits of probiotic supplements in reducing death and disease in preterm neonates. Given the totality of the evidence, additional placebo-controlled trials are unnecessary if a suitable probiotic product is available.

## abstract

**OBJECTIVE:** Systematic reviews of randomized, controlled trials (RCTs) indicate lower mortality and necrotizing enterocolitis (NEC) and shorter time to full feeds after probiotic supplementation in preterm (<34 weeks' gestation) very low birth weight (VLBW; birth weight <1500 g) neonates. The objective of this study was to update our 2007 systematic review of RCTs of probiotic supplementation for preventing NEC in preterm VLBW neonates.

**METHODS:** We searched in March 2009 the Cochrane Central register; Medline, Embase, and Cinahl databases; and proceedings of the Pediatric Academic Society meetings and gastroenterology conferences. Cochrane Neonatal Review Group search strategy was followed. Selection criteria were RCTs of any enteral probiotic supplementation that started within first 10 days and continued for ≥7 days in preterm VLBW neonates and reported on stage 2 NEC or higher (Modified Bell Staging).

**RESULTS:** A total of 11 ($N = 2176$), including 4 new ($n = 783$), trials were eligible for inclusion in the meta-analysis by using a fixed-effects model. The risk for NEC and death was significantly lower. Risk for sepsis did not differ significantly. No significant adverse effects were reported. Trial sequential analysis) showed 30% reduction in the incidence of NEC ($\alpha = .05$ and .01; power: 80%).

**CONCLUSIONS:** The results confirm the significant benefits of probiotic supplements in reducing death and disease in preterm neonates. The dramatic effect sizes, tight confidence intervals, extremely low *P* values, and overall evidence indicate that additional placebo-controlled trials are unnecessary if a suitable probiotic product is available. *Pediatrics* 2010;125:921–930

Mortality and morbidities such as necrotizing enterocolitis (NEC), late-onset sepsis (LOS), and feeding difficulties as a result of immature bowel function are a major issue in preterm, especially extremely preterm (<28 weeks' gestation) neonates worldwide. Probiotics may prevent NEC by promoting colonization of the gut with beneficial organisms, preventing colonization by pathogens, improving the maturity and function of gut mucosal barrier, and modulating the immune system (eg, TLR4 receptor, nuclear factor-κB, inflammatory cytokines) to the advantage of the host.[1,2] Many clinical trials have evaluated the safety and benefits of probiotic supplementation in preterm very low birth weight (VLBW) neonates. Deshpande et al[3] first reported a systematic review of randomized, controlled trials (RCTs) of probiotic supplementation in preterm VLBW neonates. The results of their systematic review and meta-analysis were based on 7 trials that involved 1393 preterm VLBW neonates with gestation <33 weeks. The risk for all-cause mortality and NEC was reduced by 53% and 64%, respectively, in neonates who received probiotic supplementation compared with control group neonates. The time to achieve full milk feeds was also significantly less (by an average of ~3 days) in those who received probiotic supplementation.[3] These significant results were subsequently confirmed by 2 more systematic reviews that indicated the tremendous potential of probiotic supplementation in saving preterm neonates from death and disease.[4,5] Expert bodies such as the Cochrane Neonatal Review Group (CNRG) have concluded that except for those who weigh <1000 g (because of lack of specific data in this high-risk population), a change in practice is supported by the data.[5] Individual experts have also commented that on the basis of current data, those who wish to offer probiotic supplementation as a routine therapy for preterm neonates cannot be faulted.[6] Subsequent to these systematic reviews, 4 more RCTs (including 1 multicenter trial) that involved 783 preterm neonates have been reported.[7–10] Given the global burden related to death, NEC, LOS, and feeding difficulties in preterm VLBW neonates and the reported very significant benefits of this low-cost, simple, and easily available intervention, we aimed to update our systematic review[3] of probiotic supplementation of preterm VLBW neonates and study the implications of its results to the current clinical practice and research.[11–13]

## METHODS

Guidelines from CNRG,[14] Centre for Reviews and Dissemination,[15] and the QUOROM statement were followed for this systematic review and meta-analysis.[16] The following prespecified criteria, similar to our previous systematic review, justified inclusion of any trial in the analysis: (1) RCT involving preterm VLBW neonates (<34 weeks' gestation and birth weight <1500 g) and reporting on stage 2 NEC or higher (Modified Bell staging criteria)[17,18] and (2) enteral administration of any probiotic commenced within the first 10 days of life and continued for at least 7 days. The details of the search strategy and approach to analysis are given in the Appendix, which is published as supplemental information at www.pediatrics.org/content/full/125/5/921.

The primary outcome was efficacy of probiotic supplement in preventing stage 2 NEC or higher, safety in terms of blood culture–positive sepsis including that by the organism(s) in the probiotic supplement, and any other adverse events reported by the authors. The secondary outcomes included the time to reach full feeds (TFF; 120–150 mL/kg per day enteral feeds or as per the prestated definitions by authors) and duration of hospital stay.

Trial sequential analysis (TSA) was recently reported as a useful tool to establish when firm evidence is reached in a cumulative meta-analysis.[19] We therefore conducted TSA to evaluate whether the findings of our updated meta-analysis are conclusive.[2,20,21] Cumulative *z* curves, information size, and sequential monitoring boundaries were estimated on the basis of risk for type I error of 5% and type II error of 20%. An intervention effect of 30% for the prevention of NEC was regarded as clinically significant. Moderate heterogeneity was assumed, and a heterogeneity correction factor of 1.33 was applied. Cumulative *z* curve of each cumulative meta-analysis was calculated with a fixed-effect and a random-effect model. The monitoring boundaries were calculated by using the method reported by Reboussin et al.[22]

## RESULTS

A total of 38 potentially relevant citations were obtained by our search method. An additional citation was identified by using text words and searching related articles.[10] The selection process details are given in Fig 1. Four new trials were finally included in the updated analysis after extraction of data from the publications,[7–10] and receipt of additional data for preterm VLBW neonates who were <34 weeks' gestation.[7] One trial ($N = 20$) was subsequently excluded because of unavailability of necessary data from the author.[25] Search of other databases mentioned already did not identify any additional eligible studies. Characteristics of the 11 trials (4 new and the 7 from our previous report[3]) that were included in the analysis ($N = 2176$) are summarized in Table 1.[7–10,27–33] The quality of the trials assessed by Jadad score is sum-

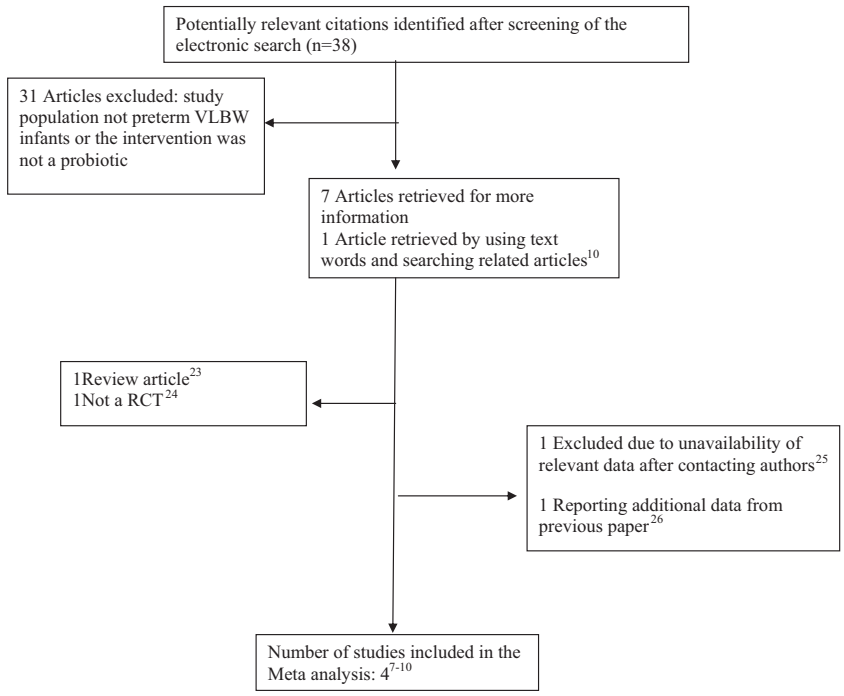


**FIGURE 1**
Flow diagram of the study selection process after screening of electronic search.

marized in Table 2.[34] The results of quality assessment were similar by using CNRG guidelines.

## Effect of Probiotics on Stage 2 or Higher (Definite) NEC

Data on definite NEC were reported by all 11 trials involving 2176 neonates[7–10,27–33] (Fig 2). A higher proportion of neonates in the control group (no probiotics) developed definite NEC compared with the probiotics group (71 [6.56%] of 1082 vs 26 [2.37%] of 1094). Meta-analysis of data by using a fixed-effects model estimated a lower risk (relative risk [RR]: 0.35 [95% confidence interval (CI): 0.23–0.55]; $P <$ .00001) of NEC in the probiotic group. There was no significant heterogeneity ($I^2 = 0\%$, $P = .57$) among the trials. Individually, only 4 trials reported significantly higher risk for NEC in the control group.[8,9,29,30] The numbers needed to treat (NNT) with probiotics to prevent 1 case of NEC was 25 (95% CI: 17–34).

## Effect of Probiotics on Blood Culture–Positive Sepsis

Meta-analysis of data from 10 trials[7–10,28–33] ($N = 2138$) estimated no significant difference in the risk for sepsis between the probiotics and control group neonates (RR: 0.98 [95% CI: 0.81–1.18] $P = .80$; Fig 3); however, there was significant heterogeneity ($I^2 = 52.1\%$, $P = .03$) among the trials. Only 1 trial reported significantly lower risk for sepsis in the probiotic group,[7] and another reported higher risk for sepsis in the probiotic group, which was not significant after adjustment for gestation and birth weight.[6]

## Effect of Probiotics on Mortality

Pooling of data from 9 trials,[7–10,28–30,32,33] ($N = 2051$) showed a reduced risk for death from all causes in the probiotic versus the control group (RR: 0.42 [95% CI: 0.29–0.62]; $P <$ .00001; Fig 4). No significant heterogeneity was noted between the trials ($I^2 = 0\%$, $P = .86$). The NNT to prevent 1 death from all causes by treatment with probiotics was 20 (95% CI: 14–34). Pooling of data ($N = 1335$) from 5 trials,[8,28,30,32,33] showed no significant difference in the risk for mortality as a result of NEC in the probiotic versus the control group (RR: 0.30 [95% CI: 0.08–1.08]). There was no significant heterogeneity among these trials ($I^2 = 0\%$, $P = 0.53$).

## Effect of Probiotics on TFF

Meta-analysis of data ($N = 936$) from 5 trials [8,9,30,32,33] showed significant reduction in TFF in the probiotic versus the control group (weighted mean difference: −5.03 days [95% CI: −5.62 to −4.44]; $P <$ .0001). There was significant heterogeneity ($I^2 = 83.3\%$, $P <$ .0001) among the trials. This difference was not significant after using the random-effects model (weighted mean difference: −2.39 days [95% CI: −5.53 to 0.75]; $P = .14$).

## Sensitivity Analysis

Only 5 of 11 included trials had a primary outcome of NEC or of death and NEC.[8,9,28–30] A sensitivity analysis of these 5 trials ($N = 1717$) showed significant reduction of NEC in the probiotic group (0.29 [95% CI: 0.17–0.49]; $P <$ .00001) and reduction in mortality(0.39 [95% CI: 0.25–0.59]; $P <$ .00001). There was no heterogeneity among all 5 trials ($I^2 = 0\%$, $P =$ .71).[8,9,28–30] Four of these 5 trials with primary outcome of NEC or of death and NEC showed significant reduction of NEC in the probiotic group[8,9,29,30]; however, no difference in sepsis was noted. All trials included in the meta-analysis had Jadad quality score ≥3 (Table 2). A sensitivity analysis based on Jadad score <3 vs ≥3 therefore was not required. The roughly symmetrical funnel plot (Fig 5) suggests that publication bias was unlikely. All results except TFF remained similar by using the random-effects model.

**TABLE 1** Characteristics of Trials Included in the Analysis

| Source | Birth Weight/GA | Probiotic Agent/s | Dosage and Duration | Type of Milk | Primary Outcome |
|---|---|---|---|---|---|
| Kitajima et al,[33] 1997 | <1500 g | BB | $0.5 \times 10^9$ organisms once daily from first feed for 28 d | MM or FM | Gut colonization by BB |
| Dani et al,[28] 2002 | <33 wk or <1500 g | LB-GG (Dicloflor) | $6 \times 10^9$ CFU once daily from first feed until discharge | MM, DM, or FM | UTI, sepsis, NEC |
| Costalos et al,[31] 2003 | 28–32 wk | SB | $10^9$/kg twice daily from first feed for 30 d | FM | Gut function and stool colonization |
| Bin Nun et al,[30] 2005 | <1500 g | BI, ST, BBB | BI $0.35 \times 10^9$ CFU, ST $0.35 \times 10^9$ CFU, and BBB $0.35 \times 10^9$ CFU once daily from first feed to 36 wk corrected age | MM or FM | NEC |
| Lin et al,[29] 2005 | <1500 g | LB-A, BI | LB-A 1004356 and BI 1015697 organisms twice daily from day 7 until discharge | MM or DM | NEC or death |
| Manzoni et al,[32] 2006 | <1500 g | LB-C (Dicloflor) | $6 \times 10^9$ CFU once daily from day 3 of life to 6 wk or discharge from NICU | MM or DM | Gut colonization by *Candia* species |
| Mohan et al,[27] 2006 | <37 wk[a] | BB-L | $1.6 \times 10^9$ CFU once daily from day 1 to day 3 $4.8 \times 10^9$ CFU once daily from day 4 to day 21 | FM | Gut colonization by BB-L and enteric pathogens |
| Stratiki et al,[7] 2007 | 27 to 37 wk[a] | BB-L | Preterm formula $1 \times 10^7$ CFU/g started within 48 h to 30 d | FM | Intestinal permeability |
| Lin et al,[8] 2008 | <34 wk and <1500 g | BBB, LB-A | $2 \times 10^9$ CFU/d for 6 wk | MM or FM | NEC or death |
| Samanta et al,[9] 2009 | <34 wk and <1500 g | BBB, BB-L, BI, LB-A | $2.5 \times 10^9$ CFU/d until discharge | MM or FM | NEC, TFF, sepsis, death, and hospital stay |
| Rougé et al,[10] 2009 | <32 wk and <1500 g | BB-LG, LB GG | $1 \times 10^8$ CFU/d until discharge | MM, DM, or FM | Enteral feed intake at day 14 |

GA indicates gestational age; BB, *Bifidobacterium breve*; LB GG, *Lactobacillus GG*; SB, *Saccharomyces boulardii*; BI, *Bifidobacteria infantis*; ST, *Streptococcus thermophilus*; BBB, *Bifidobacterium bifidus*; LB-A, *Lactobacillus acidophilus*; LB-C, *Lactobacillus casei*; BB-L, *Bifidobacterium lactis*; BB-LG, *Bifidobacterium longum*; CFU, colony-forming units; MM, mother's milk; DM, donor milk; FM, formula milk; UTI, urinary tract infection.

[a] Data for <34 weeks and <1500 g obtained by contacting the authors.

### Results of TSA

TSA results showed evidence to support a 30% reduction in the incidence of NEC ($\alpha = .05$; power: 80%; Fig 6). The cumulative *z* curve crossed the monitoring boundary. The conclusion was unchanged when $\alpha = .01$ was used. Adjusting for 1 interim analysis (first meta-analysis)[3] did not change the conclusion, because the boundary was still crossed. The results were similar for random- and fixed-effect models.

## DISCUSSION

The results of our update confirm those of the previous systematic reviews while improving their precision and further reducing the role of chance alone. The dramatic benefits in terms of reduced risk for all-cause mortality and definite NEC are sustained; however, despite the addition of 4 new trials ($N = 783$) to the existing data, there is still no evidence that probiotic supplementation reduces the risk for LOS.

The incidence of NEC (5%–6% of VLBW neonates) has not changed significantly despite advances in neonatal intensive care.[12] Definite NEC (stage 2 or higher) continues to be a potentially disastrous illness in preterm neonates, with significant mortality (at least 20%–25%) and morbidity, including need for surgery and survival with short bowel syndrome and its consequences such as recurrent sepsis and dependence on total parenteral nutrition (TPN).[12,35] Surgical NEC is associated with prolonged (>6 months) hospitalization and long-term neurodevelopmental impairment (NDI).[36–38] Results of a systematic review of observational studies indicated that the risk for long-term NDI was significantly higher in the presence of at least stage 2 NEC versus no NEC (odds ratio: 1.82 [95% CI: 1.46–2.27]) in preterm VLBW neonates. Those who required surgery were at higher risk for NDI than were those who were treated medically (odds ratio: 1.99 [95% CI: 1.26–3.14]).[38] On the basis of the length of stay, the estimated annual additional hospital charges for NEC have been reported to be as high as $216 666 per survivor in the United States.[39] Given the significant burden of NEC, the benefits of probiotics in this area are extremely significant.

Feeding difficulties that lead to prolonged deprivation of enteral feeds and dependence on TPN are a major issue in extremely preterm neonates. The lack of scientific guidelines for defining and managing signs of "feed intolerance" and the fear of NEC are associated with frequent stoppage of enteral feeds in this high-risk popula-

**TABLE 2** Jadad Score for Assessment of Trial Quality

| Parameter | Kitajima et al,[33] 1997 | Dani et al,[28] 2002 | Costalos et al,[31] 2003 | Bin Nun et al,[30] 2005 | Lin et al,[29] 2005 | Manzoni et al,[32] 2006 | Mohan et al,[27] 2006 | Stratiki et al,[7] 2007 | Lin et al,[8] 2008 | Samanta et al,[9] 2009 | Rougé et al,[10] 2009 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. Randomization | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| A. Method to generate randomization was clear and appropriate | Yes | Yes | Yes | NA | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 2. Double blind? | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| A. Was method for blinding appropriate? | N/A | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | NA | Yes |
| 3. Description of withdrawal or dropout | Yes | No | Yes | No | No | No | No | Yes | Yes | No | Yes |
| Total score | 3 | 4 | 5 | 3 | 4 | 4 | 4 | 5 | 5 | 3 | 5 |

Yes = 1 point; No = 0 points; scores: 0 = worst, 5 = best. NA indicates not available; N/A, not applicable.

tion.[13] Despite the aggressive approach to enteral and parental nutrition, postnatal growth restriction continues to remain a significant issue in this population.[40,41] Given the importance of optimal enteral nutrition in early postnatal life, the benefits of probiotics (eg, improved gastric emptying and gut barrier function, reduction in TFF) have significant implications in improving the overall prognosis of this high-risk population.[7,25] The change in results, from significant (fixed-effects model) to no significant (random-effects model) reduction in TFF, may relate to significant heterogeneity in the feeding protocols in various trials.

Given the lack of specific data on neonates who are fed exclusively breast milk/formula/mixed milk feeds, evaluation of the benefits of probiotics in the presence of different types of milk feeds is difficult. It is important to note that despite its significant benefits, preferential use of breast milk alone has not eliminated the risk for NEC significantly in preterm VLBW neonates. Although specific data are not available from all trials included in our systematic review, the reduction in all-cause mortality may relate to the significant reduction in the incidence of definite NEC and possibly TFF and severe sepsis, leading to an improvement in the general well-being.[8,42]

The reasons for the lack of reduction in the risk for LOS need to be discussed. Colonization of the gut by aberrant flora and its translocation play an important role in LOS in preterm neonates.[11,43] Probiotic microorganisms are expected to colonize the gut, compete with pathogens, improve the gut barrier function and permeability, and modulate immune function.[7,25] The gastrointestinal tract is reported to be the main reservoir of coagulase-negative *Staphylococcus* (CONS), the most frequent organism responsible for LOS in extremely preterm neonates.[44] The inability of probiotics alone to overcome the burden of LOS may thus relate to the presence of not only a single (gut) but also multiple (eg, endotracheal tubes, central venous catheters, TPN solutions, lipid infusions) sources of various pathogens (CONS, Gram-negative, fungi) in the presence of frequent exposure to broad-spectrum antibiotics, prolonged deprivation of enteral nutrition, and an immature immune system in this high-risk population.[8] Mortality from CONS sepsis is low, whereas that related to virulent pathogens (eg, Gram-negative organisms) is high.[44–46] It is not known whether the immunomodulating effects of probiotics are different in CONS versus non-CONS organisms.[47] Benefits of probiotics may thus depend on the type of microorganisms responsible for LOS and, as with any intervention, on the baseline incidence of LOS in various settings. Although probiotic sepsis has been reported in immunocompromised hosts and neonates,[48–50] it is reassuring to know that no significant adverse events, especially probiotic sepsis, have been reported in any of the trials included in our analysis despite the diversity of the populations and the settings of the trials. Nevertheless, we emphasize the need for careful surveillance not only for probiotic sepsis but also for the development of antibiotic resistance and altered immune responses in the long-term.[51,52] Although we do not have the specific data to support this, the risk for translocation of probiotic bacteria across a compromised gut barrier followed by sepsis may be higher in critically sick and/or extremely low birth weight (ELBW) neonates.

Overall, the results of our updated systematic review and meta-analysis (11 good-quality RCTs and $N = 2176$) confirm the dramatic benefits of probiotic supplements in reducing the risk for death and for definite NEC in preterm

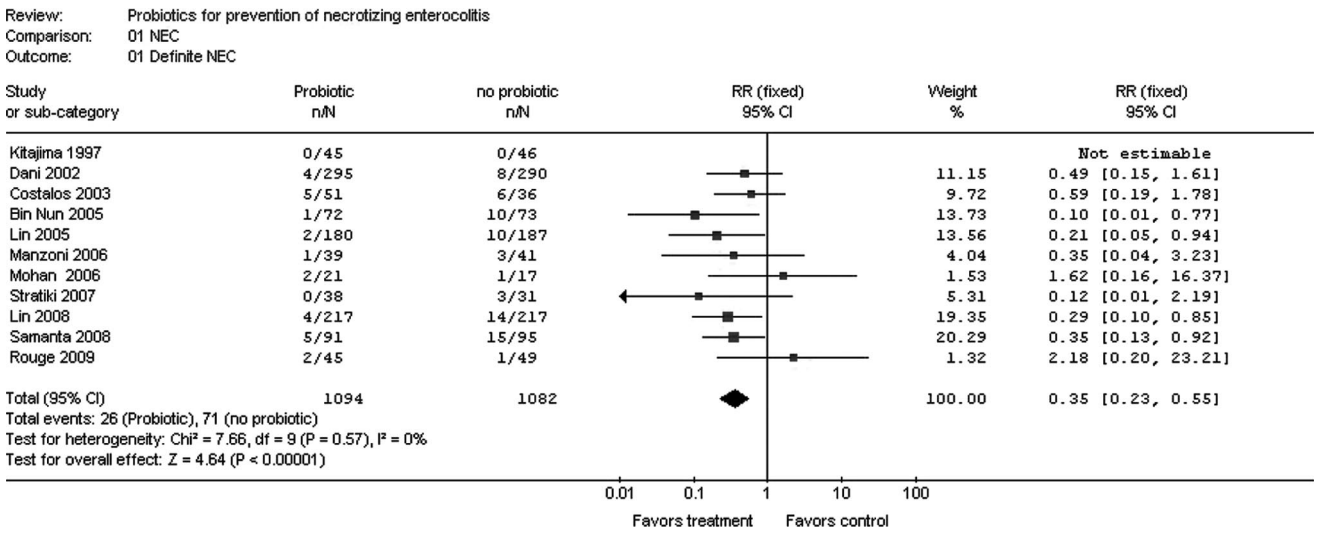


**FIGURE 2**
Effect of probiotics on NEC.

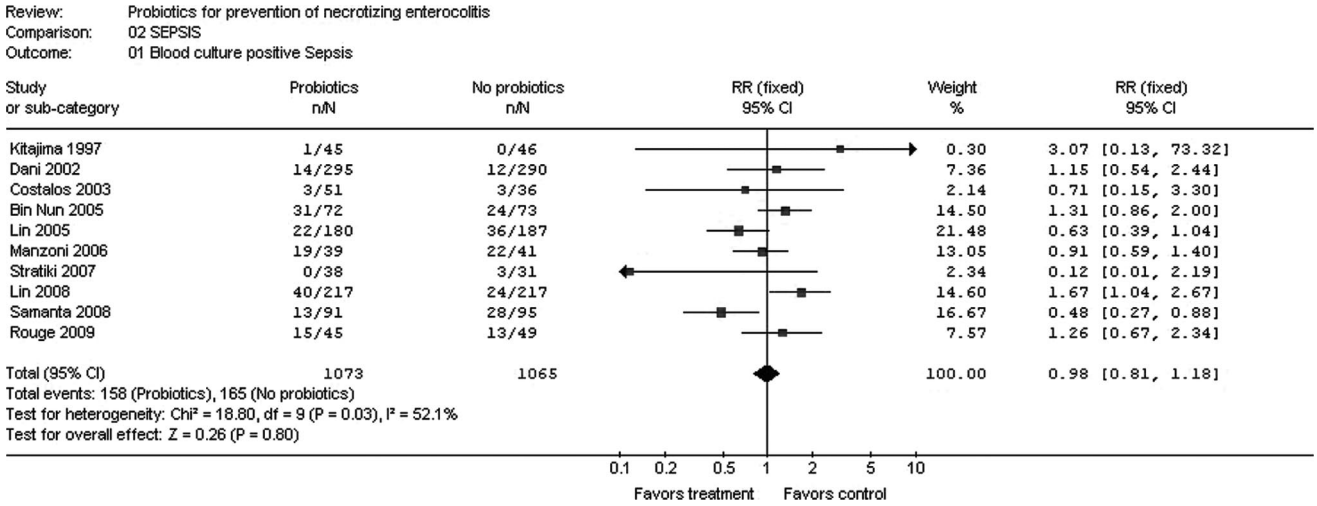


**FIGURE 3**
Effect of probiotics on blood culture–positive sepsis.

VLBW neonates. There is no evidence of a reduced risk for LOS. The significant effect size, precision, consistency of the results across all trials, extremely low *P* values almost ruling out the role of chance alone, low risk for publication bias, no statistical heterogeneity, critical areas of benefit, and the TSA conclusive of at least 30% reduction in the incidence of NEC all indicate that withholding probiotics from high-risk neonates is now almost unethical.[53]

Our findings will have a significant impact on recruitment in the current/planned placebo-controlled trials of probiotics in preterm neonates, because parents have the right to complete and up-to-date information on this topic in a transparent manner. On the basis of our results, we believe that it will be very difficult to justify the need for additional placebo-controlled trials in this population given the significant reduction in definite NEC and all-cause mortality. Moreover, given the sample size and power (Table 3) of the ongoing/planned placebo-controlled trials, it is unlikely that their individual or cumulative results will affect our results significantly. We anticipate that the lack of specific data on extremely preterm (<28 weeks' gestation) neonates and on long-term outcomes and the need to reproduce these results in a setup with possibly a higher standard of care and lower baseline incidence of death and definite NEC could still be pushed forward as the basis for more placebo-controlled trials; however, the data reported by Satoh et al[42] indicate the safety and efficacy of probiotics in ex-

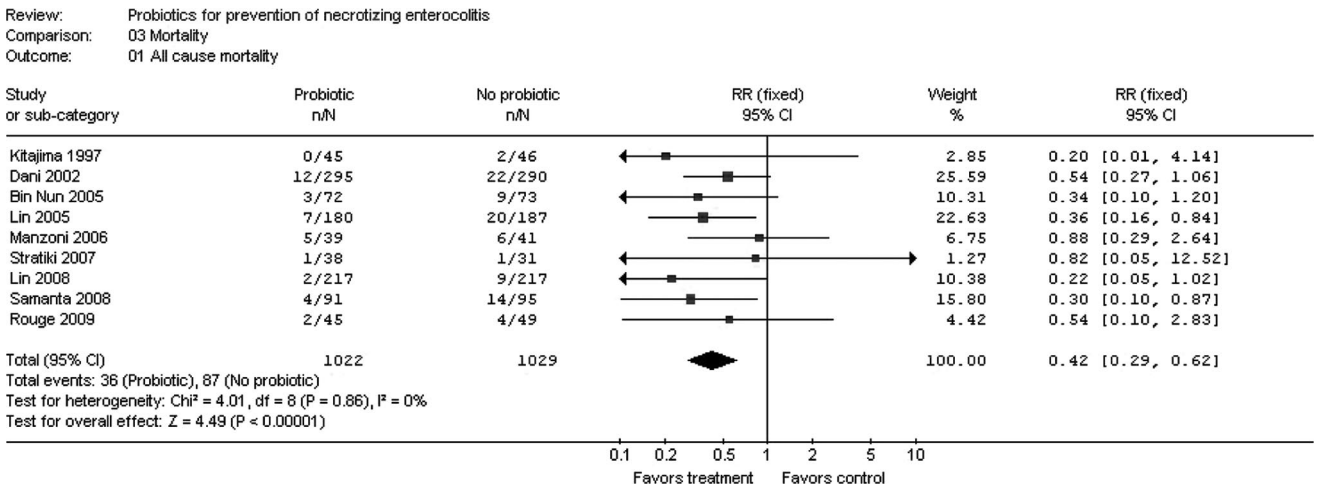


**FIGURE 4**
Effect of probiotics on all-cause mortality.

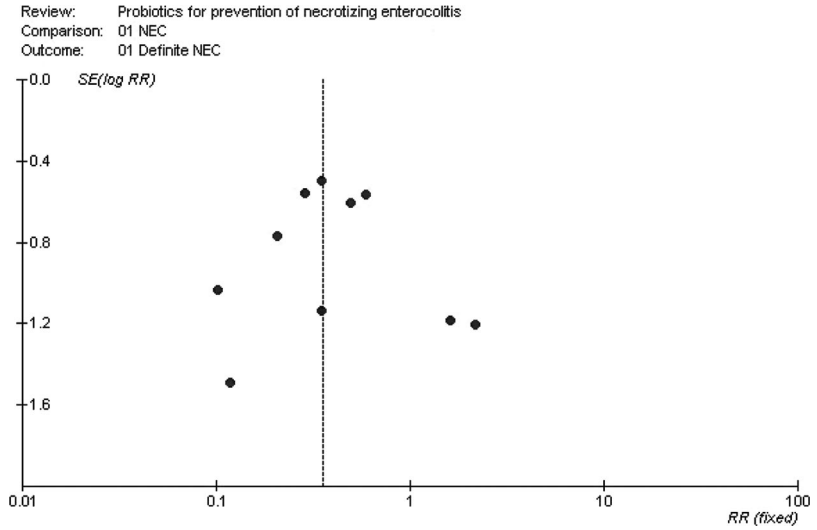


**FIGURE 5**
Funnel plot.

tremely preterm neonates. This observational study from Tokyo compared the data before and after introducing probiotics as a routine therapy in preterm neonates (epoch I [1994–1998, no probiotic]: ELBW = 101 of 226; epoch II [1999–2003, supplementation with *Bifidobacteria*]: ELBW = 220 of 338). *Bifidobacteria breve* (1 billion) supplementation was mixed in milk/formula and started as early as 7.2 hours of age and continued until discharge (37 weeks or 2.3 kg). There was significant reduction in the incidence of NEC, sepsis, and sepsis in death in epoch II versus I (NEC: 6 [2.6%] vs 0 [0%]; sepsis: 65 [28.8%] vs 70 [20.7%]; sepsis in death: 9 [13.8%] vs 2 [0.6%]). The significant benefit in NEC occurred despite the low baseline incidence of the condition. Manzoni et al[54] also recently reported the safety of routine use of probiotics in VLBW neonates ($N = 743$; mean birth weight: 1056 ± 88 g; mean gestation: 29.5 ± 1.1 weeks) during a 6-year period.

Given that probiotics reduce all-cause mortality significantly, it is important to know whether this benefit comes at the cost of an increased number of survivors with long-term NDI. Chou et al[55] recently reported the long-term neurodevelopmental outcomes of neonates (<32 weeks' gestation) in their RCT of oral probiotics for NEC. A total of 83.1% of neonates (probiotics: 153; placebo: 148) from their trial were assessed by Bayley infant developmental assessment tool (BSID-II) at 24 months' corrected age; 1 of 153 and 4 of 148 had died after discharge. There were no significant differences in growth (head circumference, length, and weight), cerebral palsy, blindness, deafness, Mental Developmental Index (<70), and Psychomotor Developmental Index (<70). Given the importance of this issue, it is critical that authors of all trials in this area report long-term neurodevelopmental outcomes of the enrolled neonates. Definite NEC and sepsis both are associated with higher risk for long-term NDI in preterm VLBW neonates.[38,56] Given the significant reduction in definite NEC and possibly severe sepsis[8] after probiotic supplementation, it is difficult to hypothesize that probiotic exposure in early postnatal life will be associated with long-term NDI in preterm neonates. A

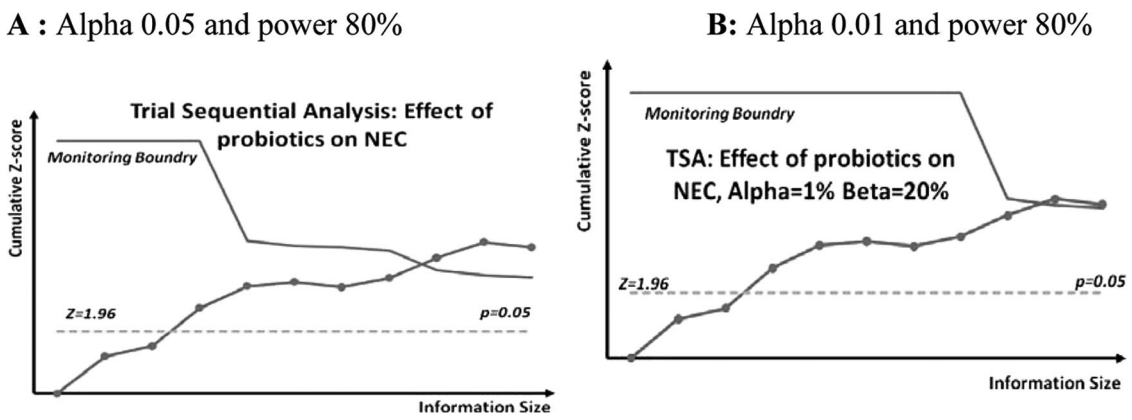


**FIGURE 6**
Trial sequential analysis.

**TABLE 3** Characteristics of Ongoing Placebo-controlled Trials of Probiotics

| Study | Primary Outcome | Inclusion Criteria | Estimated Completion | Sample Size | Effect Size | Power |
|---|---|---|---|---|---|---|
| Braga et al (Brazil)[a62] ISRCTN67165178 | NEC | 750–1500 g | Completed | 630 | NA | NA |
| Costeloe et al (UK)[63] ISRCTN05511098 | Sepsis, NEC, or death | <31 wk | 2013 | 1300 | NA | NA |
| Lozano et al (Colombia)[64] NCT00727363 | Death or neonatal sepsis | <2000 g | 2011 | 1110 | NA | NA |
| Tobin et al (Australia)[65] ACTRN12607000144415 | LOS | <32 wk | NA | 1100 | 33% | 90% |

NA indicates not available.
[a] Completed recruitment results awaited.

significantly large sample size (Table 4) would be required to document the smallest but clinically significant benefit in centers with higher standards of care and lower baseline incidence of definite NEC and death. The potentially preventable number of deaths and cases of NEC in the placebo arm will be an ethically challenging issue in conducting such trials while ignoring the totality of evidence.

## CONCLUSIONS

Considering the robustness of the evidence provided and the very significant benefits in critical areas that outweigh the potential adverse effects, we believe that probiotics should now be offered as a routine therapy for preterm neonates and that additional placebo-controlled trials are not warranted; however, selection of a safe and suitable product with documented probiotic properties and close monitoring of the target population is a must before offering this therapy as a routine in this high-risk but most deserving population.[57,58] Consistent benefits despite significant variations in probiotic strains and protocols indicate that probiotics "in general" are beneficial in this high-risk population in the context of the broader perspective of meta-analysis.[59] It is important to note that the effect of a probiotic bacterium is strain-specific and cannot be extrapolated even to other strains of the same species.[60]

Other important but as yet unanswered questions (eg, product/strain(s), dosage, duration, practicalities of administration) could easily be addressed by well-designed and tightly controlled prospective, observational studies or head-on trials of various strains/combinations/dosages/protocols etc in collaboration with the industry and regulatory agencies.[2,61] Rigorous evaluation of an available and potentially suitable product that has not been tested in this high-risk population may possibly be the only role for additional

**TABLE 4** Estimated Sample Sizes for Various Primary Outcomes in ELBW Neonates

| Primary Outcome | Incidence in Control Group (%) | Incidence in Probiotic Group (%) | % Reduction | Power | $\alpha$ | Sample Size |
|---|---|---|---|---|---|---|
| Definite NEC | 6.0[a] | 4.2 | 30 | 0.8 | .05 | 4908 |
| | 10.7[b] | 7.5 | 30 | 0.8 | .05 | 2658 |
| Death or definite NEC | 30.0[b] | 21.0 | 30 | 0.8 | .05 | 740 |
| | 30.0[b] | 25.5 | 15 | 0.8 | .05 | 2520 |

[a] Figures based on Luig et al.[55]
[b] Figures based on Hintz et al.[66]

placebo-controlled trials in this area. Current evidence makes it unlikely that parents would opt for a 50% chance of their infant's being allocated to a placebo if a suitable probiotic product were available.

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# Updated Meta-analysis of Probiotics for Preventing Necrotizing Enterocolitis in Preterm Neonates

Girish Deshpande, Shripada Rao, Sanjay Patole and Max Bulsara

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