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Benefits of probiotics in preterm neonates in low-income and medium-income countries: a systematic review of randomised controlled trials

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ABSTRACT

Objective Although there is an overall reduction in underfive mortality rate, the progress in reducing neonatal mortality rate has been very slow. Over the last 20 years, preterm births have steadily increased in low-income and medium-income countries (LMICs) particularly in sub-Saharan Africa and South Asia. Preterm birth is associated with increased mortality and morbidity, particularly in LMICs. Based on systematic reviews of randomised controlled trials (RCTs), many neonatal units in high-income countries have adopted probiotics as standard of care for preterm neonates. We aimed to systematically review the safety and efficacy of probiotics in reducing mortality and morbidity in preterm neonates in LMICs.

Design Systematic review and meta-analysis of RCTs.

Data sources Medline, Embase, Cochrane Central Register of Controlled Trials, Cumulative Index of Nursing and Allied Health Literature and E-abstracts from Pediatric Academic Society meetings and other paediatric and neonatal conference proceedings were searched in January 2017.

Eligibility criteria RCTs comparing probiotics versus placebo/no probiotic in preterm neonates (gestation <37 weeks) conducted in LMICs.

Results Total 23 (n=4783) RCTs from 4 continents and 10 LMICs were eligible for inclusion in the meta-analysis using fixed effect model. The risk of necrotising enterocolitis (NEC greater than or equal to stage II) (risk ratio (RR) 0.46 (95% CI 0.34 to 0.61), P<0.00001, numbers needed to treat (NNT) 25 (95% CI 20 to 50)), late-onset sepsis (LOS) (RR 0.80 (95% CI 0.71 to 0.91), P=0.0009, NNT 25 (95% CI 17 to 100)) and all-cause mortality (RR 0.73 (95% CI 0.59 to 0.90), P=0.003, NNT 50 (95% CI 25 to 100)) were significantly lower in probiotic supplemented neonates. The results were significant on random effects model analysis and after excluding studies with high risk of bias. No significant adverse effects were reported.

Conclusion Probiotics have significant potential to reduce mortality and morbidity (eg, NEC, LOS) in preterm neonates in LMICs.

Strengths and limitations of this study

- ► The strengths of our systematic review include its robust methodology, comprehensive nature, large sample size and exclusive focus on randomised controlled trials (RCTs) of probiotics in preterm neonates in low-income and medium-income countries.
- ► The limitations include variations in the probiotic protocols in the included RCTs. Furthermore, nearly 40% of the included trials carried a high risk of bias in many domains of assessment.

INTRODUCTION

The Unicef 2010 report showed that the global burden of underfive mortality was reduced by one-third compared with 1990s; however progress in reducing neonatal mortality has been slow.[1–3] Almost 40% of underfive deaths occur during the neonatal period and majority of these deaths occur in sub-Saharan Africa, South Asia and Oceania. An estimated 98% of all neonatal deaths occur in low-income and medium-income countries (LMICs).[4–6] Out of 135 million births each year, 3.1 million have died within the neonatal period and nearly 35% of these deaths occur in preterm neonates.[2 5] It may be perceived that prematurity is not a problem of LMICs. However, it is important to note that only 8.6% of preterm births occur in developed countries.[5] Over the last 20 years, the number of preterm births has steadily increased to 9.1 million as of 2010 in the regions of sub-Saharan Africa and South Asia. Preterm birth is associated with increased risk of mortality and morbidity including late-onset sepsis (LOS), necrotising enterocolitis (NEC), feeding difficulties and long-term neurodevelopmental impairment.[6–8] Although survival of preterm neonates has improved in some LMICs, morbidities such as NEC and LOS are still a major issue.[5 9–12] Considering the United Nation's (UN's) millennium developmental goal and the UN Secretary-General's Global Strategy for

Women's and Children's Health (2010) and its accompanying 'Every Woman, Every Child initiative, Every Newborn Action plan'(ENAP), it is important to develop cost-effective simple strategies to reduce the mortality and morbidity associated with prematurity in LMICs.[13]

WHO defines probiotics as 'live micro-organisms which when administered in adequate amounts confer a health benefit on the host'.[14] Probiotics have been shown to significantly reduce the risk of NEC, all-cause mortality, LOS and facilitate feed tolerance in preterm very low birth weight (VLBW) neonates.[15–17] The mechanisms of benefits of probiotics include gut barrier enhancement, immune response modulation (eg, TLR4 receptor, nuclear factor-B, inflammatory cytokines) and direct inhibition of gut colonisation by pathogens.[18–22] Many developed countries are already using probiotics routinely in preterm neonates for prevention of NEC.[23–32] It has been suggested that probiotics may have a role in LMICs for prevention, treatment of acute gastrointestinal diseases, particularly in children with HIV infection.[33–36] Given their simplicity and affordability, we aimed to systematically review the safety and efficacy of probiotics in reducing the risk of mortality and morbidity in preterm neonates in LMICs.

METHODS

Guidelines from the Cochrane Neonatal Review Group (http://neonatal.cochrane.org/resources-review-authors),[37] Centre for Reviews and Dissemination (http://www.york.ac.uk/crd/guidance/)[38] and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement[39] were followed for undertaking and reporting this systematic review and meta-analysis. Ethics approval was not required.

Eligibility criteria

Types of studies

Only randomised controlled trials (RCTs) were included in the review. Observational studies, narrative/systematic reviews, case reports, letters, editorials and commentaries were excluded but read to identify potential additional studies.

Types of participants

Preterm neonates born at a gestational age (GA) <37 weeks or LBW (<2500 g) or both (same criteria as the Cochrane review, 2014).[15]

Setting

Only RCTs from LMICs were included. LMICs were defined as per the World Bank guidelines which include countries with gross national income per capita of under US$12 736/year.[40]

Intervention and comparison

Enteral administration of probiotic supplement versus control (placebo/no probiotic).

Outcomes

All-cause mortality, LOS (positive blood/cerebrospinal fluid (CSF) culture on a sample collected 48–72 hours after birth), definite NEC (stage ≥II modified Bell staging)[41] and time to full enteral feeds (TFEF: 120 mL/kg/day).

Search strategy

The databases Medline searched via PubMed (https://www.ncbi.nlm.nih.gov 1966–2017), Embase (Excerpta Medica dataBASE) via Ovid (http://ovidsp.tx.ovid.com, 1980–2017), Cochrane Central Register of Controlled Trials (http://www.thecochranelibrary.com, through January 2017), Cumulative Index of Nursing and Allied Health Literature via OVID (http://ovidsp.tx.ovid.com, 1980–January 2017) and E-abstracts from the Pediatric Academic Society meetings (https://www.pas-meeting.org/about/#past, 2000–January 2017) were searched in January 2017. Abstracts of other conference proceedings such as European Academy of Paediatric Societies and the British Maternal and Fetal Medicine Society were searched in Embase. 'Google Scholar' was searched for articles that might not have been cited in the standard medical databases. Grey literature was searched using the national technical information services (http://www.ntis.gov/), Open Grey (http://www.opengrey.eu/), and Trove (http://trove.nla.gov.au/). We have also searched Literatura Latino-Americana e do Caribe em Ciências da Saúde (LILACS) and Caribmed via the BIREME/PAHO/WHO—Latin American and Caribbean Center on Health Sciences Information; PAHO, Pan American Health Organization (http://lilacs.bvsalud.org/en/) using broad terminologies Probiotics OR Probiotic Or Bifidobacterium OR Bifidobacteria OR Lactobacillus OR Lactobacilli OR Saccharomyces. We also searched ClinicalTrials.gov (https://clinicaltrials.gov), International Clinical Trials Registry Platform (http://www.who.int/ictrp/en/) and BioPortfolio (https://www.bioportfolio.com) for ongoing RCTs. The reference lists of eligible studies and review articles were searched to identify additional studies. Reviewers SR, GJ and GD conducted the literature search independently. No language restriction was applied. The non-English studies were identified by reading the recent systematic reviews of probiotic supplementation for reducing the risk of NEC[42 43] and from cross references of individual studies. Full texts of all non-English studies were obtained via University of Sydney and Department of New South Wales (NSW) health library. A research officer from the NSW Health, University of Sydney translated the articles. Attempts were made to contact the authors for additional data and clarification of methods. Only published data were used for those studies where available.

PubMed was searched using the following terminology: ((('Infant, Newborn' [Mesh]) OR ('Infant, Extremely Premature' [Mesh] OR 'Infant, Premature' [Mesh])) OR ('Infant, Low Birth Weight' [Mesh] OR 'Infant, Extremely Low Birth Weight' [Mesh] OR 'Infant, Very

Low Birth Weight' [Mesh])) AND 'Probiotics' [Majr]. It was also searched using ((('Infant, Extremely Premature' [Mesh] OR 'Infant, Extremely Low Birth Weight' [Mesh] OR 'Infant, Very Low Birth Weight' [Mesh] OR 'Infant, Small for Gestational Age' [Mesh] OR 'Infant, Premature, Diseases' [Mesh] OR 'Infant, Premature' [Mesh] OR 'Infant, Newborn, Diseases' [Mesh] OR 'Infant, Newborn' [Mesh] OR 'Infant, Low Birth Weight' [Mesh])) AND ((('Bifidobacterium' [Mesh]) OR 'Lactobacillus' [Mesh]) OR 'Saccharomyces' [Mesh]). The other databases were searched using similar terminologies. The detailed search terminology is given in online supplementary appendix 1.

Study selection

The abstracts of citations obtained from the initial broad search were read independently by reviewers SR, GJ and GD to identify potentially eligible studies. Full-text articles of these studies were obtained and assessed for eligibility by reviewers SR, GJ and GD independently, using the predefined eligibility criteria. Differences in opinion were resolved by group discussion to reach consensus. Care was taken to ensure that multiple publications of the same study were excluded to avoid data duplication.

Data extraction

Reviewers GD, SR and GJ extracted the data independently using a data collection form designed for this review. Information about the study design and outcomes was verified by all reviewers. Discrepancies during the data extraction process were resolved by group discussion. We contacted authors for additional information/clarifications.

Assessment of risk of bias

Risk of bias (ROB) was assessed using the Cochrane 'Risk of Bias Assessment Tool'.[44] Authors GD, SR and GJ independently assessed the ROB in all domains including random number generation, allocation concealment, blinding of intervention and outcome assessors, completeness of follow-up, selectivity of reporting and other potential sources of bias. For each domain, the ROB was assessed as low, high or unclear risk based on the Cochrane Collaboration guidelines.

Data synthesis

Meta-analysis was conducted using Review Manager 5.3 (Cochrane Collaboration, Nordic Cochrane Centre). Fixed effects model (FEM) (Mantel-Haenszel method) was used. Random effects model (REM) analysis was conducted to recheck the results if there was significant heterogeneity on FEM. Effect size was expressed as risk ratio (RR) and 95% CI.

Statistical heterogeneity was assessed by the χ^2 test, I^2 statistic and visual inspection of the forest plot (overlap of CIs). A P value <0.1 on χ^2 statistic was considered to indicate heterogeneity. I^2 statistic values were interpreted as per the Cochrane handbook guidelines as follows: 0% to 40%—might not be important; 30% to 60%—may represent moderate heterogeneity; 50% to 90%—may represent substantial heterogeneity; 75% to 100%—considerable heterogeneity.[37] The risk of publication bias was assessed by visual inspection of the funnel plot.[45]

Subgroup analysis

(1) Low ROB: random sequence generation and allocation concealment; (2) preterm neonates less than 34 weeks gestation or birth weight less than 1500 g; (3) where *Bifidobacterium* was part of the supplementation; (4) where *Lactobacillus* was part of the supplementation; (5) single strain probiotic were used and (6) multiple strain probiotics were used.

Summary of findings table

The key information concerning the quality of evidence, the magnitude of effect of the intervention and the sum of available data on the main outcome was presented in the 'summary of findings table' as per the Grades of Recommendation, Assessment, Development and Evaluation (GRADE) guidelines.[44]

RESULTS

The literature search retrieved 1926 potential relevant citations. After carefully reviewing the abstracts, 1814 studies were excluded: reviews: 378; observational studies: 187; commentaries: 49; case reports: 147; RCTs in adult and paediatric population: 53 and non-relevant studies: 982. Finally, 23 RCTs (n=4783) conducted in 10 different LMICs in 4 continents were included in the meta-analysis.[12 46–67] The search strategy results are given in online supplementary appendix 1. The flow diagram of study selection process is given in figure 1. The characteristics of the included studies are given in table 1. Out of the 23 included studies, single-strain probiotics were used in 11 studies, whereas 12 used multiple strains. *Lactobacillus* was part of the supplementation in 13 studies; *Bifidobacterium* was part of the supplementation in 11 studies and saccharomyces in 3 studies (table 1).

ROB of included studies

A total of 14/23 (60%) included studies were judged to have low ROB for the domain of 'random sequence generation', and (56%) were considered to have low ROB for 'allocation concealment' (table 2).

Effect of probiotics on ≥Stage II (definite) NEC

Data on definite NEC was reported by 20 trials (n=4022).[12 46–53 55 56 58–65 67] A higher proportion of neonates in the control group developed definite NEC compared with the probiotic group (65/2065 (3.1%) vs 135/1957 (6.9%)). Meta-analysis using a FEM estimated a lower risk (RR 0.46 (95% CI 0.34 to 0.61), P<0.00001) of NEC in the probiotic group. There was no significant heterogeneity (I^2=19%, P=0.22) among the trials. The numbers needed to treat (NNT) with probiotics to prevent one case of NEC was 25 (95% CI 20 to 50; figure 2).

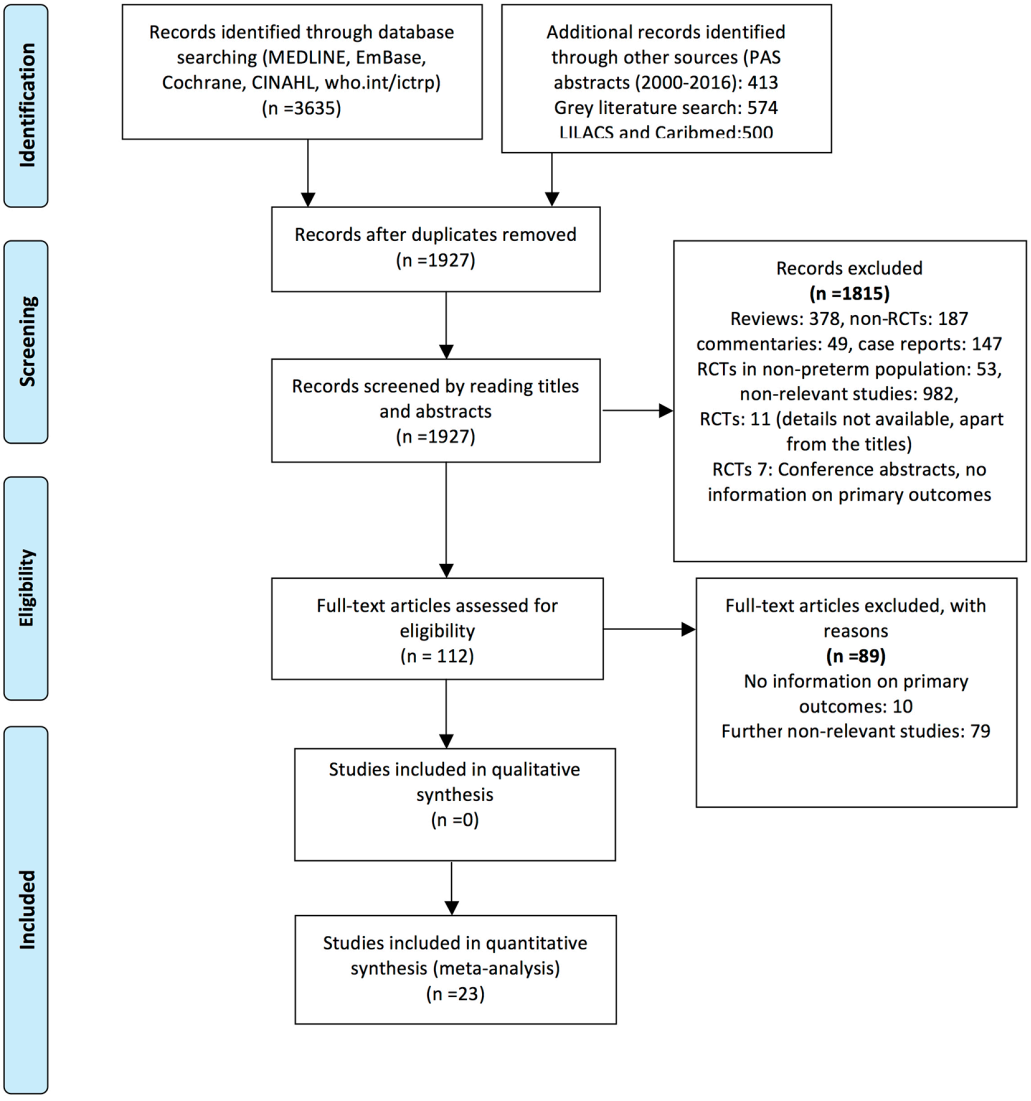

Figure 1 Flow diagram of search strategy and study selection (January 2017). CINAHL, Cumulative Index of Nursing and Allied Health Literature; LILACS, Literatura Latino-Americana e do Caribe em Ciências da Saúde; PAS, Pediatric Academy Society; RCT, randomised controlled trial.

Effect of probiotics on LOS

Data from 18 trials[12 46 47 49 51–54 56–62 64 65 67] (n=4062) showed that a higher proportion of neonates in the control group developed LOS compared with those in the probiotic group (308/2076 (14.5%) vs 358/1986 (18%)). Meta-analysis using a FEM estimated a lower risk (RR 0.80 (95% CI 0.71 to 0.91), P=0.0009) of LOS in the probiotic group. There was no significant heterogeneity (I^2=25%; P=0.16) among the trials. The NNT with probiotics to prevent one case of LOS was 25 (95% CI 17 to 50; figure 3).

Effect of probiotics on all-cause mortality

Data from 19 trials (n=4196),[12 46–49 51–54 56–65] showed reduced risk of death due to all causes in the probiotic versus control group (137/2148 (6.37%) vs 176/2048 (8.59%)). Meta-analysis using a FEM estimated a lower risk (RR 0.73 (95% CI 0.59 to 0.90), P=0.003) of death in the probiotic group. No significant heterogeneity was noted between the trials (I^2=0%; P=0.67). The NNT to prevent one death by probiotic supplement was 50 (95% CI 25 to 100; figure 4).

Effect of probiotics on TFEF

Meta-analysis of data (n=2154) from 13 trials [12 47–49 53 56 59–63 65 66] showed significant reduction in TFEF in the probiotics versus control group (MD=−3.09 days (95% CI: −3.49 to −2.69), P<0.00001). However, there was significant heterogeneity (I^2=90%, P<0.00001) among the trials. These results were hence checked by using REM and remained significant (MD=−1.95 days (95% CI: −3.44 to −0.45), P=0.01; figure 5). MD, mean difference.

Subgroup analysis

The beneficial effects continued to be observed in studies: (1) low ROB: random sequence generation and allocation concealment (table 3); (2) that only included infants with gestational age <34 weeks or birth weight

Table 1 Characteristics of included studies

Study ID	Location	Study characteristics
Awad *et al*[46]	Egypt	*Participants:* all neonates admitted to nursery, 28–41 weeks and weight 1.1–4.3 kg *Intervention and dose:* KP (*L. acidophilus*, 6×10^9 CFU) versus LP (*L. acidophilus,* 6×10^9 CFU) versus placebo *Duration of supplementation:* commenced on D1, duration NA *n=150* (60 vs 60 vs 30), *Preterm: 89* (37 vs 36 vs 16) *Type of milk:* details NA; *Type of delivery:* preterm CS: KP (57%) versus LP (56%) versus placebo (75%) *Primary outcome: all outcomes for LP versus KP versus Controls:* incidence of neonatal sepsis (18/36, 50% vs 25/37, 68% vs 12/16, 75%; P=0.251) and NEC (0/36 vs 1/37 vs5/16; P=0.000) neonates and evaluation of efficacy of a KP *Other outcome: mortality:* 4/36 (11.1%) versus 12/37 (32.4%) versus 5/16 (31.3%), P=0.076
Braga *et al*[47]	Brazil	*Participants:* preterm infants 750–1499 g *Intervention and dose:* (*L. casei* + *B. breve*: 3.5×10^7 to 3.5×10^9 CFU) versus no probiotic *Duration of supplementation:* once daily from the second day of life until day 30 *n=231* (probiotics: 119; controls: 112) *Type of milk:* EBM/PDHM; *Type of delivery:* CS 53.8% vs 49.1% *Primary outcome:* ≥Stage II NEC (0/119, 0% vs 4/112, 3.6%) *Other outcomes: LOS:* 40/119 (33.6%) versus 42/112 (37.5%); *Mortality:* 26/119 (21.8%) versus 27/112 (24.1%)
Dashti *et al*[48]	Iran	*Participants:* preterm infants 700–1800 g *Intervention and dose:* (*L. acidophilus*, *L. rhamnosus*, *B. longum*, *L. bulgaricus*, *L. casei*, *S. thermophilus*, *B. breve* and *Bifidobacterium*: total 1×10^9 CFU/sachet) versus placebo powder *Duration of supplementation:* once daily from first feed of life until discharge *n=136* (probiotics: 69; controls: 67) *Type of milk:* EBM/formula milk; *Type of delivery:* CS 82.4% versus 17.6% *Primary outcome:* ≥Stage II NEC (2/69, 2.9% vs 1/67, 1.5%) *Other outcomes: mortality:* 8/69 (11.6%) versus 4/67 (5.97%)
Demirel *et al*[49]	Turkey	*Participants:* preterm infants ≤32 weeks and ≤1500 g *Intervention and dose:S.boulardii*, 5×10^9 CFU versus no probiotic *Duration of supplementation:* NA *n=271* (probiotic: 135; controls: 136) *Type of milk:* EBM/formula; *Type of delivery:* CS 77.7% versus 83% *Primary outcome:* NEC ≥Stage II (6/135, 4.4% vs 7/136, 5.1%), P=1; *mortality:* (5/135, 3.7% vs 5/136, 3.7%), P=1 *Other outcomes: LOS:* 20/135 (14.9%) versus 21/136 (15.4%) P=0.906; *feed intolerance:* 30/135 (22.2%) versus 62/136 (46%), P<0.001
Deng and Chen[50]	China	*Participants:* 125 preterm infants, <37 weeks, <2500 g at birth *Intervention and dose: B. longum*, *L. acidophilus*, *Enterococcus faecalis*, triple viable powder oral or nasal Bifico plus powder/capsules. For birth weight <1500 g: 0.33×10^7 CFU of each probiotic two times per day and >1500 g: 0.5×10^7 of each probiotic two times per day; control: sterile warm water *Duration of supplementation:* commenced from first feed until 14 days of life *n=125* (*62* controls 33.2±2.3 weeks vs *63* probiotic group 32.4±2.8 weeks), *Type of milk:* EBM/preterm formula; *Type of delivery:* NA *Primary outcome: NEC: controls:* Bell Stage I (1/62, 1.6%), Bell Stage II (4/62, 6.5%), Bell Stage III (4/62, 6.5%) versus *Treatment* Bell Stage I (1/63,1.6%), Bell Stage II (1/63, 1.6%) *Other outcomes: LOS, mortality:* NA
Dilli *et al*[51]	Turkey	*Participants:* VLBW infants with a gestation of <32 weeks and birth weight <1500 g *Intervention and dose: B.lactis* (5×10^9 CFU) versus placebo (maltodextrin) *Duration of supplementation:* from day 8 of life, once daily until discharge or a maximum of 8 weeks *n=200* (probiotic 100; placebo: 100) *Type of milk:* EBM/formula; *Type of delivery: CS:* 35/100 (35%) versus 37/100 (37%) *Primary outcome: NEC (≥stage 2):* 2/100 (2%) versus 18/100 (18%), P<0.001 *Other outcomes: LOS:* 8/100 (8%) versus 13/100 (13%), P=0.6; mortality: 3/100 (3%) versus 12/100 (12%), P=0.003; time to full enteral feeds* (150 mL/kg/day): 18 (14–23) days versus 25 (15–37) days, P<0.001

Continued

Table 1 Continued

Study ID	Location	Study characteristics
Dutta *et al*[52]	India	*Participants:* preterm infants 27–33 weeks gestation *Intervention:* high dose (10 billion CFU: *L. acidophilus*, *L. rhamnosus*, *B. longum*, *S. boulardii*) *versus* low dose (1 billion CFU: *L. acidophilus*, *L. rhamnosus*, *B. longum*, *S. boulardii*) versus placebo (potato starch, maltodextrin) *Duration of supplementation: probiotic groups: (A):* high dose for 21 days, (C): low dose for 21 days, (B): high dose short course (D1–D14 and D15–D21) *N: probiotic (114)* versus *placebo (35)* *Type of milk:* EBM/formula; *Type of delivery:* probiotic group versus placebo: SVD (69% vs 60%), CS: data NA *Primary outcome: stool colonisation rates on D14, D21, D28 with three different probiotic regimens* (*Lactobacillus* and *Bifidobacterium* colonisation was significantly higher in groups A, B and C vs placebo, respectively. Groups A, B and C did not differ from each other. There were trends towards more CFU of *Lactobacillus* and *Bifidobacterium* per millilitre of stool in group A versus B and B versus C. Groups A and B and SPL independently predicted high *Lactobacillus* counts on day 28; groups A, B and C and SPL predicted high *Bifidobacterium* counts) *Other outcomes: LOS:* 10/114 (8.8%) versus 6/35 (17.1%), P=0.14, *mortality:* 8/114 (7%) versus 2/35 (12.7%), P=0.85; *NEC (≥stage 2)*: 6/114 (5.3%) versus 0/35 (0%), P=0.35
Fernández- Carrocera *et al*[53]	Mexico	*Participants:* preterm infants <1500 g *Intervention and dosage:* multispecies probiotic product (*L. acidophilus*+*L. rhamnosus*+*L. casei*+*L. plantarum*+*B. infantis*+*S. thermophilus*) versus no probiotic *Duration of supplementation:* from the day of commencement of enteral feeds, once daily. Actual duration: NA *n=150* (probiotics:75; controls: 75) *Type of milk:* EBM/formula; *Type of delivery:* data not available *Primary outcome:* ≥ Stage 2 NEC: 6/75 (8%) versus 12/75 (16%), P=0.142 *Other outcomes: LOS:* 42/75 (56%) versus 44/75 (58.7%), P=NA; *mortality:* 1/75 (1.3%) versus 7/75 (9.3%), P=0.063
Hua *et al*[54]	China	*Participants:* preterm infants <37 weeks *Intervention and dosage:* probiotic Jin Shuang Qi (*L. acidophilus*, *S. thermophilus*, *Bifidobacterium*) 5×10^7 CFU/day versus no probiotic *Duration of supplementation:* from the day of commencement of enteral feeds, once daily. Duration of supplementation: not clear *n=257* (probiotics:119, controls: 138) *Type of milk:* EBM/formula; *type of delivery:* CS 55.5% versus 64.5% *Primary outcome: stool colonisation by drug-resistant bacteria* (no difference in both groups, P>0.05) *Other outcome: LOS:* 2/119 (1.7%) versus 8/138 (5.8%); P=0.168, *NEC (stage NS):* 0/119 versus 2/138; P=0.501; *Mortality:* 2/119 versus 2/138
Huang *et al*[55]	China	*Participants:* preterm infants 28–32 weeks and <1500 g *Intervention and dosage: Bifidobacterium* (50 million live bacteria/capsule) 0.25×10^8 live bacteria oral/nasally two times per day versus non-treatment (control) *Duration of supplementation:* From 7 days until 14 days of age *n=183* (probiotic: 95, control: 88) *Type of milk:* Not stated; *type of Delivery:* NA *Primary outcomes: NEC:* 2/95 (2.1%), both Bell's stage 1 versus 9/88 (10.23%): Bell's stage 1:6, stage 2:2, stage 3:1 (P<0.01), body mass changes/*weight gain*†: probiotic group: 8.109±2.127 g versus control group 6.489±2.327 g (P<0.01) *Other outcomes: LOS, death; TFEF: NA, gut colonisation:* after 7 days of treatment, the two groups' intestinal bacteria and bacteria ratio of the total number of cocci and rods, the differences were statistically significant (P<0.01). Rod bacteria ratio before and after preventive treatment groups showed no significant difference (P>0.05); in the control group rod bacteria ratio difference was statistically significant (P<0.01)
Oncel *et al*[56]	Turkey	*Participants:* preterm infants ≤32 weeks and <1500 g *Intervention and dosage:L. reuteri DSM 17938* in oil-based suspension, 1×10^8 CFU/day vs placebo (oil-based suspension without probiotics) *Duration of supplementation:* from the time of first enteral feeds until discharge *n=400* (probiotics: 200; placebo: 200) *Type of milk:* EBM/preterm formula; *type of delivery:* CS 75% versus 76% *Primary outcome: probiotics versus controls: ≥ Stage 2 NEC or death:* 20/200 (10%) versus 27/200 (13.5%); P=0.27, *NEC (≥ stage 2):*8/200 (4%) versus 10/200 (5%); P=0.63 *Other outcomes: late-onset sepsis:* 13/200 (6.5%) versus 25/200 (12.5%); P=0.041; *time to full feeds*†:9.1±3.2 versus 10.1±4.3 days; P=0.006; *hospital stay**:38 (10–131) versus 46 (10–180) days; P=0.022; *feed intolerance:* 56/200 (28%) versus 79/200 (39.5%); P=0.015
Qiao *et al*[57]	China	*Participants:* preterm 28–34 weeks GA, >1000 g, <72 hours life *Intervention: Bifidobacterium*, *Lactobacillus*, *Streptococcus thermophilus*, 0.5 g per bag *Duration of supplementation:* 0.5 bag three times daily for 3 days after admission to hospital *n=287* (probiotic: 149 versus control 138) *Type of milk:* not stated; *type of delivery:* no stats on CS/type of delivery *Primary outcomes:* time to full oral feeds (7.3 days vs 16.9 days); P<0.05, time to full enteral nutrition (9.8 days vs 16.9 days); P<0.05, LOS (6.7% vs 15.2%); P<0.05, NEC (3.4% vs 10.9%); P<0.05, hospitalisation time (25.0 days vs 30.8 days); P: NA; mortality†: (6.0±4.0)% and (9.0±6.5)%; P>0.05

Continued

Table 1 Continued

Study ID	Location	Study characteristics
Rojas *et al*[58]	Columbia	*Participants:* preterm infants ≤2000 g *Intervention and dosage:L. reuteri DSM 17938*, 1×10^8 CFU, once daily versus placebo (oil-based suspension without probiotics) *Duration of supplementation:* commenced within 48 hours of life. Duration: NA *n=750* (probiotics: 372; placebo: 378) *Type of milk:* EBM/formula; *type of delivery:* VD non-instrumental: 16% (study) versus 17% (placebo), VD instrumental: 0% (study) versus 0.5% (placebo), elective CS: 18% (study) versus 17% (placebo), non-elective CS 65% (study) versus 65% (placebo) *Primary outcome: nosocomial infection and mortality:* 57/372 (15.3%) versus 67/378 (17.7%); P=0.38; *death:* 22/372 (5.9%) versus 28/378 (7.4%); P=0.41 *Other outcomes: LOS:* 24/372 (6.5%) versus 17/378 (4.5%); P=0.24; *duration of hospitalisation**: 20 (11–33) versus 20 (11–38) days; P=0.53
Roy *et al*[59]	India	*Participants:* preterm infants <37 weeks and birth weight <2500 g *Intervention and dosage:* half of the 1-gram sachet that contained *L. acidophilus* 1.25×10^9 + *B. longum* 0.125×10^9 + *B. bifidum* 0.125×10^9 + *B. lactis* 1×10^9 versus sterile water *Duration of supplementation:* commenced within 72 hours of birth for 6 weeks or until discharge *n=112* (probiotics: 56; placebo: 56) *Type of milk:* EBM; *type of delivery:* CS 83.9% versus 76.8% *Primary outcome: enteric fungal colonisation*†: 3.03±2.33 ×10^5 CFU versus 3±1.5×10^5; P=0.03 and *LOS (bacterial and fungal):* 31/56 (55.4%) versus 42/56 (75%); P=0.02 *Other outcome: TFEF*†:11.22±5.04 versus. 15.41±8.07 days; P=0.016
Saengtawesin *et al*[60]	Thailand	*Participants:* preterm (<34 weeks) and VLBW (<1500 g) infants *Intervention and dosage:* probiotic mixture (*L. acidophilus+B. bifidum* each 1×10^9 CFU/250 mg), 125 mg/kg two times per day versus Nn *Duration of supplementation:* NA *n=60* (probiotics: 31, controls:29) *Type of milk:* EBM/preterm formula; *type of delivery:* CS 67.7% versus 62% *Primary outcome: NEC ≥stage 2:* 1 (3.2%) versus 1 (3.4%); P=0.74 *Other outcomes: LOS:* 2 (6.45%) versus 1 (3.44%); P=0.53, *TFEF*†: 12.03±5.49 days versus 13.76±8.25 days (P=0.64)
Samanta *et al*[12]	India	*Participants:* preterm(<32 weeks) and VLBW (<1500 g) infants *Intervention and dosage:* probiotic mixture (*B. infantis+B. bifidum+B. longum+L. acidophilus*, each 2.5×10^9 CFU), administered two times per day versus no probiotic *Duration of supplementation:* NA *n=186* (probiotics: 91; controls: 95) *Type of milk:* EBM; *type of delivery:* CS 46.15% versus 49.47% *Primary outcomes: Incidence of NEC (≥ stage 2)*: 5/91 (1.1%) versus 15/95 (15.8%); P=0.042, *death due to NEC:* overall death: 4/91 (4.4%) versus 14/95 (14.7%); P=0.032; *feed tolerance: time to full feeds*†: 13.76±2.28 versus 19.2±2.02 days; P<0.001 *Other outcomes: LOS:* 13/91 (14.3%) versus 28/95 (29.5%); P=0.02; *hospital stay*†: 17.17±3.23 versus 24.07±4 days; P<0.001
Sari *et al*[61]	Turkey	*Participants:* preterm infants <33 weeks or birth weight <1500 g *Intervention and dosage: L. sporogenes*, 0.35×10^9 CFU, once a day versus no probiotic *Duration of supplementation:* from first enteral feed until discharge *n=221* (probiotics: 110, controls: 111) *Type of milk:* EBM/formula; *type of delivery:* CS 67.3% versus 75.7% *Primary outcomes: NEC ≥ Stage II:* 6/110 (5.5%) versus 10/111 (9%); P=0.447, *death/NEC:* 9/110 (8.2%) versus 13/111 (11.7%); P=0.515 *Other outcomes: LOS:* 29/110 (26.4%) versus 26/111 (23.4%); P=0.613, *hospital stay:* 34.5 versus 30 days; P=0.919, †: 17.3±8.7 versus 18.3±9.8 days, P=0.438, *feed intolerance:* 49/110 (44.5%) versus 70/111 (63.1%); P=0.006
Serce *et al*[62]	Turkey	*Participants:* preterm infants <32 weeks and <1500 g *Intervention and dosage: Sacch. boulardii* 0.5×10^9 CFU two times per day versus placebo (distilled water) *Duration of supplementation:* from the first enteral feed until discharge *n=208* (probiotic: 104; placebo: 104) *Type of milk:* EBM/formula; *type of delivery:* CS 80.8% versus 88.5% *Primary outcomes: stage ≥2 NEC*: 7/104 (6.7%) versus 7/104 (6.7%); P=1 *LOS:* 19/104 (18.3%) versus 25/104 (24.3%); P=0.29 *Other outcomes: death:* 5/104 (4.8%) versus 4/104 (3.8%); P=0.74, *hospital stay**: 39 (28–60) days versus 43 (29–60) days; P=0.62
Shadkam *et al*[63]	Iran	*Participants:* preterm infants 28 to 32 weeks and 1000–1800 g *Intervention and dose:* (*L. reuteri DSM 17938*: 2.0×10^7 CFU) versus distilled water *Duration of supplementation:* two times per day started once infant reached 40 mL/kg/day of feed until 120 mL/kg/day of feed *n=60* (probiotics: 30; controls: 30) *Type of milk:* EBM/formula milk; *type of delivery:* details NA *Primary outcome:* (Stage NS) NEC (2/30, 6.7% vs 11/30, 36.7%); P=0.005 *Other outcomes: LOS:* 4/30 (13.3%) versus 10/30 (33.4%); P=0.109, *TFEF*†: 12.83±4.26 versus 16.78±6.66 days; P=0.01; *mortality:* 1/30 (3.3%) versus 2/30 (6.7%); P=0.5

Continued

Table 1 Continued

Study ID	Location	Study characteristics
Tewari *et al*[64]	India	*Participants:* preterm infants <34 weeks (two groups: EPT: 27–30+6 weeks and VPT: 31–33+6 weeks) *Intervention: Bacillus clausii* (2.4×10^9 spores per day) versus placebo *Duration of supplementation:* commenced D5 in asymptomatic and D10 in symptomatic neonates and continued for 6 weeks/discharge/death/occurrence of LOS whichever was earlier *n=244* (study: EPT: 61 and VPT: 62) versus(placebo:121) *Type of milk:* EBM/PDHM; *type of delivery:* CS: EPT: 66% versus 59% and VPT: 58% versus 60% *Primary outcome: incidence of definite and probable LOS: definite LOS:* EPT: 6/61 (10%) versus 8/59 (14%); P=0.26; VPT: 2/62 (3%) versus 3/62 (5%); P=0.39; *probable LOS:* EPT: 8/61 (12%) versus 9/59 (15%); VPT: 4/62 (6%) versus 5/62 (7%) *Other outcomes: death:* EPT: 8/61 (13%) versus 9/59 (15%); P=0.84, VPT: 4/62 (7%) versus 5/62 (8%); P=0.79; *NEC (≥ stage 2):* EPT: 0/61 versus 0/59; VPT: 0/62 versus 0/62
Van Niekerk *et al*[65]	South Africa	*Participants:* preterm infants <34 weeks and birth weight 500 to 1250 g *Intervention and dosage:* Pro-52 (*L. rhamnosus* GG and *B. infantis*), 0.35×10^9 CFU of each daily versus placebo (MCT oil) *Duration of supplementation:* from the first enteral feed until day 28 of life *n=184* (probiotic: 91; placebo: 93) *Type of milk:* EBM/formula; *type of delivery:* CS 80.8% versus 88.5% *Primary outcome:* impact of probiotic supplementation on the incidence and severity of NEC in premature VLBW infants that are exposed to HIV. *NEC:* 3/91 (3.3%) versus 6/93 (6.45%) *Other outcomes: LOS:* 15/91 (16.5%) versus 10/93 (10.8%); *death:* 5/91 (5.5%) versus 6/93 (6.45%); *TFEF†: HIV exposed:* 10.19±4.055 versus 9.68±3.46 days, P=0.56 and *HIV non-exposed:* 9.63±2.42 versus 11.14±4.15 days, P=0.022
Yang *et al*[66]	China	*Participants:* 62 preterm infants <37 weeks *Intervention: B. longum, L. acidophilus, Enterococcus faecalis* triple viable powder oral or nasal Bifico plus powder/capsules (probiotics powder/capsules), Shanghai Xinyi Pharmaceutical), 0.5×10^7 CFU two times per day of each *Duration of supplementation:* from commencement of feeds until 14 days of life *n=62* (controls: 31; probiotics: 31) *Type of milk:* EBM/preterm formula; *type of delivery:* NA *Primary outcomes: NEC incidence:* 2/31 (6.45%) versus 3/31 (9.68%) versus (no mention of criteria for NEC used) *Other outcomes: sepsis, mortality, TFEF:* NA
Xu *et al*[67]	China	*Participants:* 125 neonates with a GA of 30–37 weeks and birth weight 1500–2500 g. *Intervention:S. boulardii* CNCM I-745 at a dose of 50 mg/kg (10^9 CFU) two times per day *Duration of supplementation:* 9–28 days (mean 25.3 days) *n=125* (probiotic: 63; control: 62); analysis (probiotic: 51; control: 49) *Type of milk:* EBM/formula; *type of delivery:* NA *Primary outcome:* weight gain was 16.14±1.96 g/kg/day versus 10.73±1.77 g/kg/day; P<0.05 and linear growth was 0.89±0.04 cm/week versus 0.87±0.04 cm/week; P=0.17 *Other outcome:* TFEF: 0.37±0.13 versus 1.70±0.45; P<0.01, maximal enteral feeding volume tolerated: 128.44±6.67 versus 112.29±7.24 mL/kg/day: P=0.03 and duration of hospitalisation: 23.3±1.6 versus 28.0±1.8; P=0.035

For all outcomes, results in the study/probiotic group are given first.
*Median and IQR (25%–75%).
†Mean and SD.
CFU, colony forming unit; CS, caesarean section; EBM, expressed breast milk; EPT, extremely preterm; GA, gestational age; KP, killed probiotic; LGG, *Lactobacillus rhamnosus* GG (ATCC 53103) Gorbach and Goldin; LOS, late-onset sepsis; LP, living probiotic; MCT, medium chain triglycerides; NA, not available; NEC, necrotising enterocolitis; NS, not specified; PDHM, pasteurised donor human milk; SPL, spontaneous preterm labour; SVD, spontaneous vaginal delivery; TFEF, time to full enteral feed; VD, vaginal delivery; VLBW, very low birth weight; VPT, very preterm.

Table 2 Risk of bias of the included randomised controlled trials

Author/reference	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias
Awad *et al*[46]	Unclear risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	Low risk
Braga *et al*[47]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Dashti *et al*[48]	Unclear risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	Low risk
Demirel *et al*[49]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Deng and Chen[50]	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk
Dilli *et al*[51]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Dutta *et al*[52]	Low risk	Unclear risk	Low risk	Low risk	Low risk	Low risk	Low risk
Fernández-Carrocera *et al*[53]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Hua *et al*[54]	Unclear risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	Unclear risk
Huang *et al*[55]	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk
Oncel *et al*[56]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Qiao *et al*[57]	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk
Rojas *et al*[58]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Roy *et al*[59]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Saengtawesin *et al*[60]	Low risk	Unclear risk	High risk	High risk	Low risk	Low risk	Unclear risk
Samanta *et al*[12]	Low risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk	Unclear risk
Sari *et al*[61]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Shadkam *et al*[63]	Unclear risk	Low risk	Low risk	Low risk	Unclear risk	Unclear risk	Unclear risk
Serce *et al*[62]	Low risk	Low risk	Unclear risk	Unclear risk	Low risk	Low risk	Low risk
Tewari *et al*[64]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Van Niekerk *et al*[65]	Low risk	Unclear risk	Low risk	Unclear risk	Low risk	Low risk	Low risk
Yang *et al*[66]	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk
Xu *et al*[67]	Low risk	Unclear risk	Low risk	Low risk	Low risk	Low risk	Low risk

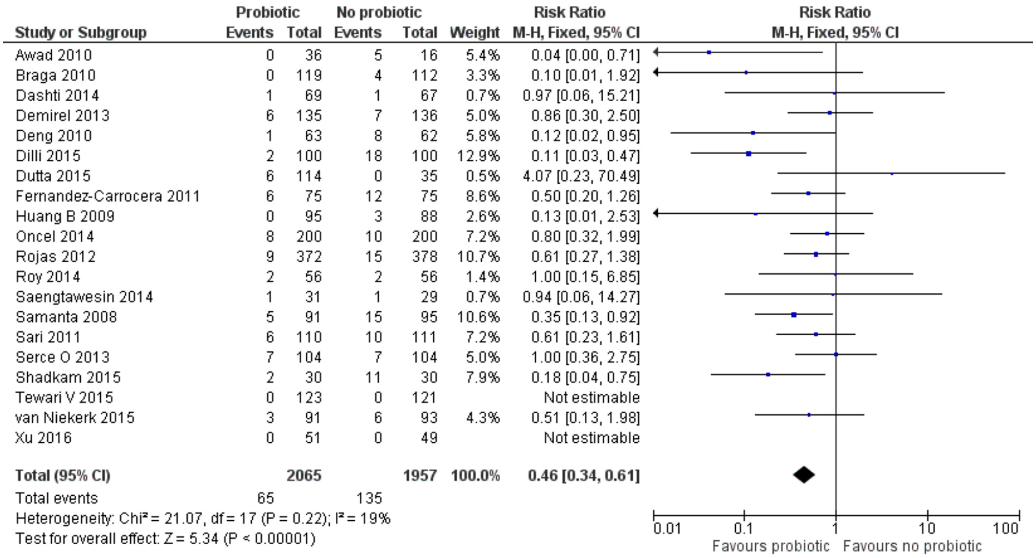

Figure 2 Forest plot: effect of probiotics on definite (≥Stage II) necrotising enterocolitis.

<1500 g; (3) where *Bifidobacterium* was part of the supplementation; (4) where *Lactobacillus* was part of the supplementation; (5) single strain probiotics were used and (6) multiple strain supplements were used; however, on REM meta-analysis, statistical significance was lost for some of these analyses (table 4). The overall evidence according to GRADE guidelines is provided as a summary of findings table (table 5). The evidence was deemed high in view of the large sample size, low risk of bias in majority (14/20) of the included studies, narrow CIs around the effect size estimate, very low P value for effect size estimate and mild statistical heterogeneity. Visual inspection of the funnel plot suggested that there was no publication bias (figure 6).

Safety

None of the studies reported any significant adverse effects including probiotic sepsis.

DISCUSSION

The results of our systematic review of 23 RCTs (n=4783) conducted in 10 LMICs across 4 continents show that probiotic supplementation in preterm neonates (born <37 weeks) significantly reduces the risk of all-cause mortality, LOS and NEC in such a set-up. The limitations of this review include variations in types of probiotics used in different studies and limitations of study qualities in few studies. The strengths of our systematic review include its robust methodology, comprehensive nature and exclusive focus on RCTs of probiotics in preterm neonates in LMICs. The limitations of our review include the variations in the probiotic protocols in the included RCTs, and the fact that nearly 40% of the included trials carried a high risk of bias in many domains of assessment.

To our knowledge, this is the first systematic review focusing on RCTs of probiotics in preterm neonates in LMICs. The summary findings as per GRADE guidelines

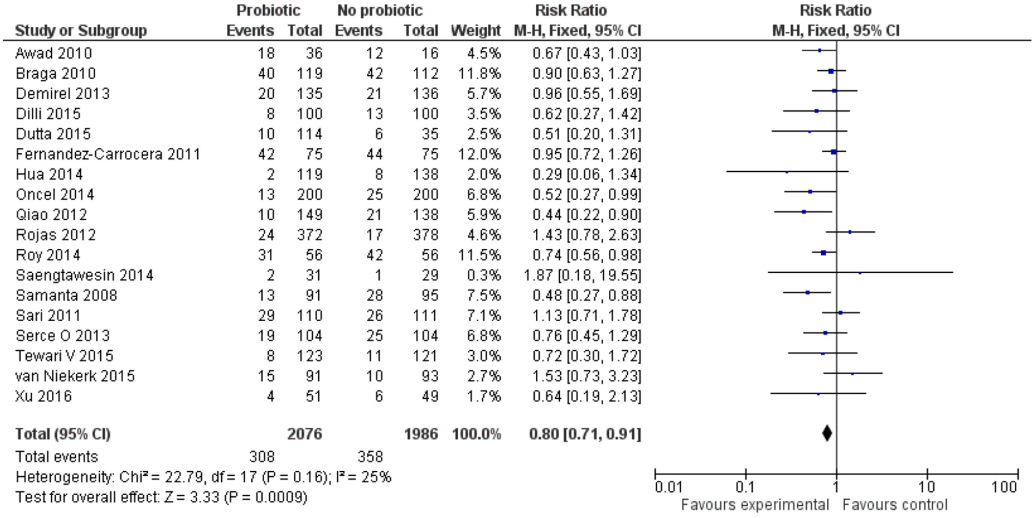

Figure 3 Forest plot: effect of probiotics on late-onset sepsis.

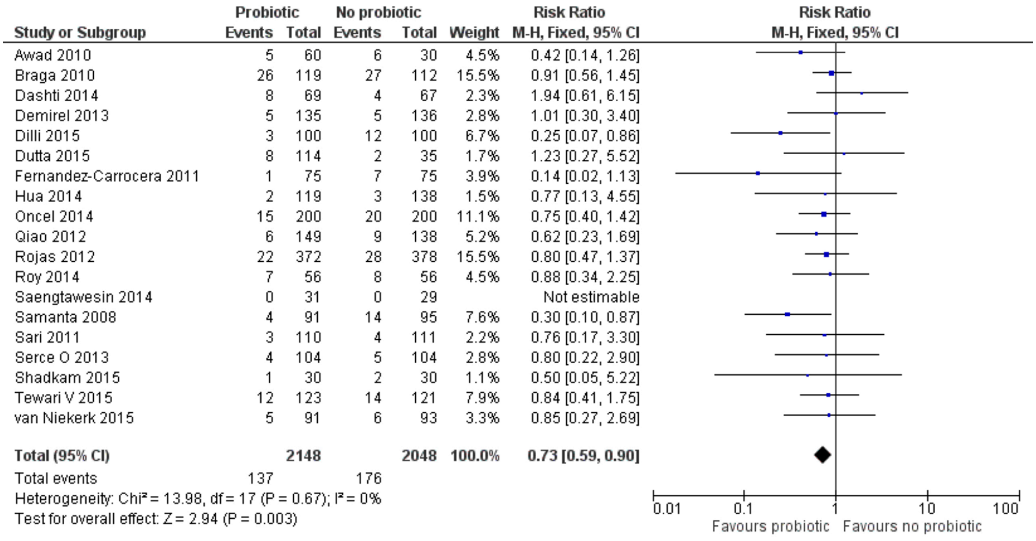

Figure 4 Forest plot: effect of probiotics on all-cause mortality.

confirm the high-quality evidence it provides (table 5). Our results are significant considering the UN's MDG4 and UN Secretary-General's Global Strategy for Women's and Children's Health (2010) and its accompanying Every Woman, Every Child initiative, ENAP and the burden of prematurity in LMICs.[4 5 13]

The incidence of prematurity is significantly increasing in LMICs compared with Europe or North America. There are issues related to reporting of preterm births and outcomes in LMICs.[68] However, the studies funded by the WHO estimate 13 million preterm births/year in LMICs with 11 million (85%) of these being concentrated in Africa and Asia, ~0.5 million each in Europe and North America (excluding Mexico) and 0.9 million in Latin America and the Caribbean.[69] The highest rates (11.9%) and number (seven million) of preterm births were in Africa and Asia, respectively. Mortality and morbidities such as LOS, NEC and feeding difficulties are major issues in preterm neonates. Although specific data from LMICs is not available, approximately one million preterm neonates die every year, predominantly due to sepsis, and long-term impairment in survivors is becoming an important issue.[70]

Consistent with our recent systematic review,[71] our results show that probiotics reduced the risk of NEC and all-cause mortality and of LOS in preterm neonates. (RR 0.81 (95% CI 0.71 to 0.92), P=0.001). The reduction of LOS by probiotics is important considering that neonatal sepsis is responsible for nearly a third all neonatal deaths in LMICs.[19 20 22 72–77]

It is important to note that the burden of NEC is as significant in LMICs as in high-income countries. The incidence and severity of NEC is higher in LMICs and includes up to 15% cases of NEC totalis with ~100% mortality.[9 12] It occurs in VLBW and ELBW neonates and in preterm neonates with higher birth weight. Lack of antenatal steroids and being small for gestational age (SGA) due to intrauterine growth restriction (IUGR) are known risk factors for NEC.[78] The reason for higher incidence of NEC in LMICs could include the higher numbers of preterm 'SGA-IUGR' births and limited coverage of antenatal steroids.[79 80] The NEC-related mortality and morbidity is almost entirely due to progression of the illness from stage II to stage III. Management of surgical NEC is difficult in LMICs considering the limited resources. Primary prevention of NEC is therefore an important strategy for reducing the health burden of the condition in LMICs. Considering the effect size with regards to reduced risk of NEC, the benefits of probiotics in LMICs could not be overemphasised.

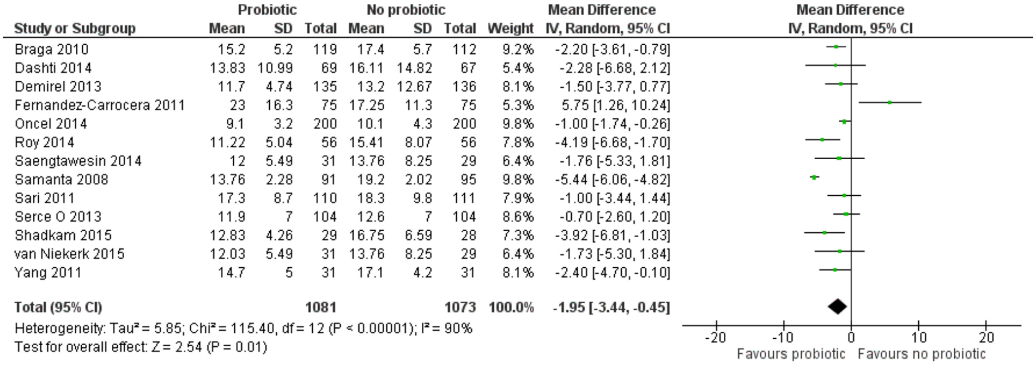

Figure 5 Forest plot: effect of probiotics on time to full enteral feeds.

Table 3 Results of the subgroup analysis (ROB)

Item	Number of studies	Sample size	RR (95% CI) (FEM)	RR (95% CI) (REM)	I^2 statistic (%)
Definite NEC: studies with low ROB on random sequence generation	14	3464	0.55 (0.40 to 0.74)	0.58 (0.42 to 0.81)	1
Definite NEC: studies with low ROB on allocation concealment	13	3035	0.48 (0.34 to 0.66)	0.52 (0.33 to 0.80)	29
LOS: studies with low ROB on random sequence generation	15	3466	0.85 (0.74 to 0.97)	0.84 (0.72 to 0.98)	18
LOS: studies with low ROB on allocation concealment	11	2839	0.86 (0.75 to 0.99)	0.85 (0.74 to 0.97)	6
All-cause mortality: studies with low ROB on random sequence generation	14	3366	0.72 (0.57 to 0.91)	0.75 (0.60 to 0.95)	0
All-cause mortality: studies with low ROB on allocation concealment	13	3073	0.76 (0.60 to 0.96)	0.78 (0.62 to 0.99)	0

FEM, fixed effect model; LOS, late-onset sepsis; NEC, necrotising enterocolitis; REM, random effects model; ROB, risk of bias; RR, relative risk.

Table 4 Results of the subgroup analysis

	Definite NEC			Late-onset sepsis			All-cause mortality		
Item	Number of studies (sample size)	RR (95% CI) (FEM)	RR (95% CI) (REM)	Number of studies (sample size)	RR (95% CI) (FEM)	RR (95% CI) (REM)	Number of studies (sample size)	RR (95% CI) (FEM)	RR (95% CI) (REM)
RCTs with gestational age <32 weeks or birth weight <1500 g	14 (2886)	0.51 (0.37 to 0.70)	0.56 (0.40 to 0.78)	11 (2470)	0.84 (0.71 to 1.01)	0.84 (0.68 to 1.04)	12 (2591)	0.75 (0.61 to 0.93)	0.78 (0.61 to 0.99)
RCTs: *Lactobacillus* was part of the supplementation	13 (2595)	0.45 (0.32 to 0.64)	0.48 (0.32 to 0.71)	12 (2979)	0.81 (0.70 to 0.93)	0.79 (0.64 to 0.97)	16 (3473)	0.70 (0.56 to 0.89)	0.73 (0.58 to 0.93)
RCTs: *Bifidobacterium* was part of the supplementation	11 (1716)	0.35 (0.22 to 0.55)	0.38 (0.23 to 0.63)	9 (1756)	0.76 (0.64 to 0.89)	0.75 (0.59 to 0.94)	12 (2173)	0.70 (0.52 to 0.93)	0.71 (0.49 to 1.03)
Single-strain probiotic supplementation	11 (2727)	0.46 (0.32 to 0.66)	0.46 (0.32 to 0.66)	9 (2446)	0.86 (0.7 to 1.04)	0.83 (0.67 to 1.03)	9 (2444)	0.70 (0.52 to 0.94)	0.71 (0.53 to 0.96)
Multistrain probiotic supplementation	9 (1333)	0.45 (0.28 to 0.73)	0.47 (0.28 to 0.78)	8 (1556)	0.76 (0.65 to 0.90)	0.75 (0.59 to 0.96)	10 (1752)	0.76 (0.56 to 1.03)	0.78 (0.54 to 1.13)

FEM, fixed effect model; NEC, necrotising enterocolitis; REM, random effects model; RR, relative risk.

Table 5 Summary of findings as per GRADE guidelines[38]

Outcome	Absolute risk: Estimate without probiotic supplementation	Absolute risk: Corresponding risk estimate with probiotic supplementation	Relative effect (RR) 95% CI	Number of participants	Quality of evidence GRADE	Comment
Late-onset sepsis	358/1986 (18%)	308/1986 (14.5%)	0.80 (0.71 to 0.91); P=0.0009, I^2=25%	3902	High	Refer note*
Mortality	176/2048 (8.6%)	137/2148 (6.4%)	0.73 (0.59 to 0.9); P=0.003, I^2=0%	4196	High	Refer note*
NEC	135/1957 (6.9%)	65/2065 (3.1%)	0.46 (0.34 to 0.61); P<0.00001, I^2=19%	4022	High	Refer note*

*Note: The evidence was deemed high in view of the large sample size, low risk of bias in majority (14/20) of the included studies, narrow CIs around the effect size estimate, very low P Value for effect size estimate and mild statistical heterogeneity.
GRADE, Grades of Recommendation, Assessment, Development and Evaluation; RR, relative risk.

The issue of implementing probiotics for preterm neonates in LMICs is complex. The options include either reconfirming their safety and efficacy in large definitive RCTs in LMICs or adopting their routine use based on current evidence. Conducting large multicentre trials and accessing proven safe and effective probiotics is difficult, especially in resource-limited set-ups.[34] Apart from the significant budget, the difficulties include regulatory hurdles, logistics of importing a probiotic product, maintaining cold chain and providing ongoing independent safety and quality control. However, there are recent examples of large RCTs conducted successfully in community settings in LMICs.[81–83] Neonatal demographic characteristics, such as gestation and IUGR, are an important issue in conducting RCTs in LMICs as they determine the risk of NEC, duration of probiotic supplementation and the cost-benefit ratio. It is also important to note that many RCTs have used different probiotic/s and probiotic activity could be strain specific.

Knowledge of the pattern of gut colonisation in preterm neonates in a given set-up is important before using probiotics for research or routine use. Dutta *et al* have reported abnormal intestinal colonisation patterns in the first week of life in VLBW neonates in their level III neonatal intensive care unit in India.[52] On day 1, 45% neonates had sterile guts, and by day 3, all were colonised predominantly by *Escherichia coli*, *Klebsiella pneumoniae* and *Enterococcus faecalis*. Only one isolate had lactobacilli and bifidobacteria were not detected during the study period. Formula feeding was associated with *E. coli* colonisation. Results of completed[82] and ongoing trials such as NCT02552706 will be important.[83]

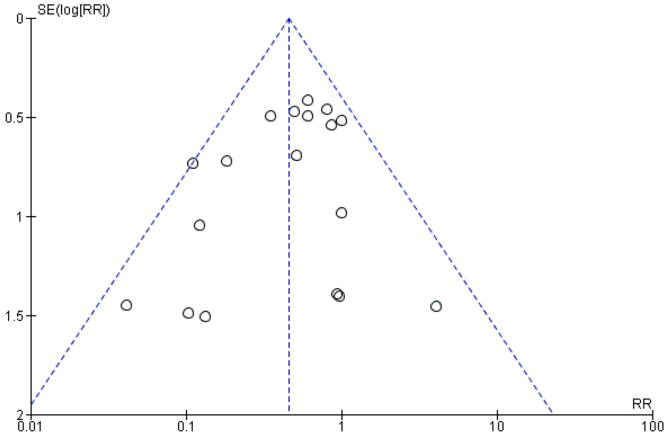

Figure 6 Funnel plot assessing publication bias. RR, risk ratio.

Probiotic sepsis, antibiotic resistance and altered immune responses in the long run are the potential adverse effects of probiotics in preterm neonates. Availability of killed or inactivated probiotic strains with clinically proven benefits may help in avoiding such adverse effects and in avoiding the need to maintain the cold chain. Awad *et al* have compared the effect of oral killed (KP) versus living *Lactobacillus acidophilus* (LP) in reducing the incidence of LOS and NEC in neonates.[46] Both LP and KP reduced the risk of NEC (absolute risk reduction (ARR): 16%, 15%, respectively) and LOS (ARR: 18%) significantly compared with placebo. LOS and NEC was reduced significantly in neonates colonised versus not colonised by *Lactobacillus* at day 7 (27.9 vs 85.9%, 0 vs 7.8%) and day 14 (48.7 vs 91.7% for LOS and 0 vs 20.8% for NEC). KP retained the benefits similar to LP on comparison between all groups. Given the global implications of these results, the benefits of inactivated/killed probiotics need to be assessed in further large definitive trials.

In summary, our results indicate that probiotics are effective in significantly reducing the risk of all-cause mortality, LOS and NEC in preterm VLBW neonates in LMICs. Considering the burden of death, disease (NEC, LOS) and suboptimal nutrition in preterm neonates in LMICs, cooperation between various stake holders (eg, industry, scientists, regulatory agencies) is warranted to either develop or to improve access to high-quality safe and effective probiotics in such set-ups. Support from organisations such as the WHO is important in providing access to probiotics for the countries (eg, sub-Saharan Africa) where most prematurity related deaths occur.

Whether probiotics could be used for research and/or routine use in preterm neonates in LMICs will depend on the national health priorities, resources and ethics.

Contributors GD conceptualised and designed the study, performed an independent literature search, selected studies for inclusion, extracted and interpreted data, assessed risk of bias of included studies, handled the meta-analysis software, oversaw translation of manuscripts in the Chinese language and wrote the first and final drafts of the manuscript. GJ performed an independent literature search, selected studies for inclusion, contacted authors for additional information where necessary, extracted and interpreted the data, checked the data entered by GD on the meta-analysis software, assessed the risk of bias of included studies and helped with the first and the final draft of the manuscript. SR performed an independent literature search, selected studies for inclusion, verified the extracted data, assessed risk of bias, interpreted data and helped with the first and the final draft of the manuscript. SP supervised the project, acted as referee author in case of differences of opinion between the first three authors, interpreted the data and supervised the first and approved the final versions of the manuscript. All authors approved the final manuscript as submitted.

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