Effects of Probiotics in Premature Infants: A Systematic Review and Network Meta-Analysis

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Abbreviations: RR: risk ratio; SUCRA: surface under the cumulative ranking curve, NEC, necrotising enterocolitis; RCT, randomised controlled trial; MD: mean difference; CI, confidence interval; CrI, credible interval; PCoA, principal coordinate analysis.

Table of Contents Summary: Probiotics have been proved to be effective in promoting premature infants' health, but the optimal usage is unknown.

Contributors' Statement:

Prof Yin and Sun conceptualized and designed the study, edited, reviewed and revised the manuscript.
Dr Chi designed the study, selected the articles, extracted and analysed the data, and drafted the initial manuscript.
Dr Li and Wang selected the articles, extracted the data, and analysed the data.
Prof Buys supervised data collection and critically edited the final manuscript.
All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Abstract

CONTEXT: Probiotics have been proved to be effective in promoting premature infants' health, but their optimal usage is unknown.

OBJECTIVE: To compare probiotic supplement methods for preterm infants.

DATA SOURCES: Systematic review and network meta-analysis of PubMed, Embase, Cochrane Collaboration Central Register of Controlled Trials and ProQuest from inception of these databases to 1 September 2019.

STUDY SELECTION: Randomized clinical trials of probiotic supplement intervention for preterm infants. Abstract, title, and full-text screening were conducted independently by 2 reviewers. The primary outcomes were all-cause mortality and the morbidity of NEC. Secondary outcomes were morbidity of sepsis, time to achieve full enteral feeding, and length of hospital stay.

DATA EXTRACTION: This systematic review and Bayesian network meta-analysis was performed following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. The Cochrane Risk of Bias tool was used to assess the quality of studies and data were pooled with random-effects model.

RESULTS: The network meta-analysis included 45 randomised controlled trials with 12,320 participants. In the primary outcomes, *Bifidobacterium* plus *Lactobacillus* was associated with lower rates of mortality (risk ratio [RR] 0.56, 95% credible interval [CrI] 0.34–0.84) and NEC morbidity (0.47, 0.27–0.79) than placebo; *Lactobacillus* plus prebiotic was associated with lower rates of NEC morbidity (0.06, 0.01–0.41) than placebo; *Bifidobacterium* plus prebiotic had the highest probability of having the lowest rate of mortality (surface under the cumulative ranking curve [SUCRA] 83.94%); and *Lactobacillus* plus prebiotic had the highest probability of having the lowest rate of NEC (SUCRA 95.62%).

LIMITATIONS: Few studies report the data of extremely low birth weight infants.

CONCLUSIONS: The efficacy of single probiotic supplements is limited, compared with combined use of probiotics. *Bifidobacterium* plus prebiotic supplementation is the optimal intervention in reducing mortality; *Lactobacillus* plus prebiotic supplementation is the optimal intervention in reducing NEC morbidity. To achieve optimal effect on premature infant health, combined use of prebiotic and probiotic, especially *Lactobacillus* or *Bifidobacterium*, is recommended.

Introduction

Despite improvements in gestation management and healthcare, preterm birth remains a common but serious pregnancy outcome.[1] Partly because of environmental factors and increasing *in vitro* fertilisation in recent years, the prevalence of preterm birth ranges from 5% to 18% across 184 countries,[2] and an estimated 15 million infants are born preterm globally.[3] These infants with an immature immune system and gastrointestinal tract are at risk of complications of premature birth, which is the leading cause of neonatal death.[4]

Recent studies suggested that the composition of infants' gut microbiota is affected by birth weight and gestational age.[5] Altered gut microbiota has been proved to be an important factor putting infants at high risk of developing necrotising enterocolitis (NEC) and sepsis, which may lead to death and lifelong physical impairment.[6, 7] It is evident that early probiotic supplementation may benefit premature infants by improving their gastrointestinal tolerance against potential pathogens and regulating the altered gut microbiota to resemble that of a term healthy infant.[8, 9]

Probiotic with or without prebiotic supplementation is a practicable method among nutrition interventions, and may support gut microbiota colonisation,[10] growth, and long-term neurological development in premature infants.[11, 12] Probiotic supplements in formula may regulate the stability and composition of premature infants' gut microbiota.[9] Recent studies[13, 14] and meta-analyses[15, 16] suggested that probiotic intervention has beneficial effects in premature infants, especially in reducing the mortality and morbidity of NEC and sepsis. Girish

et al.[17] showed that probiotics could reduce the risk of late-onset sepsis and NEC when *Bifidobacterium* or *Lactobacillus* was part of the supplementation through a subgroup analysis in pairwise meta-analysis, but the result was restricted to low and middle income countries. However, previous pairwise meta-analysis only focused on efficacy but could not find the most effective intervention method. It is suggested that there are different effects when different strains or combinations are used.[18] As numerous strains and preparations, including multi-strains without given reasons, have been used in relevant trials, probiotic usage in infants needs to be regulated by more evidence. Previous published network meta-analyses compared the effect of different strains also have several shortcomings in their methodology or design. Due to the robust methodology in the systemic study search, our study aimed to include a larger number of articles compared with one study which has similar scope and inclusion criteria.[19] In addition, the present network analysis tried to rank the efficacy of strains used by each intervention whichprevious study failed to do the ranking . [20] Thus, these studies did not point out which strain is an optimum option for infants' health, and failed to provide powerful evidence for clinical use of probiotics. In the present study, we examined the effect of probiotics in premature infants and figured out the optimal intervention through a network meta-analysis approach based on direct and indirect evidence from randomised controlled trials (RCTs).

Methods

Search strategy and selection criteria

This systematic review and Bayesian network meta-analysis was performed according to the guidelines from the Cochrane Neonatal Review Group (http://neonatal.cochrane.org/resources-review-authors), and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA) statements.[21, 22] The protocol of this study was created and registered in the PROSPERO database, with the registration number CRD42016033063.

We searched for randomised trials in the Cochrane Collaboration Central Register of Controlled Trials, MEDLINE/PubMed, and Embase, using a combination of MeSH and free text, from inception of these databases to 1 July 2020 with no language restrictions. To identify any unpublished studies, we searched degree theses in the ProQuest database. We also hand searched references of all included studies to identify review articles and related meta-analyses. Studies were identified with the following terms and their combinations: premature infant, low birth weight infant, probiotics, synbiotics (both as MeSH and free text terms), and names of common probiotics (as free text terms). Two researchers independently conducted the literature search and reviewed the titles, abstracts, and full-text papers to determine whether they met the inclusion criteria. Any conflicts were resolved through discussion with a third author.

The population of interest was premature infants who were born with a birth weight of less than 2500 g or a gestational age under 37 weeks. Probiotics or placebo was supplied to different groups allocated by randomization. Outcome variables included incidence of death, NEC,

sepsis, time to achieve full enteral feeding, and length of hospital stay. Only RCTs that compared at least two interventions or one intervention with placebo matched the eligible trial design. In addition, studies were included if they compared probiotic interventions with nystatin, which could act as an intermediate variable in further indirect comparisons.

Data extraction

Our primary outcomes were all-cause mortality and the morbidity of NEC, which was defined as cases that had reached Bell's stage 2 or higher, thereby excluding mild and doubtful cases. Secondary outcomes were morbidity of sepsis, time to achieve full enteral feeding, and length of hospital stay. If one trial includes multi-arms, the data of each arm were extracted and included in further analysis. Data were extracted directly from the articles by two independent authors using a standardised form. Disagreements were resolved by consensus, if needed, with a third author.

Data synthesis and statistical analysis

First, we performed pairwise meta-analyses with R software (version 3.5.1). We carried out random-effects model meta-analyses to obtain effect sizes for primary and secondary outcomes, and presented dichotomous outcomes as risk ratio (RR) and continuous outcomes as standardised mean difference (MD) with 95% confidence interval (CI) separately.

Second, we performed random-effects network meta-analyses of dichotomous outcomes, including mortality, morbidity of NEC, and sepsis, using a Bayesian framework with Markov

chain Monte Carlo methods in JAGS (version 4.3.0) and R software. Trials used the same intervention were merged into nodes weighted by participants, and the edges between nodes represented for the direct comparison between the two interventions. The network meta-analysis of the continuous outcome, time to full enteral feeding, was also performed in a Bayesian framework by using GeMTC (version 0.14.3). All analyses were run on four chains with 20,000 iterations per chain, including a burn-in period of 1000 runs. We used surface under the cumulative ranking curve (SUCRA) probabilities to rank the interventions for an outcome. The SUCRA probability of each intervention was expressed as a percentage of the efficacy of the intervention relative to an imaginary intervention that always turned out to be the best method.[23] The larger the surface under the curve, the better the rank of the intervention. We used principal coordinate analysis (PCoA) based on SUCRA values to display the overall ranking distribution across the five parameters via dimension reduction. Inconsistency between direct and indirect evidence, which could lead to inconsistency of the model, was assessed by the node splitting method and inconsistency plot performed with Stata.[24] The Cochrane risk of bias tool for RCTs[25] was used to assess the risk of bias of the studies. Additionally, funnel plots were used to investigate signs of publication bias. A two-sided *p* value of less than 0.05 was regarded as statistically significant.

Results

Search Results and Study Characteristics

The initial search yielded 574 articles, and 113 potentially eligible articles were retrieved in full text and 45 eligible articles were included in the final analysis (Figure 1). These 45 RCTs were performed between 2002 and 2018, and compared 14 different interventions or placebo. The characteristics of included trials and quality assessment results are presented in the appendix (Table S1). In total, 12,320 participants were included in these trials and randomly allocated into intervention groups (n = 6577) and placebo group (n = 5743). Samples were drawn from 19 different locations including America,[26-32] Asia,[33-46] Europe,[8, 47-66] Africa,[67] and Australia.[68, 69] Most RCTs included comparison of two arms (n = 41), but some included three (n = 3) or four (n = 1) arms. The duration of treatment varied from 2 weeks to 9 weeks or covered the infant's hospitalisation. Baseline parameters, including gestational age, birth weight, sex, and sample size, were similar across all the arms of the same study.

Pairwise meta-analysis outcomes

In pairwise comparisons for the primary outcomes and secondary outcomes (appendix Figure S1), no evidence of statistical heterogeneity was seen in general. The pairwise meta-analysis showed that *Lactobacillus* plus prebiotic, *Bifidobacterium* plus prebiotic, and *Bifidobacterium* plus *Lactobacillus* were associated with lower rates than placebo for mortality and NEC morbidity. *Lactobacillus* plus prebiotic was superior to placebo in sepsis morbidity;

Bifidobacterium plus prebiotic and *Bifidobacterium* plus *Lactobacillus* were superior to placebo in the time to full enteral feeding and length of hospital stay.

Network meta-analysis outcomes

Figure 2 shows the networks of eligible comparisons for primary outcomes, including mortality and NEC morbidity. The network plots of secondary outcomes, including sepsis morbidity, time to full enteral feeding, and length of hospital stay, are provided in the appendix (Figure S2). These five network plots indicate that all the interventions (except nystatin) had at least one direct comparison with placebo. The weight of each direct comparison, depending on the variance of observed effect and network structure, is presented in the contribution plots (appendix Figure S3).

Figure 3 shows the result of each intervention compared with placebo in the network meta-analysis. In terms of primary outcomes, *Bifidobacterium* plus *Lactobacillus* was associated with lower rates of mortality (RR 0.56, 95% CI 0.34 - 0.84) and NEC morbidity (RR 0.47, 95% CI 0.27 - 0.79) than placebo, and *Lactobacillus* plus prebiotic was associated with lower rates of NEC morbidity (RR 0.06, 95% CI 0.01 - 0.41) than placebo. In terms of secondary outcomes, *Lactobacillus* plus prebiotic was associated with lower rates of sepsis morbidity (RR 0.18, 95% CI 0.06 - 0.44) than placebo, and *Bifidobacterium* plus *Lactobacillus* led to a reduction of time to full enteral feeding (RR 3.97, 95% CI 1.65 - 5.74) and length of hospital stay (RR 7.30, 95% CI 0.99 - 14.13) compared with placebo.

Overall results of the network meta-analysis including pairwise comparisons of primary (Figure 4) and secondary (appendix Figure S4) outcomes between different interventions are presented in the league table. *Lactobacillus* plus prebiotic was associated with lower rates of NEC morbidity (RR 0.13, 95% CI 0.01 - 0.90) and sepsis morbidity (RR 0.21, 95% CI 0.07 - 0.53) than *Bifidobacterium* plus *Lactobacillus*. In terms of time to full enteral feeding, *Lactobacillus* plus prebiotic was superior to *Bifidobacterium* plus *Lactobacillus* (RR 4.84, 95% CI 1.90 - 13.63).

Bayesian Markov chain Monte Carlo modelling demonstrated that *Bifidobacterium* plus prebiotic had the highest probability of having the lowest rate of mortality (SUCRA 83.94%; Figure 5A), followed by *Lactobacillus* plus prebiotic (SUCRA 79.69%) and *Bifidobacterium* plus *Lactobacillus* (SUCRA 73.81%). *Lactobacillus* plus prebiotic had the highest probability of having the lowest rates of NEC and sepsis (SUCRA 95.62% and 98.85%, respectively; Figure 5B and C). *Bifidobacterium* plus *Lactobacillus* had the highest probability of being the most effective intervention in reducing the time to full enteral feeding (SUCRA 89.41%; Figure 5D) and the length of hospital stay (SUCRA 82.13%; Figure 5E). The five-dimensional graph containing all five parameters was dimension reduced to a two-dimensional graph using the PCoA method. This graph was generated to show the efficacy of different interventions of probiotics in alleviating the mortality, NEC morbidity, and sepsis morbidity, as well as reducing the time to full enteral feeding and length of hospital stay (Figure 5F). In this cluster rank plot, placebo located in the bottom right, while three interventions located in the top left, including *Bifidobacterium* plus *Lactobacillus*, *Bifidobacterium* plus prebiotic, and *Lactobacillus* plus

prebiotic; these turned out to be the most effective probiotic interventions when considering all five parameters. It is notable that *Lactobacillus* and *Bifidobacterium* supplements alone located in the middle of the plot, which meant that their efficacies turned out to be normal compared with other strains and worse than combination interventions, such as *Lactobacillus* plus prebiotic and *Bifidobacterium* plus *Lactobacillus*.

Risk of Bias

Inspection of the funnel plot (appendix Figure S5) did not show significant asymmetry, which suggests low risk of publication bias with each outcome selected, and demonstrated that no small-study effects existed. The result of the inconsistency test is presented in appendix Figure S6. There was no significant difference between direct and indirect estimates in closed loops, which ensured the assessment of network coherence for each comparison for all five parameters. We performed bias assessment for each RCT and generated a summarised graph (appendix Figure S7). Although some studies were considered high risk because of blinding of outcome assessment and incomplete outcome data, most of the included studies were at low risk of bias in all components. The overall quality of evidence contributing to network meta-analysis (appendix Figure S10) was assessed with the Grading of Recommendations Assessment, Development and Evaluation (GRADE) profiler[70] software (version 3.6).

Discussion

In this study, we included 45 studies to investigate which probiotic strain has the best effect on the health of premature infants. To our knowledge, this is the first network meta-analysis

comparing efficacy of different probiotic supplements in premature infants' health. The results suggest that the rates of mortality, NEC morbidity, and sepsis morbidity, as well as the time to full enteral feeding and length of hospital stay, may be reduced by combined use of any two of *Lactobacillus*, *Bifidobacterium*, and prebiotic.

Intestinal mucosa is a natural barrier for migration of bacteria as well as their products. This barrier may also exclude potential pathogens competitively, modify the host response to endotoxin, inhibit the colonisation of pathogens, and upregulate the immune responses.[71] It is acknowledged that premature infants are less developed in their immune system and intestinal mucosa barrier, and have a distinct gut microbiota.[72] These physical characteristics put them at risk of death and morbidity of infectious diseases, especially NEC and sepsis.[73, 74] Undoubtedly, breastmilk is the best nutrition for newborn infants.[75] However, some mothers cannot provide enough breastmilk to their preterm infants.[76] Beyond breastmilk, these infants need to intake formula or other preparations as a nutrition supplement, in which probiotic intervention could play an important role in promoting health of preterm infants.

Our results performed indirect comparisons between different strains and placebo, suggesting that the use of *Bifidobacterium* and *Lactobacillus* is effective in all the primary and secondary outcomes, which is consistent with the results of direct comparisons from previous pairwise meta-analysis. The included RCTs have estimated the effect of six different probiotic strains (*Bifidobacterium*, *Lactobacillus*, *Enterococcus*, *Saccharomyces*, *Streptococcus*, and *Bacillus*), and their combinations on the health parameters of premature infants. Notwithstanding the attempts of trials using other strains, our results showed that interventions involving

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Bifidobacterium and *Lactobacillus* have more beneficial effects in each health parameter. AlFaleh et al.[15] suggested that probiotic preparations containing either *Lactobacillus* alone or in combination with *Bifidobacterium* are effective in infants at risk of NEC, which is consistent with our results. In general, *Bifidobacterium* and *Lactobacillus* are more effective than other probiotic strains.

Further, our results demonstrated that combined use of probiotics may have better efficacy in premature infants than the single strain. As shown in Figure 5, combined use of probiotics ranks higher than single use both in each parameter and in general. This is consistent with Guthmann et al.,[77] who reported that two or more probiotic strains or a combination of *Bifidobacterium* and *Lactobacillus* achieves the best results in preterm infants. Thus, in further design of trials and clinical use, combination of probiotics and their synergistic effect should be taken into account.

In addition, our results showed that the combined use of prebiotic with *Bifidobacterium* or *Lactobacillus* was more effective in preterm infants. Previously, we conducted a pairwise meta-analysis[78] showing that prebiotic treatments may reduce the rate of mortality but have little benefit in the morbidity of NEC. Premature infants, especially those at high risk, usually receive excessive antibiotic treatment, which may dramatically affect the composition of their gut microbiota. This imbalance of gut microecology cannot be addressed by single use of probiotic or prebiotic. Previous RCTs[31, 33, 37, 52, 56] and meta-analyses[78, 79] suggested this problem could be solved by the combined use of probiotic and prebiotic, which is also mentioned as synbiotic supplementation. The synbiotic might contribute to colonisation of the probiotic. This has been

proved by stool cultures after 7 to 14 days of intervention. Manzoni et al.[56] and Underwood et al.[31] compared the stool microbiota in two groups of premature infants receiving *Lactobacillus* alone or combined use of *Lactobacillus* and prebiotic, and found that the group receiving synbiotic supplementation exhibited augmented *Lactobacillus* colonisation. Similarly, the same acceleration effect of prebiotic on *Bifidobacterium* colonisation in low birth weight infants' intestinal tract was found by Dilli et al.[52] and Chi et al.[9] Prebiotic interacts with the probiotic, leading to a synergistic effect to boost the antibacterial defence of infants' immature intestinal tract barrier.[80] In addition, prebiotic supplements have been proved to accelerate intestinal maturation in preterm infants.[81] According to the RCTs[31, 33, 37, 52, 56] and Cochrane reviews,[15, 82] combined use of probiotic and prebiotic was described to be well tolerated and safe in infants. Further, the dosage and course of treatment should be individualised according to the birth weight or gestational age of each infant. Infants weighting under 1500 g need a higher dosage and longer course to ensure the effect of prebiotic usage.[56]

Limitations

The effect of *Lactobacillus* plus *Bifidobacterium* plus prebiotic on all five parameters was not distinguishable from those of all 14 interventions. As the combined use of any two of *Lactobacillus*, *Bifidobacterium*, and prebiotic achieved a remarkable effect, the combined use of all three should have presented a cumulative effect. It is possible that *Lactobacillus* and *Bifidobacterium* target the same mechanism(s) in infants' intestinal tract, therefore excluding the cumulative effect. Alternatively, this may be attributed to the limited sample size and dosage used in relevant studies. In further study, more RCTs using *Lactobacillus* plus *Bifidobacterium*

plus prebiotic should be performed and the optimal dosage determined in consideration of both safety and efficacy. Another limitation of this study is insufficient data in extremely low birth weight infants. Only two RCTs set their inclusion criteria as infants with a birth weight of less than 1000 g. According to the results of Al-Hosni et al.[26] and Wejryd et al.,[63] probiotic supplementation is safe in infants with a birth weight less than 1000 g. Nevertheless, there is always a hypothetical risk of sepsis infection caused by probiotic intervention, especially in extremely low birth weight infants whose immune system may be significantly immature. In addition, previous guidelines[83] listed extremely low birth weight as one of the contraindications. Thus, in premature infants with a gestational age less than 27 weeks or a birth weight less than 1000 g, more evidence and studies are needed to prove the safety of probiotic supplementation.

Strengths

To our knowledge, this is the first network meta-analysis figures out the rankings of common probiotics use, and the optimal strains for in premature infants, whereas most previous pairwise meta-analysis focused on whether probiotics are effective or not. Thus, this study may provide new evidence for researchers to choose strains and methods when designing studies to give premature infants nutritional intervention individually and precisely.

Conclusion

In this network meta-analysis, we found that the efficacy of single probiotic supplements is limited. Notably, the risk of death was the lowest in premature infants who received *Bifidobacterium* plus prebiotic supplements. In addition, *Lactobacillus* plus prebiotic has the

highest probability of being the optimal intervention for reducing NEC morbidity. To achieve optimal effects on premature infants' health, combined use of prebiotic and probiotic, especially *Lactobacillus* or *Bifidobacterium*, is recommended in further study design.

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Figure Legends

Figure 1: Flowchart
Flowchart of study selection.

Figure 2: Network Graphs

Network of eligible comparisons for mortality (A), NEC morbidity (B), sepsis morbidity (C), time to full enteral feeding (D), and length of hospital stay (E). The width of lines is proportional to the number of studies comparing every pair of interventions. The size of nodes is proportional to the number of participants assigned to receive the intervention.

Figure 3: Forest plots

Forest plots of network meta-analysis of all studies for mortality, NEC morbidity, sepsis morbidity, time to full enteral feeding, and length of hospital stay. Other interventions were compared with placebo, which was the reference intervention. Circle in red means significantly in favour of comparator. PLA = Placebo. BIF = *Bifidobacterium*. BL = *Bifidobacterium* + *Lactobacillus*. BLE = *Bifidobacterium* + *Lactobacillus* + *Enterococcus*. BLSA = *Bifidobacterium* + *Lactobacillus* + *Saccharomyces*. BLST = *Bifidobacterium* + *Lactobacillus* + *Streptococcus*. BP = *Bifidobacterium* + Prebiotic. BST = *Bifidobacterium* + *Streptococcus*. LAC = *Lactobacillus*. LP = *Lactobacillus* + Prebiotic. SAC = *Saccharomyces*. BAC = *Bacillus*. NYS = Nystatin. BLP = *Bifidobacterium* + *Lactobacillus* + Prebiotic. RR = risk ratio. CrI = credible interval. MD = mean difference.

Figure 4: Efficacy of primary outcomes

Efficacy of interventions displayed by league table. Interventions are reported in alphabetical order. For the lower triangle (mortality) and upper triangle (NEC morbidity), results are the RRs (with 95% CI) in the column-defining treatment compared with the row-defining treatment. Significant results are shaded in dark colour. NEC = necrotising enterocolitis. PLA = Placebo. BIF = *Bifidobacterium*. BL = *Bifidobacterium* + *Lactobacillus*. BLE = *Bifidobacterium* + *Lactobacillus* + *Enterococcus*. BLSA = *Bifidobacterium* + *Lactobacillus* + *Saccharomyces*. BLST = *Bifidobacterium* + *Lactobacillus* + *Streptococcus*. BP = *Bifidobacterium* + Prebiotic. BST = *Bifidobacterium* + *Streptococcus*. LAC = *Lactobacillus*. LP = *Lactobacillus* + Prebiotic. SAC = *Saccharomyces*. BAC = *Bacillus*. NYS = Nystatin. BLP = *Bifidobacterium* + *Lactobacillus* + Prebiotic. RR = risk ratio. CrI = credible interval.

Figure 5: SUCRA and PCoA plot

SUCRA and PCoA plot according to the network meta-analysis. Cumulative probability indicates the ranking of efficacy on mortality (A), NEC morbidity (B), sepsis morbidity (C), time to full enteral feeding (D), and length of hospital stay (E). The larger the surface under the curve, the better the rank of the intervention being the stipulation. The overall rank distribution of these terms is shown by a dimension reduction method of PCoA plot (F). The top two

principal coordinates (PCoA1 and PCoA2) represent the maximum amount of variation presented in the dataset. SUCRA = surface under the cumulative ranking curve. PCoA = principal coordinate analysis. PLA = Placebo. BIF = *Bifidobacterium*. BL = *Bifidobacterium* + *Lactobacillus*. BLE = *Bifidobacterium* + *Lactobacillus* + *Enterococcus*. BLSA = *Bifidobacterium* + *Lactobacillus* + *Saccharomyces*. BLST = *Bifidobacterium* + *Lactobacillus* + *Streptococcus*. BP = *Bifidobacterium* + Prebiotic. BST = *Bifidobacterium* + *Streptococcus*. LAC = *Lactobacillus*. LP = *Lactobacillus* + Prebiotic. SAC = *Saccharomyces*. BAC = *Bacillus*. NYS = Nystatin. BLP = *Bifidobacterium* + *Lactobacillus* + Prebiotic.

Table 1 Characteristics of included studies

Study (year)	No. of participants	Design	Location	Inclusion criterion	Outcomes	Arms (n)	Treatment	No. of randomized	Gestational age mean (SD)	Birth Weight mean (SD)	Male (n)	Treatment duration (week)	Dosage (CFU)	Times /day
Al-Hosni (2012)	101	SB	America	LBW	①②③	2	PLA	51	25.7 (1.4)	779 (126)	22	9	5 x 10^9	1
							LAC	50	25.7 (1.4)	778 (138)	28	9	5 x 10^9	1
Bin-Nun (2005)	145	DB	Israel	LBW	①②③④	2	PLA	73	29.3 (4.3)	1111 (278)	37	6	1.1 x 10^9	1
							BST	72	29.8 (2.6)	1152 (262)	44	6	1.1 x 10^9	1
Braga (2011)	231	DB	Brazil	LBW	①②③④	2	PLA	112	29.5 (2.5)	1151 (225)	55	4	3.5 x 10^9	1
							BL	119	29.2 (2.6)	1195 (206)	58	4	3.5 x 10^9	1
Chowdhury (2016)	102	DB	Bangladesh	LBW, PTB	②④	2	PLA	50	31.68 (0.84)	1338 (98)	36	2	3 x 10^9	1
							BLP	52	31.38 (0.93)	1312 (110)	33	2	3 x 10^9	1
Costalos (2003)	87	DB	Greece	PTB	②③	2	PLA	36	31.8 (2.7)	1644 (378)	23	Till discharge	1 x 10^9	1
							SAC	51	31.1 (2.5)	1651 (470)	24	Till discharge	1 x 10^9	1
Costeloe (2016)	1310	DB	England	PTB	①②③④⑤	2	PLA	660	28 (2.59)	1043 (317)	374	Till discharge	8.5 x 10^8	1

							BIF	650	28 (2.44)	1039 (312)	370	Till discharge	8.5 x 10^8	1
Dani (2002)	385	SB	Italy	LBW, PTB	②③	2	PLA	290	30.7 (2.3)	1345 (384)	151	7	6 x 10^9	1
							LAC	295	30.8 (2.4)	1325 (361)	135	7	6 x 10^9	1
Demirel a (2013)	181	SB	Turkey	LBW, PTB	①②	2	NYS	90	28.4 (2.6)	1057 (290)	50	Till discharge	5 x 10^{10}	1
							SAC	91	29 (2.7)	1135 (253)	45	Till discharge	5 x 10^{10}	1
Demirel b (2013)	271	DB	Turkey	LBW, PTB	①②③④⑤	2	PLA	136	29.2 (2.5)	1131 (284)	66	Till discharge	5 x 10^9	1
							SAC	135	29.4 (2.3)	1164 (261)	69	Till discharge	5 x 10^9	1
Dilli (2015)	300	DB	Turkey	LBW, PTB	①②③④⑤	3	PLA	100	28.2 (2.2)	1147 (271)	58	Till discharge	5 x 10^9	1
							BP	100	28.9 (1.9)	1205 (240)	57	Till discharge	5 x 10^9	1
							BIF	100	28.2 (2.2)	1147 (271)	58	Till discharge	5 x 10^9	1
Dutta (2015)	149	DB	India	PTB	①②③	2	PLA	35	30.82 (1.72)	1252 (309)	23	3	1 x 10^{10}	1
							BLSA	114	30.87 (1.73)	1345 (284)	68	3	1 x 10^{10}	1
Fernandez-Carrocera (2013)	150	DB	Mexico	PTB	①②④⑤	2	PLA	75	31 (1.5)	1170 (159)	NA	6	2 x 10^9	1
							BLST	75	35.2 (0.9)	1090 (153)	NA	6	2 x 10^9	1

Guney-Varal (2017)	110	SB	Turkey	LBW, PTB	①②③④	2	PLA	40	29.3 (1.7)	1228 (249)	19	Till discharge	2 x 10^9	1
							BL	70	29.7 (1.9)	1729 (257)	45	Till discharge	2 x 10^9	1
Havranek (2013)	31	DB	America	PTB	③④	2	PLA	16	25.9 (1.5)	789 (129)	10	Till discharge	1 x 10^{10}	1
							BL	15	25.9 (1.3)	856 (105)	8	Till discharge	1 x 10^{10}	1
Hays a (2016)	102	DB	France	LBW, PTB	①②	2	PLA	52	29.4 (2)	1170 (233)	35	4-6	1 x 10^9	1
							BIF	50	29 (1.5)	1170 (237)	23	4-6	1 x 10^9	1
Hays b (2016)	100	DB	France	LBW, PTB	①②	2	PLA	52	29.4 (2)	1170 (233)	35	4-6	1 x 10^9	1
							BIF	48	29 (1.5)	1170 (237)	22	4-6	1 x 10^9	1
Hays c (2016)	99	DB	France	LBW, PTB	①②	2	PLA	52	29.4 (2)	1170 (233)	35	4-6	1 x 10^9	1
							BIF	47	29 (1.5)	1170 (237)	22	4-6	1 x 10^9	1
Indrio (2017)	60	DB	Italy	PTB	③④⑤	2	PLA	30	30.1 (1.2)	1407 (536)	16	4	1 x 10^8	1
							LAC	30	30.2 (1.2)	1472 (455)	15	4	1 x 10^8	1
Jacobs (2013)	1099	DB	Australia	LBW, PTB	①②③④⑤	2	PLA	551	27.8 (2)	1048 (260)	300	Till discharge	1 x 10^9	1
							BST	548	27.9 (2)	1063 (259)	272	Till discharge	1 x 10^9	1

Kanic (2015)	80	SB	Slovenia	LBW	①②③⑤	2	PLA	40	29 (2.82)	1024 (250)	27	Till discharge	1.2 x 10^7	2
							BLE	40	28 (2.22)	1104 (233)	22	Till discharge	1.2 x 10^7	2
Lin (2008)	434	DB	China	LBW, PTB	①②③⑤	2	PLA	217	NA	1077 (214)	115	6	2 x 10^9	2
							BL	217	NA	1029 (246)	122	6	2 x 10^9	2
Lin (2005)	367	DB	China	LBW	①②③⑤	2	PLA	187	28.2 (2.5)	1071 (243)	100	Till discharge	2 x 10^9	2
							BL	180	28.5 (2.5)	1104 (242)	84	Till discharge	2 x 10^9	2
Manzoni (2006)	80	DB	Italy	LBW	①②③④⑤	2	PLA	41	29.3 (4)	1174 (340)	21	6	6 x 10^9	1
							LAC	39	29.6 (5)	1212 (290)	20	6	6 x 10^9	1
Manzoni (2009)	358	DB	Italy	LBW	①②③④	3	PLA	168	29.8 (2.8)	1109 (269)	86	Till discharge	6 x 10^9	1
							LAC	39	29.6 (5)	1212 (290)	20	Till discharge	6 x 10^9	1
							LP	151	29.5 (3.2)	1138 (253)	72	Till discharge	6 x 10^9	1
Mihatsch (2010)	180	DB	Germany	PTB	②③④	2	PLA	89	26.7 (1.7)	871 (287)	47	Till discharge	2 x 10^{10}	1
							BIF	91	26.6 (1.8)	856 (251)	55	Till discharge	2 x 10^{10}	1
Nandhini (2016)	218	SB	India	LBW, PTB	①②③⑤	2	PLA	110	31.4 (1.4)	1444 (217)	NA	Till discharge	3 x 10^9	1

							BLP	108	31.6 (1.4)	1430 (209)	NA	Till discharge	3 x 10^9	1
Oncel (2015)	300	DB	Turkey	LBW, PTB	①②③④⑤	2	NYS	150	27.7 (2.5)	1051 (320)	75	Till discharge	1 x 10^8	1
							LAC	150	28.1 (2.4)	1058 (270)	82	Till discharge	1 x 10^8	1
Oncel (2014)	400	DB	Turkey	LBW, PTB	①②③⑤	2	PLA	200	27.9 (2.5)	1048 (298)	98	Till discharge	1 x 10^8	1
							LAC	200	28.2 (2.4)	1071 (274)	108	Till discharge	1 x 10^8	1
Patole (2014)	153	DB	Australia	LBW, PTB	②③④⑤	2	PLA	76	28 (1.67)	1025 (215)	41	5	3 x 10^9	1
							BIF	77	29 (1.5)	1090 (227)	41	5	3 x 10^9	1
Qiao (2017)	60	DB	China	PTB	①②③	2	PLA	30	32.1 (1.9)	1532 (412)	16	Till discharge	1.5 x 10^8	1
							BLE	30	32.4 (1.6)	1653 (476)	17	Till discharge	1.5 x 10^8	1
Rojas (2012)	750	DB	Colombia	LBW	①②③⑤	2	PLA	378	32 (2.96)	1516 (460)	185	Till discharge	1 x 10^8	1
							LAC	372	32 (2.22)	1530 (368)	186	Till discharge	1 x 10^8	1
Rouge (2009)	94	DB	France	LBW, PTB	①②③⑤	2	PLA	49	28.1 (1.8)	1057 (260)	26	Till discharge	1 x 10^8	4
							BL	45	28.1 (1.9)	1115 (251)	28	Till discharge	1 x 10^8	4
Roy (2014)	112	DB	India	LBW, PTB	①②③④⑤	2	PLA	56	32.2 (2)	1069 (365)	16	Till discharge	6 x 10^9	1

							BL	56	32 (2)	1192 (341)	14	Till discharge	6 x 10^9	1
Saengtawesin (2014)	60	SB	Thailand	LBW, PTB	①②③④⑤	2	PLA	29	30.59 (1.76)	1208 (199)	11	6	2 x 10^9	2
							BL	31	31 (1.82)	1250 (179)	19	6	2 x 10^9	2
Samanta (2009)	186	DB	India	LBW, PTB	①②③④⑤	2	PLA	95	30.14 (1.59)	1210 (143)	NA	Till discharge	2.5 x 10^9	2
							BL	91	30.12 (1.63)	1172 (143)	NA	Till discharge	2.5 x 10^9	2
Sari (2011)	221	DB	Turkey	LBW, PTB	①②③④⑤	2	PLA	111	29.7 (2.4)	1278 (282)	62	Till discharge	3.5 x 10^8	1
							LAC	110	29.5 (2.4)	1231 (262)	60	Till discharge	3.5 x 10^8	1
Serce (2013)	208	DB	Turkey	LBW, PTB	①②③④⑤	2	PLA	104	28.7 (2.1)	1162 (216)	56	Till discharge	5 x 10^8	2
							SAC	104	28.8 (2.2)	1126 (232)	51	Till discharge	5 x 10^8	2
Shashidhar (2017)	104	DB	India	LBW	①②④⑤	2	PLA	52	31 (2.1)	1190 (208)	20	Till discharge	1.3 x 10^9	1
							BLSA	52	31.2 (2.1)	1256 (185)	27	Till discharge	1.3 x 10^9	1
Sinha (2015)	1340	DB	India	LBW	①③	2	PLA	672	NA	2263 (179)	320	4	1 x 10^{11}	1
							BLST	668	NA	2261 (179)	319	4	1 x 10^{11}	1
Stratiki (2007)	75	DB	Greece	PTB	②③④	2	PLA	34	30.5 (8.15)	1500 (889)	17	Till discharge	2 x 10^7	12

							BIF	41	31 (7.41)	1500 (652)	23	Till discharge	2 x 10^7	12
Tewari (2015)	244	DB	India	PTB	①②③⑤	2	PLA	121	30 (3)	1322 (398)	58	Till discharge	2 x 10^9	1
							BAC	123	30 (3)	1313 (414)	62	Till discharge	2 x 10^9	1
Totsu (2014)	283	DB	Japan	LBW	①②③⑤	2	PLA	130	28.5 (3.3)	998 (281)	71	Reach 2000g	2.5 x 10^9	1
							BIF	153	28.6 (2.9)	1016 (289)	87	Reach 2000g	2.5 x 10^9	1
Underwood (2009)	89	SB	America	LBW, PTB	②④	3	PLA	29	29.3 (2.6)	1363 (363)	19	4	5 x 10^8	1
							LP	30	29.5 (2.6)	1394 (365)	21	4	5 x 10^8	1
							BLP	30	29.5 (2.6)	1394 (365)	21	4	5 x 10^8	1
Van Niekerk (2015)	110	DB	South Africa	LBW	②③④	2	PLA	56	29 (3)	1215 (189)	24	4	3.5 x 10^8	1
							BL	54	29 (4)	1258 (201)	29	4	3.5 x 10^8	1
Wejryd (2018)	134	DB	Sweden	LBW, PTB	①②③④	2	PLA	66	25.5 (1.3)	740 (148)	42	Reach 2000g	1.3 x 10^8	1
							LAC	68	25.5 (1.2)	731 (129)	32	Reach 2000g	1.3 x 10^8	1
Xu (2016)	125	SB	China	LBW, PTB	③⑤	2	PLA	63	33 (1.41)	1957 (51)	24	1	1 x 10^9	1
							SAC	62	33 (0.72)	1947 (54)	27	1	1 x 10^9	1

Continuous variable was represented as mean (SD). DB = double blind. SB = single blind. PTB = preterm birth. LBW = low birth weight. ① = mortality. ② = NEC morbidity. ③ = sepsis morbidity. ④ = time to full enteral feeding. ⑤ = length of hospital stay. PLA = Placebo. BIF = *Bifidobacterium*. BL = *Bifidobacterium* + *Lactobacillus*. BLE = *Bifidobacterium* + *Lactobacillus* + *Enterococcus*. BLSA = *Bifidobacterium* + *Lactobacillus* + *Saccharomyces*. BLST = *Bifidobacterium* + *Lactobacillus* + *Streptococcus*. BP = *Bifidobacterium* + Prebiotic. BST = *Bifidobacterium* + *Streptococcus*. LAC = *Lactobacillus*. LP = *Lactobacillus* + Prebiotic. SAC = *Saccharomyces*. BAC = *Bacillus*. NYS = Nystatin. BLP = *Bifidobacterium* + *Lactobacillus* + Prebiotic.