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Probiotics Reduce Mortality and Morbidity in Preterm, Low-Birth-Weight Infants: A Systematic Review and Network Meta-analysis of Randomized Trials

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Abstract

BACKGROUND & AIMS: We aimed to compare the effectiveness of single- vs multiple-strain probiotics in a network meta-analysis of randomized trials.

METHODS: We searched MEDLINE, Embase, Science Citation Index Expanded, CINAHL, Scopus, Cochrane CENTRAL, BIOSIS Previews, and Google Scholar through January 1, 2019, for studies of single-strain and multistrain probiotic formulations on the outcomes of preterm, low-birth-weight neonates. We used a frequentist approach for network meta-analysis and the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach to assess the certainty of evidence. Primary outcomes included all-cause mortality, severe necrotizing enterocolitis (NEC) (Bell stage II or more), and culture-proven sepsis.

RESULTS: We analyzed data from 63 trials involving 15,712 preterm infants. Compared with placebo, a combination of 1 or more *Lactobacillus* species (spp) and 1 or more *Bifidobacterium*

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Supplementary Material

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spp was the only intervention with moderate- or high-quality evidence of reduced all-cause mortality (odds ratio [OR], 0.56; 95% confidence interval [CI], 0.39–0.80). Among interventions with moderate- or high-quality evidence for efficacy compared with placebo, combinations of 1 or more *Lactobacillus* spp and 1 or more *Bifidobacterium* spp, *Bifidobacterium animalis* subspecies *lactis, Lactobacillus reuteri*, or *Lactobacillus rhamnosus* significantly reduced severe NEC (OR, 0.35 [95% CI, 0.20–0.59]; OR, 0.31 [95% CI, 0.13–0.74]; OR, 0.55 [95% CI, 0.34–0.91]; and OR, 0.44 [95% CI, 0.21–0.90], respectively). There was moderate- or high-quality evidence that combinations of 1 or more *Lactobacillus* spp and 1 or more *Bifidobacterium* spp and *Saccharomyces boulardii* reduced the number of days to reach full feeding (mean reduction of 3.30 days [95% CI, reduction of 5.91–0.69 days]). There was moderate- or high-quality evidence that, compared with placebo, the single-species product *B animalis* subsp *lactis* or *L reuteri* significantly reduced duration of hospitalization (mean reduction of 13.00 days [95% CI, reduction of 22.71–3.29 days] and mean reduction of 7.89 days [95% CI, reduction of 11.60–4.17 days], respectively).

CONCLUSIONS: In a systematic review and network meta-analysis of studies to determine the effects of single-strain and multistrain probiotic formulations on outcomes of preterm, low-birth-weight neonates, we found moderate to high evidence for the superiority of combinations of 1 or more *Lactobacillus* spp and 1 or more *Bifidobacterium* spp vs single- and other multiple-strain probiotic treatments. The combinations of *Bacillus* spp and *Enterococcus* spp, and 1 or more *Bifidobacterium* spp and *Streptococcus salivarius* subsp *thermophilus*, might produce the largest reduction in NEC development. Further trials are needed.

Keywords

Commensal; Microbiota; Supplement; Newborn

Preterm birth, defined as birth before 37 weeks of gestation, affects nearly 10% of pregnancies and is the leading cause of perinatal morbidity and mortality in the United States.[1] Preterm infants are at increased risk of sepsis, death, and lifelong neurodevelopmental and cognitive impairment.[2] The most significant acquired disease of the gastrointestinal tract in preterm infants is necrotizing enterocolitis (NEC), which is characterized by bowel necrosis in variable depths and locations but most often affects the terminal ileum and proximal colon.[3] Survivors are at increased risk of short bowel syndrome, parenteral nutrition–associated liver disease, pulmonary hypertension, and developmental delay.

The mechanisms by which NEC develops are poorly understood. Numerous studies report fecal microbiota alterations in preterm infants who have NEC and in those who go on to develop NEC[4]; however, it remains unclear whether these gut microbiota alterations contribute to or simply result from the pathogenesis of NEC. Dozens of trials of microbiome-targeting therapies have tested various agents for their ability to prevent morbidity and mortality in preterm infants.

Probiotics are live microbes which, when consumed in adequate amounts, confer a health benefit on the host.[5] Probiotics might reduce the risk of sepsis and NEC by multiple mechanisms, including suppression of inflammation through the nuclear factor-κB signaling

pathway, up-regulation of host anti-inflammatory genes, alleviation of hypoxemic injury, production of short-chain fatty acids to lower intestinal pH and support intestinal epithelial cell function, suppression of pathogenic bacterial growth including Enterobacteriaceae via niche exclusion and antimicrobial metabolites, strengthening intestinal barrier function, and regulation of host immunity.[6,7] A 2014 Cochrane review[8] concluded that probiotics prevent severe NEC and all-cause mortality in preterm infants, although the most effective formulations have yet to be identified. To build on this growing evidence base, we performed a network meta-analysis (NMA) to assess the relative effectiveness of various single-strain and multistrain probiotic formulations for critical clinical outcomes among preterm, low-birth-weight neonates.

Materials and Methods

We produced this NMA as a secondary analysis of an unpublished systematic review and protocol based on the protocol registered with PROSPERO (CRD42018085566).[9,10] The results of this analysis inform the "American Gastroenterological Association Institute Technical Review on the Role of Probiotics in the Management of Gastrointestinal Disorders."[11]

Search Strategy and Selection Criteria

Detailed methods have been published elsewhere.[9,10] In brief, we conducted searches for relevant randomized controlled trials (RCTs) in MEDLINE, Embase, Science Citation Index Expanded and Social Sciences Citation Index, CINAHL, Scopus, Cochrane Central Register of Controlled Trials (CENTRAL), BIOSIS Previews, and Google Scholar from inception through January 1, 2019 (see the "Search Strategies" section in the Supplementary Materials). No language restrictions were imposed. We reviewed reference lists from eligible trials and related reviews for additional eligible RCTs.

Pairs of reviewers independently screened the titles and abstracts of all identified studies and, subsequently, assessed the eligibility of the full texts of potentially eligible studies. Reviewers resolved discrepancies through discussion, or, if needed, by adjudication from a third reviewer (BS). We selected RCTs featuring single- or multiple-strain probiotics (defined as living bacteria) for the prevention of morbidity or mortality in preterm (gestational age, <37 weeks) and/or low birth weight (birth weight, <2500 g) infants. We excluded studies that enrolled term infants or included both term and preterm infants, unless data for preterm infants were reported separately or >80% of infants were preterm. We also excluded studies that enrolled infants once they achieved full enteral feed or enrolled infants with early-onset sepsis, feed intolerance, or NEC.

Data Abstraction and Risk-of-Bias Assessment

Pairs of reviewers assessed the risk of bias and extracted the following data, independently and in duplicate: (1) general study information (author's name, publication year, country of origin, and funding source), (2) study population details (sample size; mean gestational age; birth weight; percentage of caesarean births; and percentage of infants fed exclusively with mother's, donor's, or formula milk), (3) details of the intervention and comparison (eg,

probiotics species and strains, dosage, time of initiation, and duration of therapy), and (4) outcomes (severe NEC [stage II or greater based on the Bell criteria],[12,13] all-cause mortality, culture-proven sepsis, duration of hospitalization, time to establish full enteral feeds (days), and feed intolerance).

We used a modified Cochrane risk-of-bias instrument[14,15] that addresses the following issues: random sequence generation, allocation concealment, blinding of study participants (in the case of our study, infants' parents), blinding of health care providers, blinding of data collectors and outcome assessors/adjudicators, and incomplete outcome data (studies with loss to follow-up of 5% or more of randomized infants were considered at high risk of bias).

Data Synthesis and Statistical Methods

For each direct paired comparison, we calculated the odds ratios (ORs) and associated 95% confidence intervals (CIs) for dichotomous outcomes.[10] For continuous outcomes, we calculated weighted mean differences with corresponding 95% CIs. We used the median baseline risk from the control arm of eligible trials to calculate the risk difference using MAGICapp. We used methods described by the *Cochrane Handbook*[16] and Hozo et al.[17] to estimate the mean and standard deviation where median, range, and sample size were reported and to impute the standard deviation if the standard error or deviation for the differences were not reported. We merged the following comparisons into a single intervention node called *placebo*: infants receiving formula, parental nutrition, control, or no treatment, and infants receiving placebo.

Initially, we performed conventional pairwise meta-analysis using a DerSimonian-Laird random-effects model for all comparisons with 2 RCTs or more. We assessed heterogeneity between RCTs for each direct comparison with visual inspection of the forest plots and the I^2 statistic. We then performed frequentist random-effects NMA under a consistency model using the methodology of multivariate meta-analysis, assuming a common heterogeneity parameter.[18,19] We used the commands and syntax provided in the network suite prepared by Chaimani and Salanti[20] and White et al.[18,19] We did not perform NMA when there were fewer than 10 studies for an outcome or when the number of studies was less than number of interventions (nodes) because of concerns with the model fitness. We investigated small study effect using the Harbord test for binary outcomes and the Egger test for continuous outcomes in all direct comparisons with at least 10 RCTs.[21]

We evaluated the presence of incoherence (also called *inconsistency*) by comparing direct evidence with indirect evidence using the node-splitting method.[22,23] We also confirmed the coherence assumption in the entire network using the design-by-treatment model (global test) as described by Higgins et al.[24] We estimated ranking probabilities using the surface under the cumulative ranking curve (SUCRA), mean ranks, and rankograms. We used Stata, version 15.1 (StataCorp, College Station, TX) for data preparations and analyses.

Assessing the Certainty of Evidence

We rated the certainty of evidence for each network estimate using the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) approach.[25–27] Initially, we rated the certainty of each direct comparison; according to established GRADE

guidance, the starting point for certainty across the body of RCTs is high but may be rated down based on limitations in risk of bias, imprecision, inconsistency, indirectness, and publication bias.[25] Then, we rated the certainty of the indirect evidence, with a focus on the dominant lowest-order loop.[26] We rated the certainty of indirect evidence as the lowest certainty of the contributing direct comparisons. Finally, we rated the certainty of network estimates. We considered the relative contribution of direct and indirect evidence to the network estimate when rating the certainty. We considered rating down the certainty in the network estimate if there was incoherence between the indirect and direct estimates or an imprecise treatment effect.[26,27]

Summary of Results

To optimize the interpretation of NMA findings, we applied a novel approach in which we categorized the interventions—from the most effective to the least effective—based on the effect estimates obtained from the NMA and their associated certainty of evidence. For each outcome, we created groups of interventions as follows.

- Group 1: the reference intervention (placebo) and interventions with no evidence of difference compared to placebo (ie, 95% CI includes the null value), which we refer to as *among the least effective*.
- Group 2: interventions superior to placebo but not superior to any other of the intervention(s) superior to placebo, referred to as *inferior to the most effective, but superior to the least effective*.
- Group 3, interventions that proved superior to at least 1 intervention in group 2, which we call *among the most effective*.

We then divided all 3 categories into 2 categories: those with moderate- or high-certainty evidence relative to placebo and those with low- or very-low-certainty evidence relative to placebo.

Results

Additional results can be found in the Supplementary Figures 1–15 and Supplementary Tables 1–12.

Description of the Evidence

We identified 7562 records through our literature search, of which we included 96 publications from 87 studies. We excluded 16 studies of prebiotics and synbiotics (eg, studies not reporting probiotic and placebo arms) and 8 studies that did not report any of our target outcomes,[28–35] leaving 63 eligible RCTs involving 15,712 infants[36–98] (see the "References to Trials Included in the Network Meta-analysis" section of the Supplementary Materials). We did not exclude multiarm trials (ie, trials with >2 arms) of prebiotics or synbiotics if they had a probiotic arm and a placebo/control/no treatment arm. Figure 1 provides details of the study selection process.

Across the included trials, the median of the average birth weight was 1204 g (interquartile range, 1062–1433), and the median of the average gestational age was 30.1 weeks (IQR,

28.7–31.3). The “Characteristics of Participants of Trials Included in the Network Meta-analysis” section in the Supplementary Materials summarizes the characteristics of infants included in the eligible trials.

The majority of multiple-strain probiotic products contained *Lactobacillus* species (spp) together with *Bifidobacterium* spp (28 of 32 studies). Of the 31 studies of a single-strain probiotic, 15 studies used a product containing *Lactobacillus* spp. In the Supplementary Materials, the “Treatment Characteristics of Trials and Outcomes Included in the Network Meta-analysis” section presents the characteristics of the treatments used, and the “Networks of Treatment Comparisons” section presents the networks of eligible treatment comparisons for each outcome.

Of the 63 studies, 39 were judged to be at low risk of bias for sequence generation and allocation concealment, 44 studies proved to be at low risk of bias for blinding of infants’ parents/caregivers and study personnel, 35 proved to be at low risk of bias for masking of outcome assessments, and 57 proved to be at low risk of bias in terms of missing participant outcome data. The “Summary of Risk-of-Bias Assessments for Included Trials” section of the Supplementary Materials provides additional details.

All-Cause Mortality

All-cause mortality was reported in 52 studies involving 14,003 infants (Supplementary Figure 1). Of the 22 available direct comparisons, in 10 comparisons, 2 or more studies were available for conventional pairwise meta-analysis in which the I^2 was 0 in 7 comparisons and <50% for the remaining comparisons (Supplementary Table 1). The results of all direct comparisons proved similar to the NMA estimates (Supplementary Table 7). We did not observe any statistically significant global or loop-specific incoherence (Supplementary Figure 6).

High-certainty evidence showed that combinations of *Lactobacillus* spp and *Bifidobacterium* spp reduced all-cause mortality (OR, 0.56; 95% CI, 0.39–0.80; high certainty; risk difference [RD], −2.2%; 95% CI, −3.1 to −0.1) compared to placebo (Figure 2 and Table 1). No other statistically significant difference was identified between the remainder of the treatments and placebo (Figure 2 and Table 1 and Supplementary Table 1). Table 2 provides details of active treatment probiotic combinations and strains showing effectiveness compared to placebo. Supplementary Table 12 and Supplementary Figure 11 provide details of rankings and SUCRA values (see the “SUCRA and Cumulative Probability Plots” section in the Supplementary Materials).

Necrotizing Enterocolitis Stage II or Higher

We included 56 RCTs with 12,738 infants involving 19 preventive therapies (Supplementary Figure 2). Of the 22 direct comparisons, 10 involved 2 studies or more. Heterogeneity was substantial (I^2 = 53. 6%) for the comparison *Bifidobacterium* spp and *Streptococcus salivarius* subspecies (subsp) *thermophilus* with placebo. The results of all direct comparisons proved similar to the NMA estimates (Supplementary Tables 2 and 8). We did not observe any statistically significant global or loop-specific incoherence (Supplementary Figure 7).

Among the studies with high- or moderate-certainty evidence relative to placebo, combinations of *Lactobacillus* spp and *Bifidobacterium* spp (OR, 0.35; 95% CI, 0.20–0.59; high certainty; RD, −4.1%; 95% CI, −5.1 to −2.6), *Bifidobacterium animalis* subsp *lactis* (OR, 0.31; 95% CI, 0.13 to 0.74; high certainty; RD, −4.4%; 95% CI, −5.6 to −1.6), *Lactobacillus reuteri* (OR, 0.55; 95% CI, 0.34–0.91; moderate certainty; RD, −2.8%; 95% CI, −4.2 to −0.5), and *Lactobacillus rhamnosus* (OR, 0.44; 95% CI, 0.21–0.90; moderate certainty; RD, −3.5%; 95% CI, −5.0 to −0.5) significantly reduced severe NEC (Figure 2 and Table 1).

Among the studies with low or very low certainty, the combinations *Bacillus* spp and *Enterococcus* spp (OR, 0.23; 95% CI, 0.08–0.63; low certainty; RD, −4.9%; 95% CI, −5.9 to −2.3), *Lactobacillus* spp and *Bifidobacterium* spp and *Enterococcus* spp (OR, 0.28; 95% CI, 0.16–0.49, low certainty; RD, −4.9%; 95% CI, −5.4 to −3.2), and *Bifidobacterium* spp and *S salivarius* subsp *thermophilus* (OR, 0.38; 95% CI, 0.19–0.75; low certainty; RD, −3.9%; 95% CI, −5.2 to −1.5) significantly decreased the likelihood of severe NEC when compared to placebo (Figure 2 and Table 1). Figure 2 and Table 1, show the comparative effectiveness and the certainty for all pairwise comparisons. Supplementary Table 12 and Supplementary Figure 12 provide details of rankings and SUCRA values.

Culture-Proven Late-Onset Sepsis

Culture-proven sepsis was reported in 48 RCTs involving 12,258 infants (Supplementary Figure 3) with 19 direct comparisons. Our analysis did not show statistical evidence of incoherence either in the design-by-treatment interaction model (global test) or loop-specific models (Supplementary Figure 8). Heterogeneity in 5 of the 10 direct comparisons with 2 or more studies was substantial (I^2 > 50% for the comparisons of *L reuteri*; *Lactobacillus* spp, *Bifidobacterium* spp, and *Saccharomyces boulardii*; *Lactobacillus* spp, *Bifidobacterium* spp, and *Enterococcus* spp; *Lactobacillus* spp and *Bifidobacterium* spp; and *Bifidobacterium* spp and *S salivarius* subsp *thermophilus* vs placebo) (Supplementary Table 3). The results of NMA were, however, similar to the direct comparisons (Supplementary Table 9).

None of the probiotic products showed statistically significant reduction in the likelihood of culture-proven late-onset sepsis (Figure 2). Three combinations of products—*Lactobacillus* spp and *Bifidobacterium* spp; *Lactobacillus* spp, *Bifidobacterium* spp, and *S boulardii*; and *Lactobacillus* spp, *Bifidobacterium* spp, *Enterococcus* spp—showed nonsignificant effects with very-low-certainty evidence toward benefit (Figure 2 and Table 1). Supplementary Table 12 and Supplementary Figure 13 provide details of rankings and SUCRA values.

Feed Intolerance

Feed intolerance was reported in 12 RCTs involving 2963 infants with 10 direct comparisons, of which 2 comparisons had 2 contributing trials and 1 comparison had 3 contributing trials. The design-by-treatment interaction model showed evidence of incoherence. This was limited to the only available closed loop of *L reuteri*, *L rhamnosus*, and placebo. Heterogeneity in the comparison of *L reuteri* vs placebo was substantial (I^2 = 87.2) (Supplementary Table 4). because of the observed incoherence and small number of studies, we did not perform NMA. The 2 comparisons of *S boulardii* vs placebo and *L*

rhamnosus vs placebo were not statistically significant (OR, 0.42; 95% CI, 0.18–1.00; I^2 = 26.0%; low certainty and OR, 0.47; 95% CI, 0.10–2.26; I^2 = 35.8%; low certainty, respectively); however, *L reuteri* may result in reduction of feed intolerance compared to placebo (OR, 0.32; 95% CI, 0.12–0.89; I^2 = 87.2%; very low certainty) (Figure 2 and Table 1).

Continuous Outcomes

The "GRADE Presentation of Secondary Outcomes" section of the Supplementary Material provides detailed results of NMA of continuous outcomes, and Figure 2 summarizes these results. The 35 studies (7579 infants) that reported time to reach full enteral feed involved 12 interventions in 13 direct comparisons (Supplementary Figure 4). We did not observe any statistically significant global or loop-specific incoherence (Supplementary Figure 9). Of 9 direct comparisons with 2 or more studies, 5 had substantial heterogeneity (I^2 > 50%) (Supplementary Table 5).

Among the studies with high- or moderate-certainty evidence relative to placebo, only combinations of *Lactobacillus* spp and *Bifidobacterium* spp and *S boulardii* (mean difference [MD], −3.30; 95% CI, −5.91 to −0.69; moderate certainty) reduced the mean number of days to reach full feeds (Figure 2 and Table 1). Among the studies with low or very low certainty, *L reuteri* (MD, −2.62; 95% CI, −4.53 to −0.71; low certainty) and combinations of *Lactobacillus* spp and *Bifidobacterium* spp (MD, −2.15; 95% CI, −3.78 to −0.51; low certainty) significantly decreased the number of days to reach full enteral feeding compared to placebo (Figure 2 and Table 1). In the comparisons of combinations of *Lactobacillus* spp and *Bifidobacterium* spp and *Enterococcus* spp, the NMA results showed no statistical reduction in number of days to reach full feed compared to placebo, whereas the direct evidence from 2 RCTs showed moderate-certainty evidence of benefit (MD, −2.47; 95% CI, −4.63 to −0.32) (see the "GRADE Presentation of Continuous Outcomes" section of the Supplementary Materials). Supplementary Table 12 and Supplementary Figure 14 provide details of rankings and SUCRA values (see the "SUCRA and Cumulative Probability Plots" section of the Supplementary Materials).

The 31 studies (7539 infants) that reported duration of hospital stay involved 13 interventions with 14 direct comparisons (Supplementary Figure 5). Our analysis did not show statistical evidence of incoherence either in the design-by-treatment interaction model (global test) or loop-specific models (Supplementary Figure 10). Of the 6 direct comparisons involving 2 or more RCTs, 3 had substantial heterogeneity (I^2 > 50%) (Supplementary Table 6). Supplementary Table 11 provides the results of all pairwise comparisons. Only the single-strain probiotics *B animalis* subsp *lactis* (MD, −13.00; 95% CI, −22.71 to −3.29; moderate certainty) or *L reuteri* (MD, −7.89; 95% CI, −11.60 to −4.17; moderate certainty) were statistically more effective than placebo in reducing the duration of hospitalization (Figure 2 and "GRADE Presentation of Continuous Outcomes" section of the Supplementary Materials). Supplementary Table 12 and Supplementary Figure 15 provide details of rankings and SUCRA values (see the "SUCRA and Cumulative Probability Plots" section of the Supplementary Materials).

Discussion

In this systematic review and NMA comparing the effectiveness of different probiotic combinations for the prevention of mortality and morbidity in preterm infants, we found, across a number of outcomes, that few single- and multiple-strain probiotics are more effective than placebo, with no difference in the evidence of harms (ie, sepsis). High-certainty evidence indicates that combinations of 1 or more *Lactobacillus* spp and 1 or more *Bifidobacterium* spp are best for the prevention of all-cause mortality and stage II NEC (potentially averting 22 deaths and 41 cases of stage II NEC out of 1000 patients), and moderate-certainty evidence indicates that *B animalis* subsp *lactis, L reuteri*, and *L rhamnosus* prevent stage II NEC (averting 44, 28, and 35 cases of stage II NEC out of 1000 patients, respectively) (Figure 2). The combination of 1 or more *Lactobacillus* spp and 1 or more *Bifidobacterium* spp and *S boulardii* best reduces time to full enteral feeding; however, *L reuteri*, and combinations of *Lactobacillus* spp and *Bifidobacterium* spp, may also be effective. Finally, moderate-certainty evidence suggests that *B animalis* subsp *lactis*, as well as *L reuteri*, reduces the duration of hospital stay. Low-certainty evidence suggests that *Bacillus* spp and *Enterococcus* spp; *Lactobacillus* spp, *Bifidobacterium* spp, and *Enterococcus* spp; and *Bifidobacterium* spp and *S salivarius* subsp *thermophilus* prevent stage II NEC (averting 49, 46, and 39 cases of stage II NEC out of 1000 patients, respectively). Currently, there is no evidence for benefit of the remaining probiotic formulations. We did not observe any effect modification for birth weight, gestational age, feeding with breast milk, or birth type.

Our review has a number of strengths. To our knowledge, it is the most comprehensive systematic review on this topic to date, including all available literature from English and non-English RCTs for comparative assessments of the effects of prebiotics, probiotics, and synbiotics. The review is based on analyses using sophisticated statistical models that considered both NMA effect estimates and probability rankings. The review uses the GRADE approach for assessing the certainty in the NMA effect estimates and provides an innovative, transparent presentation of our findings. This presentation captures, in a single summary table, the relative performance of each treatment on each outcome, categorized by the certainty of the evidence (Figure 2).

The most important limitation of our study is that for almost all outcomes, the network of interventions is sparse, with most trials comparing probiotic formulations to placebo (ie, limited direct evidence comparing the effects of different probiotic species/strains with each other). In addition, the limited testing of single- or multiple-strain probiotics (other than *Lactobacillus* spp and *Bifidobacterium* spp) may have contributed to the inability to determine, with moderate or high certainty, evidence of benefit, harm, or no effect. Furthermore, it is unclear whether these results might be extrapolated to centers that routinely administer donor human breast milk, because these programs are associated with a decreased baseline risk of NEC.[99] Importantly, NEC is difficult to diagnose given its variable clinical presentations, and the modified Bell criteria are quite broad and not always interpreted the same way by different clinicians. NEC risk is also modified by antimicrobial and acid suppression therapy, which often were not reported in the individual trials. Despite

these drawbacks inherent to this particular outcome measure, beneficial effects were noted with multiple probiotic formulations.

Many of the trials in the published literature, and thus included in our review, empirically select the most tested probiotics (eg, *Lactobacillus* spp and *Bifidobacterium* spp) rather than selecting strains or combinations of strains based on biological plausibility. Although this may be due to many unknowns about the mechanisms causing NEC, what is known about NEC could help researchers make more informed decisions about strains to test. Although different microbial strains within the same genus and species can have different effects on the host, not every published trial describes the specific treatment at the strain level. Furthermore, manufacturers can change both the microbial and nonmicrobial components of commercially available probiotic products over time, and the viability of the live microbes is rarely reported. Although this study differentiated between different species and strains (when reported) of probiotics, there was not enough information given in most trials to extend the examination to nonmicrobial components of the therapies. As our knowledge of microbial functional genomics and strain-level differences continues to deepen, future studies must be as descriptive as possible regarding probiotic formulations and the rationale for their selection. Finally, standard doses and frequencies of probiotic administration have yet to be established, so we chose not to further divide the evidence base according to how the products were administrated.

Recently, 2 strain-specific meta-analyses and an NMA addressed probiotic effectiveness, and their results are consistent with our findings for probiotics. The reviews also addressed possible variability in effectiveness of probiotic strains/species. Athalye-Jape et al examined the effects of *L reuteri* DSM 17938[100] and *Bifidobacterium breve* M-16V[101] in RCTs and nonrandomized studies involving preterm infants and found no significant benefits for *B breve* M-16V on severe NEC, late-onset sepsis, all-cause mortality, and time to reach full enteral feedings. By contrast, the investigators reported significant reductions in length of hospital stay, time to reach full feedings, and duration of hospitalization, as well as nonsignificant reductions in the incidence of severe NEC and all-cause mortality with *L reuteri* DSM 17938. Furthermore, an NMA addressing the strain-specific effects of probiotics in 51 RCTs provided evidence that a combination of strains (multiple-strain probiotics) are usually better than any single-strain probiotics, but the paucity of studies addressing particular strains or combinations of strains limited inferences regarding comparative effectiveness.[102] These findings were confirmed in a recent systematic review, [103] suggesting the continued importance in conducting and reporting on rigorous studies of underreported strains or combinations.

It should also be noted that safety data in the majority of RCTs included in this analysis do not report adverse event and safety outcomes with the same level of rigor that is required in RCTs that test pharmacologic agents. Although this problem pertains to trials of probiotics, which are considered dietary supplements rather than pharmaceuticals, for any clinical condition,[104] this concern may be especially relevant to the fragile population included in this review. In 1 recent example, a preterm infant receiving a probiotic died of intestinal mucormycosis resulting from possible contamination in the manufacturing process.[105] Although the primary concern of live microbe administration, intestinal barrier translocation

leading to sepsis, is decreased by several probiotic formulations, sound clinical judgment should be exercised.

Conclusion

Moderate- to high-certainty evidence shows the superiority of combinations of 1 or more *Lactobacillus* spp and 1 or more *Bifidobacterium* spp over alternative single- and multiple-strain probiotic treatments. The 2 combinations of *Bacillus* spp and *Enterococcus* spp and of *Bifidobacterium* spp and *S salivarius* subsp *thermophilus* may provide the largest reduction in the development of NEC, but this is supported by only low to very low certainty of evidence. Multicenter and large RCTs should be prioritized to distinguish between the efficacy of single- and multiple-strain probiotics among preterm infants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflicts of interest

These authors disclose the following: Adam V. Weizman has served on an advisory board for AbbVie, Ferring, Janssen, and Takeda and as a speaker for AbbVie and Janssen. Behnam Sadeghirad received funding from Mitacs Canada and Accelerate Internship, in partnership with Nestlé Canada, to support his graduate student stipend. Mitacs is a national, not-for-profit organization that has designed and delivered research and training programs in Canada working with universities, companies, and both federal and provincial governments. The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The remaining authors disclose no conflicts.

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Abbreviations used in this paper:

CI	confidence interval
GRADE	Grading of Recommendations Assessment, Development and Evaluation
MD	mean difference
NEC	necrotizing enterocolitis
NMA	network meta-analysis
OR	odds ratio
RCT	randomized controlled trial
RD	risk difference

spp	species
subsp	subspecies
SUCRA	surface under the cumulative ranking curve

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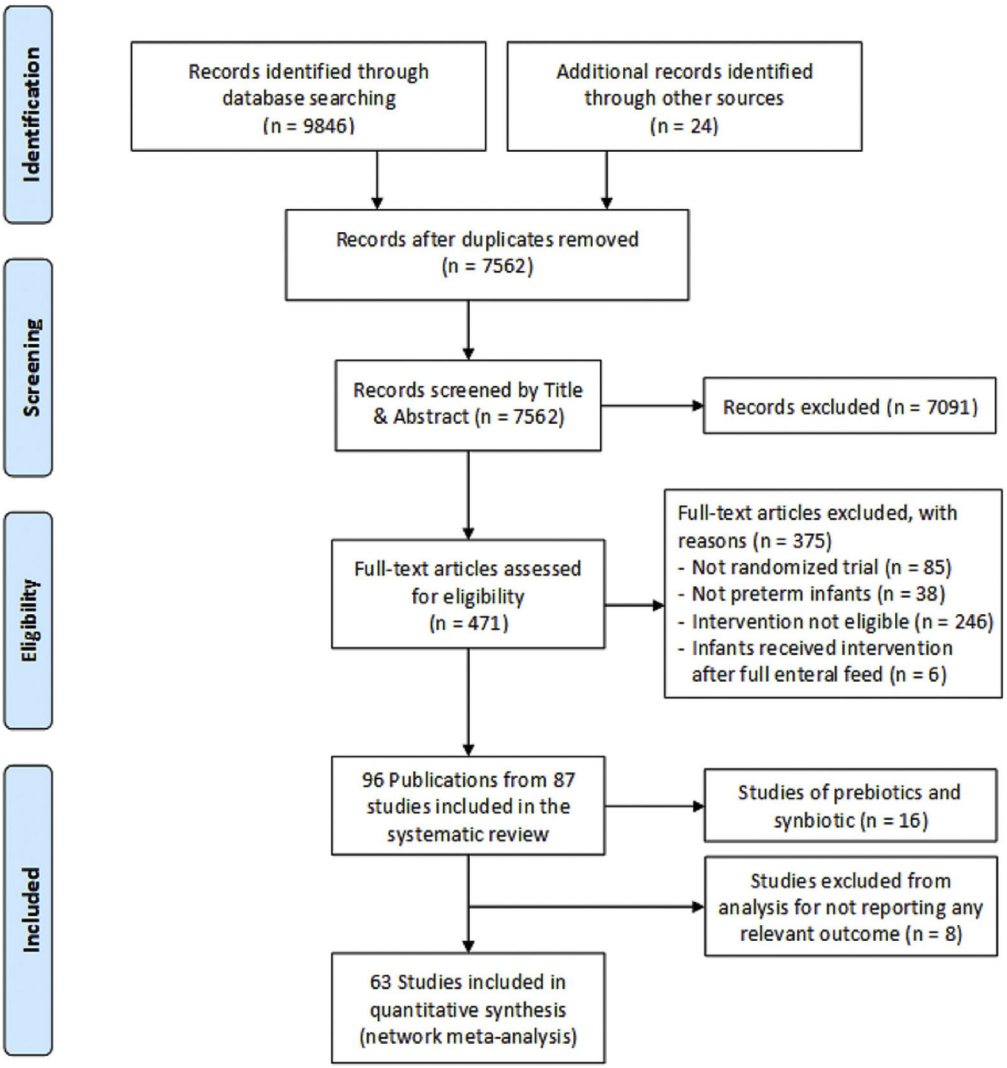

Figure 1.
Flow diagram for study selection.

	All-cause Mortality OR (95% CI)	NEC (stage ≥ II) OR (95% CI)	Culture proven sepsis OR (95% CI)	Feed intolerance OR (95% CI)	Reduction in days to reach full feed MD (95% CI)	Reduction in days of hospitalization MD (95% CI)
***Lactobacillus* spp and *Bifidobacterium* spp**	**0.56 (0.39,0.80)**	**0.35 (0.20,0.59)**	0.87 (0.60,1.27)	-	**-2.15 (-3.78,-0.51)**	-2.84 (-6.21,0.54)
Bifidobacterium animalis* subsp. *lactis	0.43 (0.16,1.15)	**0.31 (0.13,0.74)**	0.73 (0.38,1.43)	0.10 (0.00,2.29)	-	**-13.00 (-22.71,-3.29)**
Lactobacillus reuteri	0.77 (0.51,1.17)	**0.55 (0.34,0.91)**	0.71 (0.41,1.26)	0.26 (0.06,1.10)	**-2.62 (-4.53,-0.71)**	**-7.89 (-11.60,-4.17)**
Lactobacillus rhamnosus	0.84 (0.33,2.12)	**0.44 (0.21,0.90)**	0.84 (0.45,1.57)	0.75 (0.11,5.35)	0.02 (-3.29,3.32)	-1.85 (-7.62,3.91)
***Lactobacillus* spp and *Bifidobacterium* spp and *Enterococcus* spp**	0.78 (0.23,2.62)	**0.28 (0.16,0.49)**	0.43 (0.17,1.07)	0.23 (0.02,3.07)	-	-6.00 (-19.53,7.53)
Bifidobacterium* spp and *Streptococcus salivarius* subsp. *thermophilus	0.84 (0.51,1.40)	**0.38 (0.19,0.75)**	1.04 (0.52,2.06)	-	-1.35 (-4.66,1.95)	-2.75 (-10.00,4.50)
***Bacillus* spp and *Enterococcus* spp**	0.95 (0.02,48.18)	**0.23 (0.08,0.63)**	-	-	-	-
Lactobacillus* spp and *Bifidobacterium* spp and *Saccharomyces boulardii	1.05 (0.51,2.17)	0.73 (0.29,1.85)	0.54 (0.28,1.04)	0.47 (0.04,5.04)	**-3.30 (-5.91,-0.69)**	-3.20 (-8.38,1.98)
Lactobacillus acidophilus	0.29 (0.03,3.12)	1.00 (0.02,53.66)	-	-	-	20.70 (-12.55,53.95)
B. animalis* subsp. *lactis* and *Bifidobacterium longum* subsp. *longum	0.39 (0.04,4.18)	1.42 (0.37,5.42)	0.77 (0.23,2.57)	-	-	-
B. longum* subsp. *longum	0.77 (0.11,5.35)	0.25 (0.03,2.30)	0.75 (0.23,2.50)	-	-	-
Lactobacillus* spp and *Bifidobacterium* spp and *S. salivarius* subsp. *thermophilus	0.40 (0.12,1.30)	0.42 (0.16,1.13)	0.68 (0.35,1.30)	0.68 (0.06,7.70)	5.75 (-0.33,11.83)	7.25 (-5.83,20.33)
Bifidobacterium adolescentis	0.93 (0.02,47.20)	0.13 (0.01,2.51)	-	-	-	-
Bacillus coagulans	0.91 (0.38,2.15)	0.58 (0.20,1.65)	1.15 (0.41,3.21)	0.47 (0.04,5.02)	-1.00 (-5.78,3.78)	4.50 (-4.33,13.33)
Bifidobacterium bifidum	4.31 (0.20,90.52)	0.85 (0.02,43.14)	0.49 (0.13,1.85)	-	-1.10 (-5.31,3.11)	-0.60 (-13.61,12.41)
Bacillus clausii	0.83 (0.37,1.87)	0.98 (0.14,7.10)	0.70 (0.20,2.45)	0.81 (0.06,11.00)	-	-
Bifidobacterium breve	0.92 (0.63,1.34)	0.92 (0.64,1.32)	0.87 (0.48,1.55)	-	-1.53 (-4.30,1.24)	1.18 (-5.88,8.24)
S. boulardii	1.01 (0.46,2.23)	0.81 (0.42,1.55)	0.77 (0.40,1.45)	0.53 (0.08,3.40)	-1.02 (-3.64,1.61)	-1.86 (-6.65,2.92)

NOTE. OR = odds ratio; MD = mean difference. Results are the mean difference, or odds ratio, and associated 95% confidence intervals (95% CIs) between the intervention and placebo from the network meta-analysis. Mean difference values < 0 indicates the treatment is superior to placebo. An OR less than 1 indicates the treatment is superior to placebo; Underlined numbers in bold represent statistically significant results.

Table legends and description of color gradients:

	Statistically significant difference with placebo and at least one other tx	Statistically significant difference with placebo	Statistically no difference with placebo
High or moderate certainty evidence	Among the most effective	Inferior to the most effective, but superior to placebo	No more effective than placebo
Low or very low certainty evidence	May be among the most effective	May be inferior to the most effective, but superior to placebo	May be no more effective than placebo

Figure 2.

NMA results sorted based on GRADE certainty of evidence and treatment effectiveness for the comparisons of active treatments vs placebo for each outcome.

Table 1.

NMA Results Sorted Based on GRADE Certainty of Evidence for the Comparisons of Active Treatments vs Placebo for Binary Outcomes

Outcome	Certainty of evidence	Classification	Intervention	OR (95% CI) vs placebo	RD per 1000 (95% CI)
All-cause mortality	High (moderate to high)	Among the most effective	*Lactobacillus* spp and *Bifidobacterium* spp	**0.56 (0.39–0.80)**	**22 fewer (31 fewer to 10 fewer)**
		Among the least effective	*Lactobacillus* spp, *Bifidobacterium* spp, and *S salivarius* subsp *thermophilus*	0.40 (0.12–1.30)	30 fewer (45 fewer to 14 more)
			B animalis subsp *lactis*	0.43 (0.16–1.15)	29 fewer (43 fewer to 7 more)
			L reuteri	0.77 (0.51–1.17)	11 fewer (25 fewer to 8 more)
			L rhamnosus	0.84 (0.33–2.12)	8 fewer (34 fewer to 52 more)
			B clausii	0.83 (0.37–1.87)	8 fewer (32 fewer to 41 more)
			B breve	0.92 (0.63–1.34)	4 fewer (18 fewer to 16 more)
			S boulardii	1.01 (0.46–2.23)	0 fewer (27 fewer to 57 more)
	Low (low to very low)	May be among the least effective	*L acidophilus*	0.29 (0.03–3.12)	36 fewer (50 fewer to 93 more)
			B animalis subsp *lactis* and *B longum* subsp *longum*	0.39 (0.04–4.18)	31 fewer (49 fewer to 134 more)
			B longum subsp *longum*	0.77 (0.11–5.35)	11 fewer (46 fewer to 174 more)
			Lactobacillus spp, *Bifidobacterium* spp, and *Enterococcus* spp	0.78 (0.23–2.62)	11 fewer (39 fewer to 73 more)
			Bifidobacterium spp and *S salivarius* subsp *thermophilus*	0.84 (0.51–1.40)	8 fewer (25 fewer to 19 more)
			B coagulans	0.91 (0.38–2.15)	4 fewer (31 fewer to 53 more)
			B adolescentis	0.93 (0.02–47.20)	2 fewer (50 fewer to 672 more)
			Bacillus spp and *Enterococcus* spp	0.95 (0.02–48.18)	2 fewer (50 fewer to 672 more)
			Lactobacillus spp, *Bifidobacterium* spp, and *S boulardii*	1.05 (0.51–2.17)	2 more (25 fewer to 54 more)
			B bifidum	4.31 (0.20–90.52)	3 fewer (50 fewer to 668 more)
NEC (stage II or more)	High (moderate to high)	Among the most effective	*B animalis* subsp *lactis*	**0.31 (0.13–0.74)**	**44 fewer (56 fewer to 16 fewer)**
			Lactobacillus spp and *Bifidobacterium* spp	**0.35 (0.20–0.59)**	**41 fewer (51 fewer to 26 fewer)**
		Inferior to the most effective/superior to the least effective	*L rhamnosus*	**0.44 (0.21–0.90)**	**35 fewer (50 fewer to 5 fewer)**
			L reuteri	**0.55 (0.34–0.91)**	**28 fewer (42 fewer to 5 fewer)**
		Among the least effective	*B longum* subsp *longum*	0.25 (0.03–2.30)	48 fewer (63 fewer to 73 more)

Outcome	Certainty of evidence	Classification	Intervention	OR (95% CI) vs placebo	RD per 1000 (95% CI)
			S boulardii	0.81 (0.42–1.55)	12 fewer (37 fewer to 32 more)
			B breve	0.92 (0.64–1.32)	5 fewer (22 fewer to 19 more)
	Low (low to very low)	May be among the most effective	*Bacillus* spp and *Enterococcus* spp	**0.23 (0.08–0.63)**	**49 fewer (59 fewer to 23 fewer)**
			Lactobacillus spp, *Bifidobacterium* spp, and *Enterococcus* spp	**0.28 (0.16–0.49)**	**46 fewer (54 fewer to 32 fewer)**
			Bifidobacterium spp and *S salivarius* subsp *thermophilus*	**0.38 (0.19–0.75)**	**39 fewer (52 fewer to 15 fewer)**
		May be among the least effective	*B adolescentis*	0.13 (0.01–2.51)	56 fewer (64 fewer to 83 more)
			Lactobacillus spp, *Bifidobacterium* spp, and *S salivarius* subsp *thermophilus*	0.42 (0.16–1.13)	37 fewer (54 fewer to 8 more)
			B coagulans	0.58 (0.20–1.65)	26 fewer (51 fewer to 38 more)
			Lactobacillus spp, *Bifidobacterium* spp, and *S boulardii*	0.73 (0.29–1.85)	17 fewer (45 fewer to 49 more)
			B bifidum	0.85 (0.02–43.14)	9 fewer (64 fewer to 685 more)
			B clausii	0.98 (0.14–7.10)	1 fewer (55 fewer to 265 more)
			L acidophilus	1.00 (0.02–53.66)	0 fewer (64 fewer to 723 more)
			B animalis subsp *lactis* and *B longum* subsp *longum*	1.42 (0.37–5.42)	25 more (40 fewer to 208 more)
Culture-proven late onset sepsis	High (moderate to high)	Among the least effective	*B animalis* subsp *lactis*	0.73 (0.38–1.43)	38 fewer (92 fewer to 54 more)
			S boulardii	0.77 (0.40–1.45)	32 fewer (88 fewer to 56 more)
			L rhamnosus	0.84 (0.45–1.57)	22 fewer (80 fewer to 70 more)
	Low (low to very low)	May be among the least effective	*Lactobacillus* spp, and *Bifidobacterium* spp, and *Enterococcus* spp	0.43 (0.17–1.07)	84 fewer (127 fewer to 9 more)
			Lactobacillus spp, *Bifidobacterium* spp, and *S boulardii*	0.54 (0.28–1.04)	66 fewer (108 fewer to 5 more)
			B bifidum	0.49 (0.13–1.85)	74 fewer (135 fewer to 100 more)
			Lactobacillus spp, *Bifidobacterium* spp, and *S salivarius* subsp *thermophilus*	0.68 (0.35–1.30)	45 fewer (97 fewer to 38 more)
			B clausii	0.70 (0.20–2.45)	42 fewer (122 fewer to 157 more)
			L reuteri	0.71 (0.41–1.26)	41 fewer (87 fewer to 33 more)
			B longum subsp *longum*	0.75 (0.23–2.50)	35 fewer (117 fewer to 162 more)
			B animalis subsp *lactis* and *B longum* subsp *longum*	0.77 (0.23–2.57)	32 fewer (117 fewer to 168 more)

Outcome	Certainty of evidence	Classification	Intervention	OR (95% CI) vs placebo	RD per 1000 (95% CI)
			Lactobacillus spp and *Bifidobacterium* spp	0.87 (0.60–1.27)	18 fewer (57 fewer to 35 more)
			B breve	0.87 (0.48–1.55)	18 fewer (76 fewer to 67 more)
			Bifidobacterium spp and *S salivarius* subsp *thermophilus*	1.04 (0.52–2.06)	5 more (69 fewer to 121 more)
			B coagulans	1.15 (0.41–3.21)	20 more (87 fewer to 70 more)
Feed intolerance	Low (low to very low)	May be among the least effective	*B animalis* subsp *lactis*	0.10 (0.00–2.29)	239 fewer (272 fewer to 190 more)
			Lactobacillus spp, *Bifidobacterium* spp, and *Enterococcus* spp	0.23 (0.02–3.07)	195 fewer (268 fewer to 263 more)
			L reuteri	0.26 (0.06–1.10)	186 fewer (254 fewer to 19 more)
			B coagulans	0.47 (0.04–5.02)	124 fewer (261 fewer to 381 more)
			Lactobacillus spp, *Bifidobacterium* spp, and *S boulardii*	0.47 (0.04–5.04)	124 fewer (261 fewer to 382 more)
			S boulardii	0.53 (0.08–3.40)	108 fewer (246 fewer to 288 more)
			Lactobacillus spp, *Bifidobacterium* spp, and *S salivarius* subsp *thermophilus*	0.68 (0.06–7.70)	70 fewer (254 fewer to 470 more)
			L rhamnosus	0.75 (0.11–5.35)	54 fewer (236 fewer to 395 more)
			B clausii	0.81 (0.06–11.00)	40 fewer (254 fewer to 531 more)

Table 2.

Detailed Active Treatment Probiotic Combinations and Strains Showing Effectiveness Compared to Placebo

Probiotic combinations	All-cause mortality	NEC (stage ≥ II)	Reduction in days to reach full feed	Reduction in days of hospitalization
Lactobacillus spp and *Bifidobacterium* spp	• *L rhamnosus* ATCC 53103 + *B longum* subsp *infantis* • *Lactobacillus casei* + *B breve* • *L acidophilus* + *B longum* subsp *infantis* • *L acidophilus* + *B bifidum* • *L rhamnosus* ATCC 53103 + *B longum* subsp *longum* Reuter ATCC BAA-999 • *L acidophilus* + *B bifidum* + *B animalis* subsp *lactis* + *B longum* subsp *longum*	• *L rhamnosus* ATCC 53103 + *B longum* subsp *infantis* • *L casei* + *B breve* • *L rhamnosus* + *L acidophilus* + *L casei* + *B longum* subsp *infantis* + *B bifidum* + *B longum* subsp *longum* • *L acidophilus* + *B longum* subsp *infantis* • *L acidophilus* + *B bifidum* • *L rhamnosus* ATCC 53103 + *B longum* subsp *longum* Reuter ATCC BAA-999 • *L acidophilus* + *B bifidum* + *B animalis* subsp *lactis* + *B longum* subsp *longum*	• *L casei* + *B breve* • *L rhamnosus* + *L acidophilus* + *L casei* + *B longum subsp infantis* + *B bifidum* + *B longum subsp longum* • *L acidophilus* + *B bifidum* • *L acidophilus* + *B bifidum* + *B animalis subsp lactis* + *B longum subsp longum*	—
Bifidobacterium animalis subsp *lactis*	—	• B animalis subsp lactis • *B animalis subsp lactis* DSM 15954	—	• B animalis subsp lactis
Lactobacillus reuteri	—	• *L reuteri* DSM 17938 • *L reuteri* ATCC 55730	• *L reuteri* DSM 17938 • *L reuteri* ATCC 55730	• *L reuteri* DSM 17938 • *L reuteri* ATCC 55730
Lactobacillus rhamnosus	—	• *L rhamnosus* ATCC 53103 • *L rhamnosus* ATC A07FA • *L rhamnosus* LCR 35	—	—
Lactobacillus spp, *Bifidobacterium* spp, and *Enterococcus* spp	—	• *L acidophilus* + *B longum subsp longum* + *Enterococcus faecalis* • *Lactobacillus gasseri* PTA-5845 + *B longum subsp infantis* PTA-5843 + *Enterococcus faecium* PTA-5844	—	—

Probiotic combinations	All-cause mortality	NEC (stage ≥ II)	Reduction in days to reach full feed	Reduction in days of hospitalization
		• *L acidophilus* + *B longum subsp longum* + *E faecium* • *L acidophilus* + *B longum subsp infantis* + *E faecalis*		
Bifidobacterium spp and *S salivarius* subsp *thermophilus*	—	• *B longum subsp infantis* + *B bifidum* + *S salivarius subsp thermophilus* • *B longum subsp infantis* DSM 33361 + *B animalis subsp lactis* DSM 15954 + *S salivarius subsp thermophilus* TH-4	—	—
Bacillus spp and *Enterococcus* spp	—	• *B subtilis* + *E faecium*	—	—
Lactobacillus spp, *Bifidobacterium* spp, and *S boulardii*	—	—	• *L rhamnosus* + *L acidophilus* + *B longum subsp longum* + *S boulardii* • *L acidophilus* + *B bifidum* + *S boulardii*	—