Probiotics and Preterm Infants: A Position Paper by the European Society for Paediatric Gastroenterology Hepatology and Nutrition Committee on Nutrition and the European Society for Paediatric Gastroenterology Hepatology and Nutrition Working Group for Probiotics and Prebiotics

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ABSTRACT

More than 10,000 preterm infants have participated in randomised controlled trials on probiotics worldwide, suggesting that probiotics in general could reduce rates of necrotising enterocolitis (NEC), sepsis, and mortality. Answers to relevant clinical questions as to which strain to use, at what dosage, and how long to supplement are, however, not available. On the other hand, an increasing number of commercial products containing probiotics are available from sometimes suboptimal quality. Also, a large number of units around the world are routinely offering probiotic supplementation as the standard of care despite lacking solid evidence. Our recent network meta-analysis identified probiotic strains with greatest efficacy regarding relevant clinical outcomes for preterm neonates. Efficacy in reducing mortality and morbidity was found for only a minority of the studied strains or combinations. In the present position paper, we aim to provide advice, which specific strains might potentially be used and which strains should not be used. In addition, we aim to address safety issues of probiotic supplementation to preterm infants, who have reduced immunological capacities and occasional indwelling catheters. For example, quality reassurance of the probiotic product is essential, probiotic strains should be devoid of transferable antibiotic resistance genes, and local microbiologists should be able to routinely detect probiotic sepsis. Provided all safety issues are met, there is currently a conditional recommendation (with low certainty of evidence) to provide either *Lactobacillus rhamnosus* GG ATCC53103 or the combination of *Bifidobacterium infantis* Bb-02, *Bifidobacterium lactis* Bb-12, and *Streptococcus thermophilus* TH-4 in order to reduce NEC rates.

Key Words: *Bifidobacterium*, *Lactobacillus*, microbiome, necrotizing enterocolitis, premature neonate, preterm infant, probiotics, sepsis

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What Is Known

- Probiotics might be a potential therapy for preterm infants to reduce morbidity and mortality.
- Only a limited number of different strains have shown preliminary potential effectiveness.

What Is New

- We provide advice, which specific strains might potentially be used and which strains should not be used for preterm neonates.
- Several safety issues are addressed to which probiotic products and their supplementation for preterm infants should fulfil.

Infants born prematurely have high rates of mortality, septicaemia, and gastrointestinal morbidities, such as necrotising enterocolitis (NEC). The exact aetiology of these morbidities is unknown, but include intestinal immaturity with increased permeability and an immature immune system (1–3). Enteral tolerance is frequently reduced in preterm infants, and most require parenteral nutrition. Feeding preterm infants nonpasteurized own mother's milk is the best feeding strategy to reduce neonatal mortality and many morbidities (4).

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Over the last 2 decades, certain probiotic strains, either single or in combination, have been administered in clinical trials in an attempt to reduce NEC and late-onset sepsis, and to improve feed-related outcomes, such as time to full feeds. Whilst multiple potential mechanisms of how probiotics may exert their beneficial effect have been postulated (5–8), very few, if any, mechanistic studies exist in this patient group. Results of individual trials have varied, but almost all systematic reviews and meta-analyses have shown positive effects on reducing the incidence of a range of adverse outcomes when studies with different strains are combined and analysed as a single group (9–23). Importantly, however, long-term neurodevelopmental follow-up has neither shown beneficial nor detrimental effects of probiotics in preterm neonates in a recent meta-analysis based on 5 studies in 1637 infants (24).

Whilst many have strongly argued for their routine use (25–28), other groups including the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) and the American Academy of Pediatrics (AAP), have been more cautious, noting some of the major limitations in many of the studies, methodological differences in study design, and pointing out that probiotic efficacy may vary widely (29–36). This was emphasised by a recent high-quality study in the UK that showed no effect for a specific strain of *Bifidobacterium breve* (BBG-001) on mortality or NEC in a large group of preterm infants (37). The importance of strain specificity is further exemplified by the fact that within the species *Escherichia coli*, certain strains may cause haemolytic uremic syndrome (strain O157:H7), whereas others are considered probiotic supplements (strain Nissle 1917). On the other hand, several probiotic genera or species share underlying mechanistic characteristics that are beneficial (38), which would favour the argument of pooling the data of several strains together. So far, heterogeneity of organisms and dosing regimens studied have prevented strain-specific treatment recommendations from being made.

Recently, the ESPGHAN Working Group for Probiotics and Prebiotics published a document using a network meta-analysis (NMA) approach to identify strains with greatest potential efficacy for preventing major neonatal morbidities in preterm infants (39). Following this publication, the ESPGHAN Committee on Nutrition and the ESPGHAN Working Group for Probiotics and Prebiotics aimed to develop a document that might serve as a guide for the possible use of probiotics in preterm infants.

METHODS

An ESPGHAN Position Paper addresses a topic for which guidance is necessary but there is only limited scientific evidence, and therefore, the recommendations are mostly based on expert opinion. A writing consensus group was convened to support the development of this document. This group included experts in the fields of neonatology, paediatric gastroenterology, and nutrition. All members of the group disclosed any potential conflicts of interest. No funding for the development of this document was received.

Defining the Clinical Questions

The first stage of the development of this position paper involved specifying the clinical questions:

1. Are probiotics safe enough for administration to preterm infants?

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2. Should probiotics be used in preterm infants? If yes, which probiotics (single or combinations) should be used in what dose?
3. Are combinations of species more effective than the use of a single strain to reduce the risk of NEC (stage 2 or 3)?
4. Which dose of a probiotic strain or combination of strains should be administered?
5. What should be the duration of administering probiotics?
6. Is it appropriate to administer other strains than those studied in large well-conducted randomised controlled trials (RCTs)?

Methodology for Synthesis and Grading of Recommendations

The panel decided as a primary starting point that any recommendations for the use of probiotics should be specified at strain level because of the strain-specific effects. This relates particularly to clinical question 2 and means that for studies in which the probiotic was only specified at the species level (without strain designation), no recommendation could be derived. Furthermore, it was decided that recommendations should be based primarily on the results from RCTs, and that evidence from cohort studies is only used for interpretation and discussion of the recommendation. Thus, our recently published probiotic strain-specific systematic review and NMA (39) could form the direct basis. Although in our previous paper, we defined prematurity as a gestational age (GA) of less than 37 weeks' gestation, the recommendations posed here are only applicable to infants being born at less than 32 weeks' gestation. Furthermore, the panel decided that we could only make proper recommendations on the use of a specific intervention that was tested in RCTs with adequate cumulative power for at least 1 of the 3 outcomes of particular interest, namely mortality, NEC, or late-onset sepsis. This reduces the chance of making recommendations based on type 1 errors (false positive). Sample size calculations are depicted in Table 1 for each of the 3 outcome domains, with an α of 0.05 and 1-β of 0.80. The panel acknowledges that proposed reductions in mortality, NEC stage $\geq$2, or late-onset sepsis are arbitrary. We, however, deliberately chose high baseline rates and optimistic reductions in order to achieve realistic sample sizes. This translated to a minimum of 247 infants (per group) corresponding to a sepsis reduction from 25% to 15% with 80% power, being the least number of infants that needed to be studied before recommendations were made. For each outcome domain, we assessed power separately to take this into account when formulating the recommendations (by downgrading certainty of evidence on imprecision, see below).

To grade the recommendations, the GRADEpro software was used, developed by the Grading of Recommendations, Assessment Development and Evaluations Working Group (40). GRADE assesses evidence quality by grading risk of bias, inconsistency, indirectness, and imprecision each as not serious, serious, or very serious. On the basis of these assessments any observed risk reduction is categorized as high, moderate, low, or very low certainty of evidence. The GRADEpro system offers 2 categories for the strength of the final recommendation (strong or conditional). The strength of a recommendation was graded as strong when the evidence showed a clear benefit or absence of benefit of the intervention based on moderate or high certainty of evidence. The strength of a recommendation was graded as conditional when the trade-offs were less certain, either because of the low certainty of evidence or as the evidence suggested that desirable and undesirable effects were closely balanced.

Unfortunately, only clinical questions 1 and 3 could be answered from systematic PICO (population, intervention, comparison, outcome) questions where RCTs assessed our patient group of interest. Regarding clinical question 2, the final proposed recommendations for strains (single or combinations) are based on the combined evidence on mortality, NEC stage 2 or 3, and late-onset sepsis rates, together with its quality grading (certainty assessment) and are depicted in GRADE tables. Effect sizes are reported as a relative risk (RR) versus placebo with its 95% credible interval (CrI). For each recommendation, we provide the dose (or the range in which it was used) of the probiotic strains (single or combination) that exerted the effect in the available studies.

To answer clinical question 3, additional models were constructed in our NMA database using the same methodology as previously used (39). First, we compared placebo versus administration of a single probiotic strain/species and versus multiple strains/species on the incidence of NEC stage 2 or 3. Second, we compared placebo versus administration of any single/multiple Lactobacillus probiotic(s), versus any single/multiple Bifidobacterium probiotic(s), versus the combination of any Lactobacillus and Bifidobacterium probiotics. Effect sizes are reported as a RR versus placebo with its 95% CrI. As these analyses are not strain-specific, these data are only hypothesis-generating. Therefore, these recommendations were rated as conditional and based on very low certainty of evidence.

The other clinical questions (1, 4, 5, and 6) are each discussed based on the known literature (mainly case series and the expertise of the authors). Because this is regarded as indirect evidence, these recommendations were also rated as conditional and based on very low certainty of evidence.

Probiotic Nomenclature

For the remainder of this manuscript, probiotic species are truncated at their genus: *Bifidobacterium, Escherichia, Lactobacillus, Saccharomyces,* and *Streptococcus* are denoted by *B, E, L, S,* and *Str*, respectively. In addition, subspecies (subsp) names are truncated as well: *Bifidobacterium animalis* subsp *lactis* is denoted as *B lactis*; *Bifidobacterium longum* subsp *infantis* as *B infantis*; *B longum* subsp *longum* as *B longum*, and *Streptococcus salivarius* subsp *thermophilus* as *Str thermophilus*. Over the past decades. multiple reclassifications in taxonomy have been proposed and designations in the historical publications may no longer be accurate. We therefore adhered to the latest nomenclature we were aware of, so that for example *B bifidum* Bb-12 is designated as *B lactis* Bb-12 (41). Although in our recent NMA, we analysed some strains together because of their relative resemblance, we here chose to be truly strain-specific. Results from the *Lactobacillus reuteri* DSM 17938 strain are thus separated from *L reuteri* ATCC 55730, and *B lactis* B94 is now separated from *B lactis* Bb-12.

Document Review

The manuscript and the recommendations were drafted first by the writing committee of the group (Chris H.P. van den Akker,

TABLE 1. Sample size calculations for each outcome domain ($\alpha = 0.05$; 1-$\beta = 0.80$; 2-sided)

Outcome domain	Proposed reduction	Required sample size (No. per group)
Mortality	7.5 → 5.0%	1465
NEC stage ≥2	10 → 5.0%	431
Late onset sepsis	25 → 15%	247

NEC = necrotising enterocolitis.

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Johannes B. van Goudoever, Hania Szajewska, and Raanan Shamir). Then, several other members of the author group (Magnus Domellöf, Nicholas D. Embleton, Iva Hojsak, Alexandre Lapillonne, and Walter A. Mihatsch) reviewed and discussed the evidence, reviewed the drafted recommendations, and reached a consensus on the strength of each recommendation. As a next step of the consensus development process, the manuscript with its draft recommendations was then submitted for review to the other members of the ESPGHAN Committee on Nutrition and the ESPGHAN Working Group for Probiotics and Prebiotics (Roberto Berni Canani, Jiri Bronsky, Cristina Campoy, Mary S. Fewtrell, Nataša Fidler Mis, Alfredo Guarino, Jessie M. Hulst, Flavia Indrio, Sanja Kolaček, Rok Orel, Yvan Vandenplas, and Zvi Weizman). Then, the finalized manuscript was sent to all aforementioned people together with an invitation to vote the recommendations. The ideal was to reach 100% consensus, but 85% agreement was considered acceptable as is proposed by the general ESPGHAN Guideline Development Group. All of the comments were considered, and revisions were made in response to peer-reviewer's comments until the desired 85% threshold was reached. If consensus was not reached within a maximum of 3 voting rounds, the recommendation was not accepted. A finalised document was submitted to the ESPGHAN Council for peer review before publication.

Updating

The group will monitor new publications and evidence made available and decide whether and when it is necessary to update the recommendations. In any case, the results will be reviewed within 5 years from publication.

SUMMARY OF EVIDENCE, INTERPRETATION, AND RECOMMENDATIONS

Are Probiotics Safe Enough for Administration to Preterm Infants?

Probiotics may theoretically be responsible for at least 5 types of side effects: systemic infections, deleterious metabolic activities, excessive immune stimulation, antibiotic resistance gene transfer, and gastrointestinal side effects, such as intestinal gas formation (42,43). Most of the RCTs conducted in preterm infants or other patient groups, however, did not adequately monitor or report these side effects (44). Other safety issues might more be related to quality control of the probiotic supplementation. Several issues will be elaborated below.

Probiotic sepsis in premature infants could be particularly important, as they represent an immunocompromised patient group. Furthermore, probiotic bacteraemia may be hard to detect with classic culture methods especially in single paediatric culture bottles, as strictly anaerobic strains are difficult to grow. Yet, multiple case reports have described single or multiple cases of bacteraemia (sometimes in conjunction with NEC) in premature infants (45). In particular, *B infantis* (46–49) and *L rhamnosus* GG (50–55) bacteraemia have been described in premature neonates, but other cultured probiotic strains include *L reuteri* (56), *Saccharomyces boulardii* (57,58), *B breve* BBG-001 (59), and *E. coli* Nissle 1917 (60). Probiotic bacteraemia may occur not only because of intestinal translocation, but also because of contamination from probiotic preparation and subsequent line handling. Especially if probiotics are prepared on the ward from powder sachets or capsules that are opened, probiotic spills and contamination may occur to other surface areas, medications, or intravenous catheter sites, or cross colonisation to other infants on the neonatal ward (61,62). Although the cross colonisation may not necessarily be seen as an adverse effect (in case of a safe product), it illustrates how easily living organisms may spread and warrants extreme caution when preparing and supplementing a probiotic supplement. This is exemplified in a recent paper on 3 cases of *L rhamnosus* GG bacteraemia in preterm neonates (55), in which only 1 infant actually received the particular probiotic strain, whereas the other 2 infants (who also had a central line) were only hospitalised in the same room as where other infants were supplemented with probiotics. Simultaneously, a study was published describing 6 cases of *L rhamnosus* GG bacteraemia out of a cohort of 522 patients on a paediatric intensive care unit who also received a probiotic supplement containing *L rhamnosus* GG (63). None of the infected infants were immunocompromised or had known bowel disintegrity, but contamination of their central line was suspected. Similarly, because of the risk of contamination, the European Medicine Agency even amended a contra-indication to the use of *S boulardii* in patients (not specifically neonates) who are critically ill or are immunocompromised, or those who have a central venous catheter (64).

Examples of deleterious metabolic activities include increased D-lactate and biogenic amines production or bile salt hydrolysis activity affecting cholesterol metabolism and lipid uptake. Because of a complete lack of data in infants and children on the latter examples, only the issue of D-lactate is elaborated on here as this has been studied in older infants. Whereas some Lactobacilli strains produce mainly L-lactate, many produce a mixture, and some predominantly produce D-lactate. From the Lactobacilli that are described in the next clinical question, *L rhamnosus* GG ATCC 53103 produces almost only lactate in its L-isoform, but fermentation by *L reuteri* DSM 17938 or *L acidophilus* NCDO 1748 (ATCC 4356, LA37, or NCIMB 30316) yields larger proportions of D-lactate. Although the amount of D-lactate that is produced may quantitatively be relatively small, D-lactate is difficult to dispose of after enteral uptake, which could be even more problematic in premature infants (65). Not only do most premature infants already have the tendency to be acidotic, D-lactate cannot routinely be measured in blood gases, making it very difficult to suspect or detect. In healthy 6-month old infants (66), children (67), or adults (68), D-lactate formation by probiotics is probably not much of a clinical issue. However, in term-born infants elevated urinary D-lactate concentrations were found in the first 2 weeks of life after being fed a *L reuteri* DSM 17938-containing formula (69), although there were no signs of blood acidosis. Yet, several case reports have appeared describing D-lactate acidosis in short bowel syndrome infants (70,71). To avoid any risks, it is stated in the Codex Alimentarius that if probiotics are added to infant formulas, they may only contain L-lactate-producing cultures (72). On the other hand, *L reuteri* DSM 17938 has been reviewed as GRAS (generally recognized as safe) for the use in term infant formula by the Food and Drug Administration (FDA) (73). In premature infants, the issue of D-lactate has not been systematically researched, but it seems prudent to select only those Lactobacilli that are predominantly L-lactate producers in preterm infants, until further specific safety data is available in this specific patient group. This may be especially important in infants during kidney failure or with short bowel syndrome (e.g. after extensive NEC surgery).

Although there are some indications that meconium is not sterile (74), these findings are challenged by others (75). Yet, the vast majority of gastrointestinal colonisation of the microbiome occurs in the weeks after birth (76). Albeit premature infants on a NICU by definition have an abnormal colonization because of an immature immune system, less parental skin-to-skin contact, and frequent antibiotic exposure, supplementing 1 or few probiotic strains soon after (preterm) birth influences colonization as well.

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It is currently unknown if this effect only lasts during supplementation or has longer influences and if any effect is positive in later life with potential excessive immune stimulation or allergy in susceptible individuals, although this has not been systematically researched. Augmentation of natural killer activity, T-cell functions, and cytokine production are some of the plausible mechanisms underlying the immune regulatory activities of probiotics (43,77).

The gut harbours simultaneously with its microbiome, a natural reservoir of antibiotic resistance genes, which appear to increase upon increased antibiotic exposure (78,79). This can be beneficial during administration of antibiotics, as it preserves some protection to the bacterial microbiome. Also, many commercially available probiotics carry some antibiotic resistance genes (80,81). As long as these genes in probiotic products are not transferable through plasmids to other more pathogenic bacteria, these risks are probably limited. Yet, there are several examples of probiotic strains with potentially transferable genes (82–85). Especially under antibiotic pressure such as on the NICU, risks of horizontal gene transfer might be higher and contribute to increased antibiotic resistance (86–88). A vancomycin-resistant enterococcus outbreak on a Turkish NICU was linked to the provision of certain probiotics in a recent study (89), although another report showed no greater antibiotic resistome in infants that had received probiotics (90). As most preterm infants will receive concomitant antibiotics during some period on their NICU stay, it is prudent to select only those probiotic strains with known safety profile on gene transfer (91).

Other potential gastrointestinal side-effects of probiotics, such as intestinal gas formation are even less studied, especially in premature infants. Therefore, and because potential adverse effects are probably less severe than potential benefits, these will not be addressed here further.

Probiotics are usually marketed as nutritional supplements rather than as drugs and, thus, form an unregulated market where manufacturers may change product contents and/or the production process without properly addressing these issues (92). Previously, the ESPGHAN has also called for more stringent controls of the production of probiotics, especially in premature neonates (93,94). Ascertaining product safety and quality is of specific concern here, as preterm infants frequently have the need for indwelling catheters and nasogastric tubes, and they do not have an adequate immune response. For example, a fatal case of gastrointestinal mucormycosis in a preterm infant has been described following contamination of a combination of 3 probiotic strains (95). The caveats in quality control of probiotics should thus be more stringent to ensure that the probiotic content as mentioned on the label meets the actual content throughout the shelf life of the product, while no contamination is present. Several reports, however, show that product labels on commercial or medical probiotic products frequently do not match actual contents in terms of species identity and bacterial count, or contained contamination with nonprobiotic bacteria (96–98). Even in a more recent report, only 1 out of 16 tested commercial probiotic products (including those marketed specifically for infants) contained the correct probiotics at subspecies level as claimed on the product label (99). Ensuring correct product identity at strain level is essential, not only during research but also during actual clinical implementation, in order to match achieved trial results to clinical practice (100). Probiotic products for premature infants should, therefore, be manufactured according to current Good Manufacturing Practice (cGMP) guidelines. In addition, manufacturers should provide certificates of compliance and analysis to be able to address at least strain identity, purity, viability at end of shelf life, and antibiotic susceptibility and resistance profiles.

Because of all of these potential safety and quality issues, we suggest that if a NICU is implementing probiotics as part of standard care, parents must be actively informed. Communication on the potential benefits and risks of probiotic administration is best undertaken face to face and supplemented with the use of written materials appropriate to the local context.

Recommendations

1. The panel conditionally recommends that in case of implementing a probiotic product, the local microbiologists should be informed and they should confirm the ability to routinely detect probiotic bacteraemia/fungaemia with standard culture methods (very low certainty of evidence).
2. The panel conditionally recommends not to provide probiotic strains, which produce D-lactate, as its potential risk or safety has not been adequately studied in preterm infants and remains uncertain (very low certainty of evidence).
3. The panel conditionally recommends only the use of strains devoid of any plasmids containing transferable antibiotic resistance genes (very low certainty of evidence). This information should be confirmed and provided by the manufacturer.
4. The panel conditionally recommends only the use of probiotic products manufactured according to cGMP to ensure correct strain identity with lack of contamination (very low certainty of evidence). Certificates of analysis should address at least strain identity, purity, viability, and antibiotic susceptibility and resistance profiles.
5. The panel conditionally recommends to provide parents of preterm infants with sufficient information so they can understand the potential benefits and risks of probiotic administration (very low certainty of evidence). Communication is best undertaken face to face and supplemented with the use of written materials appropriate to the local context.

Should Probiotics Be Used in Preterm Infants? If Yes, Which Probiotics (Single or Combinations) Should be Used in What Dose?

The following probiotic strains (or combination of strains) fulfilled the criteria of being defined at strain level and were tested in at least 247 infants (per group) in RCTs: *B breve* BBG-001 (YIT4010), *L reuteri* DSM 17938, *L rhamnosus* GG ATCC 53103, *S boulardii* CNCM I-745, the combination of *B bifidum* NCDO 1453 with *L acidophilus* NCDO 1748 (ATCC 4356, LA37, or NCIMB 30316), and the combination of *B infantis* Bb-02, *B lactis* Bb-12, and *Str thermophilus* TH-4. In our previous NMA (39), we analysed results from the strains *B lactis* Bb-12 and B94 together, yielding reduced NEC rates. These results, however, were largely based on the single trial (101) that investigated the B94 strain in 200 infants, which is lower than the required power to assess sepsis. The *B lactis* Bb-12 strain was assessed in 219 infants (102–105) and did not result in reduced mortality or morbidity incidence, although the power was thus also lower than our threshold. As our aim was to give strain-specific recommendations, these 2 *B lactis* strains are,

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TABLE 2. GRADE table summarizing the evidence on the use of *Lactobacillus rhamnosus* GG ATCC 53013 compared with usual care in preterm infants

Certainty assessment						No. of patients		Effect		
No. of RCTs (ref)	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	*L rhamnosus* GG ATCC 53013	Usual care	Relative (95% CrI)	Absolute (95% CrI)	Certainty
Mortality 3 (106–108)	Not serious [*]	Not serious	Serious [†]	Very serious [‡,§]	None	11/273 (4.0%)	10/277 (3.6%)	RR 0.89 (0.32 to 2.30)	4 fewer per 1.000 (from 25 fewer to 47 more)	⊕◯◯◯ VERY LOW
NEC stage 2 or 3 6 (107–111,114)	Not serious [*]	Not serious	Serious [†]	Serious [‖]	None	6/706 (0.8%)	16/687 (2.3%)	RR 0.240 (0.064 to 0.670)	18 fewer per 1.000 (from 8 fewer to 22 fewer)	⊕⊕◯◯ LOW
Late-onset sepsis 8 (106–113)	Not serious [*]	Not serious	Serious [†]	Serious [§]	None	47/660 (7.1%)	50/635 (7.9%)	RR 0.80 (0.47 to 1.30)	16 fewer per 1.000 (from 24 more to 42 fewer)	⊕⊕◯◯ LOW

CrI = credible interval; NEC = necrotising enterocolitis; RR = risk ratio.
[*]Study by Romeo scored high risk for performance bias (blinding); however, in both group 0 events; therefore, overall here regarded as low risk. Study by Dani unclear risk on selection bias, as they only described "randomly assigned, by sealed envelope technique"; overall no clear risk of bias.
[†]Relatively older infants (GA around 30 weeks; BW 1150–1350 g on average) were included. Study by Romeo BW even on average 1950 g. In the study by Manzoni 2009/2014, both the control and intervention groups received bovine lactoferrin as well, in addition to placebo or LGG. This may all explain the low event rates, even in the control group.
[‡]Underpowered.
[§]Wide confidence interval.
[‖]Few events.

therefore, not further assessed. Other probiotic strains that have been previously studied in RCTs and were summarised in our prior NMA, but were not specified at strain level or did not reach the threshold of 247 infants in each group, are: *Bacillus clausii* (4 strains: O/C, N/R84, T84, and Sin8); *Bacillus coagulans* (previously *L. sporogenes*); combination of *Ba subtilis* R0179 and *E faecium* R0026; *B bifidum* OLB6378; combination of *B bifidum, B infantis, B longum,* and *L acidophilus*; combination of *B bifidum, B lactis, B longum,* and *L acidophilus*; *B breve* M-16 V, combination of *B breve* and *L casei*; combination of *B infantis* ATCC 15697 and *L acidophilus* ATCC 4356; combination of *B infantis, L acidophilus, L casei, L plantarum, L rhamnosus,* and *Str thermophilus*; combination of *B infantis* PTA-5843, *E faecium* PTA-5844, and *L gasseri* PTA-5845; combination of *B lactis* Bb-12 and *B longum* BB536; *B longum* BB536; combination of *B longum* BB536 and *L rhamnosus* GG; combination of *B longum* 35624 and *L rhamnosus* GG; combination of *B longum* R00175, *L helveticus* R0052, *L rhamnosus* R0011, and *S boulardii* CNCM I-1079; *L acidophilus* Lb; *L acidophilus* LA-5 (DSM 13241); and *S boulardii* CNCM I-3799. These probiotic strains are thus not discussed further.

The Following Strains (or Combinations of Strains) Have a Conditional Positive Recommendation

The GRADE evidence Table as to whether *L rhamnosus* GG (LGG) ATCC 53103 versus usual care should be used for preterm infants is depicted in Table 2. Mortality and sepsis did not show any clear direction in effect size, especially if the CrI is taken in consideration (very low and low certainty of evidence, respectively). Trials on sepsis (106–113) contained sufficient numbers of infants to rule out a significant beneficial effect of administration of *L rhamnosus* GG ATCC 53103, whereas the outcome on mortality was highly underpowered (106–108). The RR for NEC is, however, clearly reduced: 1507 infants studied in total (107–111,114); RR 0.240 (CrI 0.064–0.670); low certainty of evidence. Remarkably, both the control and intervention groups contained very few events (2.3% and 0.8%, respectively), whereas the NEC rate in the control groups of all 51 RCTs combined was 6.1% on average (39). In addition, mortality and sepsis rates in the studies evaluating *L rhamnosus* GG ATCC 53103 were very low. The reasons for the very low event rates in the control group could include the fact that relatively older infants were included in the 6 RCTs (mean GA ranged from 29–34 weeks; mean BW ranged from 1150–1950 g). Furthermore, in the study by Manzoni et al in 2009/2014, both the control and intervention groups received bovine lactoferrin in addition to either the placebo or LGG. This may explain the low event rates in the studies, even in the control groups, although a recent large RCT demonstrated no effect of enteral bovine lactoferrin supplemented solely (115). The number needed to treat is thus very high, despite a considerably low RR. Although our predefined sample size calculations predicted enough power with 431 infants in each arm, these calculations were performed with an expected NEC reduction from 10% to 5%. Thus, although enough infants were included in the 6 RCTs as defined in the method section, the observed reduction from 2.3% to 0.8% has only 63% power to predict a true effect in the reduction of NEC.

On the basis of the RCTs described above, the use of L rhamnosus GG ATCC 53103 at a dose ranging from 1×10^9 to 6×10^9 colony-forming units (CFU) may conditionally be recommended in preterm infants, as there is low quality evidence it might reduce NEC stage 2 or 3.

Considering evidence from non-RCTs, a pre-post cohort study in 221 infants on LGG ATCC 53103, weighing 900 g on average and who survived until discharge, could not clearly confirm a reduction in the NEC rate, as significance turned to $P = 0.07$ after adjusting for confounders (116). Another study compared morbidity and mortality rates after implementing simultaneous administration of both *L rhamnosus* GG together with bovine lactoferrin (50). In a timeframe of 11 years 835 infants, weighing approximately 1300 g on average at birth, NEC rates decreased from 3% to 1% after implementation of the combined strategy, whereas sepsis and mortality rates were unaltered. Remarkably, 2 other retrospective cohort studies reported higher NEC rates after implementing routine administration of *L rhamnosus* GG to very low birth weight (VLBW) infants. In 1 study, NEC rate (stage $\geq$2) amounted 3.2% out of 1900 infants without probiotics, and 4.6% out of 418 infants with LGG supplementation (117). In the other more recent cohort

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TABLE 3. GRADE table summarizing the evidence on the use of the combination of *Bifidobacterium infantis* Bb-02, *Bifidobacterium lactis* Bb-12, and *Streptococcus thermophilus* TH-4 compared with usual care in preterm infants

Certainty assessment						No. of patients		Effect		
No. of RCTs (ref)	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	*B infantis* Bb-02, *B lactis* Bb-12, and *Str thermophilus* TH-4	Usual care	Relative (95% CrI)	Absolute (95% CrI)	Certainty
Mortality										
2 (119,120)	Not serious [*]	Serious [†]	Not serious	Very serious [‡,§]	None	30/620 (4.8%)	36/624 (5.8%)	RR 0.73 (0.29 to 1.50)	16 fewer per 1.000 (from 41 fewer to 29 more)	⊕◯◯◯ VERY LOW
NEC stage 2 or 3										
2 (119,120)	Not serious [*]	Serious [†]	Not serious	Serious [‖]	None	12/620 (1.9%)	34/624 (5.4%)	RR 0.290 (0.073 to 0.780)	39 fewer per 1.000 (from 51 fewer to 12 fewer)	⊕⊕◯◯ LOW
Late-onset sepsis										
2 (119,120)	Not serious [*]	Serious [†]	Not serious	Serious [‡]	None	103/620 (16.6%)	113/624 (18.1%)	RR 0.98 (0.56 to 1.80)	4 fewer per 1.000 (from 80 fewer to 145 more)	⊕⊕◯◯ LOW

CrI = credible interval; NEC = necrotising enterocolitis; RR = risk ratio.
[*]Randomization procedure not clearly explained by Bin-Nun et al, yet, this did not form a clear reason to downgrade level of evidence here. In both RCTs, it was not described when outcome assessors were deblinded.
[†]Moderate to substantial heterogeneity between results from both studies (I^2 ranges from 43 to 71%).
[‡]Wide CrI.
[§]Underpowered.
[‖]Low event rates.

(465 infants without; 175 with LGG), NEC stage ≥2 incidence increased from 10% to 19% (118).

Recommendation

If all safety conditions are met, the panel conditionally recommends the use of *L rhamnosus* GG ATCC 53103 at a dose ranging from 1×10^9 CFU to 6×10^9 CFU as it might reduce NEC stage 2 or 3 (low certainty of evidence).

The GRADE evidence Table as to whether the combination of *B infantis* Bb-02, *B lactis* Bb-12, and *Str thermophilus* TH-4 versus usual care should be used for preterm infants is depicted in Table 3. Mortality and sepsis did not show any clear direction in effect size, especially if the CrI is taken in consideration (very low and low certainty of evidence, respectively). The administration of these 3 strains, however, did seem to significantly reduce rates of NEC stage 2 and 3 (RR 0.29 [0.073–0.78]). The evidence base was made up of 1 larger (119) and 1 smaller (120) RCT, with the inclusion of a total of 1244 infants with an average birth weight of approximately 1050 g.

Based on the RCTs described above, a conditional recommendation can be made for the use of a combination of *B infantis* Bb-02, *B lactis* Bb-12, and *Str thermophilus* TH-4 at a dose of 3.0 to 3.5×10^8 CFU (of each strain) in preterm infants as there is low-quality evidence it might reduce NEC stage 2 or 3.

However, a beneficial effect of these 3 strains (at a dose of 1.75 to 3.5×10^8 CFU of each strain) on reducing NEC could not be demonstrated in a retrospective cohort of 580 infants weighing approximately 1100 g on average at birth (121).

Recommendation

If all safety conditions are met, the panel conditionally recommends using the combination of *B infantis* Bb-02, *B lactis* Bb-12, and *Str thermophilus* TH-4 at a dose of 3.0 to 3.5×10^8 CFU (of each strain) as it might reduce NEC stage 2 or 3 (low certainty of evidence).

The Following Strains (or Combinations of Strains) Have a Conditional Neutral or Negative Recommendation

The GRADE evidence Table as to whether *L reuteri* DSM 17938 in a dose ranging from 4×10^7 to 2×10^8 CFU versus usual care should be used for preterm infants is depicted in Table 4. Previously, in our NMA we showed a significant reduction in NEC rates after combining the results from the *L reuteri* ATCC 55730 and DSM 17938 strains (1459 infants; 4 studies; RR 0.43 [0.16–0.98]). For mortality and sepsis rates, we could not demonstrate a reduction in our NMA. On the basis of panel discussions, however, we decided to omit the results from the single small study that used *L reuteri* ATCC 55730 (108) to be able to give truly strain-specific recommendations on the DSM 17938 strain, despite strains being very similar (82). In addition, in hindsight, one of the studies from our NMA also included stage 1 NEC, so we furthermore decided to exclude that small study as well for the NEC analysis only (122). On the other hand, three very recently published studies using the DSM 17938 strain could be added to our table (123–125). The GRADE evidence table, thus, does not represent previously published RRs from our NMA, but uses traditional RevMan forest plot-derived RRs (see also Supplemental Figure S1a–c, Supplemental Digital Content, *http://links.lww.com/MPG/B785*).

For none of the outcome domains, a irrefutably reduced event rate was noted, although the RR for reducing NEC stage ≥2 approached significance (RR 0.65 [95% CI 0.40–1.07]). If we would had added the results from the trial with the similar ATCC 55730 strain (82,108), results would not have been any different. It

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TABLE 4. GRADE table summarizing evidence on the use of *L reuteri* DSM 17938 compared to usual care in preterm infants

Certainty assessment						No. of patients		Effect		
No. of RCTs (ref)	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	*L reuteri* DSM 17938	Usual care	Relative (95% CI)	Absolute (95% CI)	Certainty
Mortality										
5 (107–110)	Not serious	Not serious	Very serious *	Very serious †,‡	None	44/717 (6.1%)	59/721 (8.2%)	RR 0.76 (0.52 to 1.11) §	20 fewer per 1.000 (from 39 fewer to 9 more)	⨁◯◯◯ VERY LOW
NEC stage 2 or 3										
5 (108–110)	Not serious	Not serious	Very serious *	Serious †	None	25/732 (3.4%)	41/739 (5.5%)	RR 0.65 (0.40 to 1.07) §	19 fewer per 1.000 (from 33 fewer to 4 more)	⨁◯◯◯ VERY LOW
Late-onset sepsis										
6 (107–110)	Not serious	Serious ‖	Very serious *	Serious †	None	69/762 (9.1%)	82/769 (10.7%)	RR 0.78 (0.49 to 1.23) §	23 fewer per 1.000 (from 54 fewer to 25 more)	⨁◯◯◯ VERY LOW

CI = confidence interval; NEC = necrotising enterocolitis; RR = risk ratio.
*Studies by Rojas et al, Shadkam et al, Cui et al, and Kaban et al included moderately preterm infants with average GA of 32, 31, 33, and 33 weeks, and average birth weight of 1500, 1400, 1700, and 1550 g, respectively. The latter 2 studies excluded infants with a birth weight below 1500 and 1000 g, respectively.
†Wide CI.
‡Underpowered.
§RRs for using *L reuteri* DSM 17938 were derived from RevMan 5.3, instead of those from the previously published network meta-analysis.
‖High heterogeneity between studies, $I^2 = 71\%$.

must be noted, however, that 4 studies investigating the use of *L reuteri* DSM 17938 included relatively larger preterm infants with average birth weights ranging from 1400 to 1700 g approximately (122,124–126). Remarkably, these RCTs showed most efficacious results from supplementing *L reuteri* DSM 17938, whereas in the 2 studies in which average birth weights amounted approximately 750 g (123) and 1050 g (127), NEC rates were not reduced (Supplemental Figure S1b, Supplemental Digital Content, *http://links.lww.com/MPG/B785*).

On the basis of the RCTs described above, no recommendation can be made in either direction for using *L reuteri* DSM 17938 at a dose ranging from 4×10^7 to 2×10^8 CFU in preterm infants (very low to low certainty of evidence).

The panel also noted 2 epoch cohort studies. The first analysed 311 infants (232 before and 79 after introduction) weighing on average 750 g and showed highly significant results, as the NEC rate decreased from 15.1% to 2.5% after *L reuteri* DSM 17938 administration (6×10^7) was routinely initiated (128). Sepsis rates were not different between both epochs. Another recent study including those born <33 weeks gestation compared 330 infants who did not receive probiotics to 1027 infants who received *L reuteri* DSM 17938 after a policy change (129). NEC rates were significantly reduced amongst all subgroups (also those <26 weeks), but nosocomial sepsis and mortality rates were unaltered.

Recommendation

The panel concludes that *no recommendation* can be made in either direction regarding the use of *L reuteri* DSM 17938 in preterm infants to reduce the risk of mortality, NEC stage 2 or 3, or sepsis (very low certainty of evidence). Additionally, *L reuteri* DSM 17938 is a partially D-lactate-producing strain for which there is insufficient safety data available in preterm infants.

The GRADE evidence Table as to whether the combination of *B bifidum* NCDO 1453 (currently reclassified as *B longum*) with *L acidophilus* NCDO 1748 (ATCC 4356, LA37, or NCIMB 30316) versus usual care should be used for preterm infants is based on 2 studies (130,131) and depicted in Table 5. Very low certainty evidence showed that mortality rates were lower in the probiotics group. Yet, NEC rates only showed a trend towards reduced risk, whereas the point estimate for sepsis rates showed an increased risk.

Based on the RCTs described above, no recommendation can be made in either direction for using the combination of *B bifidum* NCDO 1453 with *L acidophilus* NCDO 1748 (ATCC 4356, LA37, or NCIMB 30316) at a dose of 1×10^9 CFU (of each strain) in preterm infants (based upon very low-to-moderate certainty of evidence).

Evidence from 2 recent nonrandomised trials show conflicting results. A large pre-post implementation cohort study (n = 1288 before and n = 673 after) that used these 2 strains found no reduction in rates of mortality, NEC, or sepsis after correction for confounders (132). A study, however, with similar design and strains (n = 170 before and 3 = 346 after) found a doubling of NEC rates after implementation, but a 16% reduction in late-onset sepsis rates (133).

Recommendation

The panel concludes that *no recommendation* can be made in either direction regarding the use of the combination of *B bifidum* NCDO 1453 (currently reclassified as *B longum*) with *L acidophilus* NCDO 1748 (ATCC 4356, LA37, or NCIMB 30316) in preterm infants to reduce the risk of mortality, NEC stage 2 or 3, or sepsis (very low-to-moderate certainty of evidence). Additionally, *L acidophilus* NCDO 1748 (ATCC 4356, LA37, or NCIMB 30316) is a partially D-lactate-producing strain for which there is insufficient safety data available in preterm infants.

The GRADE evidence table as to whether *B breve* BBG-001 (YIT4010) in a dose of 7×10^8 CFU versus usual care should be

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TABLE 5. GRADE table summarizing the evidence on the use of the combination of *Bifidobacterium bifidum* NCDO 1453 and *L acidophilus* NCDO 1748 (ATCC 4356, LA37, or NCIMB 30316) compared with usual care in preterm infants

Certainty assessment						No. of patients		Effect		
No. of RCTs (ref)	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	*B bifidum* NCDO 1453 and *L acidophilus* NCDO 1748 (ATCC 4356, LA37, or NCIMB 30316)	Usual care	Relative (95% CrI)	Absolute (95% CrI)	Certainty
Mortality										
2 (130,131)	Not serious *	Not serious	Serious †	Very serious ‡,§,‖	None	2/248 (0.8%)	9/246 (3.7%)	RR 0.160 (0.019–0.740)	31 fewer per 1000 (from 36 fewer to 10 fewer)	⊕◯◯◯ VERY LOW
NEC stage 2 or 3										
2 (130,131)	Not serious *	Not serious	Not serious	Very serious ‡,§,‖	None	5/248 (2.0%)	15/246 (6.1%)	RR 0.290 (0.065–1.100)	43 fewer per 1.000 (from 57 fewer to 6 more)	⊕⊕◯◯ LOW
Late-onset sepsis										
2 (130,131)	Not serious *	Not serious	Not serious	Serious §	None	42/248 (16.9%)	25/246 (10.2%)	RR 1.50 (0.66–3.30)	51 more per 1.000 (from 35 fewer to 234 more)	⊕⊕⊕◯ MODERATE

CrI = credible interval; NEC = necrotising enterocolitis; RR = risk ratio.
*The study by Saengtawesin et al was not fully blinded. Only the medical doctors were blinded, the nurses and investigators were not. Despite this, we did not rate risk of bias as serious because of small study size, outcomes were not subjective (especially mortality and culture-proven sepsis), and no appreciable differences between groups in this study.
†In the study by Lin et al, a very low mortality in both groups is reported. Only infants who survived to start enteral feeding were, however, eligible. This excluded 98 infants who died before the initiation of probiotics (or placebo).
‡Underpowered.
§Wide CrI.
‖Low event rates.

used for preterm infants is depicted in Table 6. There appears no clear direction in effect on any of the described outcomes (mortality, NEC stage $\geq$2, and sepsis). The evidence is derived from a single, large, well performed RCT in 1310 infants with a median GA of 28 weeks and higher than average event rates of NEC and sepsis (37).

Recommendation

The panel conditionally recommends against using *B breve* BBG-001 to reduce the risk of mortality, NEC stage 2 or 3, or sepsis (low-to-moderate certainty of evidence).

The GRADE evidence Table as to whether *S boulardii* CNCM I-745 versus usual care should be used for preterm infants is depicted in Table 7 (134–138). None of the 3 outcomes show a clear direction of effect when credible intervals are considered, although both the outcomes on mortality and NEC were underpowered.

On the basis of the RCTs described above, no recommendation can be made in either direction for using *S boulardii* CNCM I-745 at a dose ranging from 1×10^9 to 5×10^9 CFU in preterm infants (based upon very low to low certainty of evidence).

We found only 1 small cohort study that included only preterm infants with birth weight between 1 and 2 kg and in which this strain was investigated (139). Mortality and NEC stage 2 rate amounted 10.3% and 7.7%, respectively, in the 39 infants without probiotics and 0% in the 46 infants who had received the *S boulardii*.

Regarding safety, the European Medicine Agency recently amended a contra-indication to the use of *S boulardii* in patients with a central venous catheter, in critically ill patients, or in immunocompromised patients because of a risk of fungaemia (64).

Recommendation

The panel does not recommend the routine use of *S boulardii* for safety reasons (in line with the position of the European Medicine Agency which contraindicates the use of *S boulardii* in patients with a central venous catheter, in critically ill patients, or in immunocompromised patients because of a risk of fungaemia) as well as lack of evidence of efficacy (very low to low certainty of evidence).

Are Combinations of Species More Effective Than the Use of a Single Strain to Reduce the Risk of NEC (Stage 2 or 3)?

Several classic meta-analyses have shown decreased morbidity rates after supplementing with multiple strains versus a single strain (18,21–23). These meta-analyses, however, were not genus-specific, species-specific, or strain-specific. Therefore, it is not appropriate to extrapolate or determine whether the beneficial effect in the 'multiple strain' group was because of the chance that more effective strains were used in that group versus the strains used in the 'single strain' group. It mainly comes down to which strain is used. Use of a single strain with proven effectiveness is likely to be more efficacious than use of a combination of strains without proven effectiveness. On the other hand, a combination of 2 or more independently proven efficacious strains may be more

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TABLE 6. GRADE table summarizing the evidence on the use of *Bifidobacterium breve* BBG-001 compared with usual care in preterm infants

Certainty assessment						No. of patients		Effect		
No. of RCTs (ref)	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	*B breve* BBG-001	Usual care	Relative (95% CrI)	Absolute (95% CrI)	Certainty
Mortality										
1 (37)	Not serious	Not serious	Not serious	Very serious *,†	None	54/650 (8.3%)	56/660 (8.5%)	RR 0.97 (0.36 to 2.50)	3 fewer per 1000 (from 54 fewer to 127 more)	⊕⊕◯◯ LOW
NEC stage 2 or 3										
1 (37)	Not serious	Not serious	Not serious	Serious *	None	61/650 (9.4%)	66/660 (10.0%)	RR 0.92 (0.24 to 3.50)	8 fewer per 1000 (from 76 fewer to 250 more)	⊕⊕⊕◯ MODERATE
Late-onset sepsis										
1 (37)	Not serious	Not serious	Not serious	Serious *	None	186/650 (28.6%)	206/660 (31.2%)	RR 0.91 (0.42 to 2.00)	28 fewer per 1000 (from 181 fewer to 312 more)	⊕⊕⊕◯ MODERATE

CrI = credible interval; NEC = necrotising enterocolitis; RR = risk ratio.
*Wide confidence interval.
†Underpowered.

efficacious than a single efficacious strain, provided no antagonistic mechanisms exists.

Figure 1 shows additional models run from the database in our NMA (39) to gather more formal evidence. It is shown, that from all neonatal trials combined, there is no a priori advantage of administering multiple strains versus a single strain. Also, there appears to be no benefit of selecting a specific genus (Bifidobacterium or Lactobacillus) or a combination of these 2.

Thus, these data, although not-strain specific, do not support the notion that administration of multiple strains or combinations of species (from a different genus) is more effective than the administration of a single probiotic strain.

Recommendation

The panel conditionally recommends that when considering the use of probiotics, a strain (or combination of strains) with proven effectiveness and established safety profile should be selected, rather than focussing on administering multiple strains from different genera (very low certainty of evidence).

Which Dose of a Probiotic Strain or Combination of Strains Should Be Administered?

The administered dose of probiotic strains used in premature neonates differed widely, as can be seen in our database of 51 RCTs (39). Usually, doses were in the range of 10^8 to 10^9 CFU, but doses ranging from as low as 10^5 CFU (140) to as high as 10^{10} CFU (103) have also been used. Even between trials investigating the same strain, administered doses varied widely in different trials. In the

TABLE 7. GRADE table summarizing the evidence on the use of *Saccharomyces boulardii* CNCM I-745 compared with usual care in preterm infants

Certainty assessment						of patients		Effect		
of RCTs (ref)	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	*S boulardii* CNCM I-745	Usual care	Relative (95% CrI)	Absolute (95% CrI)	Certainty
Mortality										
2 (134–135)	Not serious	Not serious	Not serious	Very serious *,†	None	10/239 (4.2%)	9/240 (3.8%)	RR 0.92 (0.32 to 2.30)	3 fewer per 1.000 (from 26 fewer to 49 more)	⊕⊕◯◯ LOW
NEC stage 2 or 3										
5 (134–138)	Serious ‡	Not serious	Serious §	Very serious *,†	None	22/421 (5.2%)	20/325 (6.2%) ǁ	RR 0.66 (0.24 to 1.60)	21 fewer per 1.000 (from 47 fewer to 37 more)	⊕◯◯◯ VERY LOW
Late-onset sepsis										
5 (134–138)	Serious ‡	Not serious	Serious §	Serious *	None	47/421 (11.2%)	55/325 (16.9%) ǁ	RR 0.75 (0.42 to 1.30)	42 fewer per 1.000 (from 98 fewer to 51 more)	⊕◯◯◯ VERY LOW

CrI,= credible interval; NEC = necrotising enterocolitis; RR = risk ratio.
*Wide CrI.
†Underpowered.
‡Study by Zhang et al was not blinded.
§Studies by Costalos et al, Xu et al, and Zhang et al included more moderately preterm infants, on average approximately 33 weeks GA.
ǁStudy by Zhang et al did not include a control group that could be compared with; in the NMA, only a head-to-head comparison with another probiotic strain was included.

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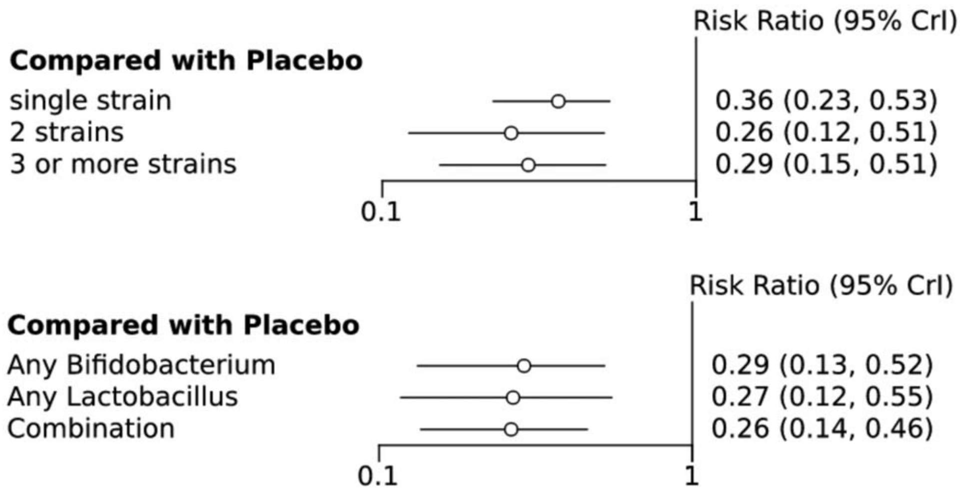

FIGURE 1. Relative effect plots depicting risk ratios on reducing necrotising enterocolitis stage 2 or 3 after supplementing (A) a single probiotic strain, 2 strains, or 3 or more strains, versus placebo care; and (B) one or more *Bifidobacterium* strains, one or more *Lactobacillus* strains, or a combination of the 2 versus placebo care.

6 trials that studied the effects of the strain *B lactis* Bb-12 (102–105,119,120), the supplemented dose differed 600-fold, ranging from 2.0×10^7 to 1.2×10^{10} CFU. For other well-studied strains, the administered doses differed less; from 2×10^8 to 6×10^9 CFU (107–114,141,142) for *L rhamnosus* GG, from 4×10^7 to 2×10^8 CFU (122–127,143) for *L reuteri* DSM 17938, and from 1×10^9 to 5×10^9 CFU for *S boulardii* I-745 (134–138).

A small trial (n = 149) in total showed no clear differences in colonization between dosing either 1×10^9 or 1×10^{10} CFU of the same strain daily (144). Another trial, however, showed that administration of a daily dose of 1×10^9 CFU of 2 probiotic strains was more effective in terms of colonization than a weekly or bi-weekly dose of the same strains (145).

Recently, a systematic review was published on dose-responses of probiotics in different clinical settings and patient groups (146). Only for antibiotic-associated diarrhoea was a dose-response observed, although this was not strain-specific and analysed with all probiotic strains simultaneously. For NEC, no such relation could be demonstrated in preterm infants. The author, however, also notes that this issue is highly understudied throughout all clinical settings.

Apart from designated doses on the product label, it is well-known that actual viable bacterial counts are frequently much lower, sometimes only a few percent of what is claimed on the product packaging (80,96). Suppliers of probiotics should thus always provide reports on the number of viable bacterial counts in their product including a stability analysis. In addition, probiotic viability is highly affected whether it is dissolved in water, breast milk or formula (147). In conclusion, data do not support the notion that a higher dose of probiotics is more effective than a lower dose and the optimal dose for most species and strains remains undetermined.

Recommendation

The panel conditionally recommends that, if probiotics are administered, to use similar doses as applied in relevant RCTs (very low certainty of evidence). Probiotic products should be accompanied with formal quality reports that ascertain product viability until the end of shelf life.

What Should be the Duration of Administering Probiotics?

This issue has not been systematically researched. The times after birth at which probiotics are started vary widely, as well as the total duration of probiotic administration (39). Several studies started probiotics immediately after birth, whereas others waited for up to a week after birth. In some studies, probiotic administration was stopped after 2 weeks. In most studies, however, probiotic administration lasted 4 to 6 weeks or up until discharge.

With strain-proven efficacy, it would be common sense to administer probiotics before and during the period when NEC risk is the highest, so relatively fast following birth. Yet, it is unknown if very early administration of a high dose of 1 or more probiotic strains might be harmful, when ‘natural’ (breast milk driven) colonisation is just beginning, and when the immune system is underdeveloped, and gastrointestinal barrier function is impaired by inadequate tight junctions and reduced mucus layer.

Data do not provide clear evidence as to when probiotic supplementation should be started or ceased. The rationale though does exist, that prolonged use may prevent more ‘natural’ colonisation and/or that the risk:benefit ratio might be the lowest when used in the period when NEC risk is the highest.

Recommendation

The available data do not clearly indicate an optimal start or length of treatment. The panel conditionally recommends individual units determine treatment duration based on the population who will receive them and their ongoing risk of diseases, such as NEC (very low certainty of evidence).

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Is it Appropriate to Administer Other Strains Than Those Studied in Large Well-conducted Randomised Controlled Trials?

It has been suggested that based on the consistently decreased risk of NEC in RCTs using variable probiotic regimens, it is time we accept that commonly used probiotic strains share pathways of benefits providing 'non-specific' protection (27,38). Our recent NMA clearly, however, shows that the results of RCTs on different probiotic strains largely differ with regard to the 3 analysed outcomes (39). Whether this is truly a reflection of strain-specific benefits (8,32,33,148), internal and external study validity, or a power issue remains to be elucidated. Considering the vulnerable patient population, and aforementioned potential safety and product quality issues, however, only high-quality, safe, and evidence-based strains can be recommended for clinical use.

Recommendation

The panel conditionally recommends that in the clinical setting, the use of a single strain or combination of strains should be practise-based on positive results from well-conducted RCTs (very low certainty of evidence). In research settings, however, it is appropriate to test new strains or new combinations of strains.

DISCUSSION

The gastrointestinal-related intervention that is both the most safe and efficacious in reducing morbidity and mortality would absolutely be to stimulate the use of unpasteurised own mother's milk. However, especially in NICUs with a high NEC incidence, the use of prophylactic probiotic therapy might be considered as well. This position paper aimed to provide some guidance on which probiotic strains have proven efficacy while addressing safety issues as well. Others have also come up with the 10 golden rules of safe introduction of probiotics (100). Unfortunately, current available evidence appears only marginally enough to conditionally recommend 1 or 2 therapeutic options that are evidence-based on RCTs. We only advise the routine use of certain strains of probiotics that have been shown to be safe and efficacious and that have been studied in a large number of VLBW infants. Thus, there is still a need for well-designed and carefully conducted RCTs, with relevant inclusion/exclusion criteria and adequate sample sizes. We specifically encourage undertaking trials that aim to include extremely premature infants (particularly those <26 weeks gestation), as these infants are relatively understudied so far. Whilst these infants have the highest risk of NEC, the risk of harm from probiotics might be the greatest as well. Such trials should define the optimal doses and intake durations, as well as providing more information about the long-term safety of probiotics. Probiotic products that are used should be submitted to systematic quality control procedures by the respective authorities to confirm the viability and identify the strain-level(s) of the active ingredient(s). As most of the trials published so far have been company-funded, independent trials, preferentially financed jointly by national/governmental/European Union bodies and other international organisations, would be desirable. Finally, long-term follow-up is warranted, not only from a neurodevelopmental perspective (24) but also regarding safety and immunity (35).

Another major problem in many of the RCTs is the definition of NEC. Probably, only surgically proven NEC is a reliable outcome and this should always be separately reported in future trials. In quite a few of the trials, blinding is an important issue and stage 2 NEC is not an exact diagnosis.

Other open questions not addressed here and in many studies are the optimal matrix of the probiotic supplement (powder, capsules, or liquids) as well as the concomitant feeding strategy (own or donor human milk or formula), despite the fact that they may affect outcomes. The times at which probiotics are added to either human milk or formula could affect strain viability at the time of ingestion, for example (147,149). Other reviews have suggested that probiotics might be more effective in infants fed human milk, rather than preterm formula (9,16,22), despite that human milk itself already lowers the incidence of sepsis and NEC. Whether this finding is a coincidence of having clustered nonefficacious strains in formula-fed infants versus more efficacious strains in the human milk-fed group, or whether there is a biological rationale remains unknown. One of the explanations could be that the human milk-fed infants respond better to probiotics because of the fact that only human milk contain human milk oligosaccharides (HMOs) from which Bifidobacteriae benefit, especially *B infantis* (150). Whether there is a further potential difference in effectiveness between own mother's milk or donor milk remains unknown. Although donor milk still contains HMOs, all beneficial bacteria that fresh human milk normally harbours (151) are destroyed in the process of pasteurization. On the other hand, mothers with antibiotics have less Bifidobacteriae in their milk as well (152). Yet, in a recent Cochrane, it was not recommended for mothers of preterm infants to use probiotics (153).

Exciting new areas of research are the study of killed (ghost) probiotics or closely related postbiotics, which might still harbour beneficial immunological effects but eliminates the risk of, for example, sepsis or contamination (154–157).

RESEARCH GAPS

The following additional clinical and research questions were also posed and voted upon with high agreement (>85%):

1. Placebo-controlled studies on promising specific strains for different outcomes are still needed, as no single strain has been studied in individual adequately powered studies. These studies could be conducted by head-to-head comparisons in trials that include a placebo arm.
2. Appropriately designed and powered studies that determine the optimal dosing, optimal time of initiation, and duration treatment of effective probiotics are needed.
3. The number of extremely preterm infants (<28 weeks GA) and infants with a birth weight below 1000 g included in the current studies is limited, whereas NEC and mortality rates are the highest in that population. Studies specifically focussed on these groups are needed. Within the future studies, stratification should be based on the quality of the enteral feeding (own mother's milk, donor milk, or formula).
4. The efficacy and safety of different modes of administration (powder, liquid, added to formula by manufacturer) should be a topic of investigation.
5. Long-term safety including the effects of probiotic administration on metabolic, endocrine, immunological, and behavioural parameters should be a topic of investigation.
6. In-hospital safety of used probiotics should be assessed by determination of "probiotic sepsis rates" by a microbiology department that is equipped to evaluate these infections.
7. Attention should be paid to characteristics of the population studied. Gender, ethnicity, region of birth, composition of diet, and antibiotic use are just a few factors that might have an impact on the safety and efficacy of specific strains.

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Recommendations

1. The panel conditionally recommends that in case of implementing a probiotic product, the local microbiologists should be informed and they should confirm the ability to routinely detect probiotic bacteraemia/fungaemia with standard culture methods (very low certainty of evidence).
2. The panel conditionally recommends not to provide probiotic strains, which produce D-lactate, as its potential risk or safety has not been adequately studied in preterm infants and remains uncertain (very low certainty of evidence).
3. The panel conditionally recommends only the use of strains devoid of any plasmids containing transferable antibiotic resistance genes (very low certainty of evidence). This information should be confirmed and provided by the manufacturer.
4. The panel conditionally recommends only the use of probiotic products manufactured according to cGMP to ensure correct strain identity with lack of contamination (very low certainty of evidence). Certificates of analysis should address at least strain identity, purity, viability, and antibiotic susceptibility, and resistance profiles.
5. The panel conditionally recommends to provide parents of preterm infants with sufficient information so they can understand the potential benefits and risks of probiotic administration (very low certainty of evidence). Communication is best undertaken face to face and supplemented with the use of written materials appropriate to the local context.
6. If all safety conditions are met, the panel conditionally recommends the use of *L rhamnosus* GG ATCC 53103 at a dose ranging from 1×10^9 CFU to 6×10^9 CFU as it might reduce NEC stage 2 or 3 (low certainty of evidence).
7. If all safety conditions are met, the panel conditionally recommends using the combination of *B infantis* Bb-02, *B lactis* Bb-12, and *Str thermophilus* TH-4 at a dose of 3.0 to 3.5×10^8 CFU (of each strain) as it might reduce NEC stage 2 or 3 (low certainty of evidence).
8. The panel concludes that *no recommendation* can be made in either direction regarding the use of *L reuteri* DSM 17938 in preterm infants to reduce the risk of mortality, NEC stage 2 or 3, or sepsis (very low certainty of evidence). Additionally, *L reuteri* DSM 17938 is a partially D-lactate producing strain for which there is insufficient safety data available in preterm infants.
9. The panel concludes that *no recommendation* can be made in either direction regarding the use of the combination of *B bifidum* NCDO 1453 (currently reclassified as *B longum*) with *L acidophilus* NCDO 1748 (ATCC 4356, LA37, or NCIMB 30316) in preterm infants to reduce the risk of mortality, NEC stage 2 or 3, or sepsis (very low to moderate certainty of evidence). Additionally, *L acidophilus* NCDO 1748 (ATCC 4356, LA37, or NCIMB 30316) is a partially D-lactate-producing strain for which there is insufficient safety data available in preterm infants.
10. The panel conditionally recommends against using *B breve* BBG-001 to reduce the risk of mortality, NEC stage 2 or 3, or sepsis (low-to-moderate certainty of evidence).
11. The panel does not recommend the routine use of *S boulardii* for safety reasons (in line with the position of the European Medicine Agency, which contraindicates the use of *S boulardii* in patients with a central venous catheter, in critically ill patients, or in immunocompromised patients because of a risk of fungaemia) as well as lack of evidence of efficacy (very low to low certainty of evidence).
12. The panel conditionally recommends that whenever considering the use of probiotics, a strain (or combination of strains) with proven effectiveness and established safety profile should be selected, rather than focussing on administering multiple strains from different genera (very low certainty of evidence).
13. The panel conditionally recommends that, if probiotics are administered, to use similar doses as applied in relevant RCTs (very low certainty of evidence). Probiotic products should be accompanied with formal quality reports that ascertain product viability until the end of shelf life.
14. The available data do not clearly indicate an optimal start or length of treatment. The panel conditionally recommends individual units determine treatment duration based on the population who will receive them and their ongoing risk of diseases, such as NEC (very low certainty of evidence).
15. The panel conditionally recommends that in the clinical setting, the use of a single strain or combination of strains should be practise-based on positive results from well-conducted RCTs (very low certainty of evidence). In research settings, however, it is appropriate to test new strains or new combinations of strains.

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