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REVIEW ARTICLE

Effectiveness of probiotics in the prophylaxis of necrotizing enterocolitis in preterm neonates: a systematic review and meta-analysis☆

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KEYWORDS
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Abstract
Objective: To elucidate the benefits of using probiotics in the prevention of necrotizing enterocolitis (NEC) and its complications in preterm newborns.
Method: This was a systematic review of randomized controlled trials, which included studies retrieved from three databases (MEDLINE, Embase, and LILACS), using a combination of the terms (necrotizing enterocolitis) AND (probiotics).
Results: 11 randomized trials were included, totaling 2,887 patients, 1,431 in the probiotic group and 1,456 in the control group. There was a reduction in the incidence of NEC (NNT = 25), overall death (NNT = 34), and neonatal sepsis (NNT = 34) in the probiotic group compared to the control group. Patients that received probiotic supplementation had lower food reintroduction time ($p < 0.001$) and hospitalization time ($p < 0.001$) when compared to those not receiving probiotics. There was no difference in mortality caused by NEC.
Conclusion: In premature newborns, the use of probiotics is effective as a prophylaxis for NEC and its complications.

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PALAVRAS-CHAVE
Enterocolite necrosante;
Probióticos;
Prematuros

Eficácia dos probióticos na profilaxia de enterocolite necrosante em recém-nascidos prematuros: revisão sistemática e meta-análise

Resumo
Objetivo: Elucidar os benefícios do uso de probióticos na prevenção de enterocolite necrosante (ECN) e de suas complicações em recém-nascidos prematuros.
Método: Revisão sistemática de ensaios clínicos randomizados, que incluiu pesquisas efetuadas em três bases de dados (MEDLINE, EMBASE e LILACS), utilizando a combinação dos termos (*necrotizing enterocolitis*) AND (*probiotics*).
Resultados: Foram incluídos 11 ensaios clínicos randomizados, totalizando 2.887 pacientes, sendo 1.431 no grupo Probiótico e 1.456 no grupo Controle. Houve redução na incidência de ECN (NNT = 25), de morte global (NNT = 34) e sepse neonatal (NNT = 34) no grupo Probiótico em relação ao grupo Controle. Pacientes alimentados com suplementação de probióticos tiveram tempo de reintrodução alimentar (p < 0,001) e de hospitalização (p < 0,001) menor quando comparados aos que não receberam. Não houve diferença na mortalidade causada por ECN.
Conclusão: Em recém-nascidos prematuros, o uso de probióticos é eficaz na profilaxia de ECN e de suas complicações.

Introduction

Necrotizing enterocolitis (NEC) is multifactorial disease that results from the interaction between the loss of integrity of the intestinal mucosa and the host response to this damage. It is determined by intestinal ischemia, mucosal damage, edema, ulceration, and passage of air or bacteria through the wall, resulting in necrosis of the mucosa and intestinal wall.

The main preexisting factors that cause increased risk for developing NEC are prematurity, enteral feeding, and colonization by pathogenic microorganisms such as *Escherichia coli*, *Klebsiella*, *Clostridium perfringens*, *Staphylococcus epidermidis* and *Rotavirus*.[1]

This is the most prevalent emergency of the gastrointestinal tract in the neonatal period.[2] Its incidence is highly variable, affecting 2% to 22% of newborns with very low birth weight (< 1,500 g).[2,3]

The occurrence of NEC is inversely related to gestational age at birth, due to the physiological intestinal immaturity of preterm neonates. Therefore, probiotics, a group of organisms capable of improving this clinical picture, have been studied in order to fight disease progression.

Probiotics were first described in the literature by Lilly and Stillwell in 1965, as growth-promoting factors produced by certain microorganisms. Recently, they have been described as living organisms which, when included in the diet in adequate amounts, can bring health benefits to the host.[4] As microorganisms able to colonize the digestive tract by adhering to the intestinal epithelium, producing antimicrobial substances, and modulating the immune response and host metabolism, probiotics have been discussed regarding their usefulness for preterm infants.[5,6]

This study aimed to elucidate the benefits of probiotics in the prevention of NEC and its complications in preterm infants.

Methods

Study identification and selection

In order to perform a systematic analysis of the available evidence on the efficacy of probiotics in the prevention of NEC, a literature search strategy was used, which included searches carried out in MEDLINE, Embase, and LILACS. The searches were completed in May 2012.

The MEDLINE search was performed through PubMed (www.ncbi.nlm.nih.gov/pubmed) and was adapted by using the terms (necrotizing enterocolitis) AND (probiotics). The same strategy was used in the Embase database. For LILACS, the terms (enterocolite) AND (probióticos) were used.

Also, a manual search was conducted through the references of pre-selected studies and published reviews on the subject.

Inclusion and exclusion criteria

Study design: only randomized and controlled trials (phase III studies) were included.
Patients: premature newborns (< 34 weeks of gestational age) and/or very low birth weight (< 1,500 g at birth) regardless of gestational age. Studies that included patients with more than 34 weeks of gestational age and 1,500 g at birth and those in which it was not possible to establish these limits were excluded from the analysis.
Intervention: Newborns who received supplementation with probiotics (regardless of the nature, mode of preparation, and dose) added to enteral nutrition with human milk and/or formula;
Control: Newborns who received only enteral nutrition with human milk and/or formula.

Analyzed outcomes

The outcomes analyzed were incidence of NEC ≥ Bell stage II,[7] overall mortality, mortality from NEC, sepsis incidence, time to reintroduction of oral feeding, and hospitalization.

Methodological quality and internal validity

A detailed assessment of quality of the studies was conducted, aiming to evaluate the strength of evidence and the validity of their inclusion in this review. The Jadad scale[8] was used, and only studies with a score equal to or greater than 3 were included in this review.

The individual characteristics of each study included in the review were analyzed according to the Consolidated Standards of Reporting Trials (CONSORT) recommendations.[9]

Statistical analysis

All data were analyzed by intention to treat, thus the study participants were assessed in groups to which they were randomized regardless of treatment and protocol irregularities. The possible losses to follow-up were considered unfavorable clinical outcome.

The measures of effectiveness or damage expressed in absolute numbers were analyzed by the difference in absolute risk, adopting a confidence interval of 95%. For all statistically significant results, the numbers needed to treat (NNT) and numbers needed to harm (NNH) were calculated.

For the analysis of continuous data, the differences of weighted means between the groups were used. The studies that did not express data as means and their respective standard deviations were not included in the analyses.

Heterogeneity and sensitivity analysis

Inconsistencies between trials were estimated using the chi-squared test for heterogeneity, and quantified using the I^2 test. A value above 50% was considered significant.

A sensitivity analysis was performed, including only studies that obtained results with power > 80%.

Results

Study selection

268 studies were retrieved through electronic searches (MEDLINE = 190; Embase = 73, and LILACS = 5). Of these, 18 randomized and controlled trials were selected to be read in full.[10-27] Six studies were identified through manual search, as they did not fit the strategy used[28,29] or were indexed in another database.[30-33] After this phase, eight studies were excluded because they did not meet inclusion criteria: five studies did not evaluate the study population;[10,14,19,25,29] one study did not assess the selected outcomes;[18] and two studies were classified as Jadad < 3.[13,28] Four studies were not included because they were published in the Chinese language, which made data comprehension and extraction impossible.[30-33]

Thus, this review included data from twelve randomized and controlled trials,[11,12,15-17,20-24,26,27] totaling 2,907 patients, with 1,441 in the probiotics group and 1,466 in the control group.

Primary study description

Data on the interventions evaluated in each primary study are described in Table 1.

Effect of probiotics on necrotizing enterocolitis

In the probiotics group, the incidence of NEC stage ≥ 2 was 3.2%, whereas in the control group it was 7.2%. There was a decrease in the absolute risk by 4.0% (95% CI: 0.02 to 0.06, $p < 0.001$, $I^2 = 37\%$; Figure 1) and it was necessary to treat 25 patients to obtain this benefit.

Effect of probiotics on mortality

The mortality rate in the study group was 5.5%, whereas in the control group it was 8.4%. Probiotics reduce the absolute risk of death by 3.0% (95% CI: 0.01 to 0.05, $p < 0.002$; $I^2 = 59\%$; Figure 1) and it was necessary to treat 34 patients to obtain this benefit.

When excluding the study that generated high heterogeneity,[12] the effect achieved in the previous analysis is sustained ($p < 0.002$ and $I^2 = 14\%$).

Only five primary studies analyzed mortality from NEC.[12,15,20,24,27] There was no statistical difference between the probiotic and placebo groups (2.6% *vs*. 3.0%, $p = 0.64$, $I^2 = 0\%$; Figure 1).

Effect of probiotics on sepsis

All studies analyzed the incidence of neonatal sepsis as the outcome. Patients receiving probiotics had a lower incidence of sepsis when compared to those not receiving them, but with no significant difference (17.9% *vs*. 20.6%, 95% CI: 0.00 to 0.05, $p = 0.05$, $I^2 = 57\%$; Figure 1).

The same effect is obtained when excluding the study that generated significant heterogeneity[21] ($p = 0.32$, $I^2 = 21\%$).

Effect of probiotics on time to oral feeding reintroduction

Eight primary studies evaluated the time to oral feeding reintroduction.[15,17,20,21,23,24,26,27] The patients that received supplementation with probiotics had oral feeding reintroduction, on average, three days earlier than the control group (95% CI: 2.78 to 3.69 days, $p < 0.001$). However, this result is related to a high heterogeneity ($I^2 = 94\%$).

Effect of probiotics on time of hospitalization

Six primary studies assessed the duration of hospitalization in a neonatal intensive care unit.[11,16,20,22,23,27] Two studies

Table 1 Data on interventions evaluated in primary studies.

Study	Probiotic agent	Dose and duration	Milk
Millar et al.[11]	Lactob GG	10^8 CFU twice a day, for 14 days	Human and/or formula
Dani et al.[12]	Lactob GG	$6x10^9$ CFU once a day until discharge	Human and/or formula
Bin-Nun et al.[15]	Bifidobac infantis Bifidobac bifidum Strepto thermophilus	$3.5x10^8$ CFU of each, once a day up to corrected gest. age of 36 weeks	Human and/or formula
Lin et al.[16]	Lactob acidophilus Bifidobac infantis	10^6 organisms of each, twice a day from seventh day until discharge	Human
Manzoni et al.[17]	Lactob casei	$6x10^9$ CFU once a day, from third day until six weeks or until discharge	Human
Lin et al.[20]	Lactob acidophilus Bifidobac bifidum	$2x10^9$ CFU once a day up to six weeks	Human and formula
Manzoni et al.[21]	Lactob rhamnosus	$6x10^9$ CFU once day, for four to six weeks or until disacharge	Human and/or formula
Rouge et al.[22]	Bifidobac longum Lactob rhamnosus	10^8 CFU once a day until discharge	Human and/or formula
Samanta et al.[23]	Bifidobac infantis Bifidobac bifidum Bifidobac longum Lactob acidophilus	$2.5x10^9$ CFU once a day until discharge	Human
Mihatsch et al.[24]	Bifidobac lactis	$1.2x10^{10}$ CFU/kg once a day, until six weeks	Human and/or formula
Braga et al.[26]	Bifidobac breve Lactob casei	$3.5x10^7$ to $3.5x10^9$ CFU once a day, from second to 30th day or until discharge	Human
Sari et al.[7]	Lactob sporogenes	3.5x108 CFU once a day, until discharge	Human and/or formula

provided data as medians and were not included in the meta-analysis.[11,27] Patients who received probiotics stayed, on average, six days less in the hospital (95% CI: 5.12 to 7.09 days, $p < 0.001$, $I^2 = 88\%$) when compared to those who did not.

Power of primary studies

The power established in each primary study, regarding each outcome, is described in Table 2.

Analysis of sensitivity

Through the analysis of sensitivity, including only studies that had statistical power greater than 80%, patients who received probiotics had a lower incidence of NEC (NNT = 13). There was no difference in overall mortality, mortality from NEC, and the incidence of sepsis between the groups.

Discussion

Although NEC is still a major challenge in neonatology, much information has been obtained to elucidate its pathogenesis, allowing a better study of its management and prevention. Special attention has been given to supplementation with probiotics for preterm infants, especially those with very low birth weight, in an attempt to reduce the incidence of this disease.

Probiotics are living microorganisms offered as nutritional supplements that act in the intestine of the host organism by regulating the local bacterial flora. They act by improving gastrointestinal permeability and increasing the resistance of the mucosa against bacterial penetration. Regarding the protection mechanisms, they: (i) increase the resistance of the intestinal barrier against the passage of bacteria and their toxins, (ii) modify the host response in relation to microbial products, (iii) increase the mucosal response to IgA, (iv) produce bactericidal substances, and (v) competitively exclude potential pathogens.[5,6]

This review aimed to assess the best evidence available in the literature to elucidate the benefits of probiotics in preterm neonates. Only randomized and controlled trials with well-defined protocols were included, to control possible biases as much as possible. The validity of the results can be potentially compromised due to different doses and preparation methods of the intervention being studied. Non-inclusion of the four studies published in the Chinese language, for which it was not possible to perform critical analysis, must also be considered.

The set of results showed, with consistent data, that enteral administration of probiotics reduced the incidence of severe cases of NEC, mortality, and sepsis, as well as

Study	Probiotics Events	Total	Control Events	Total	Risk Difference Weight	F-M, fixed, 95% CI
Incidence of ECN						
Bin-Nun et al.[15]	1	72	10	73	5.0%	−0.12 [−0.21, −0.04]
Braga et al.[26]	0	122	4	121	8.4%	−0.03 [−0.07, 0.00]
Dani et al.[12]	4	295	8	290	20.3%	−0.01 [−0.04, 0.01]
Lin et al.[16]	2	180	10	187	12.7%	−0.04 [−0.08, −0.01]
Lin et al.[20]	9	222	18	221	15.4%	−0.04 [−0.09, 0.00]
Manzoni et al.[17]	1	39	3	41	2.8%	−0.05 [−0.14, 0.05]
Manzoni et al.[21]	0	151	10	168	11.0%	−0.06 [−0.10, −0.02]
Mihatsch et al.[24]	4	93	5	90	6.3%	−0.01 [−0.08, 0.05]
Rouge et al.[22]	2	45	1	49	3.3%	0.02 [−0.05, 0.10]
Samanta et al.[23]	5	91	15	95	6.4%	−0.10 [−0.19, −0.02]
Sari et al.[27]	17	121	20	121	8.4%	−0.02 [−0.12, 0.07]
Subtotal (95% CI)		**1431**		**1456**	**100.0%**	**−0.04 [−0.06, −0.02]**
Total of envents	45		104			
Heterogeneity: Chi^2 = 15.85, df = 10 (p = 0.10); I^2 = 37%						
Overall effect test: Z = 4.97 (p < 0.00001)						
Overall mortality						
Bin-Nun et al.[15]	3	72	8	73	5.0%	−0.07 [−0.15, 0.02]
Braga et al.[26]	26	122	27	121	8.4%	−0.01 [−0.11, 0.09]
Dani et al.[12]	0	295	2	290	20.3%	−0.01 [−0.02, 0.00]
Lin et al.[16]	7	180	20	187	12.7%	−0.07 [−0.12, −0.02]
Lin et al.[20]	7	222	13	221	15.4%	−0.03 [−0.07, 0.01]
Manzoni et al.[17]	5	39	6	41	2.8%	−0.02 [−0.17, 0.01]
Manzoni et al.[21]	6	151	12	168	11.0%	−0.03 [−0.08, 0.02]
Mihatsch et al.[24]	4	93	2	90	6.3%	0.02 [−0.03, 0.07]
Rouge et al.[22]	2	45	4	49	3.3%	−0.04 [−0.13, 0.06]
Samanta et al.[23]	4	91	14	95	6.4%	−0.10 [−0.19- −0.02]
Sari et al.[27]	14	121	14	121	8.4%	0.00 [−0.08, 0.08]
Subtotal (95% CI)		**1431**		**1456**	**100.0%**	**−0.03 [−0.05, −0.01]**
Total of events	78		122			
Heterogeneity: Chi^2 = 24.38, df = 10 (p = 0.007); I^2 = 59%						
Overall effect test: Z = 3.17 (p = 0.002)						
Mortality due to ECN						
Bin-Nun et al.[15]	0	72	3	73	9.1%	−0.04 [−0.09, 0.01]
Dani et al.[12]	0	295	2	290	36.6%	−0.01 [−0.02, 0.00]
Lin et al.[20]	7	222	7	221	27.7%	−0.00 [−0.03, 0.03]
Mihatsch et al.[24]	3	93	1	90	11.4%	0.02 [−0.02, 0.06]
Sari et al.[27]	11	121	11	121	15.1%	0.00 [−0.07, 0.07]
Subtotal (95% CI)		**803**		**795**	**100.0%**	**−0.00 [−0.02, 0.01]**
Total of events	21		24			
Heterogeneity: Chi^2 = 3.66, df = 4 (p = 0.45); I^2 = 0%						
Heterogeneity: Chi^2 = 15.85, df = 10 (p = 0.10); I^2 = 37%						
Overall effect test: Z = 4.97 (p < 0.00001)						
Incidence of sepse						
Bin-Nun et al.[15]	31	72	24	73	5.0%	−0.10 [−0.06, −0.26]
Braga et al.[26]	40	122	42	121	8.4%	−0.02 [−0.14, 0.10]
Dani et al.[12]	14	295	12	290	20.1%	−0.01 [−0.03, 0.04]
Lin et al.[16]	22	180	36	187	12.6%	−0.07 [−0.14, −0.00]
Lin et al.[20]	25	222	28	221	15.2%	−0.01 [−0.07, 0.05]
Manzoni et al.[17]	19	39	22	41	2.8%	−0.05 [−0.27, 0.17]
Manzoni et al.[21]	7	151	29	168	10.9%	−0.13 [−0.19, −0.06]
Mihatsch et al.[24]	30	93	30	90	6.3%	−0.01 [−0.15, 0.13]
Millar MR.[11]	0	10	0	10	0.7%	−0.00 [−0.17, 0.17]
Rouge et al.[22]	15	45	13	49	3.2%	0.07 [−0.12, 0.25]
Samanta et al.[23]	13	91	28	95	6.4%	−0.15 [−0.27, −0.04]
Sari et al.[27]	40	121	36	121	8.3%	−0.03 [−0.08, 0.15]
Subtotal (95% CI)		**1441**		**1466**	**100.0%**	**−0.03 [−0.05, −0.00]**
Total of envents	256		104			
Heterogeneity: Chi^2 = 23.10, df = 11 (p = 0.02); I^2 = 52%						
Overall effect test: Z = 1.96 (p < 0.05)						

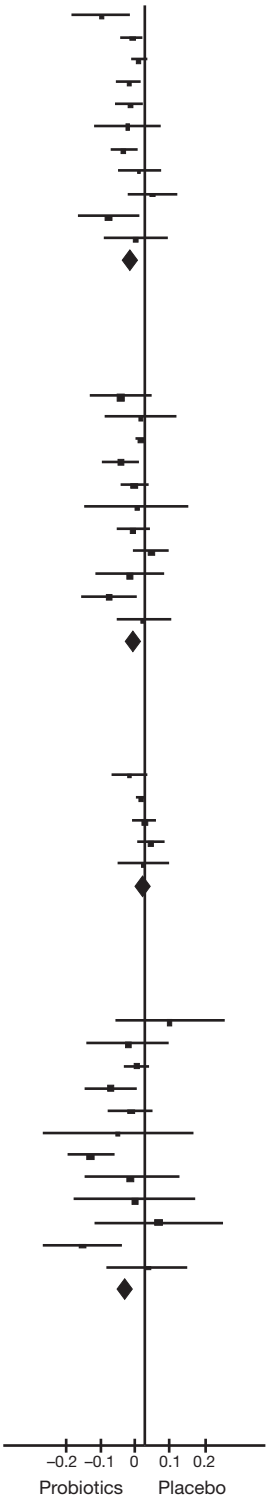

Figure 1 Meta-analysis analyzing the efficacy of probiotics in preterm neonates. 95% CI, 95% confidence interval; df, degrees of freedom; ECN, necrotizing enterocolitis; F, female; I^2, heterogeneity test; M, male.

Table 2 Power (1-b) established in primary studies.

Study	NEC	Overall mortality	Mortality due to NEC	Sepsis
Millar et al.[11]	*	*	*	NA
Dani et al.[12]	40.9%	40.6%	40.6%	8.3%
Bin-Nun et al.[15]	85.0%	35.7%	40.2%	23.5%
Lin et al.[16]	60.8%	71.9%	*	45.6%
Manzoni et al.[17]	12.5%	3.9%	*	6.2%
Lin et al.[20]	42.6%	33.1%	1.1%	9.4%
Manzoni et al.[21]	86.8%	21.3%	*	92.6%
Rouge et al.[22]	12.1%	12.4%	*	9.1%
Samanta et al.[23]	68.5%	72.4%	*	70.3%
Mihatsch et al.[24]	8.9%	12.0%	15.9%	2.6%
Braga et al.[26]	48.7%	3.1%	*	4.7%
Sari et al.[27]	9.3%	1.0%	1.1%	7.0%

NA, not applicable; NEC, necrotizing enterocolitis.
*Outcome not assessed.

presenting a shorter time until oral feeding reintroduction and shorter hospitalization stay. Although the numbers needed to treat in relation to NEC prophylaxis (NNT = 25) and mortality (NNT = 34) are relatively high, these can be counterbalanced by the high incidence of premature births, especially in countries with socioeconomic and cultural problems, and also by the easy handling and low costs related to probiotics. Considering the extremely fragile patients, susceptible to infections, complications and comorbidities, it is believed that these supplements, when available, deserve more attention.

Based on the available data, it can be inferred that probiotics are another useful tool in pediatric clinical practice. However, further studies are needed to assess the best preparation methods and doses, as well as the types of probiotics to be used.

Some reviews on the subject have been published in recent years, and similarly to the present study, showed the benefits of probiotic supplementation.[34-36] Small differences regarding methodological issues are found among these publications; for instance, regarding the search strategy and databases used, gestational age (27-37 weeks), and weight of the newborn. Nonetheless, there was a decrease in the incidence of NEC in all analyses. Wang et al.,[36] in the last review published on the subject, were the first to attempt to stratify the data regarding the species of probiotics. Both *Lactobacillus* and *Bifidobacteria* were found to be effective.

Some authors consider the available evidence as sufficient for the adoption of this type of therapy into routine practice, and claim that new studies on the subject are unnecessary and also unethical. Others are more cautious and claim that the studies have methodological flaws, that the safety of probiotics in relation to the invasion of microorganisms in the intestinal mucosa is not fully established, and that the methods of preparation are very heterogeneous.[37]

Four clinical trials registered with ClinicalTrials.gov including approximately 1,500 patients are under progress; in the future, they must be included in a data update, and may thus elucidate the benefits obtained so far.

Moreover, it should be clarified that only two selected studies had sufficient power to confirm the results.

Conclusion

The synthesis of evidence shows that supplementation with probiotics reduces the incidence of severe NEC in premature infants.

Conflicts of interest

The authors have no conflicts of interest to declare.

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