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The use of probiotics in the prevention of necrotizing enterocolitis and its role in somatic growth in preterm infants: A 10-year review

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Abstract: *Introduction:* The use of probiotics has been extensively studied and has shown promising results in reducing the incidence and severity of NEC in preterm infants. The WHO recommendations state that probiotics may be considered for a human-milk-fed very preterm infant. However, the guideline development group was not able to make a recommendation on the type, formulation, dose, timing, or duration of probiotic administration due to insufficient evidence and leave their choice based on clinical judgment. This review aims to provide information regarding probiotic strains, dose, and duration of administration.

Method: A comprehensive systematic literature review was conducted using an electronic database, PubMed. PubMed was searched for Randomized Controlled Trials that had been published within a duration of ten years, published in English, and included newborns.

Results: Half of the included studies showed that the use of probiotics was associated with a reduction in the incidence of NEC. Probiotics were also associated with improved short-term weight gain.

Conclusion: Probiotics appear to be effective in preventing NEC and promoting growth and development in preterm infants. It is important to consider the factors that alter the preterm microbiota. Therefore, there is still a gap in the microbiota analysis of breast milk from mothers who had preterm deliveries.

Keywords: Probiotics, Necrotizing Enterocolitis, Newborn,

Growth

Résumé: *Introduction :* L'utilisation de probiotiques a été largement étudiée et a montré des résultats prometteurs dans la réduction de l'incidence et de la gravité de l'ECUN chez les prématurés. Les recommandations de l'OMS indiquent que les probiotiques peuvent être envisagés pour les grands prématurés nourris au lait maternel. Cependant, le groupe d'élaboration des lignes directrices n'a pas été en mesure de formuler une recommandation sur le type, la formulation, la dose, le moment ou la durée de l'administration des probiotiques en raison de l'insuffisance des données probantes et a laissé le choix se fonder sur le jugement clinique. Cette revue vise à fournir des informations sur les souches probiotiques, la dose et la durée d'administration.

Méthode: Une revue systématique et exhaustive de la littérature a été réalisée à l'aide d'une base de données électronique, PubMed. PubMed a été consulté pour les essais contrôlés randomisés qui avaient été publiés sur une période de dix ans, en anglais, et qui incluaient des nouveau-nés.

Résultats: La moitié des études incluses ont montré que l'utilisation de probiotiques était associée à une réduction de l'incidence de la NEC. Les probiotiques ont également été associés à une amélioration de la prise de poids à court terme.

Conclusion: Les probiotiques semblent être efficaces dans la prévention de l'ECUN et la promotion de la croissance chez les prématurés. Il est important de

prendre en compte les facteurs qui modifient le microbiote des prématurés. Par conséquent, l'analyse du microbiote du lait des mères ayant

accouché avant terme présente encore des lacunes.

Mots clés: Probiotiques, Entérocolite ulcéro-nécrosante, Nouveau-né, Croissance

Introduction

A preterm infant has an immature gastrointestinal tract (GIT) that affects its functionality.¹ The GIT of a premature newborn continues to develop postnatally in an environment where feeds are intermittently administered, in contrast to intrauterine nutrition, where nutrients are continuously supplied. This significant change has an impact on postnatal GIT development.¹ Immature GIT has impaired mucosal barrier functions and increases the risk of inflammation, dysbiosis, and the development of necrotizing enterocolitis.²

Intestinal dysbiosis is an alteration in the intestinal microbiota associated with the disease. Dysbiosis in a preterm infant may be attributed to maternal microbiota, type delivery, feeding type, prolonged use of antibiotics, and host microbiota.^{3,4} In preterm infants, the microbiota supports the growth and operation of the gut immune system. The use of probiotics has been reported as an early intervention to enhance the intestinal environment and increase gut bacteria colonization.^{5,6}

Probiotics are formulations that contain live microorganisms (bacteria or fungi) given by enteral route and colonize the mucosal surface of the human gastrointestinal tract, modulate the intestinal microbiome, and promote mucosal barrier functions.⁷ Their benefits include improved gut epithelial barrier function, enhanced mucosal IgA responses, increased production of anti-inflammatory cytokines, improved maturation of the immune system in the newborn, suppressed pathogenic and promoted beneficial microbes in the gut.⁸ Preterm infants have reduced species diversity of microbiota compared to term infants.⁹

The WHO recommendations for the care of preterm or low-birth-weight infant states that probiotics may be considered for a human-milk-fed very preterm infant. However, the guideline development group was not able to make a recommendation on the type, formulation, dose, timing, or duration of probiotic administration due to insufficient evidence and leave their choice based on clinical judgment.⁷

This review aims to provide a global overview of probiotics regarding their use in the prevention of necrotizing enterocolitis, and their role in growth and development in preterm infants.

Method

In this review, PubMed was searched for randomized controlled trials that had been published within a duration of ten years (2013-2022), published in English, and included newborns (birth to 1 month). PubMed is a free

search engine accessing primarily the MEDLINE database of references and abstracts on life sciences and biomedical topics. Search queries were: (probiotics AND (necrotizing enterocolitis)) AND (growth). Studies reporting the use of Probiotics in the prevention of necrotizing enterocolitis and its role in growth and development in preterm infants were included.

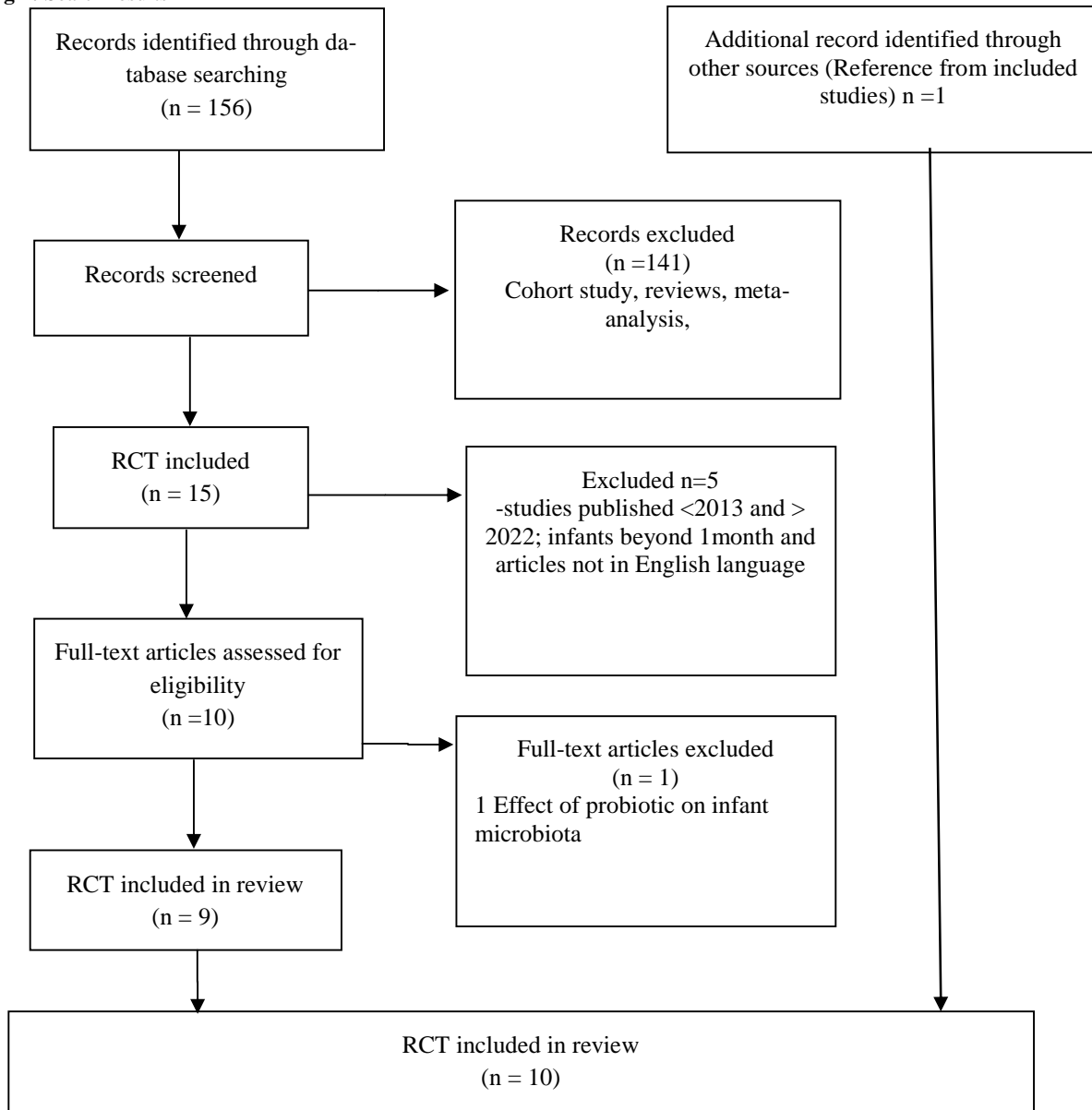
Results

A total of 10 RCTs were included in this review as shown in figure 1. Globally, the use of probiotics has been extensively studied. Table 1 includes the characteristics of the studies in this review. Five RCTs have evaluated the effect of probiotics on the incidence of NEC. Among those studies, 60% of them showed a significant reduction in NEC. The other five RCTs have evaluated the effect of probiotics on growth. Only two RCTs report improvement of weight gain in the probiotics group and hence have a positive impact on somatic growth. Information including the type, strain, dose, formulation, and duration of probiotics was extracted from the included studies (Table 1). Studies population were premature infants based on their weight and gestation age. The majority (9 studies) studied intervention included the use of probiotics versus placebo. Few studies compare the use of single strain versus multispecies (Table 1)

The Use of Probiotics in the Prevention of NEC

A study that includes a single strain (*Bifidobacterium breve* strain BBG-001) versus placebo, showed no evidence of benefit for the prevention of NEC in the probiotics group.¹⁰ This was among the largest trials including a total of 1310 babies in both arms. Cross-colonization of the placebo arm was reported to be controlled. In a study evaluating Single strain versus multispecies probiotics on necrotizing enterocolitis showed a similar incidence in both arms.¹¹ One RCT that included a total of 110 babies reported that using probiotic strains in combined multi-strain and multispecies forms at higher doses until 36 postmenstrual age (PMA) had positive effects on gastrointestinal complications, sepsis, and mortality in premature infants.¹² However, the incidence of NEC in the probiotics group was 4% compared to 12% in the control group in a study done by Shashidhar et al.¹³ The incidence of NEC was also seen to be zero in 100 babies who received probiotics, compared to the placebo group, whereby among 100 babies, five babies got NEC.¹⁴

Fig 1: Search results



The Role of Probiotics in the Growth and Development of Preterm Infants

Few studies evaluate the use of probiotics in reducing the time for full feeds, as well as daily weight gain. Hays S evaluated the effect of probiotics on Growth and found that there were no statistically significant differences seen between the groups in relation to the mean body weight, length, or head circumference.¹⁵ However, Sowden et al revealed that the probiotic group reached full feeds earlier than the placebo group and regained their birthweight earlier than the placebo group.¹¹⁶ Totsu et al and Wejryd et al evaluated the effect of probiotics on growth in preterm infants. They found no difference between the probiotics and placebo groups.^{17,18} Xu L et al evaluated the use of *saccharomyces boulardii* in 125 formula-fed preterm infants and reported that *S. Saccharomyces* at a dose of 50 mg/kg twice a day improved weight gain.¹⁹

Type of Probiotics

Various probiotics have been used in neonates but the most commonly employed are species of *bifidobacterial* and *lactobacillus*. The majority of RCT interventions consist of a multi-strain probiotic and are reported to be a safe and cost-effective way of preventing NEC and feeding intolerances in premature neonates.^{11,12,13,14, 16}

Table 1: Characteristic of studies

S/N	Author, year	Country	Study population	Intervention	-Type, strain, -Dose, formulation, and duration of probiotics	Outcomes -Primary -Secondary	Results
1	Costeloe K et al, 2016. [12]	England	Newborns 23-30-week's gestation	Probiotics or Placebo	- <i>B. breve</i> strain BBG-001 - 1 ml of <i>B. breve</i> BBG-001 (6.7 × 10 ⁷ to 6.7 × 10 ⁹ CFU per dose, daily until 36 weeks' PMA - provided in single-dose sachets as a powder, freeze-dried with maize starch	- an episode of NEC Bell stage 2 in any baby; and death. - *stool colonization with <i>B. breve</i> - Prevalence of NEC	- No evidence of benefit for the primary outcome and does not support the routine use
2	Gómez - Rodríguez G et al, 2019. [13]	Mexico	Newborns - BWT 700g - 1500 g -from less than 33 GA	Single strain versus multispecies	- <i>L. acidophilus boucardii</i> strain 1 x 10 ⁹ CFU or - multispecies probiotic which contains 1 x 10 ⁹ [CFU] of <i>L. acidophilus</i> , 4.4 x 10 ⁸ CFU of <i>L. rhamnosus</i> , 1.0 x 10 ⁹ CFU of <i>L. casei</i> , 1.76 x 10 ⁸ CFU of <i>L. plantarum</i> , 2.76 x 10 ⁷ CFU of <i>B. infantis</i> , and 6.6 x 10 ⁵ CFU of <i>Streptococcus thermophilus</i>	- NEC incidence was similar between groups (0% vs. 2.2%)	
3	Güney-Varal et al, 2017. [14]	Turkey	Newborns (32 week and 1500 gram	Probiotic (group 1) versus Placebo (group 2)	-Multi-combined probiotics of <i>L. rhamnosus</i> 4.1x10 ⁸ CFU + <i>L. casei</i> 8.2x10 ⁸ CFU + <i>L. plantarum</i> (4.1x10 ⁸ CFU) + <i>B. animalis</i> 4.1x10 ⁸ CFU - 383 mg of <i>fructooligosaccharides</i> and 100 mg of <i>galactooligosaccharides</i> as the prebiotic - set as 2x1 sachets	- The incidence of NEC and the mortality rate were found to be significantly lower in Group 1	
4	Hays S et al, 2016. [15]	France	-GA of 25 - 31 weeks, -BWT 700 g - 1600 g	Probiotics (with subgroups) versus placebo	-subgroups: P1 received <i>B. lactis</i> , -P2 received <i>B. longum</i> , and P3 received <i>B. lactis</i> and <i>B. longum</i>	- Postnatal growth (body weight, length, or head circumference)	- No statistically significant differences were seen
5	Shashidhar A et al, 2017. [17]	India	newborns with a BW of 750-1499 g	Probiotics versus Placebo	- <i>L. acidophilus</i> , <i>L. rhamnosus</i> , <i>B. longum</i> and <i>Saccharomyces boulardii</i> -once a day at a dose of 1.25x10 ⁹ CFU from the time of initiation of enteral feeds till discharge	Outcome measure: Time to reach full enteral feeds (150 mL/kg/ day). -NEC	-The mean time to reach full enteral feeding was low in probiotic -There was a trend towards lower NEC in the probiotic group (4% vs. 12%). -The probiotic had an improvement on the Z-score change in weight, potentially decreasing post-natal growth restriction -Growth during the stay was not different between groups.
6	Sowden M et al, 2022. [18]	South Africa	Neonates, with a BW 750-1500 g and below 37 GA	A probiotic or placebo	Labnic™ consists of <i>L. acidophilus</i> (0.67 billion CFU, <i>B. bifidum</i> 0.67 CFUs, and <i>B. infantis</i> 7 CFUs). -The standard dose of 0.2 ml once daily for 28 days, providing 2 billion CFUs per day	Growth and time to reach full feeds	-Feeding tolerance and growth parameters were similar in the two groups Probiotics improved weight gain, improved feeding tolerance, and had no adverse effects in preterm infants >30 weeks old
7	Totsu S et al, 2014. [19]	Japan	VLBW infants (birthweight	Probiotics (B) versus Placebo (P)	-Probiotic supplement, 2.5 × 10 ⁹ viable cells of <i>B. bifidum</i> /500 mg, -supplied as a freeze-dried powder in dextrin -The probiotic was approximately divided in half, and each portion was suspended in 0.5 mL warm water, breast milk, or infant formula Daily supplementation of <i>L. reuteri</i> (1.25 × 10 ⁸ bacteria/day) or placebo started within 3 days and continued until gestational week 36	-Incidence of morbidity and somatic growth before discharge -feeding tolerance -* growth rate	
8	Wejryd E et al, 2019. [33]	Sweden	ELBW (<1000 g) infants, GA week 28	Probiotics versus Placebo	-The probiotic <i>Saccharomyces boulardii</i> CNCM I-745 (S. Boulardii) -50 mg/kg twice daily -in Formula-fed	-Short-term growth parameters	
9	Xu L et al, 2016.[20]	China	newborns with a GA of 30 - 37 weeks and BWT 1500 - 2500 g	Probiotics versus No probiotics	Labnic™ consists of <i>L. acidophilus</i> (0.67 billion CFU, <i>B. bifidum</i> 0.67 CFUs, and <i>B. infantis</i> 7 CFUs). -The standard dose of 0.2 ml once daily for 28 days, providing 2 billion CFUs per day. Started oral feeds	-Time to reach full feeds -Incidence of NEC	shortens the time to reach full feeds, reduces the development of feeding intolerances, and incidence of NEC compared to a placebo
10	Sowden M et al, 2022. [47]	South Africa	750-1500 g and <37 weeks	Probiotics versus Placebo			

B. Bifidobacterium; BWT: Birth weight; CFU: Colony Forming Unit; GA: Gestation Age; L. Lactobacillus; LOS: Late-Onset Sepsis; NGT: Nasogastric Tube; NEC: Necrotizing Enterocolitis; PMA: Post Menstrual Age; RCT: Randomized Controlled Trials;

Formulation and Dose of Probiotics

The formulation and dose of probiotics can vary depending on the intended use and the specific strains of bacteria used. Probiotic preparations come in various forms: capsules, suspensions, powders, and combined into probiotic food. A multi-species preparation was reported in the form of drops.¹⁶ Another study included a probiotic group that received a multicomponent probiotic formulation of *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, and *Saccharomyces boulardii*. They were provided in the form of powdered sachets.¹³ Gomez et al evaluated multi-species probiotics in powder presentation form which were mixed in the feeds.¹¹ while Costeloe K et al and Totsu S et al provided probiotics in single-dose sachets as a powder, freeze-dried with maize starch.^{10, 17}

The appropriate dose of probiotics can vary depending on the strain (s) used, the intended use, and the individual's age and health status. The World Health Organization recommends a minimum daily dose of 1 billion colony-forming units (CFUs) of probiotics for general health promotion, but higher doses may be needed for specific health conditions.⁷

Time and Duration of Giving Probiotics

Early administration of probiotics soon after oral feeds started is recommended for better outcomes. In a study done by Güney-Varal et al, probiotics started when enteral feed per feed exceeded 2mls until the infant was discharged.¹² In another study, probiotics were started on day 5 after enteral trophic stimulation followed by increments of 20ml/kg/day.^[11] Administration of probiotics is not commenced if a neonate is nil per os.¹⁶

A longer duration of probiotics use seems to be beneficial. After the first dose, *B. bifidum* was given to infants twice a day until the body weight reached 2000 g.¹⁷ Sowden et al evaluated an outcome after probiotics use for 28 days.¹⁶ Wejryd E et al probiotics started within 3 days and continued until 36 corrected gestational weeks¹⁸ and Shashidhar A et al probiotics started from the time of initiation of enteral feeds till discharge.¹³ All these studies showed the benefit of probiotics after their use for a long time.

Adverse Effects

One of the adverse effects of probiotics is probiotic translocation and sepsis, due to compromised gut integrity. However, six studies reported no adverse effects associated with the administration of probiotics.^{15,20,13,16,17,19}

Discussion

Human milk is composed of probiotic bacteria. However, it has been reported that maternal use of antibiotics during pregnancy significantly reduced *Bifidobacterium*

and *Lactobacillus* spp.²¹ A study by Meyer MP et al showed that exposure to intrapartum antibiotics was associated with NEC.²² Studies of the microbiota of breast milk from mothers of preterm infants are limited, however, it has been reported that *Bifidobacterium* spp. were detected in significantly lower levels in mothers with preterm delivery.²³ Probiotic supplementation may assist in establishing a normal non-pathologic flora by preventing the binding of pathogenic bacteria to the enterocyte.²⁴

A narrative review of randomized controlled trials, observational studies, systematic reviews, and meta-analysis gather mounting evidence which supports the use of probiotics to decrease the risk of NEC in preterm infants.^[25] The current review, results showed that the use of probiotics reduces the risk of NEC. However, the optimum type of probiotic supplement and the long-term effects need further study.²⁶ The majority of RCTs in this review reported multiple strains of probiotics to appear as the most feasible and effective strategy for the prevention of NEC in preterm infants.^{11, 12, 13, 16} The finding was similar in two meta-analyses that supported the use of combination probiotics of both *Bifidobacterium* and *Lactobacillus* to prevent NEC and reduce mortality in moderately preterm infants.^{27,28} However, further clinical trials are required to focus on which probiotic combinations are most effective.²⁹

Probiotic supplementation may be associated with short-term weight gain, but the long-term effect, effects on length, head circumference, growth, and neurodevelopment are still questionable.³⁰

As described by the WHO, to have a beneficial health effect, probiotic preparations should contain a minimum number of live bacteria (colony-forming units), i.e., at least 106 cfu/g.^[7] Probiotics in fluid suspensions, however relatively easy to produce, are the least stable form of probiotics with the shortest shelf-life. In the current study, probiotics in liquid form were mixed with a stabilizer.^{16,14} Probiotics in solid forms, such as capsules and powders, are more stable and can be stored for a longer period of time than fluid suspensions.^[31]

Conclusion

The use of probiotics in the prevention of the prevalence of NEC has been widely and safely used with a positive impact. However, accepting whether the use of probiotics should be encouraged also in human-milk-fed infants or if this intervention should be directed towards exclusively formula-fed infants should be explored more. Human milk feeding provides symbiotic properties to the developing gut. However, it is important to consider the factors that alter the preterm microbiota. Therefore, there is still a gap in the microbiota analysis of breast milk from mothers who had preterm deliveries.

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