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Assessing the safety and efficacy of probiotics in improving the gut microbiome of premature infants

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ABSTRACT

Introduction and Purpose

The gut microbiome is crucial to infant health and development. For premature infants, microbiome development differs from full-term babies, posing long-term health risks. Factors such as delivery method, feeding, antibiotic treatment, and neonatal intensive care unit (NICU) environment significantly influence the microbiome of premature infants. Probiotics, especially Bifidobacterium strains, show promise in enhancing health and reducing risks of necrotizing enterocolitis (NEC) and late-onset sepsis (LOS).

Material and Methods

This review is based on articles from the PubMed database, covering the years 2004-2023, using keywords: gut microbiome, premature infants, probiotic, microbiome development, and neonatal intensive care unit.

Results

Current research indicates probiotics, particularly Bifidobacterium strains, positively impact the gut microbiome of premature infants, improving immunity and metabolism, and providing protection against NEC and LOS. Multi-strain probiotic preparations combining Bifidobacterium and Lactobacilaceae strains appear promising, but further research is needed to fully understand their effects. Addressing food intolerance (FI) in premature infants is another potential application of microbiome-focused therapies.

Conclusions

While probiotics show promise in improving the microbiome of preterm infants, comprehensive research on single and multi-strain probiotics and their specific mechanisms of action is needed. Controversies persist regarding the safety, FDA regulation, and selection of specific probiotics and formulations. There is no consensus on the optimal timing for introducing probiotics. Further research is required to optimize probiotic selection and determine the best benefits. Enhanced standardization and oversight are essential to ensure safe and effective use of probiotics in neonatal healthcare.

Keywords: gut microbiome, premature infants, probiotic, microbiome development, neonatal intensive care unit

Introduction

The gut microbiome plays a key role in shaping infants' health and development, influencing their immune system, growth and overall well-being. Many factors influence the colonization of the baby's intestines by microorganisms, including the mother's microflora, the method of delivery (cesarean section or vaginal delivery), the method of feeding (breastfeeding or formula feeding), and the baby's gestational age at birth. The development of an infant's gut microbiome typically follows a well-defined, step-by-step pattern, and deviations from this pattern can lead to long-term health problems.

Preterm infants born before 37 weeks of gestation (GA) experience a distinct set of microbial colonization patterns during the first weeks and months of life before eventually converging with those of infants born at approximately 40 weeks postmenstrual age (PMA).[1] However, preterm infants are exposed to frequent clinical interventions., which may change their gut microbiome. A stay in the neonatal intensive care unit exposes them to changing feeding patterns, frequent use of antibiotics, and a controlled microbial environment, leading to continuous enrichment of antibiotic resistance genes and altered microbial populations. These differences in the microbiome may increase the risk of immunological and metabolic diseases later in life, making modulation of the gut microbiome an important disease prevention strategy in premature infants.[2]

The degree of prematurity at birth is directly related to neonatal morbidity and mortality. Extremely premature infants, born before 32 weeks of age, are particularly susceptible to necrotizing enterocolitis (NEC), a severe necroinflammatory disease that affects approximately 7% of very low birth weight infants.[3] NEC often requires surgery and can result in lifelong complications. Another problem in premature infants is late-onset sepsis (LOS), which affects over 35% of premature infants.[4] Premature birth is associated with increased intestinal permeability, potentially allowing gut bacteria to enter the bloodstream, a risk factor for LOS.[5] Researches have shown that probiotic strains, especially those of the Bifidobacterium genus, can significantly change the composition of the intestinal microbiome of premature infants. These changes accelerate the maturation of the microbiome, making it more similar to that of full-term infants and leading to health-enhancing changes in immune and metabolic factors. Some probiotic strains may even become a permanent part of a premature baby's microbiome, suggesting potential long-term benefits. Interestingly, the presence of Bifidobacterium in the intestines of premature infants appears to provide protection against LOS. As a result, the infant gut microbiome is considered a target for the prevention of both NEC and LOS. Additionally, nutritional intolerance (FI) is a common problem in premature infants, delaying their transition to full enteral nutrition and prolonging hospital stay.[6] Given the impact of gut bacteria on intestinal balance, microbiome-focused therapies may offer opportunities for improved nutritional support in the intensive care unit.

Despite significant advances in neonatal care, there is limited compelling evidence for the effectiveness of therapies that manipulate the gut microbiome to reduce morbidity and mortality in premature infants. Probiotics and prebiotics are of great interest in neonatology, but changing the composition and function of the microbiome must be approached with caution. This review examines the current situation, focusing on the maturation patterns of the gut microbiome in preterm compared to full-term infants and the impact of probiotic supplementation on the microbiome of preterm infants. It presents the mechanisms by which probiotics can influence health, promote change, and discusses the use of probiotics as a therapeutic strategy in early life.

Necrotizing Enterocolitis (NEC)

Necrotizing enterocolitis (NEC) is a severe gastrointestinal disease affecting primarily premature infants, characterized by intestinal necrosis and bacterial translocation into the bloodstream, often resulting in sepsis. Mortality rates range from 15% to 45%, with long-term complications such as short-bowel syndrome, growth restriction, and neurological deficits. Factors like dysbiotic gut microbes, immune reactivity, and genetic predispositions contribute to NEC. Breastfeeding offers some protection against the condition. Clinical signs include abdominal distension, feeding intolerance, and systemic symptoms like temperature instability. Diagnosis is supported by radiographic findings such as pneumatosis intestinalis (Figure 1). The Bell staging system is used to assess NEC severity (Table 1). Treatment typically involves antibiotics like ampicillin, gentamicin, vancomycin, and metronidazole, although prolonged use can disrupt the microbiome and increase NEC risk. Gut rest, intravenous nutrition, and bowel function assessment are also crucial. NEC complications extend beyond the intestines, affecting the lungs and brain. Recurrence occurs in 4-10% of cases, and post-NEC intestinal stricture is common. Short bowel syndrome (SBS) is a severe consequence with high morbidity. NEC is linked to neurodevelopmental impairments, including cerebral palsy and cognitive deficits.[7]

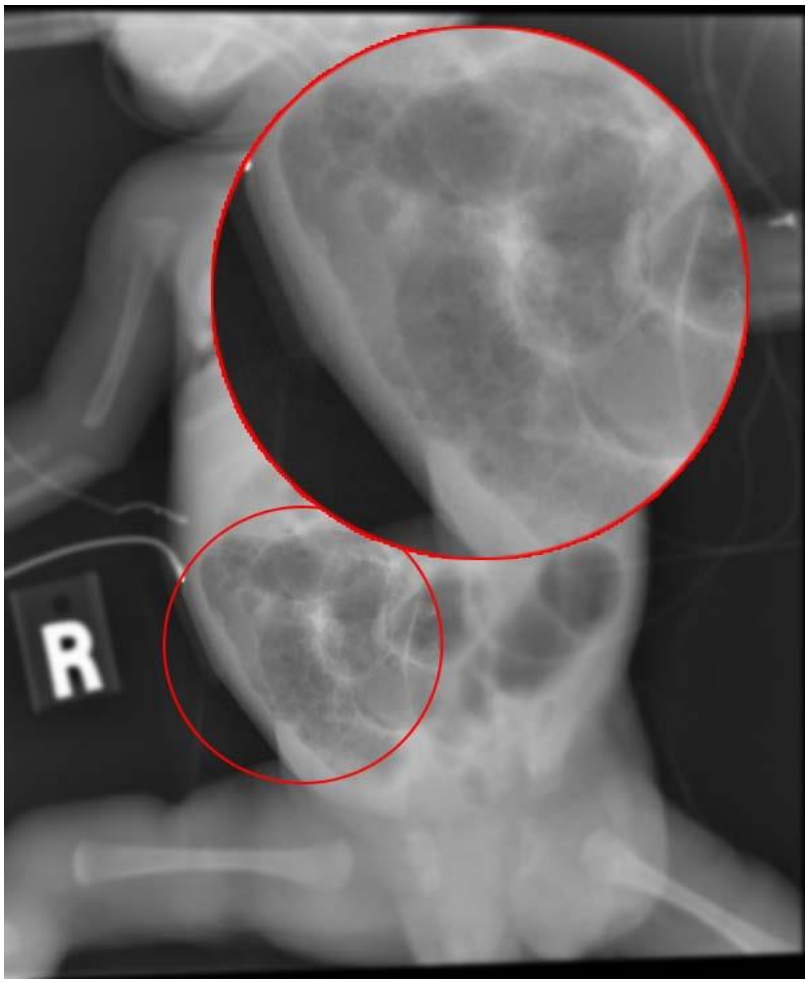

Figure 1. Radiograph of a baby with necrotizing enterocolitis

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Stage I	Stage II	Stage III
• Suspicion of NEC, • Nonspecific symptoms such as mild abdominal distension. hemodynamic instability, bradycardia, and apnea.	• Confirmed NEC • Significant abdominal distension • Radiological evidence of pneumatosis intestinalis • Metabolic acidosis • Trombocytopenia	• Advanced NEC • Shock symptoms, systemic manifestations • Severe metabolic acidosis • DIC • Radiological evidence as in stage 2 plus free intraperitoneal air indicating intestinal perforation.

Table 1. The Bell staging system (acc to Kawalec W, Grenda R, Kulus M, Pediatrics, Warsaw 2020, PZWL Medical Publishing, 2nd edition, volume I, page 238)

Development of the microbiome in the intestines of premature infants

The development of the intestinal microbiome in premature infants follows a unique pattern, shaped by various factors. Postnatal colonization of the infant's gastrointestinal tract is a dynamic process characterized by the sequential formation of microbial ecosystems. This process begins with pioneer species that change the intestinal environment and influence the timing and success of subsequent microbial species. As more species arrive, the microbial community changes, leading to a diverse community dominated by strict anaerobes, usually occurring at around 3 years of age. [8]

Unlike full-term infants, which are initially colonized by microorganisms found in the mother's vaginal secretions or stool during vaginal delivery or through the skin during cesarean section delivery, preterm infants are colonized primarily by nosocomial microorganisms. Several factors contribute to this distinction, including a higher incidence of cesarean sections, frequent use of antibiotics, delayed initiation of enteral nutrition, and longer hospital stays. The development of the intestinal microbiome of a premature infant is closely related to postmenstrual age (PMA). Early microbial communities are characterized by a dominance of Staphylococcaceae, followed by a shift towards communities rich in Enterococcaceae and Enterobacteriaceae, before reaching a dominance of Bifidobacteriaceae or Clostridiaceae around week 40 PMA, depending on the type of feeding (human milk or formula). Although alpha diversity increases over time, it generally remains lower than in full-term infants.[9,10]

In addition to bacteria, the fungal microbiome (mycobiome) in premature infants shows stochastic changes. Candida sp. dominate mainly in the first weeks or months of life, becoming dominant with Saccharomyces spp. with the introduction of solid foods. The viral microbiome (virome) in premature infants is less studied, but it shows similar stochastic changes and high individual variation. [11]

The development of the intestinal microbiome of a premature infant is additionally influenced by anatomical and functional differences in the gastrointestinal tract resulting from developmental immaturity.

These differences include shorter crypts and villi, decreased production of mucus and antimicrobial peptides due to fewer or immature goblet cells and Paneth cells, increased intestinal permeability, increased oxygen levels resulting from delayed enteral feeding and decreased colonization by strict anaerobes, and decreased motility and hormone secretion intestinal problems, partly due to long-term parenteral nutrition. Premature infants are also considered immunocompromised, making them more susceptible to serious infections and gastrointestinal conditions such as late-onset sepsis (LOS) and NEC. The complex interaction of these factors makes premature babies susceptible to the development of an abnormal microbiome and is subject to characteristic developmental processes. [12]

The use of probiotics in premature infants - single and multi-strain probiotics

Probiotics, i.e. preparations of live microorganisms with presumed health benefits, are increasingly used in early intervention strategies for premature infants. These interventions have shown significant positive effects on the development of the intestinal microbiota and the gastrointestinal environment in premature infants, bringing them closer to full-term infants in terms of the development of the intestinal immune system. Probiotics effectively regulate the intestinal microflora, thus contributing to the prevention of necrotizing enterocolitis (NEC), improving feeding tolerance and reducing mortality among premature infants. [13,14]

Despite demonstrated benefits, insight into the immunomodulatory effects of probiotics in preterm infants remains limited. Available evidence suggests that probiotics exert their immunomodulatory effects through three main mechanisms: modification of the gut microbiota composition and improvement of the intestinal environment, stimulation of immunity through probiotic-derived antigenic signals, and production of beneficial metabolites. [15]

The use of single-strain probiotics versus multi-strain probiotics in premature infants is still a subject of ongoing research. It is not entirely clear how multi-strain probiotics may provide additional or synergistic benefits that exceed those of single-strain probiotics. Furthermore, some mechanisms, such as human milk oligosaccharide (HMO) utilization, are strain-specific, which influences the selection of probiotic strains for clinical use in neonatal intensive care units (NICUs).[16]

Probiotics containing Bifidobacterium strains have shown great potential to improve intestinal health in premature infants. These probiotics offer various benefits, including nutrient metabolism, formation of cross-feeding networks, production of antimicrobial peptides, and competitive exclusion of pathogens.[17] It is worth noting that some strains of Bifidobacterium are excellent at metabolizing complex carbohydrates present in breast milk, known as human milk oligosaccharides (HMOs) , which makes them particularly suitable in probiotic preparations for premature infants.[18,19]

At the strain level, it is clear that not all Bifidobacterium species have the same ability to colonize the infant gut and induce changes in the microbiome. [20] Multi-strain probiotics have shown greater effectiveness in increasing Bifidobacterium abundance and creating more resistant and interconnected microbial communities. This collaboration benefits species with limited HMO metabolism capabilities. [21]

Supplementation with Bifidobacterium strains leads to functional changes in the intestines, such as increased concentrations of short-chain fatty acids (SCFA), especially acetate. This metabolic change contributes to lowering intestinal pH and strengthening the defense mechanisms of the intestinal epithelium against pathogens. Additionally, supplementation with Bifidobacterium strains has been associated with reduced pro-inflammatory status. However, the effect may vary depending on the specific strain used, highlighting the need for further research to optimize probiotic formulations to obtain the greatest benefit for premature infants.[22-24]

Lactobacilaceae transiently colonize the infant intestine and provide competitive pathogen exclusion, antitoxic effects, immunomodulatory effects, and limited HMO metabolism. Although they are not dominant members of the gut microbiome, they do play a role in probiotic supplements.[25-27]

Functionally, knowledge about the effects of Lactobacilaceae-based probiotics on premature infants is limited. One study suggests that these probiotics may act through competitive exclusion processes rather than HMO metabolism and immune modulation. Further research is needed to better understand the role of lactobacilli in the intestines of premature infants. [28,29]

Multi-type probiotic preparations, often containing strains of Bifidobacterium and Lactobacilaceae, are often studied for their potential benefits in premature infants. These preparations are considered due to the possibility of synergistic effects and broader health benefits due to the inclusion of microorganisms with different functional abilities. However, they also pose challenges in identifying the most effective probiotic candidates. [30,31]

Studies of multigeneric probiotics have shown that some strains, especially Bifidobacterium species, are more likely to persist in infants' guts and increase in abundance, while others, such as Lactobacilaceae, tend to disappear over time. [29]

In summary, multigeneric probiotic formulations, especially those combining Bifidobacterium and Lactobacilaceae strains, have shown promise in promoting microbiome maturation and reducing inflammation in premature infants. However, the specific strains used may have different effects and further research is needed to optimize the selection of probiotics for the best results.

Safety

The controversy surrounding the routine prophylactic administration of probiotics to premature infants has sparked a contentious debate. Although some advocates recommend the use of probiotics based on meta-analyses of various studies, concerns have been raised about their safety and effectiveness. These concerns include the lack of an appropriate FDA-approved probiotic product and the potential risks associated with dietary supplements, including reported side effects and increased mortality. [32] The choice of specific probiotic formulas is another area of uncertainty. The effectiveness of different strains and combinations of probiotics may vary, and there is no standardized approach to selecting the most appropriate preparation for a given clinical situation. Determining the optimal time to introduce probiotics remains a matter of debate. It is not widely established whether probiotics should be introduced prophylactically, during or after antibiotic treatment, or in response to specific clinical conditions.

Concerns about the safety of probiotics continue to be a significant point of discussion. These concerns include potential problems with contamination, side effects such as systemic infections and overactivation of the immune system, transfer of antibiotic resistance genes, and metabolic activities that may be harmful. Ensuring the quality and purity of probiotic products, as well as regulating their production and distribution, is a critical aspect that lacks a standardized framework. In summary, the use of probiotics in the clinical setting is characterized by a lack of consensus on patient selection, formulation, timing, and safety considerations. These issues highlight the need for further research, standardized guidelines, and increased oversight to ensure the safe and effective use of probiotics in various health care scenarios. [12,33]

Summary

The gut microbiome plays a key role in infants' health and development, influencing their immune system, growth and overall well-being. Various factors, including gestational age, mode of delivery, and feeding, influence microbial colonization in infants. Premature infants follow a distinct colonization pattern but face obstacles due to clinical interventions, potentially increasing the risk of future health problems. Therefore, modulation of the gut microbiome becomes an important strategy for disease prevention in premature infants. Extremely premature infants, born before 32 weeks of age, are particularly susceptible to conditions such as necrotizing enterocolitis (NEC) and late-onset sepsis (LOS). It has been shown that probiotics, particularly Bifidobacterium strains, can positively impact the gut microbiome, promote improved immunity and metabolism, and even provide protection against LOS. Addressing food intolerance (FI) in premature infants is another potential application of microbiome-focused therapies. Although probiotics have shown promise in improving the microbiome of preterm infants, there is a need for more comprehensive research on single and multi-strain probiotics and their specific mechanisms of action. There is also much controversy surrounding the use of probiotics in premature infants. There are concerns about safety and the lack of FDA regulation regarding probiotic products. The choice of specific probiotics and their formulation remains a matter of debate. There is also a lack of consensus on when to introduce probiotics. It is worth emphasizing that different probiotic strains may produce different effects, requiring further research to optimize probiotic selection and determine optimal benefits. Multi-strain probiotic preparations combining Bifidobacterium and Lactobacilaceae strains seem promising, but require further research to better understand their effects. The controversy surrounding the use of probiotics in premature infants highlights the need for further research, development of guidelines, and increased oversight to ensure the safe and effective use of probiotics in health care.

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References

1. Healy DB, Ryan CA, Ross RP, Stanton C, Dempsey EM. Clinical implications of preterm infant gut microbiome development. Nature Microbiol. 2022;7(1):22–24. doi: 10.1038/s41564-021-01025-4.
2. Thänert R, Sawhney SS, Schwartz DJ, Dantas G. The resistance within: antibiotic disruption of the gut microbiome and resistome dynamics in infancy. Cell Host Microbe. 2022 May 11;30(5):675-683. doi: 10.1016/j.chom.2022.03.013.
3. Alsaied A, Islam N, Thalib L. Global incidence of Necrotizing Enterocolitis: a systematic review and Meta-analysis. BMC Pediatr. 2020;20(1):344. doi: 10.1186/s12887-020-02231-5.
4.Madan JC, Salari RC, Saxena D, Davidson L, O'Toole GA, Moore JH, Sogin ML, Foster JA, Edwards WH, Palumbo P, Hibberd P. Gut microbial colonisation in premature neonates predicts neonatal sepsis. Arch Dis Child Fetal Neonatal Ed. 2012;97(6):F456–462. Doi: 10.1136/fetalneonatal-2011-301373.
5. Carl MA, Ndao IM, Springman AC, Manning SD, Johnson JR, Johnston BD, Burnham C-A, Weinstock ES, Weinstock GM, Wylie TN, Mitreva M, Abubucker S, Zhou Y, Stevens HJ, Hall-Moore C, Julian S, Shaikh N, Warner BB, Tarr PI. Sepsis from the Gut: the enteric habitat of bacteria that cause late-onset neonatal bloodstream infections. Clin Infect Dis: Official Publ Infect Dis Society America. 2014;58(9):1211–1218. doi: 10.1093/cid/ciu084.
6. Beck LC, Masi AC, Young GR, Vatanen T, Lamb CA, Smith R, Coxhead J, Butler A, Marsland BJ, Embleton ND, Berrington JE, Stewart CJ. Strain-specific impacts of probiotics are a significant driver of gut microbiome development in very preterm infants. Nat Microbiol. 2022;7(10):1525–1535. doi: 10.1038/s41564-022-01213-w.
7. Duess JW, Sampah ME, Lopez CM, Tsuboi K, Scheese DJ, Sodhi ChP, Hackam DJ, Necrotizing enterocolitis, gut microbes, and sepsis. Gut Microbes. 2023; 15(1): 2221470. doi:10.1080/19490976.2023.2221470
8. Chang C-S, Kao C-Y. Current understanding of the gut microbiota shaping mechanisms. J Biomed Sci. 2019;26(1):59. doi: 10.1186/s12929-019-0554-5.
9. Azad MB, Konya T, Persaud RR, Guttman DS, Chari RS, Field CJ, Sears MR, Mandhane PJ, Turvey SE, Subbarao P, Becke AB, Scott JA, Kozyrskyj AL. Impact of maternal

intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: a prospective cohort study. BJOG. 2016;123(6):983–993. doi: 10.1111/1471-0528.13601.
10. Korpela K, Blakstad EW, Moltu SJ, Strømmen K, Nakstad B, Rønnestad AE, Brække K, Iversen PO, Drevon CA, de Vos W. Intestinal microbiota development and gestational age in preterm neonates. Sci Rep. 2018;8(1):2453. doi: 10.1038/s41598-018-20827-x.
11. Samara J, Moossavi S, Alshaikh B, Ortega VA, Pettersen VK, Ferdous T, Hoops SL, Soraisham A, Vayalumkal J, Dersch-Mills D, Gerber JS, Mukhopadhyay S, Puopolo K, Tompkins TA, Knights D, Walter J, Amin H, Arrieta M-C. Supplementation with a probiotic mixture accelerates gut microbiome maturation and reduces intestinal inflammation in extremely preterm infants. Cell Host Microbe. 2022;30(5):696–711.e5. doi: 10.1016/j.chom.2022.04.005.
12. Mercer EM, Arrieta M-C. Probiotics to improve the gut microbiome in premature infants: are we there yet?. Gut Microbes. 2023 Jan-Dec;15(1):2201160. doi: 10.1080/19490976.2023.2201160.
13. Robertson C, Savva GM, Clapuci R, Jones J, Maimouni H, Brown E, Minocha A, Hall LJ, Clarke P. Incidence of necrotising enterocolitis before and after introducing routine prophylactic *Lactobacillus* and *Bifidobacterium* probiotics. Arch Dis Child Fetal Neonatal Ed. 2020 Jul;105(4):380-386. doi: 10.1136/archdischild-2019-317346.
14. Morgan RL, Preidis GA, Kashyap PC, Weizman AV, Sadeghirad B; McMaster Probiotic, Prebiotic, and Synbiotic Work Group. Probiotics Reduce Mortality and Morbidity in Preterm, Low-Birth-Weight Infants: A Systematic Review and Network Meta-analysis of Randomized Trials. Gastroenterology 2020 Aug;159(2):467-480. doi: 10.1053/j.gastro.2020.05.096.
15. Xiang Q, Yan X, Shi W, Li H, Zhou K. Early gut microbiota intervention in premature infants: Application perspectives. J Adv Res. 2023 Sep:51:59-72. doi: 10.1016/j.jare.2022.11.004.
16. Ouwehand AC, Invernici MM, Furlaneto FAC, Messora MR. Effectiveness of multi-strain versus single-strain probiotics. J Clin Gastroenterol. 2018;52(Supplement 1):S35–40. doi: 10.1097/MCG.0000000000001052.
17. De Vuyst L, Leroy F. Cross-feeding between bifidobacteria and butyrate-producing colon bacteria explains bifdobacterial competitiveness, butyrate production, and gas production. Int. J. Food Microbiol. 2011 Sep 1;149(1):73-80. doi: 10.1016/j.ijfoodmicro.2011.03.003.
18. Garrido D, Ruiz-Moyano S, Lemay DG, Sela DA, German JB, Mills DA. Comparative transcriptomics reveals key differences in the response to milk oligosaccharides of infant gut-associated bifidobacteria. Sci Rep. 2015 Sep 4:5:13517. doi: 10.1038/srep13517.
19. Gueimonde M, Margolles A, De Los Reyes-Gavilán CG, Salminen S. Competitive exclusion of enteropathogens from human intestinal mucus by *Bifidobacterium* strains with acquired resistance to bile — A preliminary study. Int. J. Food Microbiol. 2007 Jan 25;113(2):228-32. doi: 10.1016/j.ijfoodmicro.2006.05.017.
20. van Best N, Trepels-Kottek S, Savelkoul P, Orlikowsky T, Hornef MW, Penders J. Influence of probiotic supplementation on the developing microbiota in human preterm neonates. Gut Microbes. 2020 Nov 9;12(1):1-16. doi: 10.1080/19490976.2020.1826747.
21. Chang H-Y, Chen J-H, Chang J-H, Lin H-C Lin C-Y, Peng C-C. Multiple strains probiotics appear to be the most effective probiotics in the prevention of necrotizing

enterocolitis and mortality: an updated meta-analysis. PLoS One. 2017 Feb 9;12(2):e0171579. doi: 10.1371/journal.pone.0171579.
22. Aw W, Fukuda S. Protective effects of bifidobacteria against enteropathogens. Microb Biotechnol. 2019 Nov;12(6):1097-1100. doi: 10.1111/1751-7915.13460.
23. Lin PW, Myers LE, Ray L, Song SC, Nasr TR, Berardinelli AJ, Kundu K, Murthy N, Hansen JM, Neish AS. Lactobacillus rhamnosus blocks inflammatory signaling in vivo via reactive oxygen species generation. Free Radic Biol Med. 2009 Oct 15;47(8):1205-11. doi: 10.1016/j.freeradbiomed.2009.07.033.
24. Kumar A, Wu H, Collier-Hyams LS, Kwon YM, Hanson JM, Neish AS. The bacterial fermentation product butyrate influences epithelial signaling via reactive oxygen species-mediated changes in cullin-1 neddylation. J Immunol. 2009 Jan 1;182(1):538-46. doi: 10.4049/jimmunol.182.1.538.
25. Nowak A, Kuberski S, Libudzisz Z. Probiotic lactic acid bacteria detoxify N-nitrosodimethylamine. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2014;31(10):1678-87. doi: 10.1080/19440049.2014.943304.
26. Iwabuchi N, Yonezawa S, Odamaki T, Yaeshima T, Iwatsuki K, Xiao J-Z. Immunomodulating and anti-infective effects of a novel strain of Lactobacillus paracasei that strongly induces interleukin-12. FEMS Immunol Med Microbiol. 2012 Nov;66(2):230-9. doi: 10.1111/j.1574-695X.2012.01003.x.
27. Shi J, Zhao G, Huang X, Li X, Ma Y, Yang K. Effects of Lactobacillus rhamnosus Supplementation on Growth Performance, Immune Function, and Antioxidant Capacity of Newborn Foals. J Equine Vet Sci. 2023 Oct:129:104501. doi: 10.1016/j.jevs.2023.104501.
28. Thongaram T, Hoeflinger JL, Chow J, Miller MJ. Human milk oligosaccharide consumption by probiotic and human-associated bifidobacteria and lactobacilli. J Dairy Sci. 2017 Oct;100(10):7825-7833. doi: 10.3168/jds.2017-12753
29. Larke JA, Kuhn-Riordon K, Taft DH, Sohn K, Iqbal S, Underwood MA, Mills DA, Slupsky CM. Preterm infant fecal microbiota and metabolite profiles are modulated in a probiotic specific manner. J Pediatr Gastroenterol Nutr. 2022;75(4):535–542. doi: 10.1097/MPG.0000000000003570.
30. Timmerman HM, Koning CJM, Mulder L, Rombouts FM, Beynen AC. Monostrain, multistrain and multispecies probiotics--A comparison of functionality and efficacy. Int J Food Microbiol. 2004 Nov 15;96(3):219-33. doi: 10.1016/j.ijfoodmicro.2004.05.012.
31. Chi C, Li C, Buys N, Wang W, Yin C, Sun J. Effects of probiotics in preterm infants: a network meta-analysis. Pediatrics. 2021;147:e20200706. doi: 10.1542/peds.2020-0706.
32.Neu J. Routine probiotics for premature infants: let's be careful! J Pediatr. 2011;158(4):672–674. doi: 10.1016/j.jpeds.2010.11.028.
33. DeVeaux A, Ryou J, Dantas G, Warner BB, Tarr PI. Microbiome-targeting therapies in the neonatal intensive care unit: safety and efficacy. Gut Microbes. 2023 Jan-Dec;15(1):2221758. doi: 10.1080/19490976.2023.2221758.