dietetics

Review

A Narrative Review of the Tale of the Dysbiotic Microbiome in the Preterm Neonate

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Abstract: Background: Researchers have established that the preterm neonate is born with an immature gastrointestinal tract. The preterm neonate is thus susceptible to various complications often seen in the neonatal intensive care unit, e.g., feeding intolerances, necrotizing enterocolitis, and hospital-acquired bloodstream infections. These complications can be life-threatening, and if survived, can have an unfavorable effect on the neonate's growth and development. Aim: The aims of this narrative review article were to provide an in-depth understanding of the various factors contributing to the development of the preterm neonatal microbiome. Further, we reviewed gastrointestinal microbiome dysbiosis and its potential role in the development of feeding intolerances, necrotizing enterocolitis, and hospital-acquired bloodstream infections. Lastly, we described the potential role of probiotics in this vulnerable population. Methods: A PubMed database search was conducted identifying articles that describe the development and function of the neonatal microbiome, the role of gastrointestinal dysbiosis, and the development of neonatal complications as well as the role of probiotics in gastrointestinal dysbiosis. Results: Various maternal, neonatal, and environmental factors play a role in the development of gastrointestinal dysbiosis in the preterm neonate. This can lead to feeding intolerances, necrotizing enterocolitis, and hospital-acquired bloodstream infections. Discussion: The pathogenesis of the development of short-term complications in the preterm neonate can be linked to the immaturity of the host immune system as well as alterations seen in the intestinal microbiome. There is a growing body of evidence that probiotics can play a role in preventing dysbiosis and thus complications observed in the preterm neonate. However, the optimal combination of probiotic strains and dosage still needs to be identified.

Keywords: microbiome; preterm neonate; dysbiosis; probiotics

Citation: Sowden, M.; van Niekerk, E.; Bulabula, A.N.H.; van Weissenbruch, M.M. A Narrative Review of the Tale of the Dysbiotic Microbiome in the Preterm Neonate. *Dietetics* **2023**, *2*, 308–320. https://doi.org/10.3390/dietetics2040022

Academic Editor: Andriana Kaliora

Received: 8 September 2023
Revised: 16 October 2023
Accepted: 19 October 2023
Published: 24 October 2023

1. Introduction

The development of the intestinal innate immune system starts in the fetus and then matures in the neonate after birth [1]. Due to an early birth, the preterm neonate is born with an immature intestinal immune system characterized by a deficiency of intestinal innate immune cells and a low production of certain cytokines [1]. Premature neonates further present with an immature gastrointestinal system in terms of motility, digestion and absorption, circulatory regulation, intestinal epithelial barrier, and immune functions [2]. The preterm gastrointestinal system is thus vulnerable to delayed motility and gastric emptying, with subsequent stasis of the intestinal luminal content, leading to bacterial overgrowth [2]. Increased permeability of the immature epithelial mucosal barrier can arise from low levels of protective intestinal mucus and its peptides, decreased tight junctions between epithelial cells due to immaturity (probably due to the interactions of occludins

and claudins), reduced secretory immunoglobulin A (IgA), and the decreased regenerative capability of the immature gastrointestinal tract (GIT) [2,3]. This can lead to tissue damage, e.g., intestinal ischemia, hyperosmolar injury, increased permeability, possible translocation of microbes or their toxins, and subsequent inflammation [2–7]. Furthermore, preterm neonates experience a lower stomach hydrogen output, which with the use of H2 blockers, can lead to the proliferation of pathogens [8,9].

The immature GIT is thus susceptible to feeding intolerances, which is frequently observed as an inability to digest enteral feeds, with increased gastric residuals, abdominal distension, and emesis. The delay in full enteral feeds is unavoidably associated with the use of total parenteral nutrition (TPN) and a subsequently higher risk of central line-associated bloodstream infections (CLABSI) [10]. Gestational age at birth has an inverse relationship with complications, such as feeding intolerance, the development of necrotizing enterocolitis (NEC), and a higher risk of hospital-acquired bloodstream infection (HA-BSI) (formerly described as late-onset sepsis (LOS)) [11,12].

Probiotics, as defined by the World Health Organization (WHO), are "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [13]. Probiotics can serve different functions in the host, specifically conservation of appropriate host–microbe interactions, pathogen exclusion, mucus secretion from goblet cells, enhancement of the epithelial barrier function, production of antibacterial factors, immune response modulation (e.g., enhanced mucosal IgA responses), increased production of anti-inflammatory cytokines, changes in intestinal permeability, and the activation of the host adaptive immune system [14–16].

Research has established that using probiotics has beneficial health outcomes in preterm neonates [17]. It has been confirmed that some probiotics can change the composition of the gastrointestinal microbiome [18], thereby altering disease vulnerability. *Bifidobacterium infantis,* for example, can protect the intestinal surface against pathogen penetration by activating B cells to mature into secretory IgA-producing plasma cells, which can coat the surface of the intestine [18].

This review aims to provide a better understanding of the various factors contributing to the development of the neonatal microbiome and the potential role of probiotics in the neonatal intensive care unit (NICU). We will review several aspects regarding dysbiosis in the preterm neonate.

2. Methods

A PubMed database search was conducted between 2019 to August 2023 to collect the literature. The search included randomized placebo-controlled trials, controlled clinical trials, double-blind, randomized controlled studies, meta-analyses, systematic reviews, and review articles. The following combinations of keywords were used: "neonatal microbiome" OR "premature infant microbiome" OR "dysbiosis" AND "premature infant" OR "NEC" AND "premature infant" OR "sepsis" AND "premature infant" OR "feeding intolerance" AND "premature infant" OR "probiotics" AND "premature infant" OR "probiotics" AND "NEC" OR "probiotics" AND "feeding intolerance "AND "premature infant" or "probiotics" AND "sepsis" AND "premature infant". Papers that were not randomized placebo-controlled trials, controlled clinical trials, double-blind, randomized controlled studies, meta-analyses, systematic reviews, or review articles were excluded. Articles not containing any of the specified keyword combinations mentioned above were also excluded.

3. The Microbiome

The human microbiome is a collection of microorganisms (bacteria, protozoa, fungi, viruses, and bacteriophages) and resides on the surface of and inside the body [19]. The microbiome comprises approximately 100 trillion cells, which is ten times more than the number of human cells [20]. The largest quantity of human adult microbiota resides in the GIT [2,21].

4. Development of the Microbiome in Preterm Neonates

Figure 1 provides a visual overview of various factors that play a role in the development of the microbiome in the preterm neonate. The various factors will be described in the section below, from birth and onwards.

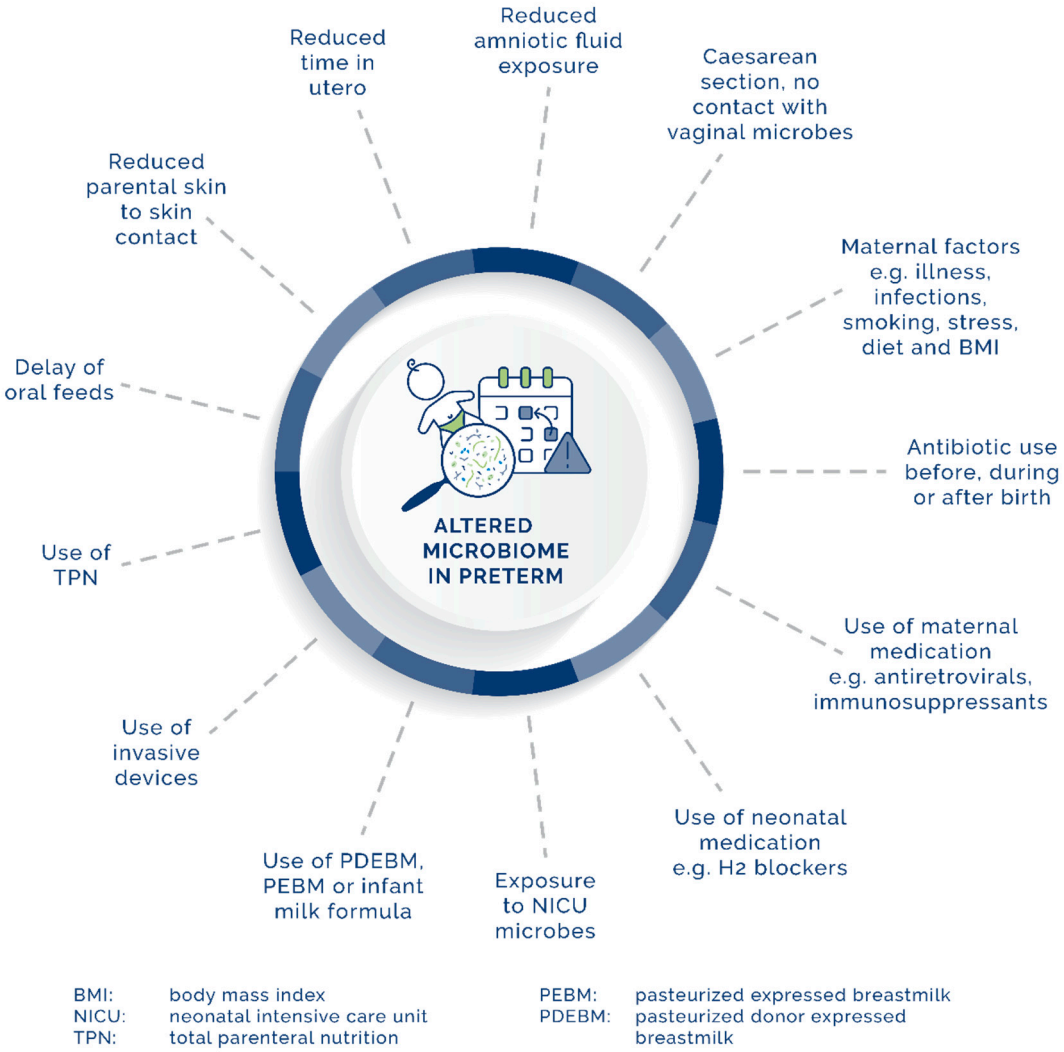

Figure 1. Maternal, neonatal, and environmental factors affecting the development of the GIT microbiome in preterm neonates.

4.1. Microbiome Development in Utero

Colonization of the developing GIT starts in utero as early as 10 weeks post-conception when amniotic fluid is swallowed [22–26]. Commensal bacteria are present in the amniotic fluid [27–29]. Microbes are also found in the placenta [22,27,30], fetal membranes, and umbilical cord blood [31]. Microbes have been detected in the meconium of neonates shortly after birth, even before the initiation of any feeds [27,28,32–38].

An in vitro model has described amniotic fluid as promoting fetal intestinal growth since amniotic fluid contains multiple trophic factors, e.g., growth factors, epidermal growth factor, and insulin-like growth factors (IGF)-1 and IGF-2 [39]. By the end of the last trimester, the fetus swallows an average of 450 mL of amniotic fluid per day [2], a crucial period that preterm neonates miss out on.

It is hypothesized that maternal intestinal antigen-presenting cells such as dendritic cells can sample and phagocytize maternal intestinal flora, carrying live commensal bacteria to the bloodstream and maternal organs. These cells can then be found in the maternal

organs such as the placenta and mammary gland. In this manner, maternal intestinal flora components may be able to cross over the placenta and be ingested by the fetus and, later, excreted in the breastmilk and ingested by the neonate [40].

The maternal microbiome affects the health of the neonate, since the maternal microbiome, linked with perinatal factors, determines the neonatal microbiome [41]. Various maternal factors may play a role in fetal microbiome development, e.g., stress, infection, illness, smoking, maternal diet, and obesity [42,43]. With respect to infection, the human immunodeficiency virus (HIV) leads to microbiome changes in the HIV-exposed but uninfected neonate, compared to the HIV-unexposed, uninfected neonate [44]. In terms of maternal diet, a high fat intake during pregnancy can lead to a reduced relative abundance of *Bacteroides*; a Western diet high in refined carbohydrates, fat, and animal protein leads to increased *Clostridium innocuum*, *Eubacterium dolichum*, *Catenibacterium mitsuokai*, and *Enterococcus* spp., and a reduced abundance of *Bifidobacteria* and *Bacteroidetes* [41,45,46]. Further, the presence of gestational diabetes can lead to a reduced relative abundance of *Prevotella* and *Lactobacillus* [45,46]. Some medications used during pregnancy, such as maternal biologics (e.g., tumor necrosis factor-α inhibitors), can cross the placental barrier, especially in the late second and third trimesters. Owing to the neonate's immature reticuloendothelial system at birth, the drug can take up to 12 months to clear. The use of these immunosuppressants can thus lead to changes in the neonate's immune system [47,48].

4.2. Microbiome Development from Birth Onwards

At birth, a neonate's gestational age has the largest influence on their microbiome diversity [36]. The presence of *Enterobacter*, *Enterococcus*, *Lactobacillus*, *Photorhabdus*, and *Tannerella* in the meconium of neonates born <33 weeks' gestation is negatively correlated with the neonate's gestational age and has been reported to provoke inflammatory responses, signifying a causative role in premature births [36].

Furthermore, lower gestational ages have been associated with a lower abundance of *Bifidobacterium*, *Bacteroides*, and *Streptococcus* [6]. One study found that the colonization of *Bifidobacteria* in very low birth weight neonates is delayed, and they only appear at a mean age of 10.6 days (versus as early as 4 days in full-term neonates). *Bifidobacteria* only became predominant at a mean age of 19.8 days in their group of preterm neonates [49].

Birth mode and associated complications also play a role. Neonates born following the premature rupture of membranes or intra-amniotic infection (chorioamnionitis) experienced microbe exposure in utero, since bacteria colonize the amniotic membrane, fetal skin, and mucosa [50]. Further, caesarean sections are associated with pathobionts, e.g., *Staphylococcus*, *Corynebacterium* and *Propionibacterium* species, compared to vaginal deliveries, which are characterized by *Lactobacillus*, *Bacteroides*, and *Bifidobacteria* [51].

After delivery, rapid changes occur in the preterm intestinal microbiome in comparison with healthy, full-term neonates. As illustrated in Figure 1, environmental factors contribute to observed changes and include the NICU, with increased exposure to HA-BSI and reduced exposure to parental bacteria, owing to delayed skin-to-skin contact in comparison with healthy full-term neonates [19]. Hospitalized neonates have higher *C. Difficile* colonization rates. Brookes et al. showed that organisms present in the early phase of colonization of the GIT have reservoirs in the NICU [52].

The feeding journey of the preterm neonate is a challenge owing to factors such as a low gastric capacity, slow initiation of enteral feeds, and low maternal breastmilk availability at birth. For these reasons, many neonates receive TPN, leading to a loss of biodiversity and an altered gut microbial colonization [2,5,32].

The type of enteral feed also influences microbiome development. When comparing breastmilk, pasteurized donor breastmilk, and infant formula, the microbial content is distinctly different [53]. Breastmilk contains a high concentration of complex human milk oligosaccharides (HMOs). After lactose and lipids, HMOs are the third most abundant component of breastmilk. HMOs exert a powerful prebiotic effect: they are resistant to the gastric pH and can reach the neonate's large intestine intact, where they modulate

the composition of the gastrointestinal microbiome and act as a carbon source for the GIT microbiota [54,55]. HMOs have anti-inflammatory properties by regulating the production of interleukin, activating lymphocytes, and blocking the adhesion of microbial pathogens to the large intestine's epithelial surface [54,56]. Breastmilk has its own core microbiome (*Streptococci*, *Lactic acid bacteria*, and *Bifidobacteria*) [18]. Apart from HMOs, breastmilk also contains lysosomes, lactoferrin, antibodies, and cytokines that stimulate the increase of *Bacteroides*, *Bifidobacterium*, and *Lactobacillus* spp. [18,26,53–55]. Moreover, breastmilk contains the peptide hormone insulin, leading to an increase in intestinal maturation [57]. Neonates who receive breastmilk have a lower microbiome diversity, but almost double the abundance of beneficial bacterial cells compared with neonates who receive infant formula [42]. Formula-fed neonates have a more diverse microbiome (*Coliforms*, *Bacteroides*, *Clostridium difficile*, and *Lactobacilli*) [58–60].

Almost all premature neonates receive antibiotics, either before, during, or after birth. Worldwide, an estimated 40% of pregnant mothers and neonates receive antibiotics for the prevention and control of infections [61]. Intrapartum antibiotic prophylaxis leads to decreased *Bifidobacterial* numbers directly after birth [18]. Empirical antibiotic therapy is often initiated in preterm neonates [61–63]. Antibiotic use early in life is detrimental to microbiome progression. It affects the timing of the progression of the microbiome, as well as the organisms present [6,44,64]. Administering antibiotics at an early age, when the immune system is still maturing, increases the risk of developing HA-BSI and NEC [65–67].

While it was previously believed that genetics influence the microbiome, new studies in twins refute this, indicating the importance (or influence) of environmental factors [18].

5. Functions of the Microbiome

The gut microbiome plays a role in almost all day-to-day human functions, including nutritional, physiological, immunological, and protective functions. The normal interaction between gut microbes and their host is a symbiotic relationship [68]. The host immune system has an intimate relationship with the gut microbiota, which affected it through various pathways, e.g., microbial components and their metabolites [69]. The innate immune system has evolved to provide mutual benefit to the host and microbiota by recognizing microbial components via specialized receptors, e.g., toll-like receptors that lead to "controlled or physiological inflammation", with the formation of tissue repair factors, antimicrobial proteins, and IgA. The toll-like receptors can recognize microbial ligands and distinguish between commensal and pathogenic bacteria. This process maintains the intestinal mucosal barrier, without which bacterial translocation and the uncontrolled absorption of complex proteins can occur [70–73].

Short-chain fatty acids produced by the gut microbes have a trophic effect on the intestinal epithelium by stimulating epithelial cell proliferation and differentiation [74–77]. The intestinal mucosa is the main crossing point between the external environment and the immune system. The gut mucosa is in constant contact with enteric microbes. On the one hand, it must provide protection against pathogenic microbes but on the other, tolerate commensal flora [78]. The intestinal epithelium cells, therefore, need to avoid the uptake of pathogenic microorganisms, antigens, and detrimental components, while also collecting antigens [79].

Moreover, the gut microbiome is involved in the correct development of gut-associated lymphoid tissue, [80] which is the largest immune organ of the body, as approximately 80% of all immunoglobulin-producing cells are found in the small bowel [79]. The microbiome contributes to the homeostasis of the intestinal immune system through pathogen displacement (competing for nutrients and cell adhesion), activation of the local immune response, and the control of local and systemic inflammation [72,74–77].

6. Dysbiosis

Dysbiosis is characterized by a change in gut microbiota, which leads to the overgrowth of potentially pathogenic bacteria and a reduction in the number of beneficial

bacteria [77,78]. Dysbiosis is associated with an inappropriate response of the innate immune system and leads to inflammation. One suggested mechanism is that the disruption of the normal neonatal intestinal bacterial flora leads to a pro-inflammatory state, allowing the translocation of pathogens across the intestinal epithelial cells [81]. This inflammation can lead to organ dysfunction, infections, and sepsis [72].

The immature immune system of a premature neonate is unable to control the overgrowth of pathogenic bacteria. Therefore, preterm neonates with dysbiosis are at a higher risk of short-term complications, e.g., feeding intolerance, NEC, and HA-BSI, as well as long-term complications such as neurodevelopmental impairment [82].

Probiotics can serve different functions in the host, i.e., conservation of appropriate host–microbe interactions, pathogen exclusion, mucus secretion from goblet cells, enhancement of the epithelial barrier function, production of antibacterial factors, immune response modulation (e.g., enhanced mucosal IgA responses), increased production of anti-inflammatory cytokines, changes in intestinal permeability, and the activation of a host-adaptive immune system [14–16]. Given their widespread availability, it was postulated that administering probiotics to premature neonates can aid in populating the intestine with normal, beneficial flora that will inhibit the growth of pathogenic flora [16].

7. Drug-Resistant Gram-Negative Bacteria (DR-GNB)

The large intestine is an important reservoir for many nosocomial pathogens, e.g., *Enterococcus species*, *Enterobacteriaceae*, *Clostridium difficile*, and *Candida* species. Premature neonates often present with an overgrowth of these pathogens if the normal gut barrier is disturbed [83,84]. During the NICU stay, the preterm is also exposed to the following factors: poor hand hygiene, overcrowding, inadequate spacing between neonate incubators, environmental colonization, inadequate cleaning of equipment in the ward, low nurse-to-patient ratios, imprudent use and prolonged courses (intrapartum and postnatal) of broad-spectrum antibiotics, and lastly, the delayed introduction of maternal breastmilk [85,86]. These microorganisms can colonize the neonatal GIT, influencing the microbiome.

Colonization of the GIT precedes invasive infection in premature neonates [84]. For example, the production of extended-spectrum beta-lactamase enzymes (ESBL-e) poses a high risk of clinical infection and morbidity in neonatal populations. ESBL-e is frequently implicated in bloodstream infections (BSI) and is a major mechanism for antimicrobial resistance. *Klebsiella pneumonia* and *Escherichia coli* are the most frequently isolated species [87].

The WHO recognizes antimicrobial resistance as a health threat of global concern owing to its associated morbidity and mortality, as well as increased healthcare costs [88]. Probiotics can colonize the GIT of humans effectively, competing with other bacteria for both nutrients and space. Probiotics promote mucosal barrier function, inhibit mucosal pathogen adherence, and interact with the innate and adaptive immune system. Further, probiotics also produce antimicrobial substances, receptor hydrolysis, and nitric oxide. In this way, probiotics can help decolonize the GIT of DR-GNB [89].

A study conducted in Cambodia showed that gastrointestinal colonization with DR-GNB occurred early during hospitalization, and that the use of a probiotic, e.g., *Lactobacillus acidophilus*, can reduce the acquisition of DR-GNB [84]. *Bifidobacterium longum subsp. infantis* can change the neonate gut microbiome and lower the abundance of common gut taxa, such as *Proteobacteria* and *Firmicutes phyla* (e.g., *Escherichia* and *Clostridium*) [90].

8. Hospital-Acquired Bloodstream Infection

The preterm neonate is at an increased risk for HA-BSI. The lower the gestational age and birth weight, the higher the risk for HA-BSI [12,91]. Other risk factors include dysbiosis, long-term use of invasive interventions, e.g., mechanical ventilation and intravascular catheterization, delay in early enteral feeding with human breastmilk, a lengthy period of parenteral nutrition, hospitalization, surgery, and underlying respiratory and cardiovascular diseases [12].

HA-BSI can further be related to microbial–host interactions, including direct bacterial translocation. Organisms that usually cause HA-BSI include coagulase-negative *Staphylococci*, *Staphylococcus aureus*, Gram-negative bacilli, and fungi [12]. A retrospective study conducted in South Africa indicated that the majority of HA-BSI infections were caused by Gram-negative bacteria, with *Staphylococcus aureus* and *Acinetobacter baumannii* as the most common organisms [92].

Neonates who develop sepsis are usually born with low microbial diversity. Premature neonates who do not develop neonatal sepsis may have a definable 'healthy microbiome'. In contrast, prolonged broad-spectrum antibiotics profoundly decrease gut microbial diversity and promote a pathogen-predominant microbiota associated with sepsis [93]. A delay in the colonization of *Proteobacteria* may cause an excessive immune response that compromises mucosal barrier integrity, resulting in inflammation, NEC, and bacterial translocation into the bloodstream, and leading to HA-BSI [65,67].

A growing body of evidence suggests that probiotics can prevent HA-BSI in premature neonates [94]. A systematic review and meta-analysis found that probiotic supplementation is safe and effective in reducing HA-BSI in preterm neonates and that the use of *Lactobacillus* species and *Bifidobacterium* species, or a combination of two or three species of probiotics, could be most beneficial [95–97].

Methods with which probiotics could reduce the incidence of HA-BSI include competitive barring of potentially pathogenic luminal bacteria and fungi by colonizing the gut, improved mucosal IgA response, modulation of gut barrier function and permeability, production of antimicrobial peptides, and upregulation of the immune response. Neonates who receive breastmilk appear to benefit even more from probiotics than formula-fed neonates. The reasons include reduced gut permeability, and thus less translocation of pathogens from the gut, intake of anti-infective agents, e.g., Lactoferrin, Immunoglobulin A, Immunoglobulin G, and Immunoglobulin M, as well as the intake of oligosaccharides which act as a prebiotic [95].

9. Feeding Intolerance

A high percentage of premature neonates experience feeding intolerance, possibly linked to an immature GIT in terms of poor gastric emptying, gastro-duodenal hypomotility, and/or duodenal gastric reflux [98,99].

Probiotic supplementation may reduce feeding intolerance. According to a systematic review and meta-analysis, 19 out of 25 trials indicated that probiotics could shorten the time to full enteral feeds, reduce observed episodes of feeding intolerance, improve weight gain and growth velocity, decrease transition time from orogastric to breast feeds, increase postprandial mesenteric flow, and shorten hospital stays [17]. Neonates supplemented with probiotics (irrespective of *Bifidobacterium* or non-*Bifidobacterium* strains, single or multiple strains, or early and late initiation of probiotics) took less time to achieve full feeds compared to the placebo groups [17].

Reducing feeding intolerance could lead to increased GIT maturity and motility through increased intestinal transit time and gastric emptying, as well as increased superior mesenteric artery flow [17].

10. Necrotizing Enterocolitis

NEC is a multifactorial, inflammatory disease of the newborn GIT and has an incidence of 7–12% in preterm neonates of less than 1500 g [18]. It is the leading cause of mortality, with mortality rates varying between 15% and 30% [78,100,101]. While the exact etiology of the development of NEC is still unknown [102,103], numerous factors have been reported to play a role [102,104–109]. These factors can lead indirectly to a disruption of intestinal mucosal integrity and to intestinal ischemia [17,106].

Most theories on NEC pathogenesis focus on dysbiosis [102], due to the strong link between the pathogenesis of NEC and abnormal bacterial colonization [105,108,109]. Changes in microbiota composition can already be observed in the preclinical phase of NEC, and

volatile fecal organic compounds can serve as a non-invasive biomarker for the detection of NEC, two to three days before the onset of its clinical symptoms [110].

A systematic review and meta-analysis showed varying effects between probiotic trials and the prevention of NEC, with no definitive conclusion drawn [109]. AlFaleh and Anabrees showed in their systematic review and meta-analysis that the administration of *Lactobacillus* species alone, as well as a mixture of probiotics, significantly reduced the incidence of severe stage II to III NEC. The administration of a mixture of probiotics also showed a reduction in the incidence of mortality. However, the administration of *Lactobacillus* or *Bifidobacterium* species alone did not reduce mortality [105]. Bi et al. also concluded after a meta-analysis that *Lactobacillus* alone reduces the incidence of NEC, but that a *Bifidobacterium* probiotic mixture could be the preferred option for NEC, sepsis, and all-cause mortality reduction in premature neonates [96].

One single-center retrospective observational study indicated that the use of a triple-species probiotic consisting of *L. acidophilus*, *B. bifidum*, and *B. longum subspecies infantis* can lead to a 4.4% reduction in NEC and an 11% decrease in HA-BSI pre- and post-implementation [111]. Another study using *Bifidobacterium infantis* and *Lactobacillus acidophilus* showed a reduction in NEC from 6.6% (85 NEC cases out of 1282 participants in the control group) to 2.7% (34 NEC cases out of 1237 participants in the probiotic group) [112].

11. Discussion

Various maternal, neonatal, and environmental factors place the preterm neonate at high risk of dysbiosis, which could increase the risk of short-term complications, e.g., feeding intolerance, NEC, and HA-BSI. These morbidities, in turn, could contribute to the development of long-term consequences of premature birth [43].

There is a growing number of studies conducted in preterm neonates using different probiotic strains and dosages. Some studies show that probiotics can play a role in preventing dysbiosis and thus avoiding neonatal complications. For example, a review by Underwood shows there is strong evidence that the use of a multi-strain probiotic in breastfed infants can prevent NEC [113], while other reviews indicate little or no effect in preventing NEC morbidity and mortality [114]. A systematic review and meta-analysis indicated that probiotic supplementation is safe and effective in reducing HA-BSI in preterm neonates. However, these authors recommend the use of *Lactobacillus* species and *Bifidobacterium* species, or a combination of two or three species of probiotics, as most beneficial [95–97].

It is important to note that different probiotic strains and dosages can have different effects. There is thus the need for large RCTs to investigate the optimal dosage and combination of probiotic strains to prevent several complications in the preterm neonate.

12. Conclusions

Various factors in the preterm neonate play a role in gastrointestinal dysbiosis. Some factors can be modified (e.g., reduced use of unnecessary antibiotics, use of formula milk) and some factors are unavoidable (e.g., preterm birth). The pathogenesis of the development of short-term complications in the preterm neonate can be linked to the immaturity of the host immune system as well as alterations seen in the intestinal microbiome. There is a growing body of evidence that probiotics can play a role in preventing dysbiosis and thus complications observed in the preterm neonate. However, the optimal combination of probiotic strains and dosage still needs to be identified.

Author Contributions: Conceptualization, M.S., M.M.v.W. and E.v.N.; data curation, M.S.; writing—original draft preparation, M.S.; writing—review and editing, M.S., M.M.v.W., E.v.N. and A.N.H.B.; visualization, M.S.; supervision, M.M.v.W., E.v.N. and A.N.H.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Xiang, Q.; Yan, X.; Shi, W.; Li, H.; Zhou, K. Early gut microbiota intervention in premature infants: Application perspectives. *J. Adv. Res.* **2022**, *51*, 59–72. [CrossRef] [PubMed]
2. Neu, J. Gastrointestinal development and meeting the nutritional needs of premature infants. *Am. J. Clin. Nutr.* **2007**, *85*, 629S–634S. [CrossRef]
3. Neu, J. Necrotizing enterocolitis: The search for a unifying pathogenic theory leading to prevention. *Pediatr. Clin. N. Am.* **1996**, *43*, 409–432. [CrossRef] [PubMed]
4. Gregory, K.; Deforge, C.E.; Natale, K.M.; Phillips, M.; van Marter, L.J. Necrotizing enterocolitis in the premature infant: Neonatal nursing assessment, disease pathogenesis, and clinical presentation. *Adv. Neonatal Care* **2011**, *11*, 155–166. [CrossRef] [PubMed]
5. Hunter, C.J.; Upperman, J.S.; Ford, H.R.; Camerini, V. Understanding the susceptibility of the premature infant to necrotizing enterocolitis (NEC). *Pediatr. Res.* **2008**, *63*, 117–123. [CrossRef] [PubMed]
6. Chernikova, D.A.; Madan, J.C.; Housman, M.L.; Zain-Ul-Abideen, M.; Lundgren, S.N.; Morrison, H.G.; Sogin, M.L.; Williams, S.M.; Moore, J.H.; Karagas, M.R.; et al. The premature infant gut microbiome during the first 6 weeks of life differs based on gestational maturity at birth. *Pediatr. Res.* **2018**, *84*, 71–79. [CrossRef]
7. Muresan, Z.; Paul, D.L.; Goodenough, D.A. Occludin 1B, a variant of the tight junction protein occludin. *Mol. Biol. Cell.* **2000**, *11*, 627–634. [CrossRef]
8. Hyman, P.E.; Clarke, D.D.; Everett, S.L.; Sonne, B.; Stewart, D.; Harada, T.; Walsh, J.H.; Taylor, I.L. Gastric acid secretory function in preterm infants. *J. Pediatr.* **1985**, *106*, 467–471. [CrossRef]
9. Guillet, R.; Stoll, B.J.; Cotten, C.M.; Gants, M.; McDonald, S.; Poole, W.K.; Phelps, D.L.; National Institute of Child Health and Human Development Neonatal Research Network. Association of H2-blocker therapy and higher incidence of necrotizing enterocolitis in very low birth weight infants. *Pediatrics* **2006**, *117*, e137–e142. [CrossRef]
10. Patel, P.; Bhatia, J. Total parenteral nutrition for the very low birth weight infant. *Semin. Fetal Neonatal. Med.* **2017**, *22*, 2–7. [CrossRef]
11. Nair, J.; Longendyke, R.; Lakshminrusimha, S. Necrotizing enterocolitis in moderate preterm infants. *Biomed. Res. Int.* **2018**, *2018*, 4126245. [CrossRef]
12. Dong, Y.; Speer, C.P. Late-onset neonatal sepsis: Recent developments. *Arch. Dis. Child. Fetal Neonatal Ed.* **2015**, *100*, F257–F263. [CrossRef]
13. Food and Agriculture Organization; World Health Organization Expert Consultation. *Evaluation of Health and Nutritional Properties of Powder Milk and Live Lactic acid Bacteria*; Food and Agriculture Organization of the United Nations: Córdoba, Argentina; World Health Organization: Geneva, Switzerland, 2001; Available online: http://pc.ilele.hk/public/pdf/20190225/bd3689dfc2fd663bb36def1b672ce0a4.pdf (accessed on 8 September 2005).
14. Gareau, M.G.; Sherman, P.M.; Walker, W.A. Probiotics and the gut microbiota in intestinal health and disease. *Nat. Rev. Gastroenterol. Hepatol.* **2010**, *7*, 503–514. [CrossRef]
15. Deshpande, G.; Jape, G.; Rao, S.; Patole, S. Benefits of probiotics in preterm neonates in low-income and medium-income countries: A systematic review of randomised controlled trials. *BMJ Open* **2017**, *7*, e017638. [CrossRef] [PubMed]
16. Olsen, R.; Greisen, G.; Schrøder, M.; Brok, J. Prophylactic probiotics for preterm infants: A systematic review and meta-analysis of observational studies. *Neonatology* **2016**, *109*, 105–112. [CrossRef]
17. Athalye-Jape, G.; Deshpande, G.; Rao, S.; Patole, S. Benefits of probiotics on enteral nutrition in preterm neonates: A systematic review. *Am. J. Clin. Nutr.* **2014**, *100*, 1508–1519. [CrossRef]
18. Bertelsen, R.J.; Jensen, E.T.; Ringel-Kulka, T. Use of probiotics and prebiotics in infant feeding. *Best Pract. Res. Clin. Gastroenterol.* **2016**, *30*, 39–48. [CrossRef]
19. Ahearn-Ford, S.; Berrington, J.E.; Stewart, C.J. Development of the gut microbiome in early life. *Exp. Physiol.* **2022**, *107*, 415–421. [CrossRef] [PubMed]
20. Zhu, B.; Wang, X.; Li, L. Human gut microbiome: The second genome of human body. *Protein Cell.* **2010**, *1*, 718–725. [CrossRef] [PubMed]
21. D'Argenio, V.; Salvatore, F. The role of the gut microbiome in the healthy adult status. *Clin. Chim. Acta* **2015**, *451 Pt A*, 97–102. [CrossRef]
22. Aagaard, K.; Ma, J.; Antony, K.M.; Ganu, R.; Petrosino, J.; Versalovic, J. The placenta harbors a unique microbiome. *Sci Transl. Med.* **2014**, *6*, 237ra65. [CrossRef]
23. Chong, C.Y.L.; Bloomfield, F.H.; O'Sullivan, J.M. Factors affecting gastrointestinal microbiome development in neonates. *Nutrients* **2018**, *10*, 274. [CrossRef] [PubMed]
24. Hitti, J.; Riley, D.E.; Krohn, M.A.; Hillier, S.L.; Agnew, K.J.; Krieger, J.N.; Eschenbach, D.A. Broad-spectrum bacterial rDNA polymerase chain reaction assay for detecting amniotic fluid infection among women in premature labor. *Clin. Infect. Dis.* **1997**, *24*, 1228–1232. [CrossRef]
25. Pettker, C.M.; Buhimschi, I.A.; Magloire, L.K.; Sfakianaki, A.K.; Hamar, B.D.; Cuhimschi, C.S. Value of placental microbial evaluation in diagnosing intra-amniotic infection. *Obstet. Gynecol.* **2007**, *109*, 739–749. [CrossRef] [PubMed]
26. Thum, C.; Cookson, A.L.; Otter, D.E.; McNabb, W.C.; Hodgkinson, A.J.; Dyer, J.; Roy, N.C. Can nutritional modulation of maternal intestinal microbiota influence the development of the infant gastrointestinal tract? *J. Nutr.* **2012**, *142*, 1921–1928. [CrossRef]

27. Collado, M.C.; Rautava, S.; Aakko, J.; Isolauri, E.; Salminen, S. Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. *Sci. Rep.* **2016**, *6*, 23129. [CrossRef]
28. Liu, C.J.; Liang, X.; Niu, Z.Y.; Jin, Q.; Zeng, X.Q.; Wang, W.X.; Li, M.Y.; Chen, X.R.; Meng, H.Y.; Shen, R.; et al. Is the delivery mode a critical factor for the microbial communities in the meconium? *EBioMedicine* **2019**, *49*, 354–363. [CrossRef] [PubMed]
29. He, Q.; Kwok, L.Y.; Xi, X.; Zhong, Z.; Ma, T.; Xu, H.; Meng, H.; Zhao, F.; Zhang, H. The meconium microbiota shares more features with the amniotic fluid microbiota than the maternal fecal and vaginal microbiota. *Gut Microbes* **2020**, *12*, 1794266. [CrossRef] [PubMed]
30. Satokari, R.; Grönroos, T.; Laitinen, K.; Salminen, S.; Isolauri, E. Bifidobacterium and Lactobacillus DNA in the human placenta. *Lett. Appl. Microbiol.* **2009**, *48*, 8–12. [CrossRef]
31. Jiménez, E.; Fernández, L.; Marín, M.L.; Martin, R.; Odriozola, J.M.; Nueno-Palop, C.; Narbad, A.; Olivares, M.; Xaus, J.; Rodríguex, J.M. Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. *Curr. Microbiol.* **2005**, *51*, 270–274. [CrossRef]
32. Dahlgren, A.F.; Pan, A.; Lam, V.; Gouthro, K.C.; Simpson, P.M.; Salzman, N.H.; Nghiem-Rao, T.H. Longitudinal changes in the gut microbiome of infants on total parenteral nutrition. *Pediatr. Res.* **2019**, *86*, 107–114. [CrossRef] [PubMed]
33. Mshvildadze, M.; Neu, J.; Shuster, J.; Theriaque, D.; Li, N.; Mai, V. Intestinal microbial ecology in premature infants assessed with non-culture-based techniques. *J. Pediatr.* **2010**, *156*, 20–25. [CrossRef] [PubMed]
34. Dominguez-Bello, M.G.; Costello, E.K.; Contreras, M.; Magris, M.; Hidalgo, G.; Fierer, N.; Knight, R. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 11971–11975. [CrossRef] [PubMed]
35. Hansen, R.; Scott, K.P.; Khan, S.; Martin, J.C.; Berry, S.H.; Stevenson, M.; Okpapi, A.; Munro, M.J.; Hold, G.L. First-Pass Meconium samples from healthy term vaginally-delivered neonates: An analysis of the microbiota. *PLoS ONE* **2015**, *10*, e0133320. [CrossRef]
36. Ardissone, A.N.; de la Cruz, D.M.; Davis-Richardson, A.G.; Rechcigl, K.T.; Li, N.; Drew, J.C.; Murgas-Torrazza, R.; Sharma, R.; Hudak, M.L.; Triplett, E.W.; et al. Meconium microbiome analysis identifies bacteria correlated with premature birth. *PLoS ONE* **2014**, *9*, e90784. [CrossRef]
37. Stinson, L.F.; Boyce, M.C.; Payne, M.S.; Keelan, J.A. The not-so-sterile womb: Evidence that the human fetus is exposed to bacteria prior to birth. *Front. Microbiol.* **2019**, *10*, 1124. [CrossRef]
38. Gosalbes, M.J.; Llop, S.; Vallès, Y.; Moya, A.; Ballester, F.; Francino, M.P. Meconium microbiota types dominated by lactic acid or enteric bacteria are differentially associated with maternal eczema and respiratory problems in infants. *Clin. Exp. Allergy* **2013**, *43*, 198–211. [CrossRef]
39. Hirai, C.; Ichiba, H.; Saito, M.; Shintaku, H.; Yamano, T.; Kusuda, S. Trophic effect of multiple growth factors in amniotic fluid or human milk on cultured human fetal small intestinal cells. *J. Pediatr. Gastroenterol. Nutr.* **2002**, *34*, 524–528. [CrossRef]
40. Elgin, T.G.; Kern, S.L.; McElroy, S.J. Development of the neonatal intestinal microbiome and its association with necrotizing enterocolitis. *Clin. Ther.* **2016**, *38*, 706–715. [CrossRef]
41. Mack, D.R. Probiotics-mixed messages. *Can. Fam. Physician* **2005**, *51*, 1455–1457, 1462–1464.
42. Groer, M.W.; Luciano, A.A.; Dishaw, L.J.; Ashmeade, T.L.; Miller, E.; Gilbert, J.A. Development of the preterm infant gut microbiome: A research priority. *Microbiome* **2014**, *2*, 38. [CrossRef]
43. Pravia, C.I.; Benny, M. Long-term consequences of prematurity. *Cleve Clin. J. Med.* **2020**, *87*, 759–767. [CrossRef] [PubMed]
44. Bender, J.M.; Li, F.; Martelly, S.; Byrt, E.; Rouzier, V.; Leo, M.; Tobin, N.; Pannaraj, P.S.; Adisetiyo, H.; Rollie, A.; et al. Maternal HIV infection influences the microbiome of HIV-uninfected infants. *Sci. Transl. Med.* **2016**, *8*, 349ra100. [CrossRef]
45. Chu, D.M.; Antony, K.M.; Ma, J.; Prince, A.L.; Showalter, L.; Moller, M.; Aagaard, K.M. The early infant gut microbiome varies in association with a maternal high-fat diet. *Genome Med.* **2016**, *8*, 77. [CrossRef]
46. Su, M.; Nie, Y.; Shao, R.; Duan, S.; Jiang, Y.; Wang, M.; Xing, Z.; Sun, Q.; Liu, X.; Xu, W. Diversified gut microbiota in newborns of mothers with gestational diabetes mellitus. *PLoS ONE* **2018**, *13*, e0205695. [CrossRef] [PubMed]
47. Bortlik, M.; Machkova, N.; Duricova, D.; Malickova, K.; Hrdlicka, L.; Lukas, M.; Kohout, P.; Shonova, O.; Lukas, M. Pregnancy and newborn outcome of mothers with inflammatory bowel diseases exposed to anti-TNF-α therapy during pregnancy: Three-center study. *Scand. J. Gastroenterol.* **2013**, *48*, 951–958. [CrossRef]
48. Dai, F.F.; Hu, M.; Zhang, Y.W.; Zhu, R.H.; Chen, L.P.; Li, Z.D.; Haung, Y.J.; Hu, W.; Cheng, Y.X. TNF-α/anti-TNF-α drugs and its effect on pregnancy outcomes. *Expert Rev. Mol. Med.* **2022**, *24*, e26. [CrossRef]
49. Sakata, H.; Yoshioka, H.; Fujita, K. Development of the intestinal flora in very low birth weight infants compared to normal full-term newborns. *Eur. J. Pediatr.* **1985**, *144*, 186–190. [CrossRef] [PubMed]
50. Tchirikov, M.; Schlabritz-Loutsevitch, N.; Maher, J.; Buchmann, J.; Naberezhnev, Y.; Winarno, A.S.; Seliger, G. Mid-trimester preterm premature rupture of membranes (PPROM): Etiology, diagnosis, classification, international recommendations of treatment options and outcome. *J. Perinat. Med.* **2018**, *46*, 465–488. [CrossRef] [PubMed]
51. Clarke, G.; O'Mahony, S.M.; Dinan, T.G.; Cryan, J.F. Priming for health: Gut microbiota acquired in early life regulates physiology, brain and behaviour. *Acta Paediatr.* **2014**, *103*, 812–819. [CrossRef] [PubMed]
52. Brooks, B.; Firek, B.A.; Miller, C.S.; Sharon, I.; Thomas, B.C.; Baker, R.; Morowitz, M.J.; Banfield, J.F. Microbes in the neonatal intensive care unit resemble those found in the gut of premature infants. *Microbiome* **2014**, *2*, 1. [CrossRef] [PubMed]
53. Lyons, K.E.; Ryan, C.A.; Dempsey, E.M.; Ross, R.P.; Stanton, C. Breast milk, a source of beneficial microbes and associated benefits for infant health. *Nutrients* **2020**, *12*, 1039. [CrossRef]

54. Singh, R.P.; Niharika, J.; Kondepudi, K.K.; Bishnoi, M.; Tingirikari, J.M.R. Recent understanding of human milk oligosaccharides in establishing infant gut microbiome and roles in immune system. *Food Res. Int.* **2022**, *151*, 110884. [CrossRef]
55. Jantscher-Krenn, E.; Bode, L. Human milk oligosaccharides and their potential benefits for the breast-fed neonate. *Minerva Pediatr.* **2012**, *64*, 83–99.
56. Zivkovic, A.M.; German, J.B.; Lebrilla, C.B.; Mills, D.A. Human milk glycobiome and its impact on the infant gastrointestinal microbiota. *Proc. Natl. Acad. Sci. USA* **2011**, *108* (Suppl. 1), 4653–4658. [CrossRef] [PubMed]
57. Mank, E.; Sáenz de Pipaón, M.; Lapillonne, A.; Carnielli, V.P.; Senterre, T.; Shamir, R.; van Toledo, L.; van Goudoever, J.B.; FIT-04 Study Group. Efficacy and safety of enteral recombinant human insulin in preterm infants: A randomized clinical trial. *JAMA Pediatr.* **2022**, *176*, 452–460. [CrossRef]
58. Wopereis, H.; Oozeer, R.; Knipping, K.; Belzer, C.; Knol, J. The first thousand days—Intestinal microbiology of early life: Establishing a symbiosis. *Pediatr. Allergy Immunol.* **2014**, *25*, 428–438. [CrossRef] [PubMed]
59. Penders, J.; Thijs, C.; Vink, C.; Stelma, F.F.; Snijders, B.; Kummeling, I.; van den Brandt, P.A.; Stobberingh, E.E. Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics* **2006**, *118*, 511–521. [CrossRef]
60. Voreades, N.; Kozil, A.; Weir, T.L. Diet and the development of the human intestinal microbiome. *Front. Microbiol.* **2014**, *5*, 494. [CrossRef]
61. Shekhar, S.; Petersen, F.C. The dark side of antibiotics: Adverse effects on the infant immune defense against infection. *Front. Pediatr.* **2020**, *8*, 544460. [CrossRef]
62. Mukhopadhyay, S.; Sengupta, S.; Puopolo, K.M. Challenges and opportunities for antibiotic stewardship among preterm infants. *Arch. Dis. Child. Fetal Neonatal Ed.* **2019**, *104*, F327–F332. [CrossRef]
63. Cotten, C.M. Adverse consequences of neonatal antibiotic exposure. *Curr. Opin. Pediatr.* **2016**, *28*, 141–149. [CrossRef] [PubMed]
64. Cho, I.; Blaser, M.J. The human microbiome: At the interface of health and disease. *Nat. Rev. Genet.* **2012**, *13*, 260–270. [CrossRef] [PubMed]
65. Mai, V.; Torrazza, R.M.; Ukhanova, M.; Wang, X.; Sun, Y.; Li, N.; Shuster, J.; Sharma, R.; Hudak, M.L.; Neu, J. Distortions in development of intestinal microbiota associated with late onset sepsis in preterm infants. *PLoS ONE* **2013**, *8*, e52876. [CrossRef] [PubMed]
66. Raba, A.A.; O'Sullivan, A.; Semberova, J.; Martin, A. Are antibiotics a risk factor for the development of necrotizing enterocolitis-case-control retrospective study. *Eur. J. Pediatr.* **2019**, *178*, 923–928. [CrossRef]
67. Torrazza, R.M.; Ukhanova, M.; Wang, X.; Sharma, R.; Hudak, M.L.; Neu, J.; Mai, V. Intestinal microbial ecology and environmental factors affecting necrotizing enterocolitis. *PLoS ONE* **2013**, *8*, e83304. [CrossRef]
68. Murgas Torrazza, R.; Neu, J. The developing intestinal microbiome and its relationship to health and disease in the neonate. *J. Perinatol.* **2011**, *31* (Suppl. 1), S29–S34. [CrossRef]
69. Hooper, L.V.; Midtvedt, T.; Gordon, J.I. How host-microbial interactions shape the nutrient environment of the mammalian intestine. *Annu. Rev. Nutr.* **2002**, *22*, 283–307. [CrossRef]
70. Sansonetti, P.J. War and peace at mucosal surfaces. *Nat. Rev. Immunol.* **2004**, *4*, 953–964. [CrossRef]
71. Hart, A.L.; Stagg, A.J.; Frame, M.; Graffner, H.; Glise, H.; Falk, P.; Kamm, M.A. The role of the gut flora in health and disease, and its modification as therapy. *Aliment. Pharmacol. Ther.* **2002**, *16*, 1383–1393. [CrossRef]
72. Tourneur, E.; Chassin, C. Neonatal immune adaptation of the gut and its role during infections. *Clin. Dev. Immunol.* **2013**, *2013*, 270301. [CrossRef]
73. Takeda, K.; Kaisho, T.; Akira, S. Toll-like receptors. *Annu. Rev. Immunol.* **2003**, *21*, 335–376. [CrossRef]
74. Guarner, F.; Malagelada, J.R. Gut flora in health and disease. *Lancet* **2003**, *361*, 512–519. [CrossRef] [PubMed]
75. Honda, K.; Littman, D.R. The microbiome in infectious disease and inflammation. *Annu. Rev. Immunol.* **2012**, *30*, 759–795. [CrossRef] [PubMed]
76. Buccigrossi, V.; Nicastro, E.; Guarino, A. Functions of intestinal microflora in children. *Curr. Opin. Gastroenterol.* **2013**, *29*, 31–38. [CrossRef] [PubMed]
77. Macpherson, A.J.; Harris, N.L. Interactions between commensal intestinal bacteria and the immune system. *Nat. Rev. Immunol.* **2004**, *4*, 478–485. [CrossRef]
78. Hill, D.A.; Artis, D. Intestinal bacteria and the regulation of immune cell homeostasis. *Annu. Rev. Immunol.* **2010**, *28*, 623–667. [CrossRef]
79. Holzapfel, W.H.; Haberer, P.; Snel, J.; Schillinger, J.; Huis in't Veld, J.H. Overview of gut flora and probiotics. *Int. J. Food Microbiol.* **1998**, *41*, 85–101. [CrossRef]
80. Shi, H.N.; Walker, A. Bacterial colonization and the development of intestinal defences. *Can. J. Gastroenterol.* **2004**, *18*, 493–500. [CrossRef]
81. Patel, R.M.; Denning, P.W. Intestinal microbiota and its relationship with necrotizing enterocolitis. *Pediatr. Res.* **2015**, *78*, 232–238. [CrossRef]
82. Rozé, J.C.; Ancel, P.Y.; Marchand-Martin, L.; Rousseau, C.; Montassier, E.; Monot, C.; le Roux, K.; Butin, M.; Resche-Rigon, M.; Aires, J.; et al. Assessment of neonatal intensive care unit practices and preterm newborn gut microbiota and 2-year neurodevelopmental outcomes. *JAMA Netw. Open* **2020**, *3*, e2018119. [CrossRef] [PubMed]
83. Donskey, C.J. The role of the intestinal tract as a reservoir and source for transmission of nosocomial pathogens. *Clin. Infect. Dis.* **2004**, *39*, 219–226. [CrossRef] [PubMed]

84. Turner, P.; Pol, S.; Soeng, S.; Sar, P.; Neou, L.; Chea, P.; Day, N.P.J.; Cooper, B.S.; Turner, C. High prevalence of antimicrobial-resistant Gram-negative colonization in hospitalized Cambodian infants. *Pediatr. Infect. Dis. J.* **2016**, *35*, 856–861. [CrossRef]
85. Anthony, M.; Bedford-Russell, A.; Cooper, T.; Fry, C.; Heath, P.T.; Kennea, N.; McCartney, M.; Patel, B.; Pollard, T.; Sharland, M.; et al. Managing and preventing outbreaks of Gram-negative infections in UK neonatal units. *Arch. Dis. Child. Fetal Neonatal Ed.* **2013**, *98*, F549–F553. [CrossRef] [PubMed]
86. Bizzarro, M.J.; Gallagher, P.G. Antibiotic-resistant organisms in the neonatal intensive care unit. *Semin. Perinatol.* **2007**, *31*, 26–32. [CrossRef]
87. Bulabula, A.N.H.; Dramowski, A.; Mehtar, S. Maternal colonization or infection with extended-spectrum beta-lactamase-producing Enterobacteriaceae in Africa: A systematic review and meta-analysis. *Int. J. Infect. Dis.* **2017**, *64*, 58–66. [CrossRef]
88. Shallcross, L.J.; Davies, S.C. The World Health Assembly resolution on antimicrobial resistance. *J. Antimicrob. Chemother.* **2014**, *69*, 2883–2885. [CrossRef]
89. Roy, A.; Chaudhuri, J.; Sarkar, D.; Ghosh, P.; Chakraborty, S. Role of enteric supplementation of probiotics on late-onset sepsis by Candida species in preterm low birth weight neonates: A randomized, double blind, placebo-controlled trial. *N. Am. J. Med. Sci.* **2014**, *6*, 50–57.
90. Casaburi, G.; Duar, R.M.; Vance, D.P.; Mitchell, R.; Contreras, L.; Frese, S.A.; Underwood, M.A. Early-life gut microbiome modulation reduces the abundance of antibiotic-resistant bacteria. *Antimicrob. Resist. Infect. Control.* **2019**, *8*, 131. [CrossRef]
91. Hansen, L.W.; Khader, A.; Yang, W.L.; Jacob, A.; Chen, T.; Nicastro, J.M.; Wang, P. Deficiency in milk fat globule-epidermal growth factor-factor 8 exacerbates organ injury and mortality in neonatal sepsis. *J. Pediatr. Surg.* **2017**, *52*, 1520–1527. [CrossRef]
92. Lloyd, L.G.; Bekker, A.; Van Weissenbruch, M.M.; Dramowski, A. Healthcare-associated infections in very low birth-weight infants in a South African neonatal unit: Disease burden, associated factors and short-term outcomes. *Pediatr. Infect. Dis. J.* **2022**, *41*, 911–916. [CrossRef] [PubMed]
93. Madan, J.C.; Salari, R.C.; Saxena, D.; Davidson, L.; O'Toole, G.A.; Moore, J.H.; Hibberd, P.L. Gut microbial colonisation in premature neonates predicts neonatal sepsis. *Arch. Dis. Child. Fetal Neonatal Ed.* **2012**, *97*, F456–F462. [CrossRef] [PubMed]
94. Dermyshi, E.; Wang, Y.; Yan, C.; Hong, W.; Qiu, G.; Gong, X.; Zhang, T. The "Golden Age" of probiotics: A systematic review and meta-analysis of randomized and observational studies in preterm infants. *Neonatology* **2017**, *112*, 9–23. [CrossRef] [PubMed]
95. Zhang, G.Q.; Hu, H.J.; Liu, C.Y.; Shakya, S.; Li, Z.Y. Probiotics for preventing late-onset sepsis in preterm neonates: A PRISMA-compliant systematic review and meta-analysis of randomized controlled trials. *Medicine* **2016**, *95*, e2581. [CrossRef]
96. Bi, L.W.; Yan, B.L.; Yang, Q.Y.; Li, M.M.; Cui, H.L. Which is the best probiotic treatment strategy to prevent the necrotizing enterocolitis in premature infants: A network meta-analysis revealing the efficacy and safety. *Medicine* **2019**, *98*, e17521. [CrossRef] [PubMed]
97. Morgan, R.L.; Preidis, G.A.; Kashyap, P.C.; Weizman, A.V.; Sadeghirad, B.; Mc Master 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**, *159*, 467–480. [CrossRef]
98. Indrio, F.; Riezzo, G.; Cavallo, L.; Mauro, A.D.; Francavilla, R. Physiological basis of food intolerance in, V.L.B.W. *J. Matern. Fetal Neonatal Med.* **2011**, *24* (Suppl. 1), 64–66. [CrossRef]
99. Fanaro, S. Feeding intolerance in the preterm infant. *Early Hum. Dev.* **2013**, *89* (Suppl. 2), S13–S20. [CrossRef]
100. Kafetzis, D.A.; Skevaki, C.; Costalos, C. Neonatal necrotizing enterocolitis: An overview. *Curr. Opin. Infect. Dis.* **2003**, *16*, 349–355. [CrossRef]
101. Yee, W.H.; Soraisham, A.S.; Shah, V.S.; Aziz, K.; Yoon, W.; Lee, S.K.; Canadian Neonatal Network. Incidence and timing of presentation of necrotizing enterocolitis in preterm infants. *Pediatrics* **2012**, *129*, e298–e304. [CrossRef] [PubMed]
102. Denning, N.L.; Prince, J.M. Neonatal intestinal dysbiosis in necrotizing enterocolitis. *Mol. Med.* **2018**, *24*, 4. [CrossRef] [PubMed]
103. Wandro, S.; Osborne, S.; Enriquez, C.; Bixby, C.; Arrieta, A.; Whiteson, K. The microbiome and metabolome of preterm infant stool are personalized and not driven by health outcomes, including necrotizing enterocolitis and late-onset sepsis. *mSphere* **2018**, *3*, e00104-18. [CrossRef] [PubMed]
104. Neu, J.; Walker, W.A. Necrotizing enterocolitis. *N. Engl. J. Med.* **2011**, *364*, 255–264. [CrossRef] [PubMed]
105. AlFaleh, K.; Anabrees, J. Probiotics for prevention of necrotizing enterocolitis in preterm infants. *Cochrane Database Syst. Rev.* **2014**, *4*, CD005496.
106. Caplan, M.S. Probiotic and prebiotic supplementation for the prevention of neonatal necrotizing enterocolitis. *J. Perinatol.* **2009**, *29* (Suppl. 2), S2–S6. [CrossRef]
107. Luedtke, S.A.; Yang, J.T.; Wild, H.E. Probiotics and necrotizing enterocolitis: Finding the missing pieces of the probiotic puzzle. *J. Pediatr. Pharmacol. Ther.* **2012**, *17*, 308–328. [CrossRef]
108. Stewart, C.J.; Marrs, E.C.; Nelson, A.; Lanyon, C.; Perry, J.D.; Embleton, N.D.; Cummings, S.P.; Berrington, J.E. Development of the preterm gut microbiome in twins at risk of necrotising enterocolitis and sepsis. *PLoS ONE* **2013**, *8*, e73465. [CrossRef]
109. Aceti, A.; Gori, D.; Barone, G.; Callegari, M.L.; Di Mauro, A.; Fantini, M.P.; Indrio, F.; Maggio, L.; Meneghin, F.; Morelli, L.; et al. Probiotics for prevention of necrotizing enterocolitis in preterm infants: Systematic review and meta-analysis. *Ital. J. Pediatr.* **2015**, *41*, 89. [CrossRef]
110. De Meij, T.G.; van der Schee, M.P.; Berkhout, D.J.; van de Velde, M.E.; Jansen, A.E.; Kramer, B.W.; van Weissenbruch, M.M.; van Kaam, A.; Andriessen, P.; van Goudoever, J.B. Early detection of necrotizing enterocolitis by fecal volatile organic compounds analysis. *J. Pediatr.* **2015**, *167*, 562–567. [CrossRef]

111. Robertson, C.; Savva, G.M.; Clapuci, R.; Jones, J.; Maimouni, H.; Brown, E.; Minocha, A.; Hall, L.J.; Clarke, P. Incidence of necrotising enterocolitis before and after introducing routine prophylactic *Lactobacillus* and *Bifidobacterium* probiotics. *Arch. Dis. Child. Fetal Neonatal Ed.* **2020**, *105*, 380–386. [CrossRef]
112. Hoyos, A.B. Reduced incidence of necrotizing enterocolitis associated with enteral administration of Lactobacillus acidophilus and Bifidobacterium infantis to neonates in an intensive care unit. *Int. J. Infect. Dis.* **1999**, *3*, 197–202. [CrossRef] [PubMed]
113. Underwood, M.A. Probiotics and the prevention of necrotizing enterocolitis. *J. Pediatr. Surg.* **2019**, *54*, 405–412. [CrossRef] [PubMed]
114. Sharif, S.; Meader, N.; Oddie, S.J.; Rojas-Reyes, M.X.; McGuire, W. Probiotics to prevent necrotising enterocolitis in very preterm or very low birth weight infants. *Cochrane Database Syst. Rev.* **2023**, *7*, CD005496. [PubMed]