Role of Probiotics in Preventing Preterm Births: Evaluating Effects on Premature Infants and Pregnant Women

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Abstract

Objective: To critically evaluate the role of probiotics in preventing preterm birth–associated complications and improving maternal and neonatal health outcomes in pregnant women and preterm infants.

Study Design: This review synthesizes the evidence from clinical trials, observational studies and systematic reviews for assessing probiotic use in pregnancy and premature births. The analysis focuses on probiotic strain specificity, single- versus multi-strain formulations, dosage, timing of administration and routes of delivery. Outcomes related to maternal vaginal health and neonatal morbidity were also examined.

Results: In preterm infants, probiotics demonstrated beneficial effects in reducing the incidence of necrotizing enterocolitis, late-onset sepsis and feeding intolerance, although efficacy varied depending on strain composition, dose and duration of consumption. Mostly, multi-strain formulations were more beneficial as compared to single-strain preparations. In pregnant women, probiotic supplementation was associated with modulation of vaginal microbiota, reduced prevalence of bacterial vaginosis and decreased Group B Streptococcus colonization. Emerging strategies such as prenatal supplementation, vaginal seeding and microbiota transplantation have shown potential in restoring microbial balance. The key challenges of using probiotics with preterm infants and pregnant women include strain-specific responses, safety concerns in vulnerable populations, an inconsistent regulatory framework and lack of long-term outcome data.

Conclusion: This review highlights the potential of probiotics in improving maternal and neonatal health and emphasizes the need for further research to overcome current challenges and enhance their accessibility in routine healthcare.

Keywords: *Probiotics; Pregnant women; Preterm infants; Necrotizing Enterocolitis.*

INTRODUCTION

The human gastro-intestinal tract (GIT) harbors trillions of microbial cells that are comprised of approximately 400 to 500 distinct bacterial species (Guarner and Malagelada, 2003; Turnbaugh *et al.*, 2007). Their distribution along GIT varies among individuals of a species and also within the same individual with time (Yang *et al.*, 2024). Microflora is scarce in the upper part of the GI tract. Unfavorable growth conditions, such as luminal secretions (acidic stomach, pancreatic juice), allow only acid-tolerant species to survive.

Gut dysbiosis has been linked to a variety of gastrointestinal disorders such as irritable bowel syndrome (IBS), inflammatory bowel disease (IBD) and metabolic conditions such as obesity and type 2 diabetes. Probiotics help to maintain the balance of gut microflora and promote the digestive health and overall well-being of an individual (Hills *et al.*, 2019). Probiotics are defined as live microorganisms which, when administered in adequate amounts, confer health benefits to the host (WHO, 2001). Probiotics are often referred to as "good bacteria" and are commonly ingested as supplements or included in traditional fermented foods. Strains of *Lactobacillus*, *Streptococcus* and *Bifidobacterium* are widely consumed as probiotics. Probiotic strains are selected for their ability to survive in the human GI tract, disease prevention or cure, and their role in food preservation and fermentation (Bansal *et al.*, 2013). Probiotics confer health benefits by improving intestinal permeability,

balancing aberrant microbiota and modulating gut microbiome, regulating secretion of proinflammatory cytokines and short-chain fatty acids (Rokana *et al.*, 2016; Dahiya *et al.*, 2017).

Probiotic strains are reported to protect against several diseases viz., urogenital infections (Ballini *et al.*, 2018), cancer (Kumar and Dhanda, 2017), lactose intolerance (Oak and Jha, 2018), oxidative stress, diabetes (Singh *et al.*, 2023), cystic fibrosis, dental caries (Guo *et al.*, 2023) acute diarrhea (Hameed and Salman, 2023), cardiovascular (He *et al.*, 2023), alzheimer (Dhami *et al.*, 2023) and crohn's disease (Carding et. al., 2015). The importance of maintaining a balanced gut microbiome extends beyond general health and can be a potential target for preterm infants (PTIs) and pregnant women.

PTIs are delivered before 37 weeks of gestation with very low birth weights (VLBW) of less than 1.5kg. Preterm births (PTB) remain a significant global health concern, with approximately 40% of births worldwide occurring prematurely (WHO). It is one of the leading causes of death in children below 5 years of age (Perin *et al.*, 2022). (Ohuma *et al.*, 2023). In 2020, an estimated 13.4 million PTIs were born, indicating a prevalence of approximately 1 in 10 births. PTIs face increased risk of mortality and morbidity due to neurodevelopmental disabilities, respiratory problems and gastrointestinal complications. Countries such as Pakistan, India, China, Nigeria and Ethiopia have the highest number of PTIs deaths (WHO). Some die because of limited access to essential care practices such as warmth, breast feeding and incubator support. Survivors suffer from complications like lifetime disability, learning disability, visual and hearing problems. Low-income countries of Southern Asia and Sub-Saharan Africa report 90% PTB deaths due to inadequate healthcare access as compared to high-income countries viz., USA.

PTIs are highly susceptible to gut dysbiosis because of their underdeveloped gastrointestinal barriers and immature immune system. The Firmicutes/Bacteroidetes (F/B) ratio is a commonly used indicator of dysbiosis (Stojanov *et al.*, 2020). Dysbiosis is associated with complications such as Sepsis (Lee *et al.*, 2021), Necrotizing Enterocolitis (NEC) and Intraventricular Haemorrhage (IVH) (Lee *et al.*, 2020a). NEC is an inflammatory bowel disease that causes necrosis of the colon and intestine (Cuna *et al.*, 2018). Infants suffering from NEC are at higher risk of poorly developed brain and associated neurodevelopmental delays (Braga *et al.*, 2011). Sepsis is a systemic inflammation marked by proliferation of pathogens in the blood of infants. Sepsis is correlated with increased hospitalization, increased morbidity and mortality of infants (Jiang *et al.*, 2020). IVH is a result of fluctuations in blood pressure and impaired cerebrovascular regulation in infants. IVH results in bleeding in the ventricular system of the brain. This prolongs their duration of hospitalization and poses PTIs at the risk of developing nosocomial infections (Lee *et al.*, 2020a). PTIs also suffer from higher respiratory distress such as pneumonia, Neonatal Respiratory Distress Syndrome and Bronchopulmonary Dysplasia. By modulating the gut microbiome, probiotics hold the potential to ameliorate these conditions in PTIs (Gritz and Bhandari, 2015). Probiotics decreased the time to achieve full enteral feeding (Aceti *et al.*, 2016), reduced the duration of stay in hospital (Sun *et al.*, 2017) and reduced mortality (Deshpande *et al.*, 2017) as compared to PTIs not given probiotic therapy.

The first documented clinical trial on the use of probiotics for infants with NEC dates back to 1999, when a neonatologist treated NICU-admitted PTIs with probiotic Infloran (mixture of *B. infantis* and *L. acidophilus*) (Hoyos, 1999). NEC is treated with a variety of probiotics containing *Lactobacillus spp., Bacillus spp.* either alone or in combination (Sharif *et al.*, 2023, Denkel *et al.*, 2016; Thomas *et al.*, 2017). Despite promising clinical outcomes, a few deaths of PTIs in hospital settings have raised concerns regarding the safety and effectiveness of probiotics in PTIs. This emphasized the need for high-quality clinical trials for the evaluation of safe administration of probiotics in PTIs, and approval for using probiotics for PTIs is yet to be awaited from the FDA.

Maternal health also plays a critical role in PTB pathophysiology. Diverse vaginal microbiota dominated by *Lactobacillus* species viz. *L. crispatus, L. iners, L. jensenii and L. gasseri* (Mehta *et al.*, 2020) were found to be associated with Full-term (FT) births (Fettweis *et al.*, 2019). This microflora play protective role, like pH regulation, hydrogen peroxide production and modulation of immune response (Lewis *et al.*, 2017). Though vaginal microbiome composition is influenced by factors like ethnicity and exposure to environmental elements like drugs, diet, microbial load and exposure to different microbes in the living ecosystem (Barrientos-Duran *et al.*, 2020). The dysbiosis of the vaginal microbiome due to a shift from dominant *Lactobacillus* to non-indigenous microbial species also contributes to PTB (Fettweis *et al.*, 2019).

With the advent of interest in probiotic interventions for PTB, this review is an attempt to fill the gap by a critical evaluation of existing literature on the potential role of probiotics in pregnant women and premature babies. This review reports the influence of probiotics on the development of gut microbiome in infants, underlying conditions of PTIs, complications in pregnant women and the contribution of mothers toward PTB.

GUT MICROBIOTA DEVELOPMENT IN PRETERM INFANTS

The gut microbiome is also known as "second-brain" (Leslie, 2016). There is an intricate network of the gut microbiome and brain through various metabolites. Gut microbiome also aids in digestion, immune system development, enteral nutrient absorption, enhances mucosal barrier function, modulates intestinal microflora and reduces intestinal permeability, resulting in reduced bacterial translocation. In PTIs, enteral nutrition supports intestinal maturation, facilitates the synthesis of otherwise unavailable nutrients like Vitamin K, generates protective factors, enhances mucosal integrity and decreases the reliance on intravenous feeding, which is the most important risk factor of infection (Thoene *et al.*, 2021)

The composition of the gut microbiome is dynamic and undergoes significant changes over time. PTIs exhibit a distinct gut microbiome when compared to FT (Toubon *et al.*, 2022), viz. decreased species diversity (Hendrick *et al.*, 2019), increased levels of harmful bacteria such as Enterobacteriaceae (*Klebsiella pneumoniae* and *Escherichia coli)* and *Clostridium difficile* and a reduced number of beneficial bacteria, viz. *Bifidobacteria* and *Lactobacilli* (Korpela *et al.*, 2018). These differences are attributed to the immaturity level of infants, mode of delivery, reduced exposure to maternal microbiota, exposure to antibiotic treatment, prolonged stay in Neonatal Intensive Care Unit (NICU) and reduced breastfeeding (Korpela *et al.*, 2018).

Factors Affecting Gut Microbiota in PTIs

The microbial community that initially colonizes the infant's gut plays a crucial role in shaping its composition and overall health during later stages of development (Fig 1).

Gestational Age

PTB are categorized into 3 subcategories on the basis of deviation from FT delivery of 38 weeks (WHO,2023):

1. Extremely preterm (EP): <28 weeks
2. Very preterm (VP): 28-32 weeks
3. Moderate to late preterm (MP): 33-37 weeks

Gestational age is a major factor affecting gut microbiota in PTIs (Aguilar-Lopez *et al.*, 2021). Survival expectancy of EP infants is relatively lower due to the limited diversity of gut microbiota as compared to FT or MP infants. Studies revealed that PTI's gut microbiome is less diverse with fewer numbers of good bacteria (*Bifidobacteria*) and a greater number of pathogens (*Enterococcus, Staphylococcus and Enterobacter*) as compared to FT infants (Yang *et al.*, 2024). Metagenome analysis of fecal samples of PTIs up to 1 year of age revealed notable differences in bacterial species that colonized their gut[Error! Reference source not found.]. (Table 1)

Table 1: Microbial diversity across gestational age categories.

Gestational Age	Dominant Genera	Key Observations
EP	▪ *Klebsiella* (14.5%) ▪ *Staphylococcus* (11.9%) ▪ *Sphingomonas* (8.7%)	Lower diversity Higher abundance of pathogens
VP	▪ *Klebsiella* (37.8%) ▪ *Sphingomonas* (8.3%) ▪ *Enterococcus* (6%)	Dominance of *Klebsiella* Reduced beneficial bacteria
MP	▪ *Klebsiella* (53.5%) ▪ *Escherichia/Shigella* (15.5%) ▪ *Clostridium* (9.4%)	Higher colonization of *Clostridium* Greater microbial diversity

Klebsiella is a gram-negative bacteria that is linked to brain damage in PTIs and reportedly increase from EP-VP-MP (Jia *et al.*, 2022; Seki *et al.*, 2021). *Enterococcus* is frequently encountered in NICUs and mainly found associated with neonatal prematurity, meningitis and sepsis (Reuschel *et al.*, 2020). Its overrepresentation disturbed colonization of normal microbiota in EP infants (Korpela *et al.*, 2018). Metagenomics studies of fecal samples also revealed increased abundance of Proteobacteria, *Firmicutes and Bacteroidetes* in order of EP-VP-MP (Yang *et al.*, 2024). An abundance of *Bifidobacterium* was inversely related to

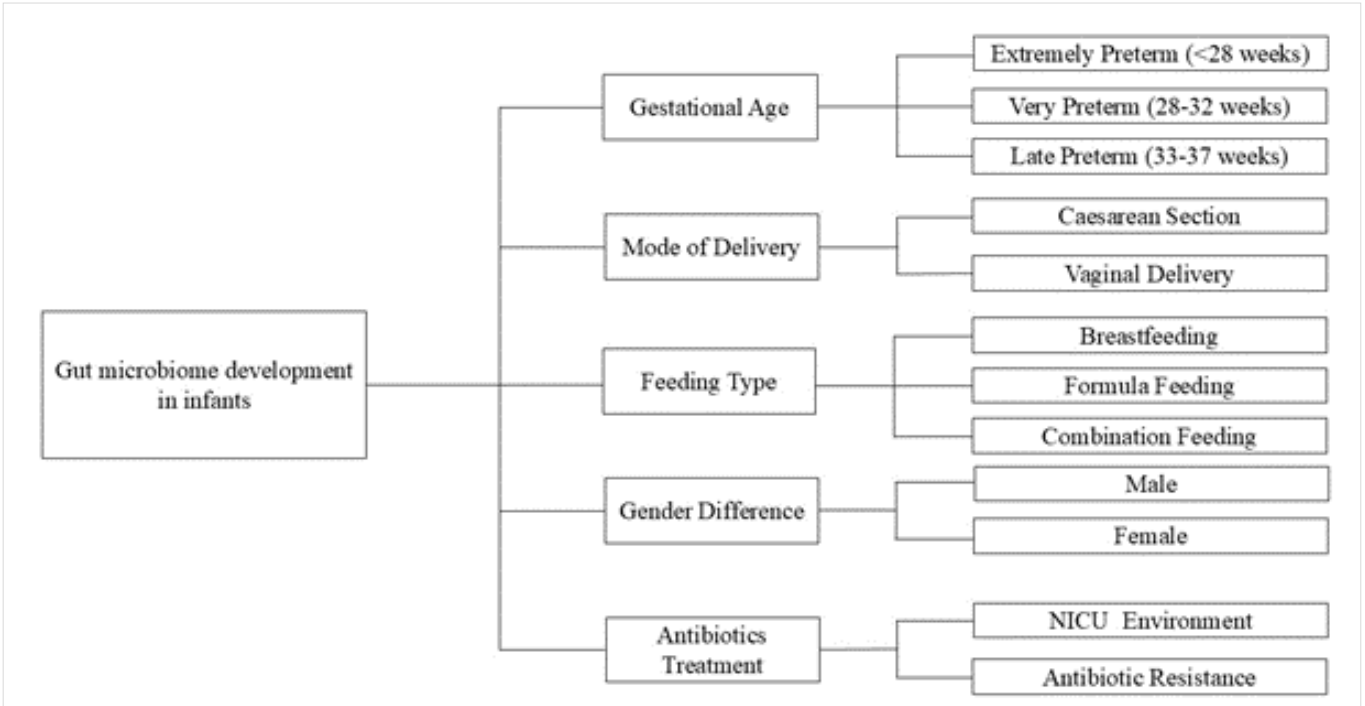

Fig. 1: Factors affecting colonization of gut microbiota in infants.

Klebsiella, thereby suggesting that probiotics can be helpful in preventing the infant gut from pathogens.

Mode of Delivery

Mode of delivery (Vaginal or Caesarean section (C-section)) is also known to influence initial colonization of gut microbiome in PTIs (Ma *et al.*, 2023; de Koff *et al.*, 2022; Korpela *et al.*, 2021).

Vaginal delivery: PTIs delivered through the vaginal route have a similar microbial composition as that of the mother's reproductive tract, i.e. a greater number of beneficial bacteria viz. *Escherichia, Bifidobacteria* and *Lactobacilli* (Reyman *et al.*, 2019). Skin, oral and nasal cavities of such infants are exposed to these bacteria that serve as the initial inoculum for colonization of the infant's gut. These early colonizers play crucial role in shaping the development of the infant gut microbiome and immune system.

C-section delivery: In contrast, infants delivered through C-section bypass exposure to maternal microbiota and exhibit delayed and altered colonization patterns. Their microbiota resembles more to that of their mother's skin (Goedert, 2016). Such infants show delayed microbial colonization, decreased microbial diversity and increased opportunistic pathogens, viz. *Enterococcus* and *Clostridium difficile* (Bokulich *et al.*, 2016). This differential microbial composition may contribute to the increased risk of NEC, sepsis and other complications observed in PTIs born via C-section. Some findings reported no significant difference in the infants micrbiome born through either mode of delivery. (Stewart *et al.*, 2017, Patel *et al.*, 2016). This might be due to the small sample size of the study, different tissues used for sampling, and type of detection method used (16s rRNA vs shotgun sequencing).

Feeding Type

Feeding type is a critical determinant of gut microbiota composition in PTIs (Pannaraj *et al.*, 2017). The feeding type with abundant genera and key benefits and limitations are summarised in Table 2.

Breast feeding: Breastfeeding is considered as the gold standard for infants' nutrition because it provides essential nutrients and bioactive components that promote the establishment of a diverse and resilient microbial community (Fehr *et al.*, 2020). Human milk contains both prebiotics (Human milk Oligosachharides, i.e., (HMOs)) and probiotics (microbes) (Vandenplas *et al.*, 2021) that influence gut microbial composition directly (vertical transmission of bacteria) as well as indirectly (by providing prebiotics).

Breast milk is composed of *Streptococcus, Staphylococcus, Propionibacterium, Lactic acid bacteria*, *Bifidobacterium* (Fernandez *et al.*, 2013) and immunomodulatory factors viz., secretory IgA (SIgA), lactoferrin and lysozyme, which strengthen an infant's immune defenses and reduce the risk of infections (Section on Breastfeeding, 2012). SIgA acts as the first line of defense in the gut mucosal barrier by binding to pathogens and preventing bacterial translocation. C-section infants have reduced levels of SIgA, but breastfeeding can increase their SIgA levels within the first three months(Chen *et al.*, 2023).

The mode of feeding can have long-term effects on the development of gut microbiome and health during infancy and beyond (Thompson *et al.*, 2015). Breastfeeding is linked to lower risk of various allergies, asthma and obesity, partly due to its beneficial effects on microbial colonization (Section on Breastfeeding, 2012). Despite its benefits, breastfeeding in PTIs is often delayed due to maternal health issues, insufficient milk production or prolonged NICUs stay, which necessitate alternative feeding strategies.

Formula feeding: Formula feed introduces a different microbial milieu to the infant's gut (Thompson *et al.*, 2015). Though modern infant formulas aim to mimic the composition of breast milk, but it lack the dynamic and bioactive components inherent to human milk. This deficiency results in lowered microbial diversity and a higher number of facultative anaerobes, viz. *Enterobacteriaceae* (associated with dysbiosis and intestinal inflammation) (Dai *et al.*, 2025). This increases their risk of developing NEC and gastrointestinal infections. Studies further indicate that formula-fed infants also exhibit a slower establishment of a protective gut microbiota with reduced colonization by *Bifidobacterium* and *Lactobacillus* (Chi *et al.*, 2021).

Formula feeding vs breast feeding: Studies have revealed distinct differences in gut microbiota composition between breastfed and formula-fed infants. In a cohort study, infants were divided into three groups: those fed on breast milk, probiotics formula and non-probiotics formula. Fecal analysis of their samples revealed increased alpha diversity in the probiotics formula group as compared to the other two groups (Chi *et al.*, 2021). Furthermore, microbial composition differed in the 1st and 6th week and stabilized at around 12 weeks. Gut bacterial load of breast-fed infants increased after two weeks of their birth (Kurath-Koller *et al.*, 2020). It is quite evident that breast milk provides more beneficial bacteria to infants than formula feed.

Combination feeding: Combination feeding involves supplementing breast milk with formula and is commonly fed

Table 2: Feeding type with their dominant genera and key benefits and limitations.

Feeding Type	Dominant Genera	Key Benefits	Key Limitations
Breast-feeding	*Bifidobacterium* *Lactobacillus*	Promotes beneficial bacteria Immune modulation	Availability may be limited
Formula Feeding	*Enterobacteriaceae* *Clostridium*	Easily accessible alternative	Lack HMOs Increased risk of Dysbiosis
Donor Human Milk	*Bifidobacterium* *Lactobacillus*	Retains some benefits of breast milk.	Pasteurization reduces biodiversity
Combination Feeding	Mixed	Provides some benefits of breast milk.	Dilutes protective effects of HMOs

to PTIs. This approach provides some of the benefits of breast milk while addressing nutritional requirements unmet by breastfeeding alone (Ma *et al.*, 2023). However, the introduction of formula can dilute the protective effects of HMOs and other bioactive compounds that results in an intermediate microbial profile, which puts this type of feeding into question (Masi *et al.*, 2020).

Donor human milk: Donor human milk is often used as an alternative when maternal breast milk is unavailable. Although it retains some of the bioactive components of breast milk, pasteurization reduces its concentration of immune-modulating factors and beneficial bacteria. Donor milk-fed PTIs exhibited microbial profiles that were better than formula-fed but less favourable than those of breastfed infants (McGuire *et al.*, 2022).

Antibiotic Treatment During NICU

PTIs undergo invasive procedures, prolonged antibiotic treatment, delayed enteral feeding and prolonged parenteral nutrition in NICUs. Along with their underdeveloped immune system, these factors can negatively affect the development of their gut microbiota (Walker and Neu, 2011). They are at risk of nosocomial infections, which necessitate the use of antibiotics as part of routine care. This treatment disrupts the colonization of beneficial bacteria in PTIs.

Studies revealed that antibiotics reduced the number of beneficial bacteria, viz. *Firmicutes* and *Actinobacteria*, and an increased level of *Proteobacteria* (involved with dysbiosis and intestinal inflammation) during the critical period of development in neonates (Madan *et al.*, 2012; Fouhy *et al.*, 2012). NICU environment itself influences gut microbiota of PTIs. Metagenomic analysis showed similarity between microbial communities present in NICU environments and those found in gut microbiota of infants receiving treatment, viz. *Staphylococcus epidermidis, Enterococcus faecalis, Pseudomonas aeruginosa and Klebsiella pneumoniae* (Brooks *et al.*, 2017). These pathogens are often resistant to antibiotics and, if dominant in the gut of PTIs, might increase the risk of infections and gut dysbiosis.

The effects of antibiotic exposure and NICU environments on gut microbiota can persist beyond the neonatal period. Analysis of microbiota of 43 PTIs revealed reduced alpha diversity and deficiencies in key genera, viz. *Clostridium* and *Rumniococcus* species in infants exposed to antibiotics, and these effects lasted up to one year (Bokulich *et al.*, 2016). Longitudinal studies tracked microbiota development until age 3 and showed similar results, i.e., antibiotic-exposed children exhibited reduced diversity and microbial communities were dominated by a single strain rather than diverse populations of the same species (Yassour *et al.*, 2016).

Gender Differences

Emerging evidence suggests that gut microbiome development also affects the gender of the infant. Female infants develop the gut microbial structure of adults earlier than male infants. Adults have a high proportion of *Firmicutes* and *Proteobacteria* in their gut. *Proteobacteria* and *Firmicutes*, *Parasutterella, Eubacterium*, *Peptoniphilus* and *Anaerosporobacter* were dominant in female infants (Ma *et al.*, 2023). In contrast, male infants predominantly possessed *Alistipes (*an anaerobic bacterium of the human GIT) (Kaur *et al.*, 2017). This suggests that the gut microbiome difference between gender originate during infancy itself. The study of Ma *et al.* (2023) was the first study where an infant's gender was found to be the primary factor in contributing to the development of gut microbiota from 1 to 6 months after birth (Ma *et al.*, 2023).

Major Complications in PTIs

PTIs are highly susceptible to health challenges due to their underdeveloped immune system and immature GI tract. Their vulnerability is increased by prolonged stays in NICUs, antibiotic exposure, delayed enteral feeding and invasive procedures. These factors disrupt gut microbiota and contribute to complications, viz. NEC, sepsis and other systemic health issues (Walker and Neu, 2011).

Necrotizing Enterocolitis: NEC is one of the most serious and life-threatening conditions in PTIs (Seghesio *et al.*, 2021). It causes intestinal inflammation (leading to bacterial invasion), cellular damage and necrosis of colon and intestine (leading to intestinal perforation, sepsis and death) (Ginglen and Butki, 2023). Many risk factors are identified for this disease, but PTB, low birth weight and formula feeding were reported to be foremost (Afzal *et al.*, 2017). Incidence of NEC varied inversely with gestational age, i.e. PTIs have more incidence of NEC than FT infants. (Battersby *et al.*, 2017; Seghesio *et al.*, 2021).

No causative agent for NEC has yet been identified, but several bacteria are associated with its onset. Early gut colonizers reportedly determine the occurrence of NEC and sepsis (Sharif *et al.*, 2020). Abundance of *Clostridium perfringens and Bacteroides dorei* in the meconium of PTIs were positively correlated with the incidence of NEC. *Clostridium spp.* and associated toxins were reported in the stool of neonates with NEC (Lee *et al.*, 2020b). *Clostridium difficile* prevented NEC by protecting against enterotoxin released by *C. perfringens* (Jangi *et al.*, 2010). Infants lacking *C. difficile* were more prone to infections and NEC (Wilson *et al.*, 1983). PTIs with an increased number of γ-proteobacteria in their gut were diagnosed after 10 days of their birth with Late-onset NEC (Heida *et al.*, 2016). An abundance of γ-proteobacteria was found to be lower before the occurrence of Late-onset NEC (Pammi *et al.*, 2017).

High prevalence of *Acinetobacter baumannii* and *Klebsiella pneumonia* was observed in Indian PTIs, frequently detected in patients infected with nosocomial treatment (in NICUs). This increased their risk of NEC, thereby leading to morbidity and mortality (Ghanchi *et al.*, 2023). Administration of probiotics decreased the number of *Acinetobacter baumannii* and *Klebsiella pneumonia* and increased the number of *Bifidobacterium* viz. *Bifidobacterium longum* and *Bacteroides thetaiotaomicron*. This suggests that probiotic supplementation fosters the proliferation of beneficial microorganisms in preterm neonates undergoing NICU admission.

Sepsis: Sepsis is a life-threatening condition caused by the body's response to an infection, characterized by tissue damage, organ failure and death. The incidence of sepsis in VLBW infants is significantly higher as compared to FT infants (Dong and Speer, 2014). Sepsis can be early-onset (within the first 72h, caused by maternal transmission) and late-onset (after 72 hours, associated with NICU-acquired infections) (Stoll *et al.*, 2010). The incidence of Late-onset sepsis varies inversely with gestational age, i.e., EP infants are more likely to develop Late-onset sepsis as compared to VP and VP to MP. This might be because EP infants must spend more time in NICUs following their birth than VP infants.

Group B *Streptococci* are a common cause of both early and late onset sepsis (Vergnano *et al.*, 2011). Prolonged antibiotic use in PTIs reduces gut microbial diversity and promotes the overgrowth of multi-drug resistant pathogens, viz. *Klebsiella pneumoniae, Enterococcus faecalis, Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Enterobacteriaceae*. These bacteria (Esaiassen *et al.*, 2017; Boghossian *et al.*, 2013).

Other health complications: These may include –

- Immune Dysfunction: PTIs exhibited delayed immune system development due to their premature birth and the absence of critical microbial exposures. Gut dysbiosis further delays the maturation of regulatory T cells, immune programming and production of immune-modulating cytokines (Nguyen *et al.*, 2016)
- Extra Uterine Growth Restriction (EUGR): Delayed feeding and nutrient malabsorption resulted in EUGR in PTIs, which increased their risk of long-term complications, viz. Obesity, type 2 diabetes and cardiovascular diseases (Lissauer *et al.*, 2015).
- Neurodevelopmental Delays: Inflammatory responses associated with NEC, sepsis and dysbiosis have been reported with increased risk of poor brain development, neurodevelopmental delays (including cerebral palsy and learning disabilities) (Braga *et al.*, 2011).

Maternal Microbiome and PTBs

The infant microbiome is shaped by microbial transfer during maternal-infant interactions. Maternal well-being and medical interventions also play a crucial role. This interplay between maternal gut microbiota and fetal immune development is a crucial determinant of neonatal health. Maternal gut-derived microbial metabolites, viz. short-chain fatty acids and immunomodulatory molecules, can cross the placental barrier and directly influence fetal immune programming (Gomez-Arango *et al.*, 2016). Conditions arising in pregnant mothers due to prolonged antibiotic usage, chorioamnionitis, vaginal dysbiosis, gestational diabetes mellitus (GDM), and preeclampsia may also affect the development of the infant microbiome (Lemas *et al.*, 2016).

Vaginal Microbiome and PTBs

Vaginal microbiome is first microbial environment encountered by a neonate during vaginal delivery. A healthy vaginal microbiome is generally dominated by *Lactobacillus* species, viz. *L. crispatus, L. gasseri, L. iners* and *L. jensenii* (Mehta *et al.*, 2020). These bacteria produce H_2O_2 and lactic acid, which lower the vaginal pH (< 4.5) and provide a protective barrier against pathogenic bacteria (France *et al.*, 2022).

Microbial shifts and vaginal dysbiosis: Alterations in the vaginal microbiome are linked to PTB. Studies reported *L iners* to be more abundant in PTI samples, while *L. crispatus and L.gaseri* dominated in term samples (Kumar *et al.*, 2021). Depletion of *Lactobacillus spp.* generally promotes the proliferation of mostly anaerobic bacteria, which results in Vaginal Dysbiosis (VD). VD is characterized by a microbial shift to anaerobic species, viz. *Bifidobacterium vaginale, Fannyhessa vaginae, Gardnerella spp., and Prevotella spp.* (Coudray *et al.*, 2020, Cheng *et al.*, 2020). This microbial shift creates a pro-inflammatory vaginal environment, which is strongly linked to PTBs (Fettweis *et al.*, 2019).

Bacterial vaginosis: Bacterial Vaginosis (BV) is the most common form of VD and a leading cause of PTBs worldwide (Javed *et al.*, 2019). Strong correlation was observed between PTBs and BV (Petricevic *et al.*, 2023). BV is characterized by reduced *Lactobacillus* species and overgrowth of anaerobic bacteria, viz. *Gardnerella, Prevotella, Atopobium, Mobiluncus, Bifidobacterium, Finegoldia, Sneathia, Leptotrichia* and novel bacteria within the *Clostridiales* order, known as BV-associated bacteria (BVAB) (Muzny *et al.*, 2018).

BVAB, including *Gardnerella vaginalis*, was reported to be a PTB signature in women of European (Tabatabai *et al.*, 2019) and Indian ancestry (Kumar *et al.*, 2021). *Finegoldia magna* (a gram-positive anaerobic cocci) was reported in women diagnosed with BV and in stool samples of PTIs (Brooks *et al.*, 2014). Moreover, higher levels of this bacterium were observed in PTIs diagnosed with NEC (Devarajalu *et al.*, 2024), thus indicating that BV can lead to NEC in PTIs.

Ethnicity and vaginal microbiota: Ancestral profiles of pregnant women revealed a relationship between PTBs and ethnicity. Women of African descent face higher PTB risk as compared to women of European (Blencowe *et al.*, 2013) and Asian ancestry (Ahrodia *et al.*, 2022). Women of African ancestry have Bacterial Vaginosis associated Bacteria 1, BVAB1(Callahan *et al.*, 2017) and 12 additional taxa, which are known to increase the chances of PTBs, while women of Asian and European ancestry have a prevalence of *Lactobacillus crispatus*, which reduces the chances of PTB (Ravel *et al.*, 2011; MacIntyre *et al.*, 2015). Taxa associated with BV were higher in PTB mothers as compared to mothers delivering FT infants (Kumar *et al.*, 2021). Non-*Lactobacillus* species dominance is the major cause of preterm labour in Black and Hispanic women (Mehta *et al.*, 2020). Meta-analysis also suggested greater bacterial diversity and lesser *Lactobacilli* in the female reproductive tract associated with PTB (Gudnadottir, 2021). Thus, ancestral profiles play a significant role in predisposing certain populations to preterm delivery.

Pregnancy Complications and Women's Health Conditions

Chorioamnionitis: It is a bacterial infection of the amniotic sac that occurs before or during labour. This condition causes increased levels of *Staphylococcus* in PTIs born to such mothers (Westaway *et al.*, 2022), possibly contributing to the risk of complications like Sepsis (Puri *et al.*, 2016).

Preeclampsia: It is a hypertensive disorder that occurs during pregnancy, and it has been associated with altered gut microbiota. Maternal gut dysbiosis correlated with the presence of oral-associated bacteria in the placenta viz. *Eillonella, Fusobacterium, Haemophilus, Granulicatella, Streptococcus, Gemella* and *Neisseria* in women with preeclampsia (Cooper *et al.*, 2024). These bacteria might disrupt immune homeostasis, thus leading to adverse neonatal outcomes.

Dysbiosis in mothers with these conditions can disrupt neonatal gut colonization. Infants born to mothers with preeclampsia reported lower levels of beneficial bacteria viz. *Escherichia/Shigella* at discharge (Westaway *et al.*, 2022). This effect is more pronounced in PTIs (Davies *et al.*, 2016), possibly due to continued maternal microbial transmission post-delivery through touch or breast-feeding.

Gestational Diabetes Mellitus: GDM is a common pregnancy complication that affects maternal metabolism and fetal development. Elevated maternal blood glucose levels facilitate the transfer of glucose to the placenta, which leads to increased fetal insulin production and secretion of Insulin-like growth factors (IGF) crucial for organ development. While this process establishes fat and glycogen reserves, it can also lead to glycemic imbalances in neonates post-birth (Mirabelli *et al.*, 2021). Persistent fetal insulin release predisposes neonates to Insulin resistance, obesity, Type-2 Diabetes mellitus and Metabolic disorders later in life (Chandrasekaran *et al.*, 2022; Desoye *et al.*, 2022)

Women with GDM exhibited a higher F/B ratio, which reflected microbial dysbiosis (Davidson *et al.*, 2021). Dysbiosis contributed to decreased α-diversity in neonates and an increased number of pro-inflammatory taxa, viz. *Escherichia* and *Parabacteroides* (Ari *et al.*, 2022).

Anxiety, Depression and Psychological Stress: Maternal psychological health during pregnancy had profound effects on both maternal and neonatal microbiomes. Anxiety, Depression and Psychological stress are common in prenatal and perinatal periods, which disrupt the maternal-gut brain axis (Trifklovic *et al.*, 2022). Neonates exposed to maternal stress in utero showed a higher risk of neurodevelopmental disorders, immune dysregulation and metabolic imbalances. Anxiety and depression during pregnancy induce preterm labour, which increases the risk of neonatal complications (Yan *et al.*, 2020).

Placental Microbiome: The Hidden Contributor

Placenta was thought to be sterile, but research revealed the presence of a diverse microbial community in amniotic fluid, umbilical cord blood, amniotic membranes and placenta. Placental microbiome is dominated by *Firmicutes, Actinobacteria, Bacteroidetes, Lactobacillus, Bifidobacterium* and *Ralstonia* (Li *et al.*, 2024). Studies also indicated that the placental microbiota is also influenced by maternal oral and gut microbiota through hematogenous transfer.

Dynamic nature of placental microbiota: During 32-34 weeks of gestation, the placental microbiome showed correlation with maternal vaginal and gut microbiota. At full term, the placental microbiota resembled the oral microbiome, including non-pathogens, viz. *Firmicutes, Proteobacteria, Bacteroidetes, Fusobacteria and Tenericutes* (Consortium *et al.*, 2012). This implies a two-way balance of microbiome, i.e., from the placenta to the gut and vice-versa.

Dysbiosis and adverse pregnancy outcomes: Placental dysbiosis is characterized by a decline in beneficial bacteria and an increase in pathogens, viz. *Burkholderia*, which is associated with PTBs (Aagard *et al.*, 2014). *Finegoldia magna* is frequently detected in the amniotic fluid of women with preterm prelabour rupture of membranes (DiGiulio *et al.*, 2010). *F. magna* also has a role in BV and PTBs (Devarajalu *et al.*, 2024 and Brooks *et al.*, 2014). Microorganisms in the placenta can migrate to the fetus (hematogenously or through direct contact with the amniotic sac). These imbalances can lead to inflammatory responses in the fetus, thereby increasing the risk of neonatal complications like NEC and sepsis (DiGiulio *et al.*, 2010).

Genetic predisposition may also cause preterm labour as specific bacteria may migrate from vaginal tissues to the amniotic membrane via complex signalling pathways involving pro and anti-inflammatory cytokines (Alamrani *et al.*, 2017).

ROLE OF PROBIOTICS IN NEONATAL CARE AND PREGNANT WOMEN

The intestinal microbiota functions as a natural protective barrier against pathogens and toxins (Gibbons *et al.*, 2021). This barrier is underdeveloped in PTIs, leading to intestinal dysbiosis, which is characterized by overgrowth of pathogens viz. *Enterobacter* and *Pseudomonas* species (Brooks *et al.*, 2018). It also makes PTIs highly susceptible to life-threatening complications, viz. NEC, Sepsis, Feeding intolerance, neurodevelopmental delays and immune dysregulation. Probiotics supplementation has been shown to regulate intestinal microbiota composition and promote intestinal mucosal barrier growth, thus enhancing resistance to pathogens (Chi *et al.*, 2021; Gibbons *et al.*, 2021; Chi *et al.*, 2020). Probiotics are also reported to play a crucial role in strengthening immune function, increasing the production of mucosal IgA, enhancing leukocyte phagocytosis and reducing the production of inflammatory cytokines (Guo and Lv, 2023; Mazziotta *et al.*, 2023). Studies also reported the efficacy of probiotics supplementation in decreasing the incidence of NEC and sepsis (Robertson *et al.*, 2020; Sun *et al.*, 2017) and reducing morbidity and mortality rates among PTIs (Morgan *et al.*, 2020).

Probiotics and Newborn Complications

Probiotic Bacteria and PTIs

In PTIs, probiotics assist in gut microbiome maturation, promote early achievement of full feeds and reduce the risk of feeding intolerance (Jalali *et al.*, 2020). But some studies

reported no significant difference in the time to reach full feeding upon probiotics supplementation (Kaban *et al.*, 2019). Oral supplementations of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* are routinely given to PTIs to develop their gut at par with FT infants. *Lactobacillus spp.* are known to create an anaerobic environment by lowering oxygen levels in the gut of PTIs (Koskella *et al.*, 2017). This is favourable for *B. bifidum* colonization. *Lactobacillus spp.* also produce bacteriocins having anti-microbial functions and thus prevents translocation of other bacterial species (Pogačar *et al.*, 2020). *Bifidobacterium* is good at colonising an infant's gut due to their ability to digest human breast milk. HMOs present in the breast milk act as prebiotics for these bacteria (Lawson *et al.*, 2020). Fecal samples from PTIs with probiotics supplementation showed relatively more breakdown products of HMOs, acetate and lactate (SCFAs) as compared to PTIs without any probiotic intervention (Liu *et al.*, 2020). These metabolites make the environment too acidic for pathogens to grow in the gut of PTIs and prevent NEC (Robertson *et al.*, 2019). Use of fermented milk product as an infant formula (containing L. paracasei) demonstrated elevated levels of specific oligotypes of Phylum *Firmicutes* (*Roseburia, Faecal bacterium* and *Blautia*) and *Bacteroides spp.*, all of these are SCFAs producers. SCFAs also have neuroprotective effects that support cognitive and motor functions in the long term (Silva *et al.*, 2020). Strains like *Bifidobacterium breve* and *Lactobacillus rhamnosus* boosted immune responses by increasing SIgA production and promoting leukocyte phagocytosis (Thomas *et al.*, 2017). Infants supplemented with *Bifidobacterium* formula also displayed slight variance, viz. reduced occurrence of *Bacteroides fragilis* and *Blautia spp.* as compared to those fed a placebo (Bazanella *et al.*, 2017). This might be due to competition in the gut ecosystem over time. *Bifidobacteria* did not persist permanently in the intestinal tract, when dose was discontinued.

Probiotics supplementation also reduced risk of sepsis by inhibiting pathogens, viz. *Staphylococcus epidermidis, Escherichia coli* and *Klebsiella pneumoniae* (Van *et al.*, 2020). Meta-analysis also revealed a 21% reduction in Late-onset sepsis among PTIs receiving probiotics and thus increasing their chances of survival (Sharif *et al.*, 2023).

Single-strain vs. Multi-strain Probiotics

Single-strain probiotics: These probiotics involve the use of a single species or strain of bacteria (Table 3). However, the efficacy of single-strain probiotics showed variability in clinical outcomes. The Probiotics in Preterm Infants Study (PiPs) trial evaluated the efficacy of *Bifidobacterium breve* BBG-001 in reducing complications, viz. NEC, late-onset sepsis and mortality in PTIs. No significant evidence supporting the protective effects of BBG-001 against complications in PTIs was observed (Costeloe *et al.*, 2016). Due to a lack of diverse mechanisms required to combat the complexity of neonatal dysbiosis and inflammation, single-strain probiotics may not be sufficient to address diverse challenges in PTIs. They may provide limited colonization benefits as seen in strains, viz. *B. breve*, which lacks genes for digesting HMOs and thus resulting in reduced ability to colonize infant gut effectively as compared to *B. infantis* and *B. bifidum* (Gotoh *et al.*, 2018). Despite their limitations, certain single strains, viz. *Lactobacillus rhamnosus* GG showed improved immune response, reduced sepsis risk and gut microbiome modulation effectively (Sharif *et al.*, 2023)

Table 3: Difference between single-strain and multi-strain probiotics.

Feature	Single-Strain Probiotics	Multi-Strain Probiotics
Mechanisms	Limited to the functionalities of one strain.	Diverse, Using complementary mechanisms.
Efficacy	Variable.	Consistently effective in reducing NEC and sepsis.
Colonization	May not persist without consistent supplementation.	Better colonization due to synergistic actions.
Clinical Evidence	Mixed outcomes; Some strains effective, others not	Strong evidence from trials like ProPrems.

Multi-strain probiotics: These probiotics combine multiple bacterial species or strains (Table 3). Studies suggest the potential benefit of multi-strain probiotics, viz. *Bifidobacterium* and *Lactobacillus* in preventing NEC in PTIs (Sharif *et al.*, 2023; Thomas *et al.*, 2017; Denkel *et al.*, 2016). Meta-analysis of 8 randomized controlled trials revealed a lower number of NEC cases when PTIs were supplemented with multi-strain probiotics (Thomas *et al.*, 2017). ProPrems Trial have demonstrated the efficacy of multi-strain probiotics in reducing NEC incidence by 54% in PTIs (Garland *et al.*, 2011). The trial used a combination of *Bifidobacterium breve* HA129, *Bifidobacterium infantis* HA116, *Bifidobacterium bidifum* HA132, *Bifidobacterium longum* HA135 and *Lactobacillus rhamnosus* HA711. Multi-strain probiotics have been shown to profoundly reduce NEC cases as compared to single-strain probiotics in the PiPs trial (Costeloe *et al.*, 2016).

The synergistic effects of multi-strain probiotics are particularly beneficial for restoring gut balance in PTIs. *Lactobacillus spp.* create an anaerobic environment required for the growth of *Bifidobacterium bifidum*, which digests HMOs and inhibits pathogens like *Klebsiella pneumoniae*. By producing SCFAs, multi-strain probiotics improve gut health, inhibit pathogen colonization and modulate immune responses (Chi *et al.*, 2020).

Probiotics for Pregnant Women

Vaginal Health and Probiotics

Probiotics in BV and PTBs: Administration of probiotics orally or vaginally has been known to encourage the proliferation of beneficial bacteria in the vaginal microbiota (Eade *et al.*, 2012). A reduced number of *Lactobacillus* was found to be associated with increased risk of BV in pregnant women. Oral or vaginal supplementation with *L. casei rhamnosus* reduced the incidence of BV in the first trimester of pregnancy (Petricevic *et al.*, 2023). Consumption of capsule formulations of dried viable *L rhamnosus* GR-1 and *L reuteri* RC-14 colonized vaginal tract effectively, and resulted in reduced overgrowth of pathogens viz.

Gardnerella vaginalis, Prevotella spp. and *Atopobium vaginae* (Petricevic *et al.*, 2008). *Lactobacillus plantarum* Lp62 showed inhibitory effects on *Gardnerella vaginalis* ATCC49154 during in-vivo and in-vitro studies (Selis *et al.*, 2021). Regular probiotic use during pregnancy helps maintain vaginal health and reduce the recurrence of BV, a major risk factor for PTBs.

Probiotics and GBS: GBS colonization in the vaginal tract is one of the leading causes of neonatal sepsis. Probiotics, particularly *L. plantarum* and *L. reuteri*, demonstrated inhibitory effects on GBS colonization by producing antimicrobial substances and increasing vaginal immunity (Wasfi *et al.*, 2018). Regular probiotic use can lower GBS prevalence and reduce the risk of neonatal complications.

Treatment with Antibiotics and Probiotics: While antibiotics are commonly prescribed for BV, they often fail to differentiate between beneficial and harmful bacteria and create a sterile environment. Probiotics supplementation during antibiotic treatment replaces pathogens in the vagina and populate themselves, thus decreasing the recurrence rate of BV as compared to antibiotic treatment alone (Liu and Yi, 2022). Probiotics administered with antibiotics reduced overgrowth of pathogens, viz. E. coli and normalized gut and vaginal microbiota post-antibiotic treatment (Kabbani *et al.*, 2017). *Bifidobacterium* levels were maintained during combined treatment, and it showed no effect on *E. coli* when it was supplemented with antibiotics (Zhong *et al.*, 2021). Concern was raised about probiotic safety during antibiotic treatment for transmission of antibiotic-resistant genes. Zhong et al (2021) confirmed that some strains, viz. *Sachharomyces boulardii* carry minimal risk of transmitting antibiotic resistance and are considered safe for use in neonates and pregnant women.

Probiotics alone had a minimal and temporary effect on gut microbiota during antibiotic treatment, which made it ineffective in preventing antibiotic-induced dysbiosis (a decrease in gut microbial diversity). The combined use of prebiotics and probiotics (Synbiotics) showed superior efficacy in restoring microbial imbalance after antibiotic treatment, as it enhanced colonization by beneficial bacteria and improved vaginal health outcomes by reducing complications like BV and PTBs (Elias *et al.*, 2023)

Probiotics and vaginal immunity: Probiotics have increased the production of sIgA and cytokines in the vagina and thus stimulating vaginal immunity as determined by Eade *et al.* (2012). These immune components strengthen the vaginal barrier and provide protection against infections, supporting maternal and fetal health during pregnancy.

Gut Microbiota and Pregnancy Outcomes

Maternal gut microbiota undergoes significant changes during pregnancy, influencing maternal health, fetal development and pregnancy outcomes. Probiotics play a critical role in maintaining microbial balance, reducing pregnancy complications such as GDM, inflammation and supporting maternal and fetal well-being.

Probiotics and GDM: Supplementing probiotics improved glucose metabolism and reduced inflammatory markers in women with GDM. Strains viz. *Lactobacillus acidophilus* and *Bifidobacterium bifidum* regulate gut microbiota and increase insulin sensitivity (Ari *et al.*, 2022). Use of probiotics as an alternative to insulin therapy reduced insulin resistance and improved lipid metabolism. Synbiotics also improved glucose control and lipid profile which underscores their efficacy in managing GDM (Mu *et al.*, 2023).

Immune modulation and inflammation: Dysbiosis associated outcomes, viz. PTBs and preeclampsia can be regulated by the immune system during pregnancy. Probiotics increased anti-inflammatory cytokines such as IL-10 while reducing pro-inflammatory markers like TNF-α and IL-6. This immune modulation helps in maintaining pregnancy and supports fetal development (Elias *et al.*, 2023). Probiotics supplementation enhanced the production of SCFAs, which improved gut barrier function, reduced inflammation and supported fetal neurodevelopment (Ari *et al.*, 2022). Improved SCFA levels are associated with better placental function and reduced risk of complications such as Intra Uterine Growth Restriction (IUGR).

Role of probiotics in Gut—Brain Axis during pregnancy: Gut-brain axis plays an important role in maternal mental health. Dysbiosis during pregnancy has been linked to anxiety, depression and stress, which negatively impact fetal development. Probiotic strains, viz. *Lactobacillus helveticus* and *Bifidobacterium longum* modulated the gut-brain axis and reduced symptoms of anxiety and depression (by increasing serotonin and GABA production) (Halemani *et al.*, 2023). Probiotics modulated the Hypothalamic-pituitary-adrenal axis and thus reduced cortisol levels and improved stress response during pregnancy (Trifkvoic *et al.*, 2022). Improved mental health contributed to better pregnancy outcomes and maternal well-being

Probiotics and prevention of maternal infections: In addition to maintaining vaginal and gut microbiota, probiotics also prevent systemic and urinary infections in pregnant women. UTIs can lead to PTBs if left untreated. Distal urethral isolates such as *L. rhamnosus* GR-1 and *L. fermentum* B-54 with RC-14 showed efficacy in preventing UTIs by inhibiting the growth of pathogens and enhancing host immune responses (Naqvi *et al.*, 2022). By reducing systemic inflammation, probiotics might prevent recurrence of UTIs and reduce the need for repeated antibiotic interventions.

Alternative Approach to Deliver Probiotics to Pregnant Women

Method of delivering probiotics ensures efficacy in improving maternal health and preventing complications such as BV, UTIs and PTBs. These approaches aim to optimize probiotic delivery for maximum microbial colonization and health benefits.

Vaginal Microbiota Transplantation (VMT): VMT is an innovative approach that involves transplanting vaginal microbiota from a healthy donor to a recipient with severe VD or recurrent BV. VMT restored microbial diversity, reduced BV recurrence and lowered PTBs in high-risk pregnancies (Vieira Batista *et al.*, 2022). Studies demonstrated that VMT increased the number of *L. crispatus*

(healthy vaginal species) and reduced pro-inflammatory and dysbiosis-associated bacteria after transplantation from the healthy vaginal microbiome and resulted in live birth (Wronding *et al.*, 2023). This technique may also benefit pregnant women undergoing C-section as it can simulate natural microbial exposure for their neonates (Mueller *et al.*, 2020).

Vaginal seeding/micro-birthing: Vaginal seeding or micro-birthing involves swabbing neonates delivered via C-section with maternal vaginal secretions. This approach aims to introduce beneficial microbes early in life, potentially enhancing the growth and development of an infant's gut microbiome (Mueller *et al.*, 2020; Lokugamage *et al.*, 2016). While this approach is beneficial, strict protocols are required to ensure the safety of both mother and child, particularly in the presence of maternal infections.

Dosage and Timing of Probiotics for PTIs and Pregnant Women

Probiotic efficacy in managing complications in PTIs and pregnant women depends on optimal dosage and timely administration. The recommended dosage and timing vary based on population, climate condition and probiotic strain. These parameters are crucial for achieving maximum therapeutic benefits while minimizing risks.

Dosage and Timing for PTIs

The dosage for PTIs typically ranges between 10^6 to 10^{10} colony-forming units (CFUs) per day. It varies according to strain and specific health outcome being targeted (Poindexter *et al.*, 2021). Panel of experts, including the American Academy of Paediatrics, Canadian Paediatric Society, European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) recommended:

- Multi-strain combinations: A combination of *B infantis*, *B lactis*, and *S. thermophilus* at 3.0 to 3.5 × 10^8 CFUs of each strain for reducing NEC and improving feeding tolerance.
- Strain-specific dosages:
  - *L rhamnosus GG: Dosage* range from 1 × 10^9 CFUs to 6 × 10^9 CFUs per day.
  - *S. boulardii*: Dosage of 5 x 10^9 CFUs per day for modulating gut microbiota and preventing infections (Xu *et al.*, 2016).

The timing of probiotic administration in PTIs could start with early supplementation or delayed supplementation. Early supplementation involves initiating probiotics within the first week of life to prevent NEC and sepsis. It supports faster colonization by beneficial bacteria, reducing dysbiosis and inflammation (Park *et al.*, 2023). However, probiotic efficacy diminishes when administration is delayed beyond two weeks, which limits their ability to prevent NEC and sepsis. Long term supplementation beyond the neonatal period is still debated. While probiotic use may sustain gut health benefits, it could increase the risk of probiotic sepsis or altered immune response.

Dosage and Timing for Pregnant Women

- *Lactobacillus* Strains: Dosage of 1 x 10^9 CFUs daily to reduce BV (Petricevic *et al.*, 2008).
- Synbiotics (*Lactobacillus acidophilus* and *Bifidobacterium bifidum*) at 1-3 x 10^9 CFUs to enhance gut microbiota diversity and reduce GDM-associated inflammation (Ari *et al.*, 2022).

Probiotic supplementation in the first trimester reduces the risk of BV and PTBs as it supports vaginal and gut-microbial balance during early fetal development (Petricevic *et al.*, 2023). Probiotics supplementation throughout pregnancy manages GDM, reduces inflammation in addition to supporting maternal and fetal health. Strain-specific safety profiles must be evaluated to prevent adverse outcomes, especially in women with underlying health conditions or antibiotic use (Xu *et al.*, 2016).

The appropriate dosage and timing of probiotics are critical for maximizing their benefits in PTIs and pregnant women. Early initiation (in PTIs) within the first week of life and in the first trimester (in pregnant women) ensures optimal outcomes.

CHALLENGES AND FUTURE PROSPECTS

Probiotics have shown promising results in neonatal care, gastrointestinal disorders and pregnancy-related conditions. Despite that, the safety of probiotic supplementation in PTIs regarding risk of sepsis, antibiotic resistance and altered immune response is still a major concern. The potential of probiotics to translocate from the gut into the bloodstream highlights the need for rigorous safety assessments and monitoring protocols.

Lack of standardized methodologies and inconsistencies in findings across studies pose a challenge in the interpretation and translation of research into clinical practice. Establishing uniform protocols, strain-specific guidelines, and validated biomarkers is crucial for advancing probiotics research. Administering probiotics in NICUs also raises ethical concerns regarding strain selection, dosing regimen and potential adverse effects in vulnerable populations.

Longitudinal studies are essential to evaluate long-term effects of probiotics on neonatal health outcomes (neurodevelopment, metabolic health and immune function). Comprehensive follow-up assessments are needed to assess the impact of probiotic interventions beyond the neonatal period and into adulthood. Despite their widespread use, no probiotics have received FDA approval for clinical use. Many probiotics remain in the trial phase, and recent reports of deaths in infants associated with illegal probiotic products underscore the urgent need for regulatory scrutiny and enforcement.

Future directions include the development of precision medicine approaches to optimize probiotic selection and dosing regimens, maximizing therapeutic efficacy and minimizing adverse effects. Engineered probiotics with enhanced therapeutic properties, viz. improved survival in the gastrointestinal tract, targeted delivery of bioactive molecules, and enhanced immunomodulatory effects, hold promise for addressing specific neonatal health challenges.

Genetically modified strains could be designed to express therapeutic proteins or metabolites with potent antimicrobial, anti-inflammatory, or neuroprotective properties. Research is needed to elucidate the mechanisms underlying the effects of probiotics on maternal gut health and neonatal outcomes and to establish evidence-based guidelines for their use during pregnancy.

CONCLUSION

Probiotics hold significant potential as therapeutic agents for promoting neonatal health and offer a range of potential benefits, including prevention of NEC, modulation of immune function, enhancement of gut barrier integrity and promotion of microbial balance. However, realizing the full potential of probiotics in neonatal care requires addressing various challenges and exploring future prospects.

Personalized probiotic interventions tailored to individual neonatal characteristics, synbiotic formulations and engineered probiotics with enhanced therapeutic properties represent promising avenues for optimizing probiotic efficacy. Longitudinal studies tracking the long-term effects of probiotic supplementation on neonatal health outcomes are essential for evaluating both efficacy and safety over time. Comprehensive follow-up assessments extending into childhood and adulthood are also necessary.

Challenges such as safety concerns, standardization of probiotic products, regulatory hurdles, and ethical considerations must be carefully addressed to ensure the safe and effective use of probiotics in neonatal populations. Collaborative efforts involving researchers, healthcare providers, policymakers, and regulatory agencies are important to advance probiotic research, translate findings into clinical practice and ultimately improve outcomes for PTIs and other neonates.

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Conflict of Interest

The authors declare that there is not any conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

Life Science Reporting

No life science threat was practised in this research.

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