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EPIGENETIC SHIFTS IN PRETERM NEONATAL MICROBIOME

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Abstract

Introduction and Study Aim: Preterm birth (<37 weeks) causes ~15 million births yearly and is a major contributor to neonatal morbidity and mortality [1]. These infants face developmental challenges, including disrupted microbial colonization and immature regulatory systems. Epigenetic modifications—heritable changes in gene expression without DNA sequence alteration—mediate environmental influences during this critical period [2]. This paper explores how the preterm microbiome and epigenetic mechanisms interact and influence health outcomes.

Brief Description of Current Knowledge: The preterm gut microbiome shows reduced diversity and more hospital-acquired bacteria [3]. Cesarean delivery, antibiotics, and lack of maternal microbes contribute to this dysbiosis. Beneficial colonizers (e.g., bifidobacteria) are reduced, while pathogens (e.g., Staphylococcus, Enterobacteriaceae) increase, linked to NEC and sepsis. Epigenetic shifts also occur rapidly in response to inflammation and stress [3]. Microbial metabolites (e.g., butyrate, folate) can alter epigenetic programming [5], and prenatal epigenetic states may shape microbial colonization [2].
Summary/Conclusions: Microbiome-epigenome interactions may shape immunity and development beyond infancy. Disruptions may imprint harmful epigenetic changes. More research is needed to clarify mechanisms and guide interventions like probiotics or epigenetic therapies.

Keywords

Gut microbiome, Preterm infants, Neonatal intensive care, Probiotics, Microbial metabolites, Intrauterine inflammation, Breastfeeding

1. Introduction

Preterm birth is a widespread global health challenge, with more than one in ten infants born prematurely each year [1]. Complications arising from prematurity (e.g. respiratory distress, infection, and neurologic injury) make it the leading cause of neonatal mortality and a major contributor to under-5 childhood mortality [1]. Survivors often face long-term health issues. A critical but underexplored aspect of preterm infant health is the interplay between early-life microbiological exposures and the infant's epigenetic development. The early neonatal period represents a window of developmental plasticity when environmental factors can have lasting effects on gene expression and physiology [2]. Epigenetic modifications – including DNA methylation, histone modification, and non-coding RNA regulation – allow environmental inputs to "program" gene activity without altering the genetic code.

One especially important environmental influence in neonates is the establishment of the gut microbiome. During a normal full-term birth, infants are colonized by maternal vaginal and intestinal microbes that help educate the immune system and support healthy organ development. In preterm neonates, however, this colonization process is often disrupted. Many are delivered via cesarean section or after intrauterine infection, spend critical weeks in neonatal intensive care units (NICUs), and receive broad-spectrum antibiotics. The result is an aberrant initial microbiome characterized by low diversity and a prevalence of microbes uncommon in term infants [3]. This dysbiotic colonization has been linked to conditions like necrotizing enterocolitis (NEC), a devastating inflammatory bowel disease primarily affecting preterms [3]. At the same time, preterm infants' immature physiological systems respond to these environmental challenges in ways that may involve epigenetic reprogramming of key genes, especially those governing immune and inflammatory responses [3]. Recent

research suggests that the microbiome and the epigenome are not independent factors but interactive players in neonatal development [2]. Microbial colonization can drive epigenetic changes in host tissues, and conversely prenatal epigenetic states (shaped by maternal conditions or exposures) can influence microbial growth niches in the infant gut [2]. This emerging paradigm implies that early microbiota-epigenome crosstalk may set the trajectory for an infant's health, influencing not only immediate neonatal outcomes but also long-term susceptibility to disorders like allergies, asthma, metabolic disease, and others [2,21]. However, many aspects of this interplay remain poorly understood. Key questions include: How do specific microbes or their metabolites alter host epigenetic marks? Can we pinpoint epigenetic mechanisms that predispose preterm infants to diseases like NEC? Which findings from animal or in vitro models translate to human preterm infants? Addressing these questions is vital, as it could open new avenues for preventative or therapeutic strategies that modify the microbiome or epigenome to improve outcomes.

The aim of this paper is to review and synthesize current knowledge on the relationship between the preterm neonatal microbiome and epigenetic shifts. We integrate microbiological and epigenetic perspectives to highlight how early microbial colonization patterns in preterm infants can affect gene regulation, and how epigenetic mechanisms may mediate or modulate critical health outcomes. We also discuss recent discoveries, current controversies, and potential clinical implications, identifying areas where further investigation is needed.

2. Review of Current Knowledge

2.1. Preterm Neonatal Microbiome: Characteristics and Influencing Factors

Microbial colonization in preterm vs. term infants: The establishment of the neonatal microbiome begins around the time of birth and continues through infancy. In healthy full-term infants born vaginally, the gut is rapidly colonized by a succession of microbes (initially facultative aerobes like *Staphylococcus*, *Streptococcus*, *Enterobacteriaceae*, followed by anaerobes such as *Bifidobacterium* and *Bacteroides*) that typically culminates in a diverse, stable microbiota by 2–3 years of age [2]. Preterm infants, in contrast, often acquire a very different microbial profile. Their gut microbiome is characterized by low species diversity, high instability, and a predominance of organisms adapted to the NICU environment [3]. Common colonizers in preterms include Proteobacteria (e.g. *Escherichia coli*, *Klebsiella*) and Firmicutes such as *Staphylococcus* (including *S. epidermidis*) and *Enterococcus*, often reflecting exposure to hospital flora. Beneficial commensals like *Bifidobacterium* (typically acquired from maternal vaginal flora and breast milk) are less prevalent or delayed in colonization [2,3]. Studies have repeatedly shown that preterm infants have a higher relative abundance of potentially pathogenic bacteria and fewer strict anaerobes compared to term infants, especially in the first weeks of life [2,6]. For example, a recent metagenomic survey of 236 preterm infants in NICUs found that Enterobacteriaceae, Staphylococcaceae, and Enterococcaceae dominate the preterm gut, and overall microbial diversity remains low during the NICU stay [6].

Several factors contribute to this dysbiosis in preterm neonates:

- Delivery mode: Vaginal delivery exposes infants to the maternal vaginal and gut microbiota, whereas cesarean section (common in preterm or medically indicated deliveries) bypasses this exposure. C-section infants, including preterms, often show aberrant colonization patterns with fewer maternal microbes and more skin or environmental organisms [2]. Notably, large epidemiological studies have linked C-section birth with higher risks of immune-mediated disorders later in childhood, potentially due to these altered early microbiomes [2].

- Antenatal factors: Intrauterine conditions can influence initial microbial seeding. It was long assumed that the fetus develops in a sterile environment, but evidence of bacterial DNA in placental tissue and preterm infant meconium suggests that in utero microbial exposure might occur [5]. Intrauterine infection or chorioamnionitis is a major cause of preterm labor and can lead to the fetus being exposed to microbial products before birth. Such exposure not only triggers preterm birth but also may prime the neonatal immune system and affect early colonization patterns. However, the concept of a "fetal microbiome" remains controversial, as some experts argue these findings could be due to contamination or non-viable bacteria. This is an area of active investigation and debate.

- Neonatal intensive care environment: Preterm babies often require intensive care in incubators, where exposure to maternal microbes is limited. Instead, their microbiome assembly is influenced by the

NICU environment – including contact with healthcare workers, surfaces, and medical devices – and frequent use of antibiotics. Early antibiotic exposure is nearly ubiquitous in very preterm infants (as prophylaxis for infection), and it dramatically perturbs gut microbiota. Broad-spectrum antibiotics can delay or suppress colonization by normal flora and allow antibiotic-resistant or opportunistic organisms to dominate [2]. For instance, preterm infants given antibiotics show lower levels of Bifidobacterium and Bacteroidetes and higher proportions of Proteobacteria. Prolonged hospitalization further exposes infants to hospital-associated microbes (like *Clostridioides difficile* or resistant staphylococci) that can colonize the gut [6].

- Feeding type: Diet is a crucial determinant of the infant microbiome. Breast milk contains not only nutrients but also maternal antibodies, human milk oligosaccharides (HMOs), and beneficial bacteria that promote colonization by bifidobacteria and other commensals. Preterm infants fed breast milk tend to have more favorable microbiota (e.g. more *Bifidobacterium* and *Lactobacillus*) compared to those fed formula [2]. In contrast, formula feeding lacks HMOs and may lead to a microbiome with more proteobacteria. Many preterms initially receive parenteral nutrition or delayed enteral feeding due to medical instability, which can further delay normal microbial colonization of the gut.

- Other factors: Lack of skin-to-skin contact (kangaroo care) can reduce transfer of parental skin microbiota. Additionally, gestational age itself plays a role – the more premature the infant, the less developed the gut environment and immune factors that help shape microbial communities. Together, these factors result in a microbiome in preterm neonates that is often described as "dysbiotic" or suboptimal for healthy development [3].

Microbiome and disease in preterm infants: Alterations in the early microbiome of preterm infants have been associated with several clinical conditions. The clearest example is necrotizing enterocolitis (NEC). NEC affects ~5–10% of very low birth weight preterm infants and involves intestinal inflammation, necrosis, and risk of systemic sepsis. Microbial imbalance is thought to be a key contributor to NEC pathogenesis. Studies have found that before the onset of NEC, infants often exhibit a bloom of Proteobacteria (Gram-negative bacteria) and a drop in diversity and beneficial Firmicutes [3]. No single pathogen consistently causes NEC, but rather a dysbiotic community triggers abnormal immune activation in an immature gut. Feeding practices influence NEC risk: exclusive breastfeeding is protective, while formula feeding increases risk, reflecting the role of microbiota in mediating these effects [3]. Similarly, early antibiotic use has been linked to higher NEC incidence, presumably by disturbing the microbiome balance [3]. Probiotic supplementation (with strains like *Bifidobacterium* and *Lactobacillus*) in preterm infants has shown efficacy in reducing NEC in multiple trials [3], reinforcing the idea that fortifying the microbiome can improve outcomes. Aside from NEC, an aberrant microbiome in preterms has been associated with sepsis (invasive infections often caused by gut-derived bacteria), poor growth, and possibly pulmonary outcomes via the gut-lung axis. As described in a gnotobiotic mouse model, transferring the gut microbiota of high-growth vs. low-growth preterm infants into germ-free mice can reproduce the growth phenotype: mice colonized with a “healthier” preterm microbiome showed better weight gain and intestinal development than those colonized with a dysbiotic microbiome [3]. This suggests that early microbial communities can influence systemic development, likely through effects on nutrient absorption, metabolism, and immune regulation. In summary, the preterm neonatal microbiome is distinct from that of term infants, characterized by dysbiosis attributable to medical and environmental factors. This altered microbiome has important implications for the infant’s health, both in the short term (risk of NEC, sepsis, growth faltering) and potentially long term (immune and metabolic programming). Importantly, these microbiome patterns do not act in isolation – they intersect with the infant’s physiological responses, including epigenetic regulation, which we explore next.

2.2. Epigenetic Landscape in Preterm Neonates

Epigenetic regulation and neonatal development: Epigenetic processes are central to how infants adapt to extrauterine life. During fetal and neonatal development, cells undergo extensive epigenetic remodeling to activate tissue-specific gene programs and respond to environmental cues [4]. The main epigenetic mechanisms include DNA methylation (typically gene-silencing when in promoters), post-translational histone modifications (which can open or compact chromatin to regulate gene expression), and regulatory non-coding RNAs (like microRNAs that modulate mRNA stability). These mechanisms enable a fine-tuned response to environmental factors such as nutrient availability, oxygen levels, and microbial signals. In preterm infants, the abrupt transition from the womb to the external environment at an earlier-than-expected developmental stage may induce unique epigenetic shifts. Evidence of epigenetic changes in preterm infants: Research indicates that preterm birth itself is associated with distinctive epigenetic profiles. For example, one study found marked DNA

methylation changes in intestinal genes during the perinatal period of preterm neonates [4]. This suggests that the stress of premature exposure (likely including oxygen, nutrition, and microbial exposure changes) triggers methylation adjustments in the gut, which could affect intestinal maturation and immunity. Another study on very preterm infants observed that those who experience significant neonatal complications (such as bronchopulmonary dysplasia, severe infections, or brain injury) show differences in DNA methylation patterns at discharge compared to those without such morbidities [9]. Specifically, differential methylation was found at sites in genes related to immune and developmental pathways, and the extent of methylation change often correlated with the number and severity of complications [9]. This supports the notion that early-life stresses leave epigenetic "scars" or signatures in the infant's genome. A major antenatal factor is intrauterine inflammation (often stemming from infection). Maternal infections and chorioamnionitis can prompt preterm labor and directly impact the fetal immune system. Fetal exposure to inflammatory cytokines has been linked to epigenetic alterations in immune-related genes. Lu and Claud (2018) report that in experimental models, prenatal exposure to bacteria or inflammatory signals resulted in DNA methylation changes in Toll-like receptor (TLR) signaling pathway genes in the fetal intestine [3]. TLRs are crucial innate immune receptors that detect microbial components. Aberrant methylation of TLR genes can alter their expression. Indeed, hypermethylation of certain TLR pathway genes (e.g. *TLR2*, *IRF1*, *IL6R*) and hypomethylation of others (*CD14*, *TLR4* regulators) was observed after in utero inflammation, correlating with altered TLR expression [3]. These epigenetic modifications persisted postnatally and were accompanied by heightened inflammatory responsiveness. Such findings illustrate how a preterm infant's epigenome might be "pre-programmed" by prenatal events in ways that modulate the response to microbes after birth. Beyond DNA methylation, other epigenetic layers are also at play. Preterm infants often endure pain, oxygen fluctuations, and nutritional instability in NICU – factors that may influence histone modification patterns or microRNA expression involved in stress-response pathways. While large-scale data on histone marks in preterm infants are limited, animal models provide clues. For example, exposure of neonatal rodents to stress or high oxygen has been shown to change histone acetylation in the brain and lungs, potentially contributing to neurodevelopmental and pulmonary outcomes. In humans, one recent epigenome-wide study identified associations between the degree of prematurity and methylation of genes linked to neurological development and white matter maturation [22], suggesting epigenetics could partly explain higher risks of neurodevelopmental delays in preterms [22]. Crucially, epigenetic changes in early life can have long-term consequences. The developmental origins of health and disease (DOHaD) concept posits that early environmental influences (including nutrition and stress) induce epigenetic modifications that persist and shape susceptibility to diseases later in life. For instance, children born preterm have higher rates of certain chronic conditions (such as asthma and metabolic syndrome) in adulthood. Epigenetic imprinting in genes related to immune regulation or metabolism may underlie these epidemiologic links. Some studies have noted that preterm infants show accelerated "epigenetic aging" or distinct DNA methylation age estimates compared to chronological age, but the implications of this are still being studied.In summary, preterm neonates undergo significant epigenetic modifications as they adapt to extrauterine life. These modifications are influenced by prenatal exposures (infection, steroids given to mature fetal lungs, etc.) and postnatal factors (nutrition, oxygen, stress). Altered epigenetic patterns have been documented in key genes, especially those involved in immune responses and development, which could affect the infant's ability to handle challenges. The next section examines how these epigenetic changes intersect with the microbial environment of the infant – the core of the microbiome-epigenome crosstalk.

2.3. Microbiome–Epigenome Crosstalk in Preterm Infants

It is increasingly evident that the gut microbiome and the host epigenome influence one another in a bidirectional relationship [2]. This crosstalk is particularly critical in early life, when both the microbiome is being established and the epigenetic landscape is highly malleable. Several mechanisms underpin this interaction: Microbial influences on the epigenome: The gut microbes produce a variety of metabolites and molecular signals that can reach host cells and alter epigenetic regulation. One prominent example is short-chain fatty acids (SCFAs), such as butyrate and acetate, produced by bacterial fermentation of dietary components. SCFAs can function as inhibitors of histone deacetylases (HDACs) and thereby increase histone acetylation in host cells, leading to a more open chromatin state and gene activation. Butyrate in particular is known to promote regulatory T-cell development via epigenetic mechanisms and strengthen gut barrier function. In the context of preterm infants, who often have lower SCFA-producing bacteria early on, an insufficient supply of these metabolites could alter normal epigenetic programming of gut immunity. Additionally, microbes synthesize vitamins (like folate and biotin) and other cofactors that feed into the host one-carbon metabolism and histone modification pathways. The early infant microbiota thus produces active metabolites such as folate, butyrate, and acetate, which can epigenetically alter gut epithelial, hepatic, and immune cells [5]. These alterations constitute a form of developmental programming; for example, microbial-driven epigenetic changes in infancy might later translate into altered risks for diseases like obesity, allergies, or inflammatory bowel disease [5].

Moreover, pattern recognition of microbial components can trigger epigenetic changes in immune cells. Colonizing bacteria continuously interact with intestinal epithelial cells and gut-associated immune cells. Commensal microbes can induce tolerogenic epigenetic patterns – e.g. increased histone acetylation at genes for anti-inflammatory cytokines – training the immune system to be less reactive to harmless antigens. Conversely, pathogenic or dysbiotic bacteria might provoke epigenetic reprogramming that skews toward pro-inflammatory gene expression. Indeed, one study demonstrated microbe-specific patterns of DNA methylation in immature human enterocytes exposed to different bacterial species: a probiotic organism and a pathogenic bacterium elicited distinct DNA methylation changes in host cells [2]. This indicates that even at the same developmental stage, the type of microbial signal can imprint unique epigenetic signatures.

Epigenetic influences on the microbiome: The crosstalk is not one-way; the host's epigenetic configuration can shape the gut habitat and thereby influence which microbes thrive. Epigenetic regulation of genes involved in mucus production, antimicrobial peptide expression, and immune receptor levels can determine the intestinal environment that microbes encounter. For instance, DNA methylation or histone modifications in the promoters of genes encoding mucins or defensins could alter their expression, changing gut niches. The earlier-mentioned study by Cortese et al. (2016) provided striking evidence of this reverse influence: they found that prenatal exposure to the synthetic glucocorticoid dexamethasone in mice induced epigenetic changes in the offspring, and those changes led to altered gut microbiota composition at 2 weeks of life [4,5]. In other words, an epigenetically modified host gave rise to a different microbial community than unexposed controls. Similarly, the work by Lu and colleagues showed that epigenetic modifications of TLR and tight-junction genes caused by intrauterine inflammation were associated with differences in early microbial colonization patterns in preterm infants [3]. The mice with TLR gene hypermethylation had distinct microbial profiles compared to those without such changes [3]. This suggests that certain epigenetic states might favor colonization by specific bacteria (perhaps by dampening inflammatory responses and allowing tolerance of commensals, or conversely by creating an inflammatory milieu that only hardy opportunists can endure).

Interplay in immune training: The concept of trained immunity offers a framework for how microbiome-epigenome interactions educate the neonatal immune system. Trained immunity refers to long-term functional reprogramming of innate immune cells (monocytes, macrophages, NK cells) after exposure to microbial stimuli, leading to an altered response to subsequent infections. This reprogramming is largely mediated by epigenetic modifications in innate immune cells. In neonates, initial colonization likely "trains" immune cells in the gut and systemically. A balanced microbiome might train innate immunity to be appropriately responsive (e.g. via epigenetic marks that enhance anti-pathogen genes but also maintain tolerance), whereas a dysbiotic microbiome could train immunity in maladaptive ways. For example, neonatal colonization with beneficial microbes could imprint monocytes with protective histone modifications that reduce later hyperinflammation, while colonization with predominantly endotoxin-producing Gram-negative bacteria could leave a persistent epigenetic memory that skews towards pro-inflammatory cytokine production. Evidence in adults shows that differences in gut microbiota correlate with differences in inflammatory gene methylation (for instance, obese individuals with an altered microbiome have higher methylation of the TLR4 gene in blood cells) [2]. In preterm infants, this area is just beginning to be explored. There is interest in whether early microbiome profiles can predict epigenetic markers of trained immunity that relate to conditions like necrotizing enterocolitis or even later-life allergy and asthma risk.

Current evidence and knowledge gaps: Current data strongly support the existence of microbiome-epigenome crosstalk in the neonatal period. The direct demonstration of microbe-induced host DNA methylation changes and host epigenome-induced microbiome shifts in model systems [2] provides a mechanistic basis for observations linking microbiota and infant health. Moreover, correlations in human studies (e.g. between low microbial diversity and methylation of immune genes, or between probiotic use and epigenetic aging markers) are starting to emerge. However, many questions remain unanswered or under investigation:

- Specific pathways: Which specific bacterial species or metabolites are the strongest drivers of epigenetic changes in preterm infants? SCFAs are one known factor, but others include polyamines, lactate, and microbially modified bile acids. Identifying key molecular mediators will help target interventions.

- Timing and persistence: When during development do microbiome-induced epigenetic changes occur, and are they transient or long-lasting? It is unknown if epigenetic marks set in the NICU period persist into childhood or are gradually overwritten as the microbiome matures. Longitudinal studies tracking infants over time are needed.

- Beneficial vs harmful programming: We need to distinguish which epigenetic changes are adaptive (e.g. promoting maturation and tolerance) versus maladaptive (predisposing to inflammation or disease). Not all changes are bad; some may be essential for normal immune development. For example, colonization likely induces epigenetic activation of genes that strengthen the gut barrier – a beneficial effect. In contrast, epigenetic suppression of certain immune regulators might predispose to NEC.

- Individual variability: Genetic factors and sex differences may influence how an infant's epigenome responds to microbes. Some infants might be more susceptible to microbiome-related epigenetic dysregulation than others. Understanding this variability could lead to personalized approaches (identifying high-risk infants who might benefit from specific probiotic or epigenetic treatments).

2.4. Clinical Implications and Future Directions

The interplay between the microbiome and epigenome in preterm infants has significant clinical implications. It suggests that some of the most serious complications of prematurity may be approached by modulating not just one factor (microbes or genes) but the interplay between the two.

Preventing and managing NEC: Given the evidence that both an abnormal microbiome and aberrant host responses (potentially epigenetically primed) contribute to NEC, interventions could target both sides of this equation. Probiotics have already shown success in reducing NEC incidence, likely by fostering a healthier microbiome that produces beneficial metabolites and competes with pathogens. It is conceivable that probiotic administration might also directly or indirectly influence epigenetic programming in the gut – for instance, by increasing butyrate production, which then modulates histone acetylation in intestinal cells to reduce pro-inflammatory gene expression. Future therapies might include postbiotics (metabolic products of microbes) like butyrate analogues to ensure the preterm gut receives appropriate epigenetic signals even if the microbiome is immature. Additionally, understanding epigenetic risk markers for NEC (such as methylation patterns in TLR or cytokine genes) could enable early identification of infants who are at high risk and might benefit most from aggressive microbiome modulation or other preventative strategies.

Long-term health and developmental outcomes: The notion that early microbiome and epigenome interactions can shape lifelong health opens possibilities for early intervention to improve adult outcomes of former preterm infants. For example, preterm birth has been associated with higher rates of asthma and allergies. If disrupted early colonization contributes via improper immune training and epigenetic imprinting, then interventions like maternal microbial modulation (ensuring mothers have healthy microbiota during pregnancy), gentle introduction of diverse microbiota after birth (while minimizing antibiotics), or even epigenome-targeted drugs could theoretically reduce later atopy or wheezing disorders. Another area is neurodevelopment: certain microbial profiles (rich in anti-inflammatory components) might favor epigenetic environments that protect the developing brain from injury. Ongoing studies are examining whether supplementation with specific microbes (like *Lactobacillus* or *Bifidobacterium*) in the NICU can influence neuroinflammatory gene expression and improve cognitive outcomes years later.

Reversibility and therapeutic targeting: A hopeful insight from recent research is that some epigenetic changes may be reversible or modifiable with changes in the environment [3]. Unlike genetic mutations, epigenetic marks can potentially be re-set. Experiments in animals have shown that an adverse epigenetic state induced in early life can sometimes be "reprogrammed" by later environmental enrichment or pharmacological agents (such as HDAC inhibitors or DNA methylation inhibitors), leading to normalized phenotype [3].. In the context of preterm infants, this means that even if an infant starts off with a dysbiotic microbiome and pro-inflammatory epigenetic programming, timely interventions might correct the course. For instance, introducing missing beneficial microbes, providing breast milk or HMO supplements, or using drugs that target epigenetic enzymes (in very controlled scenarios) could potentially mitigate the established aberrant programming. Of course, such approaches would require extreme caution and thorough research, especially any pharmacological epigenetic interventions, given the complexity of systemic effects.

Biomarkers and personalized medicine: An improved understanding of microbiome-epigenome interactions could yield novel biomarkers. For example, a particular DNA methylation signature in a blood or stool sample of a preterm infant might indicate dysbiosis or heightened risk of an inflammatory condition. This could prompt earlier nutritional or probiotic interventions in that infant. Conversely, profiling an infant's microbiome and epigenome together might help personalize antibiotic use (avoiding antibiotics in those who already show signs of low diversity and epigenetic immune suppression, unless absolutely necessary). In the

future, clinicians might routinely monitor the "omic" profile of preterm infants – including their microbiota composition and key epigenetic marks – to guide care in real time.

Controversies and future research directions: Despite growing evidence, the field is still young and faces several controversies. One debate, as noted, is the existence and importance of microbes in utero. Resolving this will require carefully-designed studies with contamination-controlled sampling of fetal environments. Another contentious point is how to disentangle effects of the microbiome from other confounders – preterm infants undergo many interventions (like oxygen therapy, medications) that can also affect epigenetics. Multi-variable models and multi-omics research will be needed to attribute changes specifically to microbiota when appropriate. Moreover, while animal models (germ-free mice, etc.) are invaluable for mechanistic insight, human studies are essential since the preterm infant's exposures and timeline are unique. Researchers are now employing integrated "omics" approaches – combining metagenomics, transcriptomics, and epigenomics – in cohorts of preterm infants [21]. These holistic studies can better capture the complex network of interactions.

In summary, recognizing the connection between epigenetic shifts and the preterm neonatal microbiome broadens our perspective on infant care. It suggests that fostering an optimal microbiome (through practices like promoting vaginal deliveries when safe, using mother's milk, judicious antibiotic use, and possibly administering probiotics) is not only immediately beneficial but could also shape the infant's gene expression patterns in beneficial ways. Conversely, it raises caution that disruptions to the microbiome might leave molecular echoes that predispose to disease. Going forward, clinical strategies that jointly consider microbial and epigenetic health – essentially treating the infant as an ecosystem – hold promise. The next decade will likely bring rapid advances in this interdisciplinary area, ultimately translating to improved outcomes for preterm infants.

3. Summary and Conclusions

Preterm infants represent a particularly vulnerable population in which environmental influences can have outsized and long-lasting effects. The composition of the early microbiome and the state of the developing epigenome are two fundamental factors that shape neonatal and future health. This review has highlighted that these factors are deeply interconnected: the preterm neonatal microbiome both drives and is shaped by epigenetic changes in the host. Preterm babies often start life with a perturbed gut microbiota, due to factors like cesarean delivery, NICU exposure, and antibiotics. This dysbiosis can contribute to conditions such as NEC by inappropriate immune stimulation. At the same time, preterm infants may have epigenetic alterations (from prenatal inflammation or postnatal stresses) that influence how their immune system and gut respond to colonizing microbes. Emerging evidence from clinical studies and experimental models supports a bidirectional relationship where microbial metabolites and signals modify host epigenetic marks, and host epigenetic programming can create an intestinal environment that selects for certain microbes [2]. The implications of these findings are profound. They suggest that early interventions to optimize the microbiome might also favorably modulate epigenetic programming, potentially improving immunity, growth, and neurodevelopment. Practices such as encouraging breast milk feeding, skin-to-skin contact, and probiotic supplementation appear beneficial and could be partially explained by their microbiome–epigenome effects. Conversely, the research urges caution in the use of broad antibiotics and other disruptions of the neonatal microbiome unless clearly indicated. On the epigenetic side, while direct clinical manipulation of the epigenome in newborns is not currently feasible, identifying epigenetic biomarkers of risk could help stratify which infants need microbiome-focused interventions most urgently.

There are still many unknowns and the field is evolving. Key areas for future research include: delineating the precise molecular pathways of microbe-epigenome interaction in humans; longitudinal studies to see how long epigenetic changes induced in the NICU persist and what they mean for adult health; and controlled trials of microbiome-modulating therapies with measurement of epigenetic outcomes. New technologies in sequencing and bioinformatics are enabling more detailed and comprehensive analysis of infant microbiomes and epigenomes than ever before. By integrating these approaches, scientists and clinicians can begin to map out how a preterm infant's early experiences become biologically embedded.

In conclusion, the concept of "epigenetic shifts in the preterm neonatal microbiome" underscores a holistic view of preterm infant health – one that bridges microbiology and gene regulation. Appreciating this interplay offers a richer understanding of disease mechanisms like NEC and opens the door to innovative strategies that target the root causes of vulnerability in preterm infants. Ensuring a healthy start for these infants may well involve nurturing their microbial partners as well as safeguarding their genomic expression landscape.

Through continued research and interdisciplinary collaboration, we can hope to translate these insights into interventions that will improve survival and long-term quality of life for the tiniest patients.

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Referenceы

1. **World Health Organization.** *15 million babies born too soon* [Internet]. Geneva: World Health Organization; 2012 May 2 [cited 2023 Oct 12]. Available from: https://www.who.int/news/item/02-05-2012-15-million-babies-born-too-soon.
2. **Indrio F, Martini S, Francavilla R, Corvaglia L, Cristofori F, Mastrolia SA, et al.** Epigenetic matters: the link between early nutrition, microbiome, and long-term health development. *Front Pediatr*. 2017;5:178. doi:10.3389/fped.2017.00178.
3. **Lu L, Claud EC.** Intrauterine inflammation, epigenetics, and microbiome influences on preterm infant health. *Curr Pathobiol Rep*. 2018;6(1):15–21. doi:10.1007/s40139-018-0159-9.

4. **Cortese R, Lu L, Yu Y, Ruden DM, Claud EC.** Epigenome–microbiome crosstalk: a potential new paradigm influencing neonatal susceptibility to disease. *Epigenetics*. 2016;11(3):205–15. doi:10.1080/15592294.2016.1155011.
5. **Underwood MA.** Development of the preterm infant gut microbiome: a research priority. *Microbiome*. 2014;2:38. doi:10.1186/2049-2618-2-38
6. **Thänert R, Schwartz DJ, Keen EC, Hall-Moore C, Wang B, Shaikh N, et al.** Clinical sequelae of gut microbiome development and disruption in hospitalized preterm infants. *Cell Host Microbe*. 2024;32(10):1822-1837.e5. doi:10.1016/j.chom.2024.07.027.
7. **Choi YS, Song IG.** Fetal and preterm infant microbiomes: a new perspective of necrotizing enterocolitis. *Clin Exp Pediatr*. 2017;60(10):307–11. doi:10.3345/kjp.2017.60.10.307
8. **Gao F, Zhang J, Jiang P, Gong D, Wang J, Xia Y, Østergaard MV, et al.** Marked methylation changes in intestinal genes during the perinatal period of preterm neonates. *BMC Genomics*. 2014;15:716. doi:10.1186/1471-2164-15-716.
9. **Everson TM, O'Shea TM, Burt A, Hermetz K, Carter BS, Helderman J, et al.** Serious neonatal morbidities are associated with differences in DNA methylation among very preterm infants. *Clin Epigenetics*. 2020;12(1):151. doi:10.1186/s13148-020-00939-7.
10. **Stilling RM, Dinan TG, Cryan JF.** Microbial genes, brain & behaviour – epigenetic regulation of the gut–brain axis. *Genes Brain Behav*. 2014;13(1):69–86. doi:10.1111/gbb.12109
11. **Sparrow S, Manning JR, Cartier J, Anblagan D, Bastin ME, Piyasena C, et al.** Epigenomic profiling of preterm infants reveals DNA methylation differences at sites associated with neural function. *Transl Psychiatry*. 2016;6(1):e716. doi:10.1038/tp.2015.210
12. **Arrieta MC, Stiemsma LT, Amenyogbe N, Brown EM, Finlay B.** The intestinal microbiome in early life: health and disease. *Front Immunol*. 2014;5:427. doi:10.3389/fimmu.2014.00427
13. **Mirpuri J, Raetz M, Sturge CR, Wilhelm CL, Benson A, Savani RC, et al.** Proteobacteria-specific IgA regulates maturation of the intestinal microbiota. *Gut Microbes*. 2014;5(1):28–39. doi:10.4161/gmic.26489
14. **Al Nabhani Z, Dulauroy S, Marques R, Cousu C, Al Bounny S, Déjardin F, et al.** A weaning reaction to microbiota is required for resistance to immunopathologies in the adult. *Immunity*. 2019;50(5):1276–88.e5. doi:10.1016/j.immuni.2019.02.014.
15. **Dahlgren AF, Pan A, Lam V, Gouthro KC, Simpson PM, Salzman NH, et al.** Longitudinal changes in the gut microbiome of infants on total parenteral nutrition. *Pediatr Res*. 2019;86(1):107–14. doi:10.1038/s41390-019-0391-y
16. **Gomez de Agüero M, Ganal-Vonarburg SC, Fuhrer T, Rupp S, Uchimura Y, Li H, et al.** The maternal microbiota drives early postnatal innate immune development. *Science*. 2016;351(6279):1296–302. doi:10.1126/science.aad2571.
17. **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(5):428–38. doi:10.1111/pai.12232
18. **Rodriguez J, Jordan S, Mutic A, Thul T.** The neonatal microbiome: implications for neonatal intensive care unit nurses. *MCN Am J Matern Child Nurs*. 2017;42(6):332–7. doi:10.1097/NMC.0000000000000375
19. **Kennedy KM, Gerlach MJ, Adam T, Heimesaat MM, Rossi L, Surette MG, et al.** Fetal meconium does not have a detectable microbiota before birth. *Nat Microbiol*. 2021;6(7):865–73. doi:10.1038/s41564-021-00904-0
20. **Dominguez-Bello MG, Godoy-Vitorino F, Knight R, Blaser MJ.** Role of the microbiome in human development. *Gut*. 2019;68(6):1108–14. doi:10.1136/gutjnl-2018-317503
21. **Lynch SV, Vercelli D. Microbiota**, epigenetics, and trained immunity: convergent drivers and mediators of the asthma trajectory from pregnancy to childhood. Am J Respir Crit Care Med. 2021;203(7):802-8. doi: 10.1164/rccm.202010-3779PP
22. **Wheater EN, Galdi P, McCartney DL, Blesa M, Sullivan G, Stoye DQ**, et al. DNA methylation in relation to gestational age and brain dysmaturation in preterm infants. *Brain Commun*. 2022;4(2):fcac056. doi:10.1093/braincomms/fcac056.