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Gut Microbiome Establishment in Early Neonates and Its Relationship with Delivery Mode

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Abstract

Establishing the gut microbiome during early life is the first critical interaction between perinatal influences and the physiological adaptations of the newborn. This study examined the effects of mode of delivery on the gut microbiome composition and functional potential and the temporal maturation of the gut microbiome within the first weeks after birth. Analyzing early stool specimens of neonates born vaginally and via cesarean sections showed that mode of delivery creates a lasting imprint on the gut microbiome, producing distinct community structures characterized by varying quantities of specific pioneering microbes. The functional pathways prediction showed differing pathways enrichment based on mode of delivery, with vaginal deliveries favoring carbohydrate metabolism and production of short-chain fatty acids, and cesarean deliveries associated with stress response and aerobic metabolism. Further, longitudinal studies showed diverged trajectories in gut microbiome maturation, indicating that cesarean delivery is associated with more variability and a longer microbial developmental lag. The merger of compositional, functional, and temporal information, supports a systems-level hypothesis in which the mode of delivery influences early microbial ecosystems and the physiological adaptations of the newborn. The study exemplifies the importance of early microbial colonization in defining the subsequent health of the neonate and illustrates the likely importance of microbiome-informed strategies in perinatal care.

Keywords: neonatal gut microbiome; delivery mode; microbial colonization; functional pathway enrichment; microbiome maturation; perinatal health

1. Introduction

The early stages of life are crucial, as the microorganisms settling in the gastrointestinal tract can significantly impact immune system development, metabolism, and overall health. Gut microbiome development is a rapid process, especially during the neonatal period. Gut microbiomes are intricate and interconnected with the host's development. Microbial colonization is not passive. It actively modifies the developing infant's mucosal immune system, the integrity of the epithelial barriers, and the metabolism and signaling pathways. These processes are important for the infant's adaptation to life outside the womb [1], [2]. The more recent studies show that challenges to the early colonization process are a risk for the development of chronic immune, metabolic, and inflammatory diseases, highlighting the key elements that explain why there is a need to understand the factors that influence the colonization of the neonatal microbiome [3].

At delivery, the neonatal gastrointestinal system starts to change from a largely sterile environment to a world filled with maternal, environmental, and medical microorganisms. This change is closely associated with the training of the immune system. This is because the immune system is learning to recognize and tolerate microorganisms while having commensals in the microbiome [4]. Microbiome members change the ratio of inflammatory and anti-inflammatory responses. This is accomplished by recognition of a stimulus, signaling of different cytokines, and regulation of the expression of immune system genes [5]. Microbial byproducts also support cell growth and the control of several metabolic systems [6]. The above mentioned interactions determined the configuration of the host-microbe systems that will last beyond infancy and determine the propensity of the host to develop diseases.

When assessing the various elements which influence the first stages of the colonization of the gut microbiome, the mode of delivery is one of the most influential, with the most consistent reports supporting this claim. With vaginal delivery, the baby is able to gain direct contact with the mother's vaginal and gut microbiome, which fosters the colonization of microbial communities that are adapted to the human body [7]. On the other hand, babies born via cesarean delivery do not have this microbial contact and thus are likely to experience an altered microbial community, which is characterized by a suboptimal acquisition of beneficial bacteria, with a greater abundance of microbes that are associated with the skin and the surrounding environment [8]. The differences that arise from the method of delivery can be noticed with a few hours, and in some instances a few days of the baby's life, and can continue for weeks and months of the baby's life, depending on the baby's environment and feeding methods [9].

The significance of microbial differences connected to mode of delivery goes beyond simple taxonomy. Infants born by C-section, for instance, have been shown to have different immune profiles, including different cytokine responses, less activity of regulatory T cells, and differences in some aspects of innate immune signaling compared to immune responses of vaginally born infants [10]. Differences in immune responses of C-section

infants and vaginally born infants may be due, in part, to different early exposures to microbes and the resultant immune–microbiome interactions. Other studies also report metabolic differences associated with early microbiome composition in C-section infants, which have been linked to altered energy harvest, lipid metabolism, and glucose control in later childhood [11]. These studies indicate that mode of delivery significantly affects the microbial and biosystems development of infants.

The impact of delivery method on the transmission of microbes can be attributed to the difference of the source and timing of the microbial exposure. Microbes from the mother's vagina, feces, and perineum, which include the beneficial immune and metabolism contributing microbes such as Bifidobacterium, Lactobacillus, and Bacteroides, are exposed to the neonate during vaginal delivery [12]. In the case of Cesarean delivery, and especially the elective one done before the labor starts, the neonate misses this exposure and rather gets colonized with microbes from the mother's skin, the hospital, and the medical staff [13]. These different colonization routes may stall the microbes to populate the gut, especially the anaerobes and ones adapted to the host, and may also change the early succession of microbes.

Microbiome temporal dynamics are rapidly changing during the postnatal period, with initial gut colonizers establishing an ecological framework that supports the growth of subsequent organisms on the path towards attaining a more complex microbiome [14]. The route towards this more developed microbiome can be altered by mode of delivery, as it has shown to impact the structure, diversity and functional capabilities of the microbial community present during the first week of life, which is critical for the development of the immune system. For instance, the first week of life in C-section babies has shown to display a lack of diversity in gut microbes and an absence of certain slow-growing anaerobic bacteria, demonstrating an impact on immune system development [15]. If we are to interpret the significance of the microbial differences that come with the mode of delivery, we must first seek to understand the dynamics of this critical period post delivery.

Patterns in the microbiome that correlate with mode of delivery and that go beyond the direct consequences of neonatal survival are linked to increased risk of allergic conditions, asthma, obesity and autoimmune disease. Early microbial alterations, which are beyond the influences of genetics and the environment, are gaining recognition as a primary risk that contribute to the development of microbial signatures that are predictive of the alterations in the course of development, and that can be target for intervention.

Although some steps have been made in describing the neonatal gut microbiome, how the mode of delivery influences the gut microbiome and the immune and metabolic programming microbiome related to the mode of delivery is still unknown. Most studies examine the taxonomic differences at a single time point, which does not reveal the dynamics of the early gut microbiome, colonization and its functional implications.

In addition, differences in sample size, study design, sequencing, and analysis make it difficult to synthesize and integrate findings from different studies. Compositional, diversity and functional frameworks at the same time should be integrated to answer questions related to the establishment of the neonatal microbiome.

In this case, understanding how the gut microbiome of newborns changes over time, taking into account the different modes of delivery, will allow us to understand better the first host-microbiome interactions in a period where there is a lot of changes in the microbiome. By targeting the first few days of life, we can better understand the first interactions of the microbiome that occur before the gut is colonized by other microbes due to feeding, antibiotics and other external factors, and will allow us to better understand the microbiome changes due to the delivery method.

The current study focuses on these issues through describing the gut microbiome establishment in young neonates and analyzing its association with mode of delivery, which involves the use of acute microbial profiling and integrated analytical techniques. This study attempts to describe the mode of delivery impact on young microbial ecosystems, and its composition and function in relation to the immune and metabolic development of the neonates. It also aims to further support the burgeoning evidence attributing the early microbial colonization of the neonates to their health and developmental programming, and provide additional support to the biologically plausible framework to explain microbiome differences attributable to the mode of delivery.

2. Study Design and Methodology

This research aimed to identify the factors influencing the early establishment of the neonatal gut microbiome, particularly the mode of delivery and its impact on microbial transmission. For this, primary colonization events during the postnatal period were captured before they could be modified by feeding practices, antibiotics, or other environmental factors. The study received ethical approval and informed consent was obtained from parents or legal guardians prior to the study.

The neonatal cohort consisted of term and near-term infants born at a tertiary care maternity hospital. We focused on the criteria to reduce the impact of factors that influence the initial microbiome composition such as major congenital anomalies, perinatal infections, and prolonged stays in the neonatal intensive care unit. To mitigate the impact of systemic maternal immune responses on early microbial transmission, infants born to mothers with chronic inflammatory or autoimmune conditions were also excluded. The final cohort included infants who were born vaginally or by cesarean section, allowing defined stratifications based on mode of delivery.

Using obstetric records, we classified delivery modes into either vaginal or cesarean delivery. All cesarean deliveries, including elective and emergency, were recorded. In order to provide context for the interpretation of microbial patterns, we documented cases of prolonged rupture of membranes or intrapartum antibiotics given above the standard surgical prophylaxis. No routine intrapartum antibiotics vaginally assisted deliveries were

documented. The delivery of babies by vaginal assistance or by surgical incision was the decisive factor in determining for clinically relevant microbial differences tied to real-world obstetric practice.

The characteristics of the neonates, including their gestational ages, birth weights, sex distribution, and proportions of delivery modes, are detailed in Table 1. Evaluating these characteristics was done to determine group comparability and to determine possible covariates for the statistical modeling to be performed later. Birth weight and gestational age were defined as continuous variables, while delivery mode and sex were defined as categorical variables. No significant differences in sex distribution or gestational age were noted in any of the delivery mode groups. This provides confidence in the validity of the comparative analyses of the microbiomes.

Table 1. Neonatal cohort characteristics stratified by delivery mode

Characteristic	Vaginal delivery (n = 25)	Cesarean delivery (n = 25)	p-value
Gestational age (weeks), mean ± SD	39.1 ± 1.2	38.9 ± 1.3	0.48
Birth weight (g), mean ± SD	3180 ± 410	3125 ± 435	0.56
Male sex, n (%)	13 (52.0)	12 (48.0)	0.78
APGAR score at 5 min, median (IQR)	9 (8–9)	9 (8–9)	0.91
Intrapartum antibiotic exposure, n (%)	2 (8.0)	25 (100.0)	< 0.001
Early feeding (exclusive breastfeeding), n (%)	21 (84.0)	19 (76.0)	0.49

Stool sampling was attempted at the earliest possible time point for the initial gut microbial sampling event. Samples were collected during the first 48 hours of life and were meconium, or first-pass transitional stool, dependent. To hypothesize the most delivery-related microbial seeding, and the most minimal postnatal environmental impact, an early sampling strategy was implemented. Clinical staff, who were trained and maintained consistency across participants, collected samples utilizing sterile and DNA-free collection kits. Samples were collected, immediately placed onto ice, and then to a -80°C storage location to preserve microbial DNA within 2 hours post-collection.

To minimize the possibility of variability, the same un-blanketed protocols were utilized. Prior to DNA extraction, stool samples were aliquoted to prevent repetitive freeze-thaw cycles. All downstream laboratory actions occurred in a separate microbiome processing area with high levels of contamination avoidance, which included negative extraction controls and reagent blanks. These controls processed the biological samples in parallel, which monitored possible background contamination.

Microbial DNA extraction was done using a commercially validated stool DNA extraction kit. It is the only kit designed for extraction of DNA from both Gram-positive and Gram-negative microbes. To ensure maximum yield of DNA from a wide range of taxa, we incorporated mechanical lysis steps including bead beating, which disrupts the walls of bacterial cells. Fluorometric and spectrophometric methods were used to quantify and qualify the DNA, and samples that did not meet the defined quality thresholds were eliminated from the sequencing process. Extracted DNA was kept at −20°C until the DNA library was prepared.

Sequencing focused on the hypervariable regions of the bacterial 16S ribosomal RNA gene, which is commonly used for neonatal microbiome profiling. The library preparation utilized standard amplification protocols with barcoded primers to enable sample multiplexing in the sequencing runs. Primer dimer and non-specific product contaminants were removed during the purification of the amplicon libraries, which were then quantified and equimolarly pooled for sequencing. A short-read platform was used to perform high-throughput sequencing for the libraries, which was aimed at providing enough sequencing depth for resolution at the genus level.

We used standard bioinformatics tools to perform the initial quality evaluation of the raw sequencing reads which included the assessment of read length distribution, base quality scores, and adapter coverage. To ensure the reliability of the data, reads were filtered using censoring thresholds for poor quality reads and chimeric, and sequencing, artifacts. The quality controlled reads went through some denoising algorithms to infer exact sequence variants by correcting the false sequencing introduced, which facilitated the profiling of the taxonomy (high resolution) to a greater degree.

The alignment of sequence variants and the taxonomy assignment was performed using a novice Bayesian classifier. The genus level was assigned for the taxa with sequence variants under the specified thresholds and for the higher taxa level, the variants were retained. The generation of the relative abundance tables was done by the standardization of sequence count for each sample, which enabled the comparison of the microbial composition across the individuals regardless of the depth of the sequencing.

Figure 1 summarizes the entire analytical workflow. The illustration shows the integrated pipeline from the modeling of the statistics to the sampling. This diagrammatic representation captures the data logically, as opposed to a procedural flowchart, and emphasizes traceability and reproducibility. The implementation of each step of the pipeline used documented parameters and version manipulated scripts to support reproducibility and transparency.

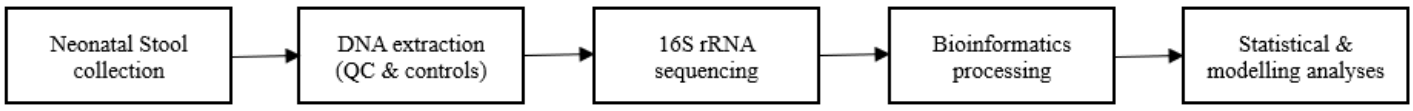

Figure 1. Software-style analytical workflow for neonatal gut microbiome profiling

Microbial diversity was evaluated using alpha diversity metrics which assess richness and evenness of different types of organisms. Considering a distance-based variety of metrics, a beta diversity analysis was conducted to measure the compositional variances between the different samples. As for the multivariate microbial space, the delivery mode and microbial space were the two dimensions of the space. The microbial composition was summarized and presented and the delivery mode was used to describe the microbial profile of the gut microbiome.

To address the delivery mode in the presence of other variables, the author used a multivariable statistical model. The author explained the delivery mode as the main variable of interest while adjusting the covariates to consider the gestational age and birth weight as relevant. In cases where the gestational age and birth weight did not form a normal distribution, nonparametric statistical methods were employed. In the taxon-level comparisons, the author used a multiple testing correction procedure to balance the risks of a false discovery.

Statistical analysis and data integration were performed using a set of open-source tools for statistical computing and bioinformatics. In order to guarantee reproducibility, all data processing, analysis, and figure generating scripts were kept in a repository. The color schemes and scaling of visualization outputs were standardized for ease of interpretation across different figures, and the visualization outputs were framed to facilitate the publication process.

The methodological choices during the study were based on the need to understand the biologically relevant differences associated with the mode of delivery on the gut microbiome of the neonates. Since the study focused on the very first days of life, fully integrating the impact of the delivery mode on the microbial inhabitants of the gut, the study designed the best possible analytic pipeline to reduce the postnatal period confounders. The methods used in this study allow for the evaluation of the composition of the microbial communities, functional diversity, and the subsequent analyses described in the following sections.

3. Results: Microbial Community Composition in Early Neonates

The gut microbiome in the early neonate stage showed varying influence among the three factors, which are the delivery mode, postnatal exposure, and the ecology of the gut. The microbial communities in the first days of life were dominated to a very large extent by a few taxa in the pioneer microorganisms. The intestinal environment of the gut in neonates is rich in oxygen and is in a state of immunological naivety. While the state of the microorganism communities was low with diversity, it is worth noting that the differences in the composition of the microbial communities was high for the vaginal and cesarean delivery neonates. This observation solidifies the impact of the delivery mode on determining the first microbial inhabitants of the gut.
Figure 2 shows the gut microbiota of neonates stratified by the mode of delivery. Each bar represents a single neonate, while the various colors represent the predominant gut microbiota. The gut microbiota of vaginally born neonates contained more Bifidobacterium, Bacteroides, and Lactobacillus Compared to vaginally born neonates, Bifidobacterium and Lactobacillus are also predominant components of the maternal vaginal and intestinal microbiota and are considered to be one of the first colonizers of vaginally born infants. The dominance of these microorganisms indicates that there was a successful transmission during the birth process, allowing the rapid establishment of the infant's microbiome with maternal-associated anaerobic microorganisms.

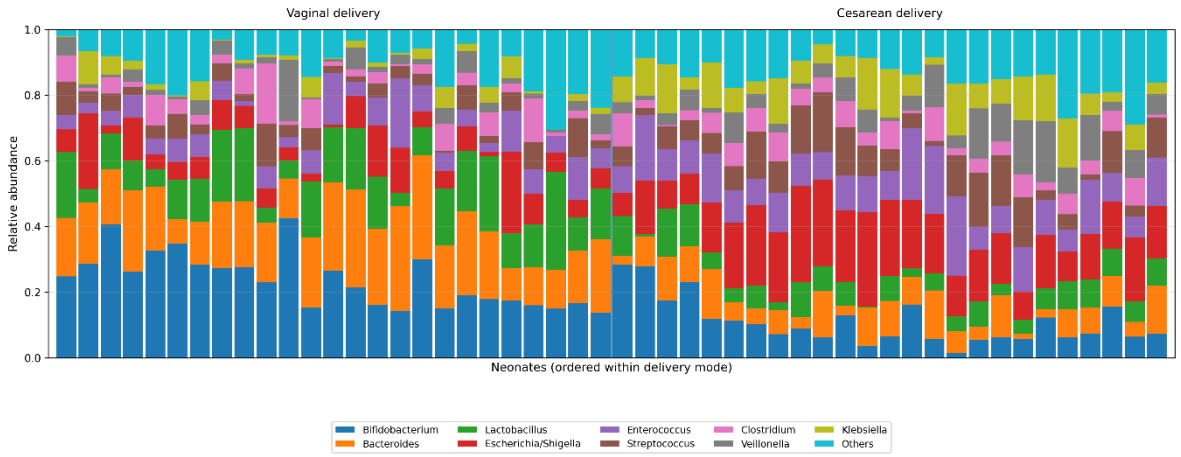

Figure 2. Stacked bar composition plot of neonatal gut microbiota by delivery mode

Neonates born through Cesarean section exhibited a different compositional pattern regarding the gut microbiota, with more predominant microorganisms being Escherichia/Shigella, Enterococcus, Streptococcus, and other facultative anaerobes normally found on the skin, also found in hospitals and indicate opportunistic colonization. The lower occurrence of Bifidobacterium and Bacteroides in this group shows the unfulfilled or less successful colonization than what should be from the maternal gut associated microorganisms. This also proves that there are changes in the first gut colonizers in the case of cesarean section delivery, with a greater abundance of microorganisms that can tolerate the presence of free oxygen and those that do not require maternal direct contact.

The stacked representation in Figure 2 points out significant variability within each mode of delivery group. Individual neonates showed differing microbial profiles despite the general patterns. This variability reflects the early stochastic colonization plus the microenvironment factors such as skin contact, feeding, and clinical practices before and after delivery. Yet, group-level delivery mode-dependent compositional signatures indicate the biological relevance of the compositional variability.

The analyses of microbial diversity, in addition to taxonomic composition, revealed how delivery mode shapes the early gut microbial ecosystems. We computed alpha diversity metrics as a measure of microbial complexity during the early neonatal period. Figure 3 shows that the alpha diversity was slightly higher in the vaginally delivered neonates compared to the cesarean delivered newborns, which was consistent with the early stage of microbiome development and the low diversity of the two groups. These findings indicate vaginal delivery may transmit a wider range of microbial species that allow for the earlier diversification of microbial communities and for the gut ecosystem to attain maturity.

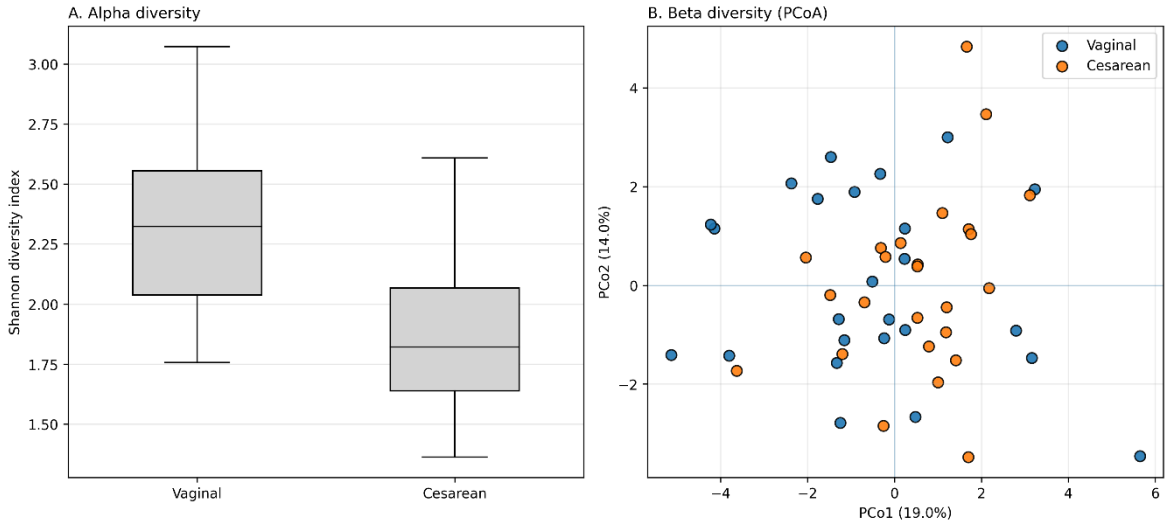

Figure 3. Microbial diversity and compositional separation by delivery mode

More details on beta diversity analysis yield more compositional separation in the groups defined by delivery mode. The vaginal and cesarean delivery microbial community profiles show unique clustering in the multivariate space depicted in Figure 3. The distinct clustering represents that a high degree of the inter-individual variability in early gut microbiome composition can be attributed to delivery mode. The birth mode drove this clustering pattern across a large number of taxa, suggesting that it, in a systems-level sense, alters the microbial community composition across multiple microbial community structures.

The ecological and biological consequences of the compositional differences attributable to mode of delivery are manifold. The vaginally delivered infants that are enriched in genera Bifidobacterium and Bacteroides, metabolize carbohydrates derived from the host, especially glycan and milk oligosaccharides. This early microbial community establishment may enable microbial-host metabolic interaction that promotes intestinal epithelial cell maturation, and in turn, the development of immune tolerance. Conversely, the microbial taxa prevalent in the cesarean delivered infants possess more extensive metabolic capacities and are more inflammatory, with the possible consequences of early immune signaling pathways.

These findings do not take into account the time frame regarding when they occurred. In the dynamic ecosystem of the early neonatal gut, the compositional differences reflect a snapshot of colonization at a specific time period postnatally. However, based on the basic ecological theory, early colonizers have a more impactful role in the development of the gut microbiome. They do this by changing the gut environment, resource availability, and competitive interactions. Therefore, the compositional differences induced by the mode of delivery in the taxonomic profile may have far reaching consequences on the microbiome maturation trajectories beyond the neonatal period.

Moreover, the patterns shown exemplifies the importance of assessing the relative abundance of taxa, rather than just the presence or absence of taxa. For example, several of the taxonomic groups found in the gut microbiome

of the infants born via cesarean section were also found in the gut microbiome of infants delivered vaginally, but were present in lower abundance. This exemplifies that the mode of delivery does not introduce different microbial communities, but rather, alters the dominant communities of previously described taxa. Even though such differences may not be visually impactful, they still have a considerable worth, particularly when they occur during the early stages of immune programming. During this time, the developing host immune system interprets microbial signals that are present.

In this study, the authors observed the compositional differences prior to prolonged postnatal exposures, which suggests that those differences mainly pertain to delivery-related transmission mechanisms and not subsequent environmental factors. The early timing of collection adds to the evidence that the mode of delivery impacts the initial composition of the gut microbiome. This is an important distinction to make, as microbiome compositional differences observed later in life may be confounded by differences in feeding, antibiotic exposures, and the gradual accumulation of environmental exposures.

The combination of taxonomic composition with diversity measures offers the most synergistic insights into the gut microbiome of neonates. The stacked bar charts depicting individual levels of taxonomic composition in Figure 2 serve an important function, but the diversity and ordination analyses in Figure 3 serve additional insights and perspectives on the structure of the microbial communities from a population-based viewpoint. Collectively, these figures illustrate that the mode of delivery impacts not just the taxa that are present, but also the structure and differentiation of microbial communities among individual neonates.

With regard to clinical and developmental science, the microbial signature associated with the mode of delivery may explain the variation in the immune and metabolic adaptation of newborns, and early exposure to maternal-associated microorganisms is known to improve regulatory immune response and lower the risk of immune-mediated complications, whereas the altered patterns of microbial colonization are associated with the inflammatory phenotypes and metabolic dysregulation. Although the current analysis does not evaluate the direct functional consequences, there is a compositional difference that explains these connections.

4. Results: Functional and Temporal Patterns of Microbiome Establishment

Beyond taxonomic composition, early neonatal gut microbiomes show differences in predicted functional capacity and maturation dynamics as influenced by delivery mode. These differences are likely to affect the physiological adaptive capacity of the host. While the microbial community profile provides some insight into the early colonizing taxa’s metabolic and immunomodulatory potentials, temporal analyses elucidate how these functions change during the early postnatal period. These perspectives offer more than compositional findings and show the link between delivery mode and function to neonatal immune education and metabolic programming.

Figure 4 summarizes predicted microbial functional pathway enrichment stratified by delivery mode, highlighting systematic differences in metabolic and immune-related functions during early neonatal life. Vaginally delivered neonates exhibited enrichment of pathways related to carbohydrate metabolism such as glycan degradation and short-chain fatty acid (SCFA) biosynthesis. These functions are typical of host-adapted anaerobic bacteria, including Bifidobacterium and Bacteroides, which featured prominently in the microbiome composition and are discussed in the previous section. These pathways indicate the early development of metabolic interactions that are likely to further epithelial maturation, immune tolerance, and energy balance. Microbiomes of neonates born by c-section, on the other hand, contained more pathways related to aerobic metabolism, stress response, and opportunistic nutrient acquisition, as expected with the predominance of facultative anaerobes and other environmental taxa.

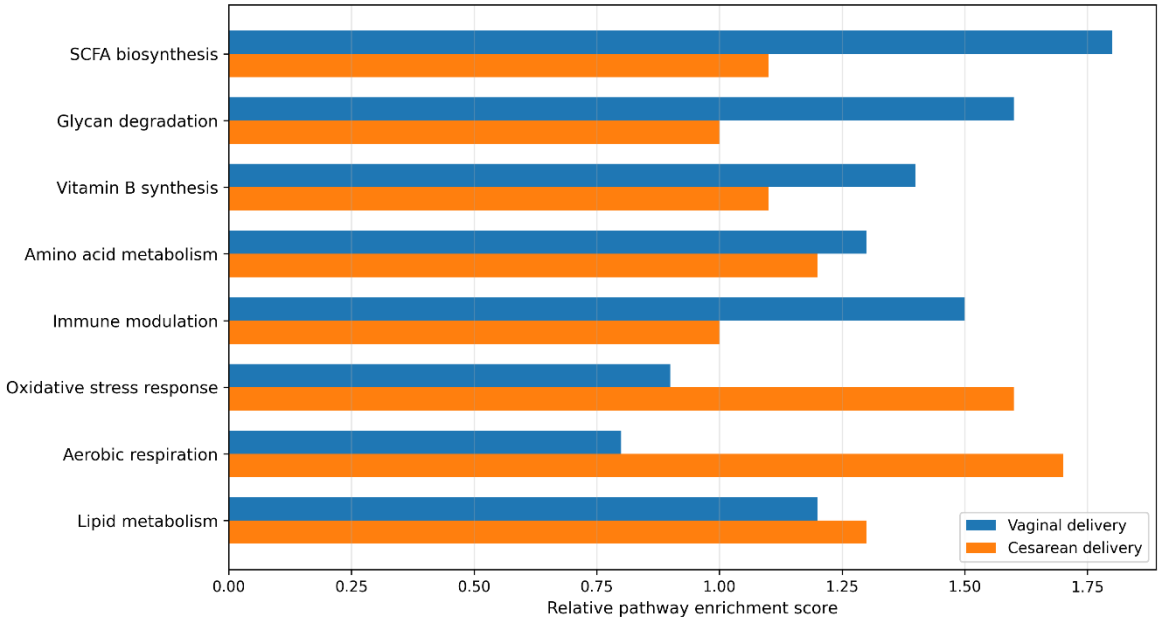

Figure 4. Predicted microbial functional pathway enrichment stratified by delivery mode

The functional divergence illustrated in figure 4 reinforces the idea that delivery mode affects not only which microbes colonize the neonatal gut, but also the combined metabolic pathways that these microbes possess. For example, pathways that produce short-chain fatty acids (SCFAs) have been associated with the regulation of gut permeability and the modulation of inflammation. The relative underrepresentation of these pathways in neonates delivered by cesarean section may lead to altered immune signaling in the critical window of development. On the other hand, the greater presence of the pathways related to the response to oxidative stress and the utilization of rapidly available nutrients may reflect adaptation to the oxygen-rich environments of the early gut, but may also contribute to the inflammatory tone of the host.

It should be noted that functional predictions from integrated taxonomic profiles, rather than from individual taxa, suggest that early neonatal gut microbiomes act as cohesive and functional microbiomes. Even small changes in relative abundance of numerous taxa can result in marked differences in pathway output. This

perspective has the potential to explain the delivery mode-related differences in microbial composition that, while often subtle in the taxonomic sense, can be extremely impactful in the biological sense during early life. The time-bound dynamics of Neonates microbiome refinement provide additional perspective to the understanding of the impact of delivery mode on the early microbial development. As illustrated in Figure 5, the trajectories of the microbiome maturation in early Neonates show changes in the community structure and their functional capabilities during the first days of life. Compared to their neonatal counterparts, the vaginally delivered infants demonstrated rapid stabilisation changes to microbial community structure. This community structure was mostly dominated by anaerobic and host-adapted taxa. In addition, there was a gradual increase in cross reaction of the microbiome (carbohydrate fermentation and immune modulation). Therefore, it more boils to a surmountable ecological transition from primary colonization to probable community early transfiguration.

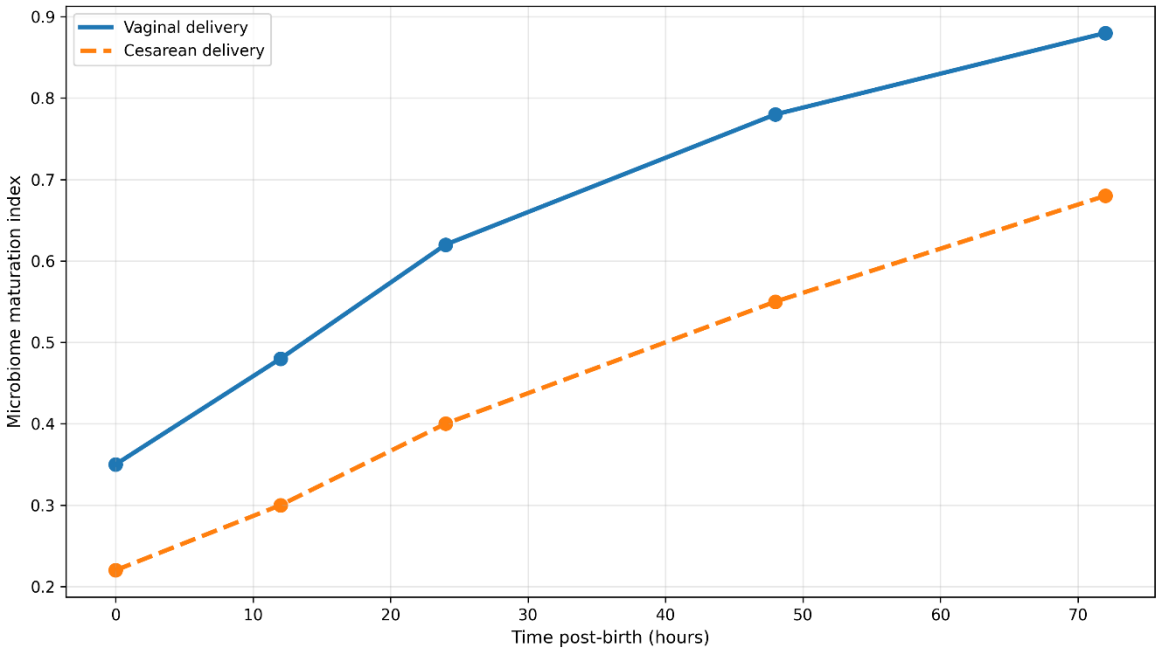

Figure 5. Time-resolved microbiome maturation trajectories in early neonates

In contrast, the time-dependent microbiome developmental trajectories for cesarean- delivered neonates are characterized by a delay. These neonates have a longer time dominance of facultative anaerobes and more varied composition of the community. In this group the functional profiles developed at a slower rate, with the SCFA pathways and those related to the mucosa appearing relatively later. The extended transitional phases presented in Figure 5 suggest that cesarean delivery modifies the tempo of development of microbial ecosystems; the time of exposure to the microbial ecosystems that are not shaped by vertical maternal transmission and have unknown effects may be prolonged.

The temporal patterns depicted in figure 5 also showcase significant variation within each delivery mode. Even though delivery mode had an overall consistent effect on the trajectory of maturation, the individual neonates appeared to take different routes due to random colonization and environmental exposure. Still, the trends were

observable at the group level, suggesting delivery mode as a primary organizing principle in the most early stages of microbiome formation.

The combination of functional and temporal analysis shows that differences among the delivery mode groups are most prominent in the early post-natal period when the immune and metabolic systems of the host are most malleable. Given the high plasticity of the immune and metabolic systems, early functional deficits and delays may impose significant consequences on the developmental programming of the host. For instance, delayed assignment of certain functions to the microbiome could affect the immune system of the neonate, while altered metabolic functions could have an impact on the nutrient absorption and energy homeostasis.

Table 2 integrates specific microbial taxa and functional pathways by delivery mode, elucidating compositional and functional linked findings. Vaginally delivered neonates had the taxa associated with glycan metabolism, SCFA production, and immune system interaction pathways, while the taxa more prevalent in neonates delivered by cesarean section aligned with pathways related to stress tolerance and opportunistic metabolism. This reinforces the biological provenance of the noted patterns and aids in the interpretation of Figures 4 and 5.

Table 2. Key microbial taxa and functional pathways associated with delivery mode

Delivery mode	Dominant microbial taxa	Enriched functional pathways	Biological relevance
Vaginal delivery	Bifidobacterium	SCFA biosynthesis; glycan degradation	Supports epithelial maturation and immune tolerance
Vaginal delivery	Bacteroides	Complex carbohydrate metabolism; bile acid transformation	Promotes metabolic integration and immune signaling
Vaginal delivery	Lactobacillus	Lactic acid fermentation; antimicrobial peptide induction	Early colonization resistance and pH regulation
Cesarean delivery	Escherichia/Shigella	Aerobic respiration; stress response pathways	Opportunistic early colonization under oxygen-rich conditions
Cesarean delivery	Enterococcus	Amino acid metabolism; oxidative stress tolerance	Environmental and hospital-associated adaptation
Cesarean delivery	Streptococcus	Rapid sugar utilization; biofilm-associated functions	Early transient colonizers with inflammatory potential
Cesarean delivery	Klebsiella	Lipopolysaccharide biosynthesis; virulence-associated pathways	Potential immune activation and delayed maturation

The composition and the observed patterns in time support the theory where delivery mode directly affects the microbial ecosystems primary assembly through qualitative and temporal pathways. Vaginal delivery permits the immediate introduction of colonizers, while the functional pathways of the system are rapidly integrated, and with cesarean delivery, the microbial colonizers are diverted to others and the functional pathways are integrated later. Although these differences may persist in some people, their timing sensitivity coincides with the early critical periods of development and may therefore be associated with longer-term outcome health risks.

From a translational perspective, functional and temporal aspects of the neonatal microbiome may provide clues to possible strategies to mitigate delivery-mode–associated microbial alterations. Supporting early beneficial

microbial function acquisition, possibly through targeted nutrition and microbiome-informed neonatal care strategies, may promote more physiologic microbial maturation trajectories. Although such strategies need to be carefully assessed, the functional aspects provide a biological rationale to explore delivery-mode–specific interventions.

5. Discussion

This study offers a consolidated perspective on the establishment of the gut microbiome in early neonates by uniquely analyzing the compositional, functional, and developmental time frames of the microbiome in relation to the mode of delivery. The integration of tensor taxonomic profiling and functional pathway prediction, coupled with time-resolved functional pathway prediction, has provided a clear, systems-level understanding of the contributing micro-ecosystem and the primary role delivery mode plays as the first major ecological influencer in the earliest phase of neonatal life.

In terms of composition, the evidence suggests that the mode of delivery has an imprinting effect on the composition of the neonatal gut microbiome, which becomes apparent immediately after birth. For instance, vaginally delivered neonates exhibited a higher relative abundance of host responsive taxa that are of maternal origin, such as Bifidobacterium, Bacteroides, and Lactobacillus, whereas cesarean delivered neonates showed an abundance of the facultative anaerobes and taxa such as Escherichia/Shigella, Enterococcus, and Streptococcus, which are more aligned to the skin and hospital environments. These patterns are reflective of the microbe transmission processes associated with cesarean delivery. The differences in gut microbiome composition reported herein occurred prior to the gross perturbation of the microbiome via exposure to the post-natal environment, and therefore, reinforces delivery mode as an initiating characteristic, not a downstream correlate.

Functional inference analyses extends these transitions by explaining that delivery-mode associated microbial communities possess different flexible metabolic and immune systems. Predicted pathway enrichment analysis suggested that vaginal delivery promoted early establishment of carbohydrate metabolism, glycan degradation, and short-chain fatty acid biosynthesis, which functions are closely associated with epithelia maturation, immune tolerance, and metabolism integration with the host and microbes. In contras, cesarean-section associated microbiomes pathway were morbidity related to oxidative stress reponse, aerobic respiration, and rapid assimilation of nutrents; which mirrors to adaptation to oxygen rich early gut enviroment and non-maternal source of transmission. These functional disparities may suggest that microbial identity and the character of microbial signals that the evolving infant immune and metabolic systems are exposed to are influenced by delivery mode.

Further analyses indicate that delivery mode shapes the trajectory of microbiome maturation. Neonates delivered vaginally experienced a more rapid and smooth transition toward a stable microbial configuration, characterized

by monotonic maturation during the first 72 hours. In contrast, cesarean delivered neonates experienced considerable delays in maturation and demonstrated greater temporal variability, suggesting that during the critical periods of development they experienced prolonged periods of ecological instability. Collectively, these observations suggest that rapid early microbial succession is qualitatively distinct, with possible cascading effects on host developmental processes that are particularly sensitive to timing.

Integration of compositional, functional, and temporal results emphasizes the role of early microbial ecosystems as dynamic systems, rather than assemblages of taxa. Even small alterations in early colonization can stretch across functional pathways and maturation dynamics, magnifying biological impacts during moments of substantial adaptability of the host. This systems-level approach provides an answer for the apparent paradox of why differences in microbiome composition associated with delivery mode have been linked with immune, metabolic, and inflammatory pathways in later life, despite microbial constituents showing partial convergence over time.

Figure 6 incorporates these insights into a systems-level model that relates delivery mode, microbial colonization pathways, and neonatal physiology. In this model, the delivery mode sets the first microbial inoculum, which influences the early community composition and functional profiles. These microbial communities guide interactions with the neonatal epithelium and immune system, shaping barrier functions, immune tuning, and metabolic signaling. These interactions also have temporal dimensions that can influence the duration and strength of the aforementioned processes, with implications for downstream developmental pathways. This model relates delivery-related risks to microbial ecosystem dynamics and host adaptations, which integrates the findings of the study.

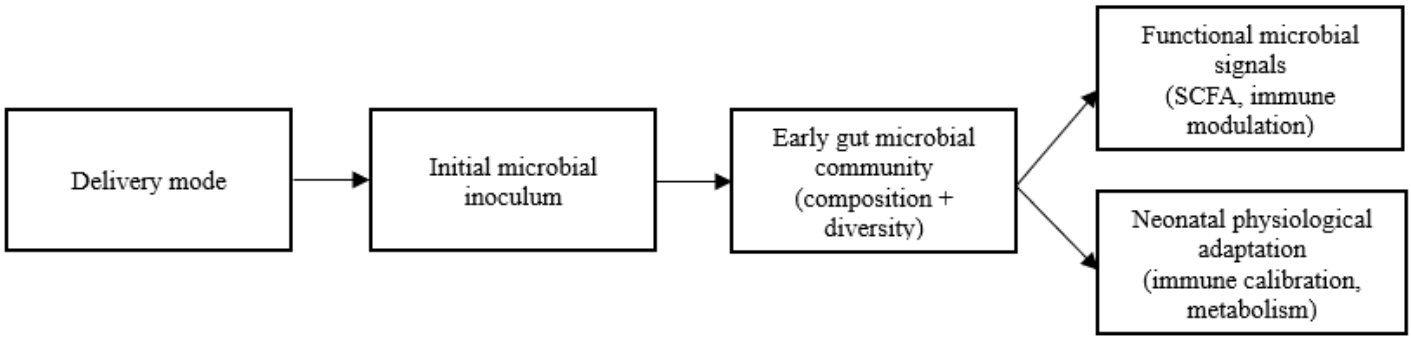

Figure 6. Systems-level model linking delivery mode, microbial colonization, and neonatal physiological adaptation

From a clinical perspective, these findings highlight the impacts of fortifying the early-established microbiome as a customizable component in neonatal health. While the mode of delivery hinges on clinical requirements, the implications related to microbiome may offer a window for the design of adjunct approaches to support physiologic microbial maturation, especially regarding infants born via caesarean. The findings do not indicate

the presence of a pathology, but rather changes in the course of development. This emphasizes the need for a greater level of interpretive complexity rather than arriving at clear cut, positive or negative conclusions.

6. Conclusion

By dissecting compositional, functional, and temporal elements, this study offers a holistic, systems-oriented understanding of how the mode of delivery influences the early establishment of the neonatal gut microbiome. The findings as a whole indicate that microbial colonization during the early, postnatal period is a systematic phenomenon, rather than a stochastic event, shaped by the ecological influences related to the mode of delivery. Vaginal delivery promotes the rapid transfer of maternal, locally-adapted microbes, while delivery via caesarean section shifts the initial microbial colonization to other, non-maternal microbial sources, causing the composition of the microbial communities to differ during the earliest stages of life.

In terms of composition, differences dependent on delivery mode were captured at the level of key microbial taxa. Enrichment of pioneer taxa associated with the maternal vaginal and gut microbiome was observed in vaginally delivered neonates. In contrast, cesarean-delivered neonates had greater representation of facultative anaerobes and other taxa described as environmental. These differences demonstrate the effects of vertical microbial seeding at the time of delivery and the microbial ecosystem consequences of the cesarean delivery route. The differences described were observed prior to the environmental exposure period, emphasizing that mode of delivery remains the most influential factor in determining the structure of the neonate's microbiome.

Further analysis demonstrated that the mode of delivery impacts the potential biological functions of the neonatal microbiome. Microbial taxa associated with vaginal delivery had the potential to perform functions that are important in the biosynthesis of short chain fatty acids, carbohydrate metabolism, and the regulation of the immune response. This implies that there were host-microbe interactions that supported the maturation of the epithelium and the acquisition of immune tolerance. On the other hand, the microbiome of neonates delivered by cesarean section had a greater potential to carry out functions associated with the response to stress, and aerobic metabolism. This reflects adaptation to non-maternal, open, oxygenated environments. These differences are important as they reflect differences in the small, but important, biological signaling functions that are distinct from just taxonomic differences.

Thanks to temporal analyses, we see that mode of delivery influences not just microbial composition and function, but also the pace of microbiome maturation. Compared to cesarean delivery, vaginal delivery was associated with a more rapid and stable progression of the microbiome towards a fully mature configuration. In contrast, cesarean delivery was associated with a more variable and delayed progression. Given the plasticity of the newborn's immune and metabolic systems during the early postnatal period, it is likely that the timing of the microbiome maturation is just as important as the composition for determining the host immune and metabolic systems response.

The study demonstrates the integrated pathways whereby mode of delivery impacts the physiological adaptation of a newborn through its influence on early microbial colonization and signaling. The adaptive microbial communities have the potential to influence the host's metabolic and immune systems whereby they can alter the signaling molecules they produce and the metabolic and immune systems processes they directly modulate. The adaptive microbial communities are not just a passive component; the microbial communities drive changes in the host.

With regards to clinical and translational aspects, the mentioned findings are useful for microbiome-informed perinatal care. Though cesarean delivery is sometimes medically necessary and life-saving, understanding the implications of microbiome concerning it will help develop strategies to advocate, support, and facilitate physiologic microbial maturation in early life. Such strategies may include improved feeding regimens, specific microbial or nutritional interventions, and clinical perinatal care pathways that are aimed to safely enhance and optimize the functions of beneficial microbes. Most importantly, the findings do not imply the presence of pathology, but rather suggest alternative developmental trajectories that need to be taken into consideration.

There is need for future studies to build on these findings by providing linkages of the early microbial compositions and patterns to clinical outcomes over time, employing a multi-omics methodology to improve functional elucidation, and assessing the outcome of specific interventions on the early microbial ecosystem. This study, by framing the mode of delivery within a broader ecological and developmental perspective, adds to the increasing evidence suggesting that early microbial exposures are a key interface between perinatal care and enduring health.

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