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Breast Milk Mitigates Microbial Differences Caused by Varying Modes of Infant Delivery Despite the C-section Microbiome Displaying an Increased Resistance to Change Over a Naturally Delivered Microbiome

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SUMMARY Early life infancy is a crucial period for shaping the microbiota, as many factors during this period are responsible for driving the assembly of the infant microbiome. Two of the most studied early-life habits and practices that have drastic impacts on the infant microbiome are the mode of infant delivery and diet. Over the years, caesarean section (C-section) delivery rates have been on the rise globally. Although microbial differences resulting from the mode of delivery become less striking later on in life, the microbiomes of C-section infants have notable differences in composition and diversity compared to those born vaginally. Our study established feed as having a stronger influence on the microbiota compared to mode of delivery. Accordingly, breast milk, to an extent, was able to mitigate differences caused by delivery, such that C-section infants supplemented with breast milk had a more similar profile to those born vaginally when compared to those fed formula. Breast milk stood out as a rare exception in its ability to modify the C-section microbiome. This is owing to the fact that the dysbiotic state commonly linked with C-section deliveries, typically displays a degree of resistance to changes induced by external factors (21, 4). This finding is clinically significant as C-section deliveries have been consistently correlated with an increased chance of developing autoimmune disorders, and breast milk being able to modify the C-section microbiome so that it more closely resembles the healthier vaginal state can be considered a therapeutic intervention. This study highlights the importance of diet as a major factor influencing the infant microbiome and establishes breast milk as the preferred feed over formula for C-sectionally delivered infants.

INTRODUCTION

The human microbiome has been established as having a major influence on biochemical and physiological pathways, affecting the health of an individual throughout their lifespan (1). The combined genetic material of the bacterial species residing within the human microbiome is estimated to contain 100-fold more genes than the human genome (1). This vast accessory genome empowers the microbiome with more flexibility through which it can afford to perform various crucial biochemical and physiological functions that the human host depends on for its survival. Some of the notable functions consist of but are not limited to dietary fibre digestion to produce SCFA with various beneficial anti-inflammatory properties (2), training and being responsible for the normal development of the host's immune system (3) and direct protection from harmful pathogens (4). Furthermore, given the importance of the cross-talk between the microbiome and the host immune system, dysbiotic imbalances in the microbiome compositions have been consistently correlated with various diseases such as asthma, obesity, and autism (5) that place major strains both on the quality of life of the affected individual and the healthcare system. Early life infancy is a crucial period for shaping the microbiota as many factors during this period are responsible for driving its assembly. These include both random chance events where the infant is exposed to a source of microorganisms, referred to as stochastic events, and deterministic factors, which are strong selective pressures that select particular community members over others (4). Besides the nature in which a specific factor drives infant microbiome composition (stochastic vs

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deterministic), the time period at which these factors exert their influence also varies. For example, maternal microbiome composition is at play as early as the prenatal period, whereas mode of delivery becomes prominent later on when the infant is born (6).

Due to the vast beta diversity among microbiomes of different individuals, the specific microbial composition that can be considered a "healthy" one is yet to be answered. However, prior relevant studies have found that greater microbial diversity (7) and the presence of certain commensal phyla such as *Firmicutes* (8) have been consistent markers of the microbiome of those living a healthy lifestyle. Further, mode of infant delivery has been implicated as an important factor in developing a healthy microbiome. Over the years, caesarean section (C-section) delivery rates have been on the rise globally as mothers may opt for a C-section over a vaginal delivery for various reasons, including decreased labour pain and medical complications (e.g. placenta previa that deem C-section the safer option) (11, 12). Although microbial differences resulting from the mode of delivery become less noticeable later on in life, the immediate microbiomes of C-section infants have notable differences in composition compared to those born vaginally. In particular, *Lactobacillus*, *Escherichia*, *Bifidobacteria*, and *Bacteroides* species are less abundant in the guts of C-section infants, while *Staphylococcus*, *Clostridium*, *Enterobacter*, and *Enterococcus* species are more abundant (13). Previous studies investigating the influences of the mode of delivery on infant microbiome dynamics have found that babies delivered via C-section exhibit reductions in all three parameters of microbial diversity compared to their vaginally delivered counterparts (17). The decreased microbial variability that the C-section microbiome commonly exhibits may be a result of the bypassed vaginal canal, which is the first and major source of exposure to colonizing microorganisms (13). This reduced diversity and the decreased numbers of mutualistic commensals can, in turn, compromise the infant's health due to the role that the microbiome has in directly stimulating the priming of the immune system. Consistent with this, there does indeed exist a correlation between C-section deliveries and an increased chance of developing diabetes, arthritis, Celiac disease, allergies, and asthma, which are all autoimmune disorders that are microbiome-mediated and consistently linked with a dysbiotic microbiota (14). Of note, the dysbiotic state common with C-section deliveries has been noted as a microbiome that is rather resistant to changes induced by microbiome influencing factors (21) which presents novel challenges when trying to rescue the microbiome from its dysbiotic state.

A group of researchers at Rutgers University demonstrated that these risks can be mitigated in C-section infants in the case that the gut microbiota recovers from its initial disrupted state (15). A common practice done in conjunction with C-section deliveries is vaginal seeding, where the infant is exposed to the mother's vaginal fluid so that the microbiome can be colonized with maternal microbes that would have normally colonized the infant during vaginal delivery (16). Breast milk supplementation has been proposed as a novel approach with the promise of offering beneficial effects on the microbiome of C-section infants (19). Breast milk not only introduces beneficial microorganisms into the microbiome, akin to probiotics, but also provides specific nutrients like human milk oligosaccharides (HMOs) that nourish and favor certain microbial populations (10). Given that, we intend to uncover whether breast milk feeding can be considered an agent of microbiota recovery for C-section infants and whether it can help to restore their microbiome to a composition similar to that of vaginally delivered infants.

METHODS AND MATERIALS

Dataset. The dataset used to produce the study was generated by Dr. Rhee at the University of California in the Department of Pediatrics, and collected stool samples collected from 325 infant-mother dyads at 2 weeks, 4 months, 6 months, 9 months, and 12 months of life. Their dietary, perinatal, and medical histories were recorded and included in the dataset. 16S rRNA sequencing was used to study gut microbial diversity and composition with the end goal of leveraging the acquired data to advance existing correlations between microbial composition and infant obesity to a causation.

QIIME2 Data processing. The Quantitative Insights Into Microbial Ecology (QIIME2) platform was used for data processing (28). DADA2 was used to denoise the data upon

demultiplexing (29). All generated reads had a read length of 150 bp. Further, all sequence positions expressed a high median quality score (i.e. median quality score > 30), which eliminated the need for further trimming or truncation of the reads. Samples which contained mitochondria and/or chloroplast sequences were filtered. Meta-data based filtering was further done to exclude "combined feeding" and to include only data pertaining to infants. Samples that contained observations labelled "non-applicable" or "not collected" as related to feed and mode of delivery were removed. ASV taxonomic classifications were determined using q2 feature-classifier function by aligning the reads to the Silva 138 database (34). The reference database was trimmed to only contain the V4 region of the 16S rRNA gene sequence with the forward and reverse primers used for PCR amplification being as follows: 515f GTGYCAGCMGCCGCGGTAA ; 806r GGACTACNVGGGTWTCTAAT (Parada et al, 2015 ; Apprill et al, 2015). A phylogenetic tree was generated and imported to R along with the ASV feature table, and taxonomy table for downstream analysis.

Diversity analysis. A rarefaction depth of 26884 was chosen for diversity analyses, which retained 3,333,616 (63.63%) features in 124 (78.48%) samples. The chosen depth was determined to ensure that a maximum number of ASVs were represented within the dataset while minimizing the number of discarded samples. Subsets of the diversity metrics were selected and had their appropriate plots regenerated in R. Alpha and beta diversity metrics were analysed using feed and mode of delivery as predictor variables. The Kruskal-Wallis test and PERMANOVA were used to determine the significance of the α- and β-diversity metrics, respectively. A phyloseq object was created using the phyloseq package in R (35) in order to combine the main outputs of the qiime2 pipeline into one object that can be used to perform downstream analysis.

Indicator species analysis. The R package indicspecies (36) was used in RStudio to perform indicator species analysis where only ASVs with an indicator value > 0.70 and $p < 0.05$ were selected and retained.

Core microbiome analysis. The R package microbiome (37) was used in RStudio to perform core microbiome on the generated phyloseq object. The prevalence and detection threshold for core microbiome were both set at 0.

DESeq analysis. The R package DESeq2 (38) was used in RStudio to perform differential abundance analysis. Both a volcano plot and a bar plot were generated to visualise the DESeq results and observe the significantly increased or decreased ASVs in comparisons where one of either mode of delivery or diet was kept constant. ASVs with unavailable adjusted p-values or log2 fold changes were also filtered out of the DESeq outputs.

RESULTS

Both feed and mode of delivery contributed to changes in the microbial community structure of infants. Beta diversity was used to observe the effect of feed and mode of delivery on the infant microbiome diversity. PERMANOVA conducted with the jaccard metric resulted in significant differences between infants that were born via a vaginal route compared to those delivered via C-section; these same differences were also present for infants that were fed with formula compared to those that were fed breast milk (Figure 1). No significant difference was observed between groups when feed and mode of delivery were considered together (i.e. breast milk + vaginal, breast milk + C-section, formula + vaginal, formula + C-section).

Different feed types were correlated with differences in microbial richness of the vaginally delivered cohort whereas C-section infants were unaffected by this change. Different alpha diversity metrics such as Shannon and Faith's PD were consistent in terms of the formula-fed cohort having a significantly higher microbial diversity than their breast milk-fed counterparts in multiple comparisons. It was also made clear that feed exerted a stronger influence on the infant microbiome than that of the mode of delivery. Infants that received the same feed had no significant differences in microbial diversity regardless of differing modes of delivery, which suggested that feed had the final say in whether two microbiomes

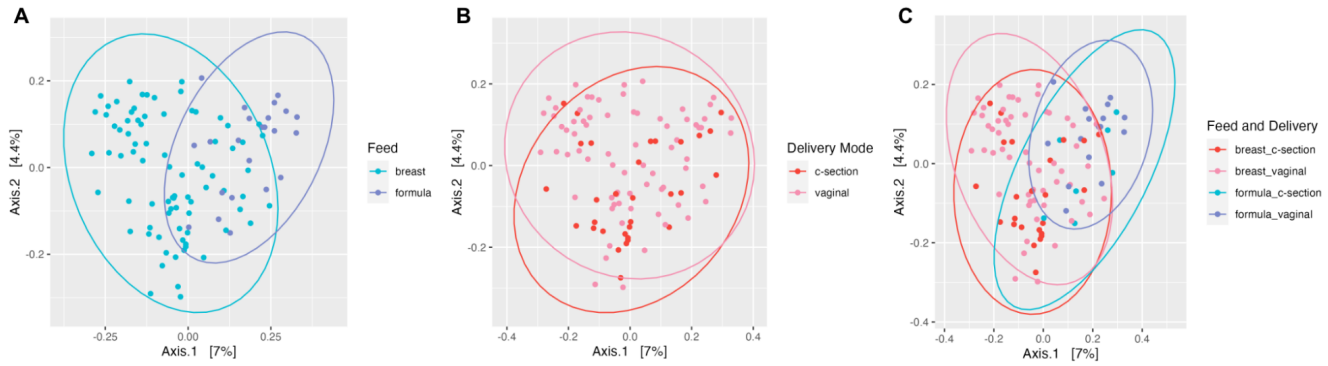

FIG. 1 Mode of delivery and diet were both factors that contributed to differences in microbial beta diversity. Beta diversity represented through PCoA plots showing Jaccard beta diversity of A) infants fed with breast milk vs those that were fed formula (P = 0.001) B) infants delivered via C-section vs those delivered via the vaginal route (P = 0.010) C) infants when both feed and mode of delivery are considered together (P = 0.396).

will end up being similar or different from one another (Figure 2). This did not hold true for modes of delivery where vaginally delivered infants receiving different feeds exhibited significant differences despite having the same mode of delivery (Figure 2). Despite these apparent differences resulting from varying diets, the effect of the diet entirely disappeared when the comparison made was between two C-sectionally delivered infants where they exhibited no differences despite receiving different feeds (Figure 2).

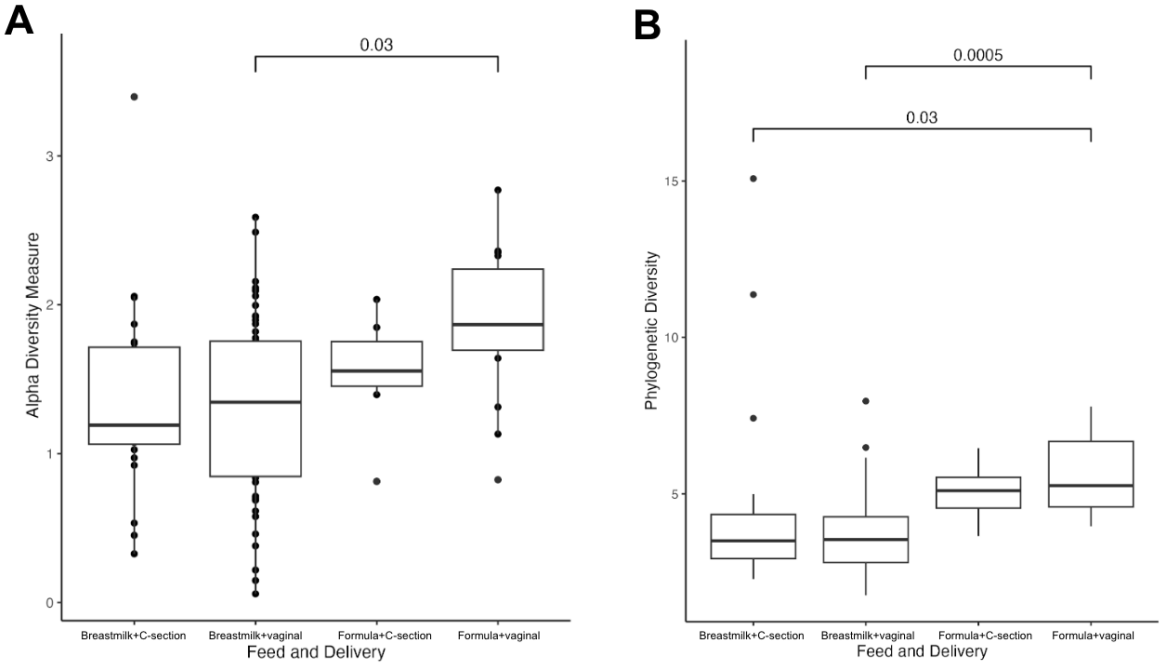

FIG. 2 Feed type contributed to changes in the microbial richness of only the vaginally delivered infants. A) Shannon alpha diversity and B) Faith's phylogenetic distance between infants with varying combinations of delivery modes and diet. A Kruskal-wallis test was used to test for significance ($P < 0.05$ was considered to be statistically significant).

Furthermore, with the exception of *Akkermansia*, our indicator taxa analysis revealed genera unique to specific diets regardless of the delivery method (Table 1), which aligns nicely with feed being the stronger microbiome modulator than mode of delivery. The four indicator genera common to both C-section and vaginally delivered infants fed with formula were: *Ruminococcus* (*gnavus group*), *Eggerthella*, *UBA1819* and *Tyzzerella*. Meanwhile, the two indicator genera common to breast milk-fed infants were *Rothia* and *Haemophilus*.

TABLE. 1 Indicator genera reveals genre that are unique to specific diets regardless of the mode of delivery. Only taxa resolved to the genus level with $p \leq 0.05$ and an indicator value above 0.70 are shown. The indicator value provides a measure of the likelihood that an organism is to be found in all the samples pertaining to a particular group or condition.

Genus	Conditions	Observed Indicator Value	P-value
[Ruminococcus]_gnavus_group	Formula, C-section Formula, vaginal	0.797	0.005
Rothia	Breast, C-section Breast, vaginal	0.758	0.025
Haemophilus	Breast, C-section Breast, vaginal	0.755	0.015
Eggerthella	Formula, C-section Formula, vaginal	0.717	0.015
UBA1819	Formula, C-section Formula, vaginal	0.709	0.010
Tyzzerella	Formula, C-section Formula, vaginal	0.703	0.005
Akkermansia	Breast, C-section Formula, vaginal	0.702	0.005

Babies fed breast milk have a higher number of unique taxa than formula fed counterparts, and breast milk partially restores microbial differences caused by varying modes of delivery. Core microbiome analysis revealed that infants who are fed breast milk have a higher number of unique taxa associated with their microbiome (Figure 3). This result

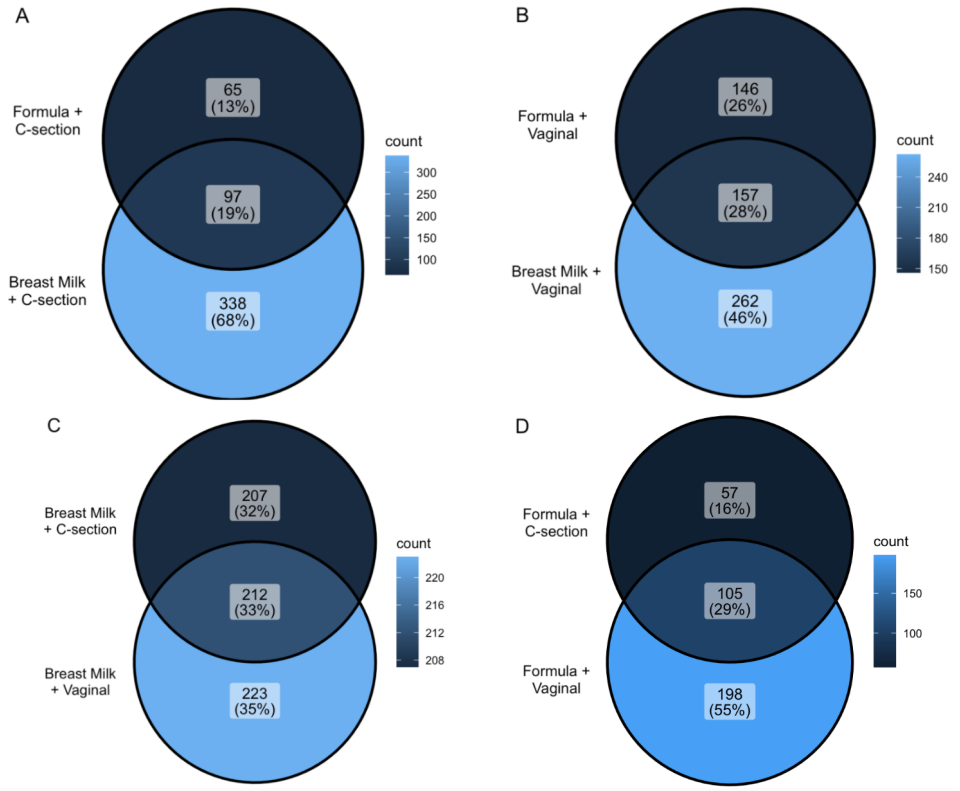

FIG. 3 Breast milk was associated with a higher number of unique taxa, and its supplementation allows for C-sectionally delivered infants to exhibit a more shared microbiome with those delivered vaginally. Venn diagrams illustrate unique and shared ASV counts between A) C-sectionally delivered infants or B) vaginally delivered infants fed with different diets (breast milk vs formula), and C) breast milk-fed infants or D) formula-fed infants born through varying modes of delivery (vaginal vs C-section).

was consistent across both the C-section and vaginally delivered cohort (Figure 3). Within the vaginal cohort, 46% of the amplicon sequencing variants (ASVs) present were unique to the breast milk group, while only 26% of the present ASV's were unique to the formula group (Figure 3B). This result was even more pronounced in the C-sectional deliveries, where 68% of the ASV's were unique to the breast milk cohort compared to a noticeably smaller 13% being unique to a formula diet (Figure 3A). Core microbiome analysis also presented us with one of the major takeaways from our study that breast milk is capable of partially restoring the microbial composition of C-sectionally delivered infants to one that would have resulted from vaginal delivery. C-section babies supplemented with breast milk have increasingly more taxa in common with those that were delivered vaginally (212 shared ASV's) (Figure 3C). On the other hand, only 105 taxa are shared between the microbiome of infants that were fed formula but had varying modes of delivery (C-section vs vaginal) (Figure 3D). Further, breast milk supplementation when compared to a formula diet, led to an upregulation of mutualistic commensal *Lactobacillus* species in the C-section cohort. Of note, *Lactobacillus* is one of the species that is underrepresented in the C-section microbiome compared to vaginal microbiome (Figure 4). Therefore, breast milk is capable of reintroducing species to the C-section microbiome that would have normally colonised the gut during vaginal delivery.

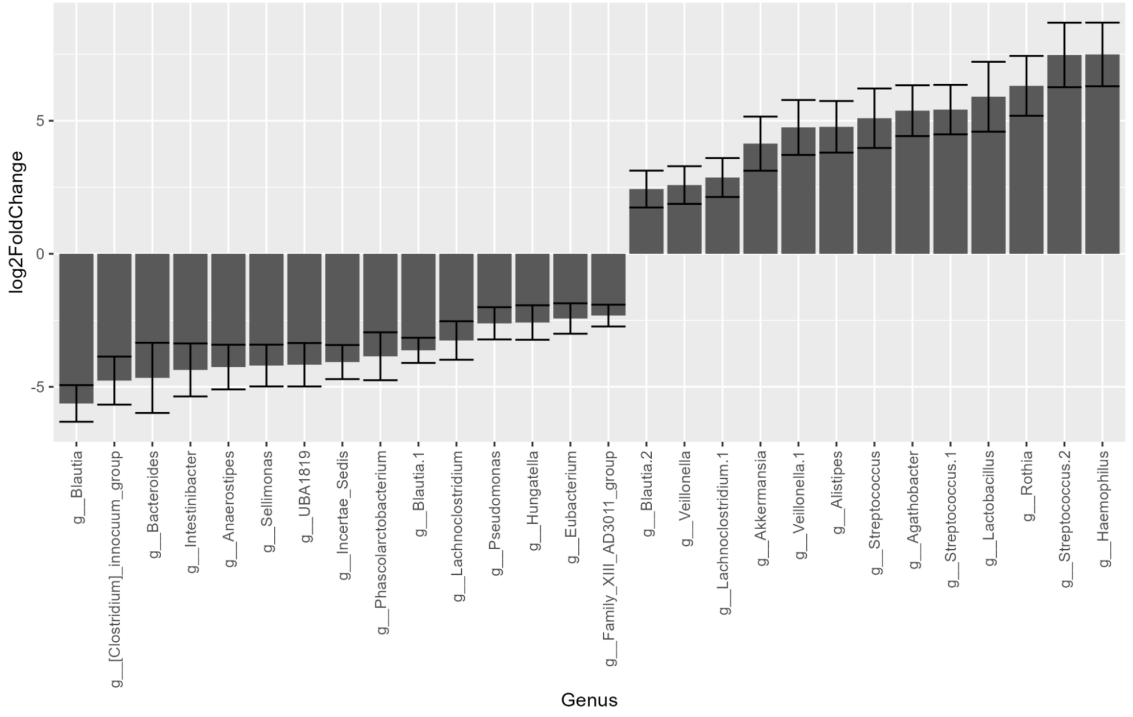

FIG. 4 Breast milk upregulated key taxa that are often depleted in C-sectionally delivered infant gut microbiota. DESeq analysis of breast milk-fed infants compared to formula-fed infants in the C-sectionally delivered cohort. Genus level taxa bar plot showing significantly increased and decreased fold changes in the breast milk-fed group compared to the formula-fed reference group.

Vaginally delivered babies were more susceptible to changes caused by feed than their C-section counterpart. Our second differential abundance analysis was congruent with our diversity analysis results, where C-section deliveries result in a microbiome that is more resistant to changes caused by external factors such as feed (Figure 5). Vaginally delivered babies had a total of 71 species up or down-regulated as a result of receiving different feeds. This was in contrast to C-section babies, where different feeds resulted in changes in abundance of only 28 species.

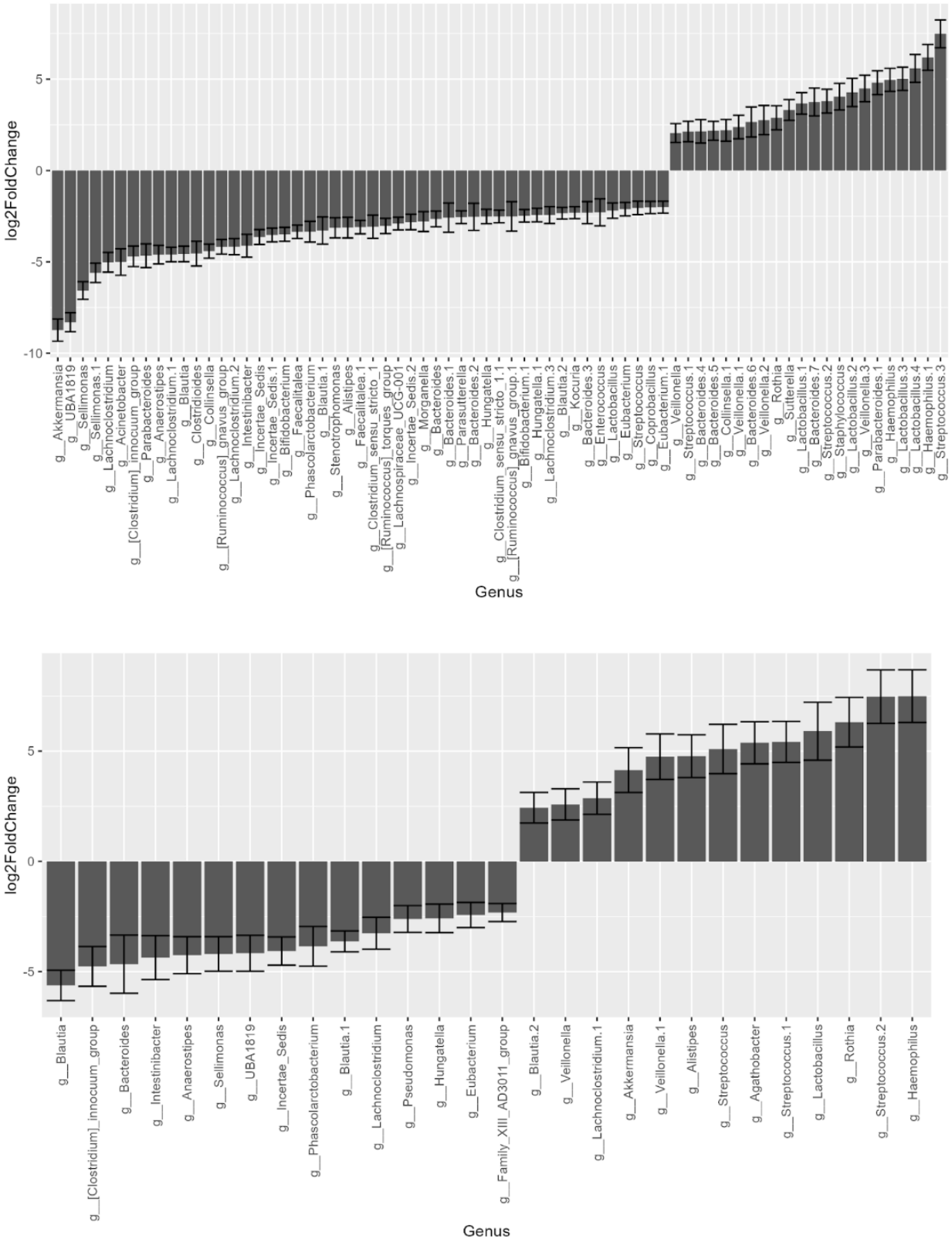

FIG. 5 The C-section microbiome was more resistant to changes caused by external factors such as diet. Top bar plot shows significantly increased and decreased fold changes in the abundance of 71 total taxa in vaginally delivered infants when fed with different diets (breast milk vs formula). Bottom bar plot shows significantly increased and decreased fold changes in the abundance of 28 taxa in C-sectionally delivered infants when fed with different diets. The formula fed cohort was set as the reference group in both comparisons.

DISCUSSION

Previous studies investigating the influences of the mode of delivery on infant microbiome dynamics have found that babies delivered via C-section exhibit reductions in all three parameters of microbial diversity compared to their vaginally delivered counterparts (17). Our results aligned with the existing literature in that we observed significant differences in microbial beta diversity resulting from different modes of delivery. Although microbial differences resulting from the mode of delivery become less striking later on in life, the role of the mode of delivery as one of the initial early life factors driving microbiome assembly cannot go unmentioned (13). This is further supported by the microbiomes of C-section infants exhibiting notable differences in composition compared to those born vaginally. In particular, *Lactobacillus*, *Escherichia*, *Bifidobacteria*, and *Bacteroides* are present in lower numbers in the guts of C-section infants, while there is an enrichment in *Staphylococcus, Clostridium, Enterobacter,* and *Enterococcus* species (13). The microbiome of C-section infants presents similarities to the maternal skin and hospital environment and displays decreased microbial variability (13). The decreased microbial variability can be explained by the C-section delivered infant bypassing the vaginal canal, which is the first and major source of exposure to colonizing microorganisms (13). This can have severe health implications as microbial communities with reduced diversity have been commonly linked with microbiota dysbiosis with subsequent risks of metabolic and autoimmune disorders following suit (17).

The role of diet as one of the critical factors influencing the microbiome dynamics was apparent in our study. Formula feeding was, on numerous occasions, associated with a higher microbial diversity when compared to breast milk and this result did not discriminate between alpha and beta diversity. Additionally, when infants were fed the same diet, they showed no diversity distinctions in their microbiome, regardless of the varying delivery methods. The same pattern did not hold true for modes of delivery where infants that were born vaginally but received different feeds exhibited microbial variations. The impact of diet on the microbiome appears to outweigh the influence of the delivery method. While the mode of delivery significantly shapes the initial composition of the microbiota at birth, dietary habits later on can override these influences induced by the mode of delivery. Indicator taxa analysis was consistent with these findings, where it revealed genera specific to a diet (breast milk vs formula) but not to mode of delivery. The four indicator genera common to both C-section and vaginally delivered infants fed with formula were: *Ruminococcus* (*gnavus group*), *Eggerthella*, *UBA1819* and *Tyzzerella*. Meanwhile, the two indicator genera common to breast milk-fed infants were: *Rothia* and *Haemophilus*. Among these genera, *Ruminococcus* had the highest indicator value. This genus has a proven association with protection against both obesity and *C. difficile* infections (24). Additionally, *Ruminoccocus* has been described to produce short-chain fatty acids (SCFAs) beneficial for epithelial cell proliferation and barrier functionality (24). *UBA1819* is categorized under the *Ruminococcaceae* family whose members produce SCFAs beneficial for the health of the digestive tract. Conversely, *Tyzzerella* has been associated with an increased risk of cardiovascular diseases (25). However, the benefits or disadvantages of an enrichment of *Eggerthella* are still relatively unknown (26). This seems to indicate that the particular formula used in this cohort has been designed to provide the aforementioned benefits while promoting some unforeseen and seemingly disadvantageous microorganisms. This finding supports the ongoing field of research where breastmilk components are being actively investigated to devise formula feed with improved functioning. An observation that was not congruent with the above takeaway where feed is the more potent influencer of the microbiome than mode of delivery, was when C-section infants had no significant differences in either alpha or beta diversity despite differences in their feed. The rather unexpected nature of this result can be justified by the resilient nature of the C-section microbiome to alterations caused by external factors. There does exist a correlation between C-section deliveries and an increased chance of developing diabetes, arthritis, Celiac disease, allergies, and asthma, which are all autoimmune disorders that are microbiome-mediated and consistently linked with a dysbiotic microbiota (14). The difficulties in developing microbiome-mediated treatment for these diseases can be explained by the resilient nature of the dysbiotic state commonly associated with C-section deliveries (21). Once dysbiosis occurs, it is very hard to shift the microbiome back to a healthy state, and in the case of a CDI (*C. difficile* infection), a complete transplant of the whole microbiome

from a healthy donor is the only available scientifically proven treatment (22). Differential abundance analysis results aligned with this observation where C-section babies, in contrast to those born vaginally, showed reduced sensitivity to dietary-induced shifts in their microbial abundance.

Our analysis revealed that infants fed with breast milk have higher numbers of unique taxa than those on a formula diet, with the observation being consistent regardless of the mode of delivery. This result can be explained by the vastly dynamic nature that breast milk composition exhibits. Breast milk composition not only varies amongst different mothers and populations but it also varies hugely over the lactation period as well as diurnally (23). This stands in striking contrast to the very narrow range of composition that formula takes on, which again is a rational insight due to it being manufactured by an established, set recipe. The vast variability in the maternal microbiome composition and nutritional components of breast milk makes for a significantly greater and wider range of organisms being unique to the breast milk-fed cohort compared to formula. Further, core microbiome analysis revealed that C-section delivered babies supplemented with breast milk had strikingly more taxa in common with vaginally delivered infants compared to those that were fed formula, hinting towards breast milk's remedifying role, where it is able to mitigate microbial differences caused by the mode of delivery. A couple observations in our study hinted towards this finding. Firstly, breast milk promoted the growth and presence of a greater number of unique taxa compared to formula. The one other study by Liu et al. (2023) that took a similar route as ours noted that certain species experienced an increase in abundance while others had reduced numbers in the intestine of C-sectionally delivered infants. This absence of specific bacterial taxa can make its absence known and manifest in the form of immunological and infectious diseases if the missing taxa are considered commensals essential to proper biological functioning. An example of this is an underrepresentation of *Lactobacillus* species in C-section populations, which can give insight into the various early-life pathologies commonly linked with C-sections (9). This arises from the favorable roles that *Lactobacillus* play in polysaccharide digestion and epithelium reinforcement, both via providing the gut colonic epithelium with energy in the form of SCFA and the anti-inflammatory role SCFAs play by stimulating T regulatory cell populations. Interestingly, differential abundance revealed an upregulation of *Lactobacillus* in the C-section cohort fed with breast milk when compared to those that were fed formula. Therefore, to an extent, we were able to determine the manner in which breast milk exerts its rectifying effect. Namely, breast milk is likely able to compensate for the reduced colonisation of specific microbial taxa that is characteristic of C-section deliveries by further reintroducing these underrepresented taxa to the gut microbiome. Differential abundance revealed breastmilk as having a greater impact in driving shifts in microbial abundance of the C-section microbiome compared to formula (Supplementary Fig S1). This finding holds particular significance in the context of the C-section microbiome, which typically displays higher resistance to alterations caused by external influences. Moreover, *Rothia* was identified as an indicator species of a breast milk exclusive diet, and it showed a rise in abundance among C-section infants who received breast milk compared to those fed formula. *Rothia* is a commensal species with the ability to detoxify immunogenic gluten proteins, potentially reducing the risk of developing celiac disease, an autoimmune condition often associated with C-section births (30). A finding that was rather odd considering the wide array of benefits that breast milk provides was that *Haemophilus* was not only an indicator species of a breast milk diet, but it also experienced an increase in numbers in C-section infants that were fed breast-milk. *Haemophilus* is commonly associated with respiratory infections and its upregulation in the infant gut microbiome warrants further studies that examine the gut-lung axis in order to make a definitive remark on the clinical significance of a normally pathogenic species having a positive correlation with breast milk feeding (33).

Limitations Several limitations may have impacted the results observed in this study. One such limitation was the reduction in sample size in the C-section delivered infants compared to their vaginally delivered counterparts. The smaller sample size in the C-section cohort compared to the vaginal group can diminish statistical power making it harder to identify significant differences, if there are any to begin with (32). This may explain the lack of

significant differences in alpha or beta diversity of the C-section infants despite receiving different feeds. Influence of external factors such as antibiotic use or masked disorders in both the infant or mother were also not removed during analysis, which could further impact the significance of the results. The reduced initial diversity in the C-section microbiome could have stemmed from antibiotic use, which was not a factor considered in this study. A previous cross-sectional study of antibiotic use in childbirth revealed that 100% of mothers would receive antibiotics when C-sections were performed (27). Conversely, only around 23.7% of vaginally delivered babies received antibiotics, with a higher proportion of vaginal deliveries receiving antibiotics if the delivery was induced (27). Applicability of the results may also be hindered by the inherent limitations that were present in the studied dataset. Even within a cohort for the same feed type, the duration of feeding was not explored. Further, factors such as race, sex, and maternal weight could have also influenced outcomes, especially if certain conditions would predispose an increased likelihood of choosing to perform a certain type of delivery method.

Conclusions Given the importance of the microbiome to human health, the research area concerned with early-life factors that drive microbiome composition during infancy is a trending one. With more mothers opting for C-section deliveries globally, the limitations of the C-section microbiome, such as a reduced microbial diversity compared to the vaginal counterpart has presented itself as a greater concern in the clinical scene. Our study found that breast milk supplementation, to an extent, was capable of dampening differences caused by varying modes of delivery, ultimately resulting in a more similar microbiome across infants born via different delivery modes. Further, as a general trend, feed was established as having a greater influence on the microbiome than mode of delivery in spite of the C-section microbiome having a rather resistant characteristic to microbial perturbations caused by dietary habits.

Future Directions Several studies have established that the mode of delivery has the greatest influence on microbial profiles and subsequent susceptibility to various infections during the first three months of life, with its influences becoming less pronounced with increased age as other factors start to play in and shape the microbiota (20). Different studies seem to contradict one another, and there are difficulties in reaching a consensus in regard to how long into the life of the infant the influences of the delivery mode seem to persist. The previously observed differences in microbial diversity being attributed to varying modes of delivery was a pattern that was independent of the age of infants in our study and persisted as long as 9 months into the infants' life. As our dataset used to generate the study did not include data on infants beyond the age of 9 months old, conducting a similar study on a cohort containing the desired data can help solidify the age at which the influence of the mode of delivery seems to become less prominent and even disappear as other microbiota-shaping factors start to play in and influence the microbiome. Further, we rationalize that the dysbiotic state of the C-section microbiome is certainly not the only characteristic that contributes to its resistant nature. Performing additional functional studies on the pathways that are up or downregulated as a result of C-section deliveries can potentially reveal additional mechanisms of resistance where microbial growth could be impacted by host-secreted metabolites within the gut (31). Additionally, due to a lack of other studies taking this specific avenue of inquiry and the few confounding factors that were not accounted for in our study (antibiotic use, race, sex), confirming the obtained result by a different cohort of infants can further serve to cement the remedying role of breast milk in C-section deliveries and potentially reveal novel mechanisms by which breast milk is able to alleviate the limitations faced by the C-section microbiome.

DATA AVAILABILITY

Bash and R scripts developed for this project are available at the following GitHub repository: https://github.com/arshiatavangar/Project_2

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CONTRIBUTIONS

Arshia contributed to the title, abstract, introduction, methods, results, discussion, limitations, conclusion and future directions. Cynthia contributed to the limitations, future directions, acknowledgements, methods, figures, results, discussion for core microbiome analysis and alpha diversity. Earl contributed to the methods, figures, results, discussion for alpha and beta diversity metrics. Kris contributed to the methods, figures, results, discussion for DESEQ analysis, indicator species analysis, and data availability. Everyone contributed to the references, formatting, and proofreading.

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