Sociodemographic Factors and Intestinal Microbiome Development in Preterm, Very Low Birth Weight Infants

Katelyn Desorcy-Scherer, PhD, RN[1,2] Monica F. Torrez Lamberti, PhD[3]
Michael Weaver, PhD, RN, FAAN[1] Graciela L. Lorca, PhD[3] Leslie A. Parker, PhD, APRN, FAAN, FAANP[1]

[1] College of Nursing, University of Florida, Gainesville, Florida
[2] School of Nursing, University of Wisconsin, Madison, Wisconsin
[3] Department of Microbiology and Cell Science, Genetics Institute, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida

Address for correspondence Katelyn Desorcy-Scherer, PhD, RN, School of Nursing, University of Wisconsin-Madison, 701 Highland Avenue, Madison, WI 53705 (e-mail: desorcy@wisc.edu).

Am J Perinatol

Abstract

Objective Preterm very low birth weight (VLBW) infants are at risk for intestinal morbidities and dysbiotic development of the intestinal microbiome. Despite the influence of sociodemographic factors on premature infant health outcomes, whether they shape the intestinal microbiome early in life is not clear. The objective was to explore the associations between race, sex, and socioeconomic status and the intestinal microbiome of VLBW infants during the first 4 weeks of life.

Study Design This was a secondary analysis of data from an ongoing randomized trial of 79 infants ≤30 weeks' gestation and ≤1,500 g. Stool samples were collected at week 1 through week 4, frozen to −80°C and analyzed by 16S rRNA sequencing of the V4 region using Illumina MiSeq. Reads were analyzed to measure α and β diversity as well as relative abundance of bacteria in the intestinal microbiome.

Results Of the 79 infants, 63 had at least one sample available. Twenty-three (37%) of infants were African American, 30 (48%) were male, and 44 (71%) had Medicaid insurance. There were no statistically significant (<0.05) differences in α diversity or β diversity, and the differential abundance analysis suggests limited patterns of distinction in the intestinal microbiome between non-African American and African American infants, male and female infants, and infants with maternal private or Medicaid insurance.

Conclusion Our results suggest race, sex, and socioeconomic status shape colonization of specific microorganisms to a limited extent. Future studies should confirm these findings and determine clinical relevance through further study of differentially abundant microorganisms and additional factors contributing to colonization patterns.

Keywords
- gut microbiome
- premature
- race
- sex
- socioeconomic status
- insurance

Key Points

- Diversity of the gut microbiome was similar between infants of varying race, sex, and socioeconomic status.
- We observed sociodemographic-linked differences in colonization of individual taxa.
- Further study is required to confirm these results and the clinical relevance of these findings.

received
November 4, 2022
accepted after revision
May 2, 2023

Thieme Medical Publishers, Inc.,
333 Seventh Avenue, 18th Floor,
New York, NY 10001, USA

DOI https://doi.org/10.1055/s-0043-1769793.
ISSN 0735-1631.

Preterm very low birth weight infants (VLBW; born ≤1,500 g) have a physiologically underdeveloped gastrointestinal system including compromised barrier function of intestinal cells, decreased intestinal motility, and delayed microbial colonization of the gut lumen.[1,2] Decreased intestinal integrity combined with high expression of immune signaling molecules predisposes the preterm infant to intestinal inflammatory injuries.[3] Necrotizing enterocolitis (NEC) is a severe inflammatory and often necrotic condition of the intestine. Approximately 9% of VLBW infants are diagnosed with NEC and mortality ranges from 20 to 35%.[4,5] Current literature suggests multiple factors contribute to NEC development including immune and gastrointestinal immaturity, enteral feeding practices, and a dysbiotic intestinal microbiome.[3,6] There is a strong scientific and clinical interest in further understanding factors that may predispose VLBW infants to morbidities of the intestine, which includes development of the intestinal microbiome.

Although evidence suggests limited microbial intestinal colonization occurs before birth, the microbiome grows expeditiously during and following delivery.[2,7,8] Several factors influence the development of the intestinal microbiome in VLBW infants, including prematurity,[2] antibiotic exposure,[9–11] mode of delivery,[12–14] feeding practices,[15–18] the hospital environment,[19,20] chorioamnionitis,[12,21] and stress.[22] Notably, whether social and demographic factors contribute to development of the intestinal microbiome in VLBW infants has not been conclusively determined, despite the influence of race, sex, and socioeconomic status (SES) on preterm infant health outcomes. Specifically, infants of Black or Hispanic race/ethnicity have higher odds of developing NEC and suffering mortality, female infants have an overall survival advantage, and postresuscitation survival is the highest for White, non-Hispanic infants of high maternal education and private insurance coverage.[23–26]

Despite the connection between social and demographic factors and preterm infant health, sociodemographic factors like race, sex, and SES have been infrequently investigated in the context of the developing gut microbiome. Most microbiome studies conducted from a sociodemographic lens have focused on healthy adult and infant populations despite the vulnerability of VLBW infants to microbiome-associated diseases. Studies conducted in adults show that socioeconomic status measured both individually and at the neighborhood level is associated with microbiome diversity and composition.[27,28] Patterns of influence extend to healthy children and infants, where evidence suggests that maternal race/ethnicity and measures of SES contribute to early-life intestinal microbiome development.[29–33] Importantly, limited studies have characterized the effects of social and demographic factors on the VLBW infant gut microbiome. Prematurity is a known determinant of the intestinal microbiome and VLBW infants are subject to a variety of care practices, including intensive antibiotic exposure and donor human milk feeding, which may distinguish the preterm intestinal microbiome from infants born at greater gestational ages and birth weights. In preterm infant cohorts, Latino ethnicity has been associated with high Firmicutes and low Gammaproteobacteria levels, opposing trends of high Gammaproteobacteria levels that have been shown to precede NEC.[14,34] Female infants have also shown higher intestinal microbiome diversity and taxon-level changes in two studies, yet others found no difference.[22,35–39] Location of familial residence, employed as an indicator of SES, also had an impact on gut microbiome diversity and colonization patterns of premature infants at 10 days and 4 months of age.[40]

Investigation into the effects of SES on the premature infant intestinal microbiome may further our understanding of factors contributing to development of dysbiosis and associated inflammatory diseases, including NEC. Here, we explore whether there is an association between race, sex, and SES and the intestinal microbiome of VLBW infants during the first 4 weeks after birth. Due to the profound impacts of social and demographic factors on preterm infant health,[24–26] we hypothesize that race, sex, and socioeconomic status are associated with in patterns of colonization in the VLBW infant intestinal microbiome.

Materials and Methods

Study Design

This study is a secondary analysis of existing data from a randomized controlled trial collected from November 2018 through October 2020. The primary goal of the parent study was to determine the relationship between feeding tube dwell time, feeding tube colonization, and health outcomes in VLBW infants. The study was approved by the University of Florida Institutional Review Board and enrollment is expected to continue through 2022.

Setting and Sample

Subjects were sampled from a 70-bed level IV neonatal intensive care unit (NICU). All infants were fed either mother's own milk (MOM) or donor human milk. Initiation, advancement, and fortification of feedings followed a standardized NICU feeding protocol and none of the infants received probiotic supplementation. Infants were included if born at ≤30 weeks' gestational age with a birth weight ≤1,500 g. Those with congenital anatomic gastrointestinal abnormalities or not expected to survive were excluded. Those needing surgery for intestinal complications related to NEC or intestinal perforation were withdrawn from the study. Infants were included in the present study if they had at least one stool sample available for microbiome analysis.

Written parental consent was obtained within 48 hours after delivery by a member of the research team. Stool samples were collected once weekly for 4 weeks from the infant's diaper by trained research staff using a sterile collection container and immediately frozen at −80°C until the time of analysis. Clinical data including race, insurance status, infant sex, gestational age, birth weight, antibiotic exposure, and feeding type were collected from electronic health records. Infant race was defined by maternal self-reported race and was recorded as either African American, Caucasian, Asian, or other. Ethnicity was documented as

either Hispanic/Latino or non-Hispanic/Latino. For the purposes of this study, race was defined as African American or non-African American, as recorded in medical records. A dichotomous classification was employed as small cell sizes prevented analysis of additional racial or ethnic groups. Type of insurance (Medicaid or private) was used as a proxy indicator for SES. Insurance coverage was considered as Medicaid if the mother received coverage by Medicaid or by both Medicaid and private insurance. Antibiotic exposure was recorded weekly as "yes" or "no" based on whether the infant received antibiotic therapy the week of each stool sample collection. Percent MOM feeding was measured by the percent of MOM fed to infants during each week of the study.

DNA Extraction and Sequencing

DNA was extracted from stool samples using QIAamp PowerFecal Pro DNA kit (QIAGEN) with the following modification: 100 µL of Protease from *Streptomyces griseus* 20 mg/mL (Sigma–Aldrich, Steinheim, Germany) was added.[41] The mixture was incubated at 37°C for 15 minutes, then the samples were processed according to the kit protocol. DNA was eluted in 50 µL of water and quantified; the concentration was standardized to 1 ng/µL before the library construction. Polymerase chain reaction (PCR) amplification of the variable V4 region was performed using 515F/806R barcoded primers for Illumina HiSeq platform[42] in 40 µL reactions containing 20 µL Phusion High-Fidelity Master Mix (New England Biolabs, Ipswich, MA), 1.2 µL DMSO, and 2µL of each primer. Duplicate PCR amplifications were pooled for each sample and cleaned with a QIAquick PCR Purification Kit (QIAGEN). A total of 250 ng of each cleaned amplicon library were submitted for sequencing at the ICBR core facility at the University of Florida. Sequencing was performed on an Illumina MiSeq with a 300-bp paired-end protocol, using single indexing. Sequencing reads were parsed by Illumina index at the sequencing center with primers and adapters removed.

Statistical Analysis

Statistical analysis and data processing of reads were done in the statistical computing environment R (v 4.1.2). Using DADA2 (v1.16.0),[43] read quality profiles were visualized and reads were trimmed 5 bases from the beginning. Error rates were inferred using Dada2's parametric error model. One sample was excluded from the analysis for low quality. Reads were merged, an amplicon sequence variant (ASV) table was constructed, and chimeras were removed. ASVs are unique DNA sequences resulting from 16S rRNA analysis of the stool samples. Utilization of ASVs in comparison to operational taxonomic units (OTUs) provides high-level resolution by preventing the need for aggregation at a specified percent similarity (~97%) for OTU definition.[44] Taxonomy was assigned using the Silva database (v.138.1), which was last updated on March 10, 2021. Demographic and clinical metadata were stored in Microsoft Excel (v.16.45). The ASV and taxonomy tables were joined with the metadata using the Phyloseq package (v.1.32.0).[45]

Reads were normalized by rarefaction before α diversity analyses to 90% of the minimum sample depth at 17,488 reads.[45] Alpha diversity was calculated using Shannon's α diversity index in Phyloseq.[45] Clinical and demographic data including the α diversity values were evaluated using descriptive statistics. Data were exported to SAS (v.9.4) for analysis with a mixed-effects model. A variance components covariance structure for the residuals was selected by Bayesian Information Criteria. Beta diversity data were nonrarefied and ordinated by nonmetric multidimensional scaling. Dissimilarity matrices were calculated using Bray–Curtis dissimilarity and comparisons were made using the Phyloseq package (v.1.32.0).[45]

Testing for taxa of differential abundance was performed in Corncob (version 0.2.0), an R package designed to model microbial sequencing data with β-binomial regression.[46] ASVs were filtered and included in the analysis if they appeared more than 5 times in 50% of the samples. The type I error significance level was set to $\alpha = 0.05$. The models included race, sex, and SES as primary variables of interest. Antibiotic exposure and MOM feeding were added as covariates with the knowledge that these clinical exposures may affect bacterial colonization. All figures were produced using ggplot2 package in R(v.3.3.2).[47]

Results

Of the 79 infants enrolled in the parent study as of October 2020, 63 infants had a total of 197 16S rRNA sequenced stool samples available for statistical analysis. One sample was removed due to poor read quality, making the total analyzed cohort 62 infants. Forty-five of the analyzed samples were from week 1, 53 from week 2, 47 from week 3, and 51 from week 4 (►Fig. 1). Twenty-three (37%) infants were African American, 30 (48%) were male, and 44 (71%) had Medicaid insurance coverage. Of the non-African American infants, 35 (56%) of the mothers' self-identified as Caucasian, 2 (3%) as

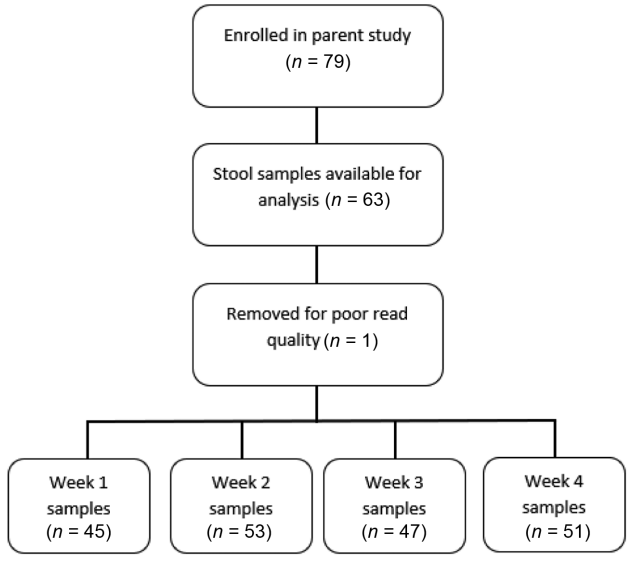

Fig. 1 Consort diagram. Consort diagram showing patient enrollment and sample analysis.

Table 1 Clinical characteristics by social and demographic factors

Frequency no. (% of total sample) or mean ± standard deviation						
Variable ($n = 62$)	Race		Sex		Insurance	
	AA	Non-AA	Male	Female	Private	Medicaid
Social and demographic factors	23 (37%)	39 (63%)	30 (48%)	32 (52%)	18 (29%)	44 (71%)
Gestational age (wk)	28.0 ± 2.0	27.9 ± 2.0	27.9 ± 2.0	28.1 ± 2.0	28.2 ± 1.9	28.0 ± 2.0
Birth weight (g)	1,042.3 ± 287.0	1,032.2 ± 291.2	1,035.3 ± 289.8	1,039.3 ± 288.4	1,046.0 ± 287.0	1,035.3 ± 289.8
Mode of delivery						
Cesarean section	13 (21%)	24 (39%)	16 (26%)	21(34%)	17 (27%)	20 (32%)
Antibiotic exposure						
Week 1	5 (8%)	9 (15%)	6 (10%)	8 (13%)	5 (8%)	9 (15%)
Week 2	3 (5%)	3 (5%)	2 (3%)	4 (6%)	3 (5%)	3 (5%)
Week 3	0 (0%)	3 (5%)	2 (3%)	1 (2%)	0 (0%)	3 (5%)
Week 4	0 (0%)	5 (8%)	4 (6%)	1 (2%)	0 (0%)	5 (8%)
MOM feeding	57.7% ± 35.0%	59.3% ± 34.6%	58.4% ± 35.1%	58.7% ± 34.5%	57.7% ± 35.3%	58.4% ± 35.1

Abbreviations: AA, African American; MOM, mother's own milk; Non-AA, non-African American.
Note: MOM feeding is the average percentage of MOM fed to infants during weeks 1 to 4.

Asian, and 2 (3%) self-identified their race as other but with Hispanic/Latino ethnicity. Demographic and clinical characteristics of the sample are in ►Tables 1 and 2. Analysis of the samples resulted in 35,728 ASVs from 11 phyla, 16 classes, 50 order, 79 family, 131 genera, and 57 species. Approximately 85% of ASVs remained after chimera removal. After filtering of the taxa, five taxa remained on weeks 1 and 2, whereas four taxa remained for week 4.

Race

Alpha and β diversity measures were similar between non-African American and African American infants. (►Table 3; ►Fig. 2). Based on our differential abundance analysis, one ASV matching to the *Staphylococcus* genus was identified as associated with race. At week 2, the model identified *Staphylococcus* as having a higher relative abundance in infants of African American race than non-African American (►Table 4; ►Fig. 3).

Sex

Alpha and β diversity were similar between infants based on sex (►Table 3; ►Fig. 2). In the differential abundance model, sex was not associated with any taxa.

Socioeconomic Status

Using insurance type as a proxy for SES, α diversity was similar between Medicaid and private insurance groups (►Table 3; ►Fig. 2). In our differential abundance analysis,

Table 2 Comparisons between social and demographic characteristics

Frequency no. (% of total sample)						
Variable ($n = 62$)	Race		Sex		Insurance	
	AA	Non-AA	Male	Female	Private	Medicaid
Race						
AA			12 (19%)	11 (18%)	4 (6%)	19 (31%)
Non-AA			18 (29%)	21 (34%)	14 (23%)	25 (40%)
Sex						
Male	12 (19%)	18 (29%)			8 (13%)	22 (35%)
Female	11 (18%)	21 (34%)			10 (16%)	22 (35%)
Insurance						
Medicaid	19 (31%)	25 (40%)	22 (35%)	22 (35%)		
Private	4 (6%)	14 (23%)	8 (13%)	10 (16%)		

Abbreviations: AA, African American; Non-AA, non-African American.
Notes: Table is a detailed representation of social and demographic characteristics within the sample. The diagonal is not provided as it represents a self-comparison.

Table 3 Results of the α and β diversity analyses

Comparison	Alpha diversity (*p*-value)	Week 1 beta diversity (*p*-value/sequential R^2-value)	Week 2 beta diversity (*p*-value/sequential R^2-value)	Week 3 beta diversity (*p*-value/sequential R^2-value)	Week 4 beta diversity (*p*-value/sequential R^2-value)
Race	0.84	0.45/0.022	0.94/0.009	0.61/0.018	0.58/0.017
Sex	0.13	0.33/0.025	0.58/0.016	0.52/0.019	0.60/0.016
Insurance coverage	0.54	0.50/0.021	0.56/0.017	0.88/0.013	0.28/0.024
MOM feeding	0.82	0.39/0.022	0.36/0.020	0.68/0.017	0.38/0.021
Antibiotic exposure	0.13	0.34/0.024	0.62/0.016	0.022[a]/0.050	0.71/0.015
Week	<0.01[a]	N/A	N/A	N/A	N/A

Abbreviations: MOM, mother's own milk; N/A, not applicable.
[a]Below the user-specified α of 0.05.
Notes: Alpha diversity *p*-values were generated using Shannon's Index and a mixed-effects model. Beta diversity *p*-values were generated using Bray–Curtis dissimilarity and PERMANOVA. Week was included in the mixed-effects model for α diversity. Beta diversity calculations were performed by week and therefore did not include week in the model. Week in β diversity results is listed as "N/A." Sequential R^2 value reported are ratios of sequential sum of squares (SS/SSTotal) in the order that they appear in the table.

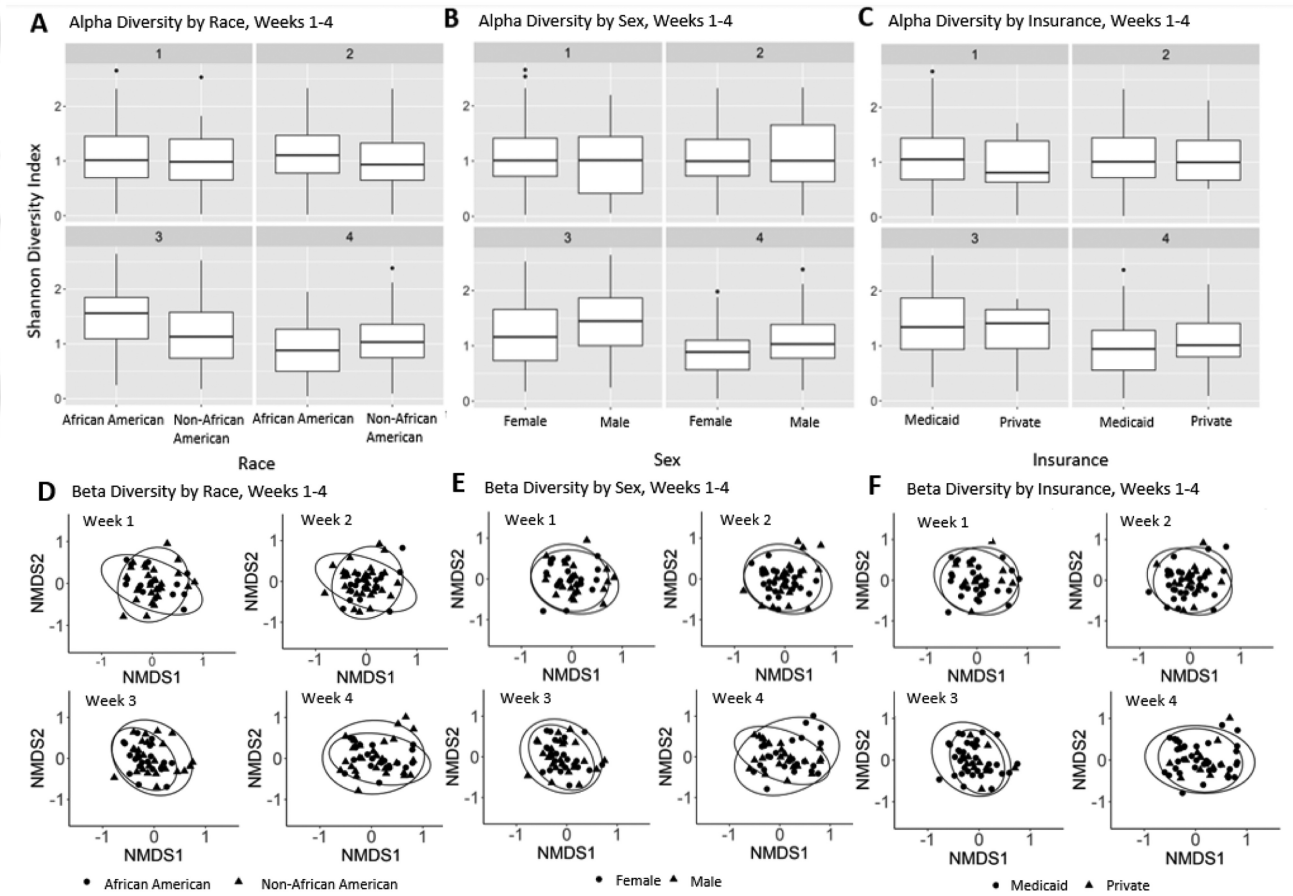

Fig. 2 Plots of α and β diversity analyses. **A–C** are boxplots of α diversity analyses at weeks 1 to 4 by race, sex, and SES. **D–F** show nonmetric multidimensional scaling (NMDS) calculated with Bray–Curtis dissimilarity on weeks 1 to 4 by race, sex, and socioeconomic status.

two ASVs were found to be associated with insurance status, *Staphylococcus* and *Escherichia–Shigella*. *Staphylococcus* was higher in private insurance groups at weeks 1 and 2 when compared with Medicaid (►**Table 4**; ►**Fig. 4**). Conversely, *Escherichia–Shigella* was more abundant in Medicaid groups at weeks 1 and 4 (►**Table 4**; ►**Fig. 5**).

Human Milk Feeding and Antibiotic Exposure

Weekly percent MOM feeding and antibiotic exposure were included in the statistical models as covariates and were associated with *Staphylococcus* and *Escherichia–Shigella* in the gut microbiome. At week 1, *Staphylococcus* and *Escherichia–Shigella* relative abundances were higher in infants

Table 4 Results of the differential abundance analyses

	Week 1			Week 2			Week 4		
	p-Value	Estimate	Std. error	*p*-Value	Estimate	Std. error	*p*-Value	Estimate	Std. error
Escherichia–Shigella	<0.001[a,b]	N/A	N/A	0.338	N/A	N/A	0.010[a,b]	N/A	N/A
Race									
Non-AA	0.137	−0.812	0.532				0.163	−0.834	0.587
Sex									
Male	0.264	−0.623	0.549				0.507	−0.341	0.510
Insurance									
Private	<0.001[a]	−3.397	0.574				<0.001[a]	−3.214	0.693
Mother's own milk	<0.001[a]	0.032	0.009				0.763	−0.002	0.006
Antibiotic exposure									
Yes	<0.001*	−1.944	0.527				0.004[a]	−2.901	0.959
Staphylococcus	0.031[a,b]	N/A	N/A	0.022[a,b]	N/A	N/A	0.275	N/A	N/A
Race									
NAA	0.070	−0.855	0.457	<0.001[a]	−2.320	0.617			
Sex									
Male	0.607	−0.277	0.534	0.189	0.778	0.476			
Insurance									
Private	<0.001[a]	1.69	0.450	<0.001[a]	2.378	0.416			
Mother's own milk	0.002[a]	−0.030	0.009	0.959	−0.001	0.006			
Antibiotic exposure									
Yes	<0.001[a]	2.029	0.492	0.417	−1.133	0.924			

Abbreviations: ASV, amplicon sequence variant; FDR, false discovery rate; N/A, not applicable; Non-AA, non-African American; std. error: standard error.

[a]Below the user-specified α of 0.05.

[b]*p*-Value indicates ASV is differentially abundant in a multitaxa model. All other *p*-values are from an individual taxon regression model. All models included race, sex, insurance, mother's own milk and antibiotic exposure. N/A based on analysis. Marked *p*-values were adjusted for 0.05 FDR among ASVs within each week by the Benjamini–Hochberg procedure. Estimates displayed are logit coefficients for expected relative taxa abundance.

with higher MOM feeding (►Table 4). Also at week 1, infants with higher antibiotic exposure had higher levels of *Staphylococcus* but lower levels of *Escherichia–Shigella* (►Table 4). We also found an association between antibiotic exposure at week 4 and lower abundances of *Escherichia–Shigella* (►Table 4).

Discussion

Results of this study suggest race, sex, and socioeconomic status exert a limited influence on the development of the VLBW infant intestinal microbiome over the first 4 weeks of life. Our findings also confirm the extreme variability and sparse colonization patterns of the gut microbiome in this population. Alpha diversity analyses revealed that the richness and evenness of bacterial communities within each sample were similar across all comparison groups including race, sex, socioeconomic status, MOM feeding, and antibiotic exposure in all weeks. Alpha diversity did vary across weeks 1 to 4 which was expected. Slow or variable increase in α diversity over time may be attributed to antibiotic exposure or prematurity within the sample population as diversity within the intestinal microbiome of preterm infants may increase at a slower rate compared with term infants.[48] Beta diversity analyses also indicated that little dissimilarity in the microbial communities was explained by race, sex, and SES. Furthermore, differential abundance of ASVs was associated with social and demographic factors yet patterns were not frequently sustained over multiple weeks. Notable exceptions are an association between higher antibiotic exposure and lower *Escherichia–Shigella* observed at weeks 1 and 4, a lower abundance of *Escherichia–Shigella* in the private insurance group at weeks 1 and 4, and a higher abundance of *Staphylococcus* in the private insurance group in weeks 1 and 2 (►Table 4).

Escherichia coli and *Shigella* are closely related members of the Gammaproteobacteria class.[49] Trends of increasing Gammaproteobacteria colonization in the preterm infant intestine have been associated with NEC development, particularly in the case of late-onset NEC (>22 d of life).[38] Excessive bacterial signaling has been implicated in NEC development through a bacterial receptor called toll-like receptor 4 (TLR-4). *Escherichia–Shigella* are part of the Enterobacteriaceae family, which express lipopolysaccharide, a TLR-4 ligand.[50] Our study identified a higher abundance of an ASV matching to *Escherichia–Shigella* to be associated with

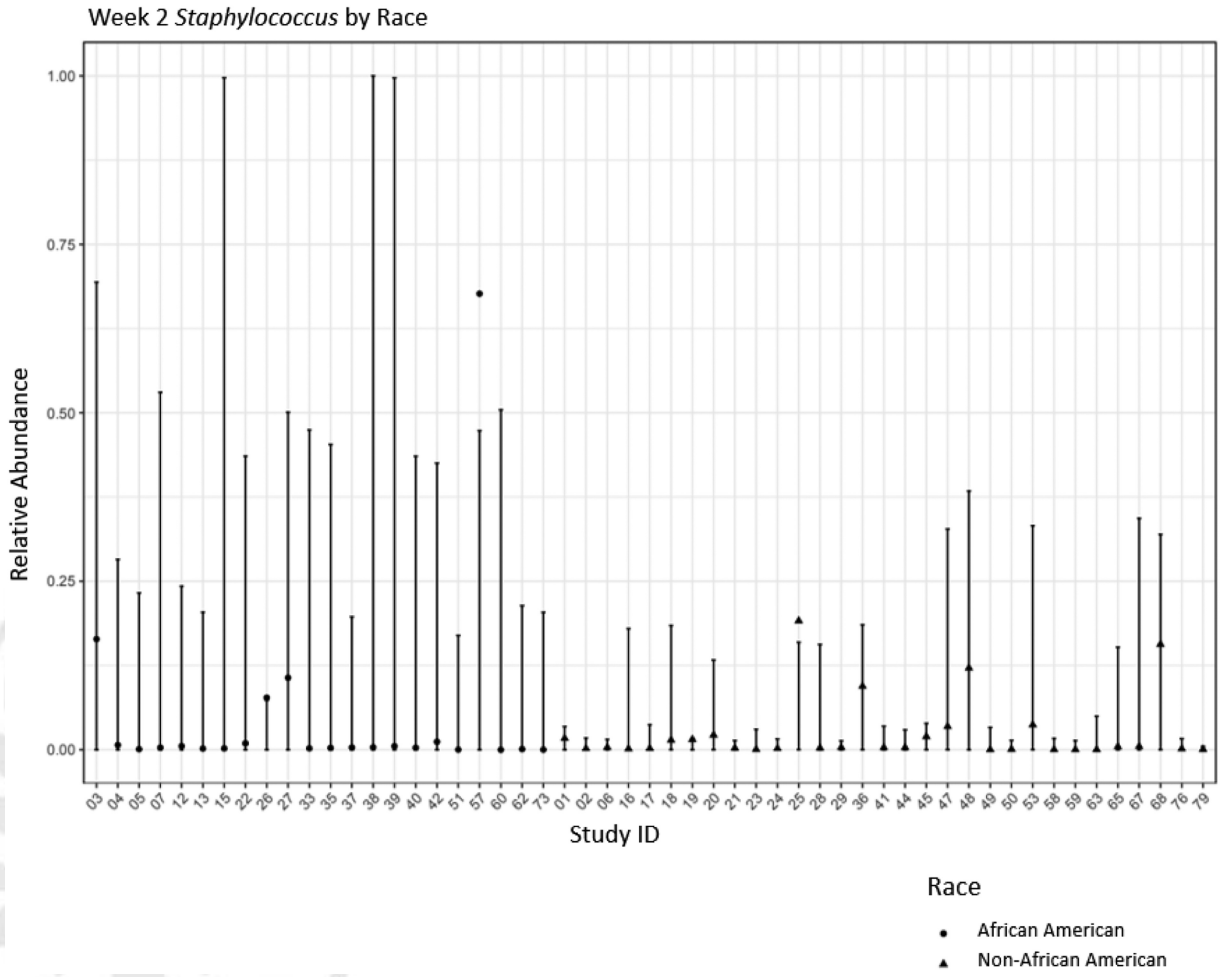

Fig. 3 Plot of week 2 *Staphylococcus* colonization by race. Plot of relative abundances (point/triangle) with 95% bootstrap prediction intervals (bars) for *Staphylococcus* at week 2.

Medicaid insurance coverage, higher MOM feeding, and no antibiotic exposure. Exactly why infants of maternal Medicaid coverage had higher levels of *Escherichia–Shigella* is not clear. High colonization of *Escherichia–Shigella* in MOM-fed infants suggest that *Escherichia–Shigella* may be part of the flora present in MOM or that MOM feeding creates an intestinal environment that is conducive to *Escherichia–Shigella* growth. Notably, human milk contains human milk oligosaccharides that limit TLR-4 signaling.[50,51] The association between high *Escherichia–Shigella* and no antibiotic exposure suggests that commonly used antibiotics in the NICU setting may exert antimicrobial activity against *Escherichia–Shigella*.

Staphylococcus is a major colonizer of the preterm infant intestinal microbiome. In patterns of preterm infant microbiota acquisition, infants begin with a gut microbiome high in *Staphylococcus* and progress through subsequent *Enterococcus*, *Enterobacter*, and *Bifidobacterium*-dominated states.[52] Although *Staphylococcus* colonization is not necessarily pathogenic, *Staphylococcus* has been identified as the causative microorganism in bloodstream infections and it has been identified as more abundant in the intestinal microbiome prior to late onset sepsis or sepsis occurring after 48 hours of age.[52] High abundance of *Staphylococcus* in the infant intestinal microbiome has been associated with prematurity, human milk feeding, and, to a limited extent, vaginal birth.[52] Notably, all but one infant (17/18) in the private insurance category of our study was born by cesarean section, which limits our ability to evaluate mode of delivery as a factor in SES associations (►**Tables 1** and **2**). Exposures to *Staphylococcus* also include the environment where cultural hygiene practices may influence transmission.[52] Our results suggest that African American race, private insurance coverage, antibiotic exposure, and donor human milk feeding may be associated with higher *Staphylococcus* abundance. While our study was not designed to examine the influence of factors beyond sociodemographic factors and select covariates, it is possible to hypothesize that the association between African American race, private insurance coverage and *Staphylococcus* colonization may be supported by varying degree of exposure to familial contact, health care interventions and the health care system. A factor to consider is skin-to-skin contact. *Staphylococcus* is present at high levels on the skin, and we can offer the hypothesis that infant gut colonization may differ if skin to skin contact was variable between sociodemographic groups. *Staphylococcus* also may colonize the gut microbiome to a greater extent in infants with higher levels of donor human milk feeding and

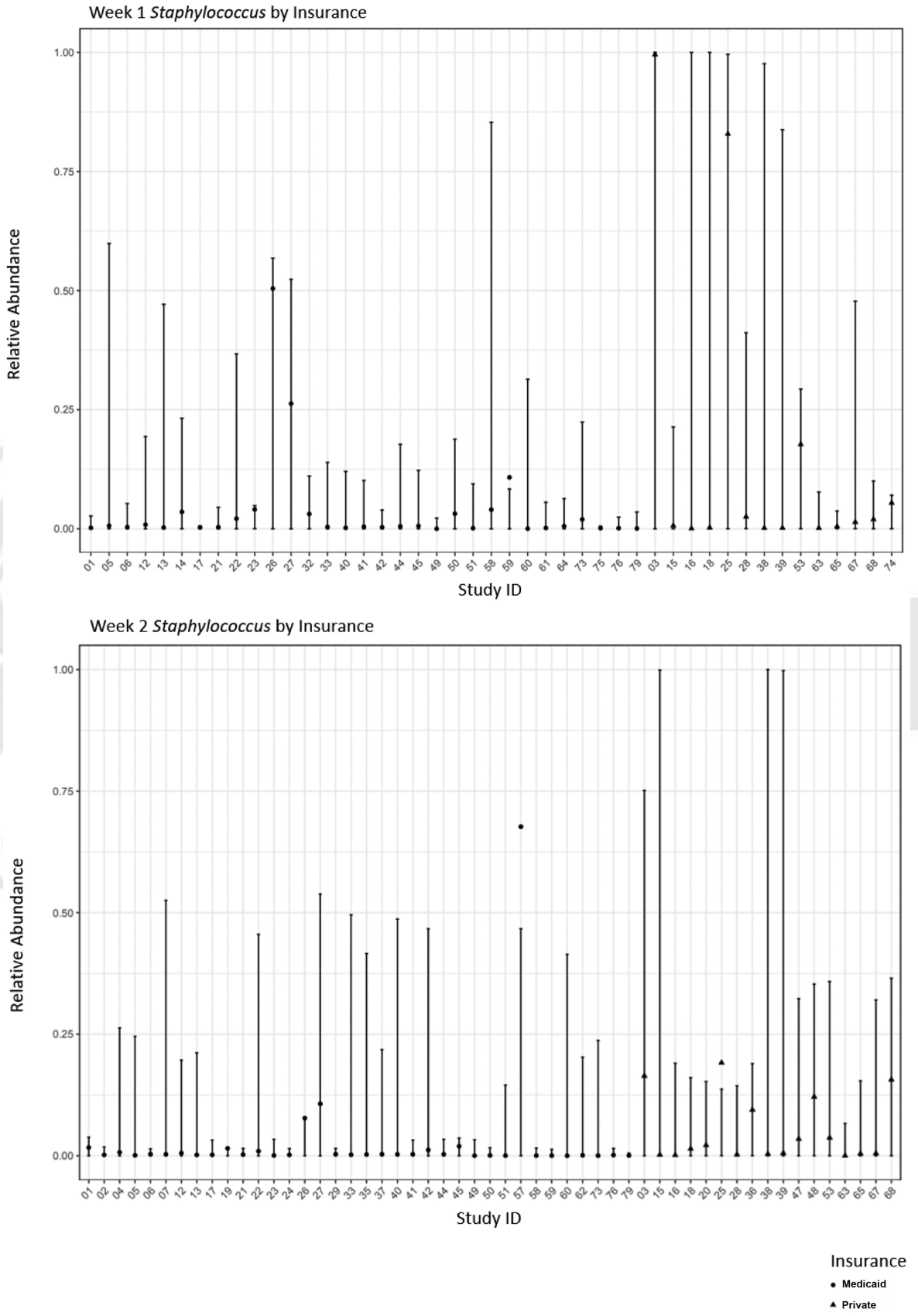

Fig. 4 Plot of weeks 1 and 2 *Staphylococcus* colonization by insurance status. Plots of relative abundances (point/triangle) with 95% bootstrap prediction intervals (bars) for *Staphylococcus* at weeks 1 and 2, separated by insurance.

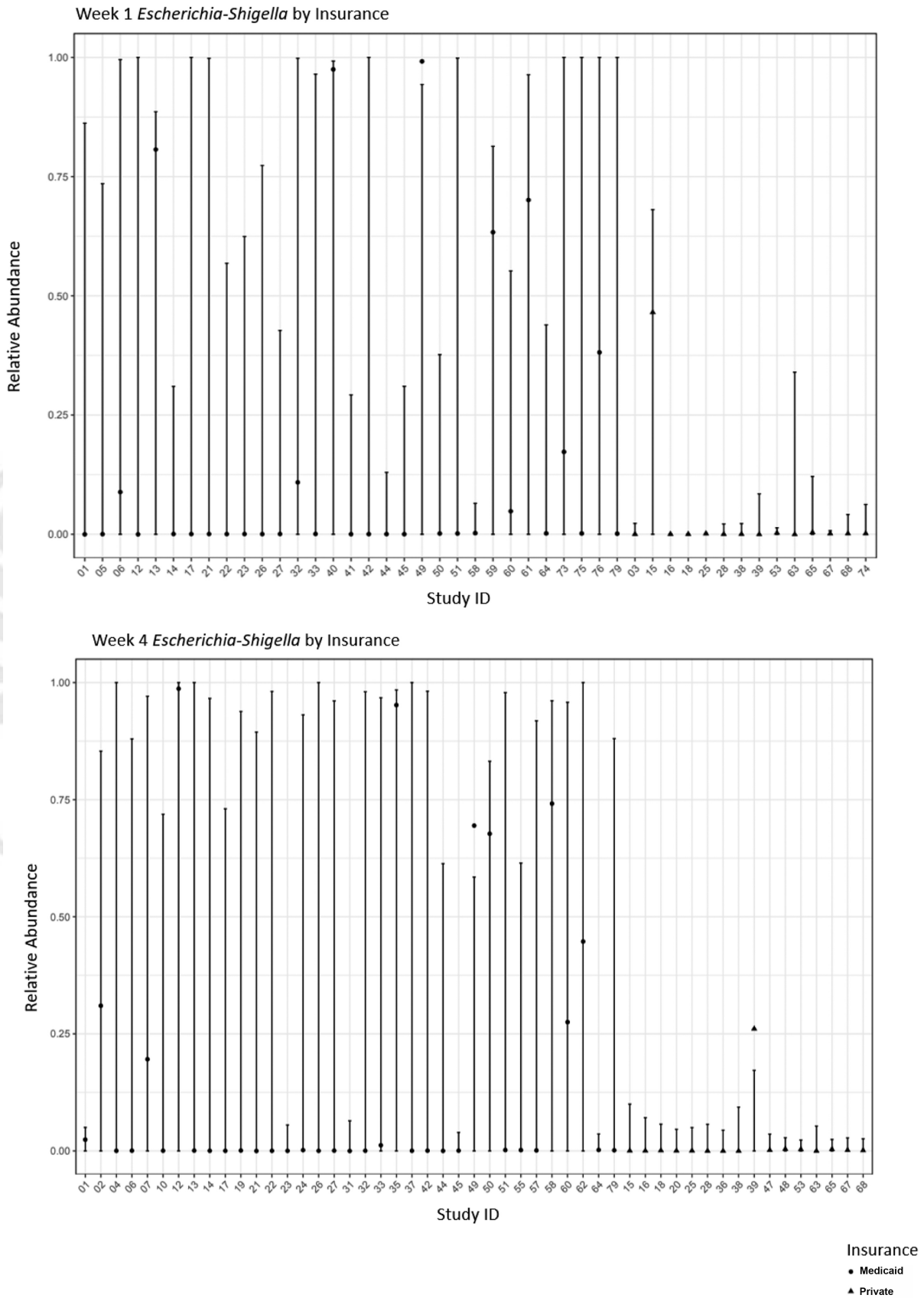

Fig. 5 Plot of weeks 1 and 4 *Escherichia–Shigella* colonization by insurance status. Plots of relative abundances (point/triangle) with 95% bootstrap prediction intervals (bars) for *Escherichia–Shigella* weeks 2 and 4, separated by insurance status.

antibiotic exposure, considering that donor human milk is sterile and antibiotics are antimicrobial agents. Sterile feedings and antibiotic exposure may ultimately reduce competition pressure, creating suitable conditions for high *Staphylococcus* colonization.

Previous studies have found that race, sex, and socioeconomic status affect the preterm infant gut microbiome development to varying extents. Latino race has been associated with lower levels of Gammaproteobacteria and higher levels of Firmicutes.[14,37] Female infants may have an intestinal microbiome with higher relative abundance of *Clostridiates*, lower *Enterobacteriales*, and *Proteus* and higher diversity when compared with male infants, although others report findings of no difference.[22,35–39] Lower SES area of residence was associated with higher α diversity, *Bifidobacterium* and *Megasphaera* at day of life 10.[40] Discordant results between the current study and previously published literature in term or healthy infants may be attributable to differences in population. This is among the few studies that have examined race, sex, and SES as determinants of the intestinal microbiome in an exclusively VLBW cohort. Infants born VLBW are physiologically immature and prematurity itself is a factor contributing to the development of the intestinal microbiome.[2] Additionally, VLBW infants are subject to a variety of other care practices with the potential to alter microbiome colonization including cesarean delivery, H2 blocker therapy, donor human milk feeding, and intensive antibiotic exposure.[53] It is possible that prematurity and care practices of VLBW infants may limit their comparability to infant cohorts with higher birth weights or gestational age at birth. The observation that the majority of the ASV associations were not sustained through multiple weeks demonstrates the extremely variable nature of the VLBW infant intestinal microbiome. In addition to the influence of prematurity and illness on the intestinal microbiome, preterm infants proceed through a patterned progression of bacterial establishment.[2,54] The preterm infant gut is also characterized by overall low diversity in comparison to term infants, potentially creating a predisposition for dominance of one or more types of bacteria, which may be transient.[2]

Limitations

This secondary analysis of data has several limitations. First, the data were collected for the primary purposes of the parent study, which has resulted in comparisons of infants based on predetermined categorical social and demographic factors. Race and SES are descriptors that cannot be fully collapsed into the categories used in this study. Prospective studies further determining the influence of these factors on microbial communities should consider study designs that allow the sampling of additional races and ethnicities as well as additional measures of SES beyond insurance coverage. Future analyses may also incorporate measures of contact such as parental time at the bedside or skin-to-skin care.

Sociodemographic comparisons are limited by the population sampled by the parent study. There are several imbalances in sociodemographic group sizes such as insurance status where 71% of the sample population reported maternal Medicaid coverage. Our study also was only able to include ASVs if they appeared more than 5 times in 50% of the samples to preserve the ability of the statistical model to produce accurate prediction intervals. Due to the extreme variation present in the developing intestinal microbiome of preterm infants, the model resulted in only 4 to 5 top taxa per week for comparison between sociodemographic groups. Additional considerations include that adding covariates like MOM feeding and antibiotic exposure to analyses may mask clinically relevant relationships between sociodemographic factors and microbiome development that exist secondary to a set of environmental conditions or exposures that are common among race, sex, and socioeconomic groups. Finally, the results of this study are exploratory, and future statistically robust studies are necessary to confirm these findings and to determine their clinical relevance.

Conclusion

Similarity in α and β diversity suggest that infant race, sex, and socioeconomic status may play a limited role in shaping the intestinal microbiome of VLBW infants. Differences in the abundance of specific microorganisms are rarely sustained throughout multiple weeks and imply differences in colonization may be transient. The clinical significance of differential microorganism abundance is not clear. The impact of dissimilarity in β diversity or differential abundance on infant health may be investigated using additional omics techniques. Future studies aiming to examine microbiome development in VLBW infants may consider utilizing metabolomics or metagenomics and confirming these results with larger sample sizes. Whether colonization patterns are sustained throughout early life may also be determined by extending the period of study beyond 4 weeks.

This submission is related to a clinical trial. The ClinicalTrials.gov Identifier is NCT03728608.

Data Sharing Statement

The data that support the findings of this study are part of an ongoing, National Institutes of Health–funded clinical trial. Data will be shared at time of publication for the parent study.

Funding

Research reported in this publication was supported by the National Institute of Nursing Research of the National Institutes of Health under award number "R01NR016964."

Conflict of Interest

None declared.

Acknowledgments

We thank Evon Scarlett-DeBose, Shannon Perry, Krista Zyskowski, and Michael Liou for their scientific, laboratory, clinical, and statistical expertise that contributed to the completion of this project.

References

1 Huda S, Chaudhery S, Ibrahim H, Pramanik A. Neonatal necrotizing enterocolitis: clinical challenges, pathophysiology and management. Pathophysiology 2014;21(01):3–12
2 Groer MW, Miller EM, D'Agata A, et al. Contributors to dysbiosis in very-low-birth-weight infants. J Obstet Gynecol Neonatal Nurs 2020;49(03):232–242
3 Underwood MA, Umberger E, Patel RM. Safety and efficacy of probiotic administration to preterm infants: ten common questions. Pediatr Res 2020;88(1, suppl 1):48–55
4 Hull MA, Fisher JG, Gutierrez IM, et al. Mortality and management of surgical necrotizing enterocolitis in very low birth weight neonates: a prospective cohort study. J Am Coll Surg 2014;218 (06):1148–1155
5 Rich BS, Dolgin SE. Necrotizing enterocolitis. Pediatr Rev 2017;38 (12):552–559
6 Rozé JC, Ancel PY, Lepage P, et al; Nutrition EPIPAGE 2 study group EPIFLORE Study Group. Nutritional strategies and gut microbiota composition as risk factors for necrotizing enterocolitis in very-preterm infants. Am J Clin Nutr 2017;106(03):821–830
7 Rackaityte E, Halkias J, Fukui EM, et al. Viable bacterial colonization is highly limited in the human intestine in utero. Nat Med 2020;26(04):599–607
8 Ardissone AN, de la Cruz DM, Davis-Richardson AG, et al. Meconium microbiome analysis identifies bacteria correlated with premature birth. PLoS One 2014;9(03):e90784
9 Dardas M, Gill SR, Grier A, et al. The impact of postnatal antibiotics on the preterm intestinal microbiome. Pediatr Res 2014;76(02): 150–158
10 Greenwood C, Morrow AL, Lagomarcino AJ, et al. Early empiric antibiotic use in preterm infants is associated with lower bacterial diversity and higher relative abundance of Enterobacter. J Pediatr 2014;165(01):23–29
11 Patel AL, Mutlu EA, Sun Y, et al. Longitudinal survey of microbiota in hospitalized preterm very-low-birth-weight infants. J Pediatr Gastroenterol Nutr 2016;62(02):292–303
12 Chernikova DA, Koestler DC, Hoen AG, et al. Fetal exposures and perinatal influences on the stool microbiota of premature infants. J Matern Fetal Neonatal Med 2016;29(01):99–105
13 Gupta RW, Tran L, Norori J, et al. Histamine-2 receptor blockers alter the fecal microbiota in premature infants. J Pediatr Gastroenterol Nutr 2013;56(04):397–400
14 Ho TTB, Groer MW, Kane B, et al. Dichotomous development of the gut microbiome in preterm infants. Microbiome 2018;6(01):157
15 Gregory KE, Samuel BS, Houghteling P, et al. Influence of maternal breast milk ingestion on acquisition of the intestinal microbiome in preterm infants. Microbiome 2016;4(01):68
16 Ford SL, Lohmann P, Preidis GA, et al. Improved feeding tolerance and growth are linked to increased gut microbial community diversity in very-low-birth-weight infants fed mother's own milk compared with donor breast milk. Am J Clin Nutr 2019;109(04): 1088–1097
17 Yee AL, Miller E, Dishaw LJ, et al. Longitudinal microbiome composition and stability correlate with increased weight and length of very-low-birth-weight infants. mSystems 2019;4(01): e00229-18
18 Parra-Llorca A, Gormaz M, Alcántara C, et al. Preterm gut microbiome depending on feeding type: significance of donor human milk. Front Microbiol 2018;9:1376
19 Brooks B, Firek BA, Miller CS, et al. Microbes in the neonatal intensive care unit resemble those found in the gut of premature infants. Microbiome 2014;2(01):1
20 Brooks B, Olm MR, Firek BA, et al. The developing premature infant gut microbiome is a major factor shaping the microbiome of neonatal intensive care unit rooms. Microbiome 2018;6(01): 112
21 Drell T, Lutsar I, Stšepetova J, et al. The development of gut microbiota in critically ill extremely low birth weight infants assessed with 16S rRNA gene based sequencing. Gut Microbes 2014;5(03):304–312
22 D'Agata AL, Wu J, Welandawe MKV, Dutra SVO, Kane B, Groer MW. Effects of early life NICU stress on the developing gut microbiome. Dev Psychobiol 2019;61(05):650–660
23 Gephart SM, Spitzer AR, Effken JA, Dodd E, Halpern M, McGrath JM. Discrimination of GutCheck(NEC): a clinical risk index for necrotizing enterocolitis. J Perinatol 2014;34(06):468–475
24 Jammeh ML, Adibe OO, Tracy ET, et al. Racial/ethnic differences in necrotizing enterocolitis incidence and outcomes in premature very low birth weight infants. J Perinatol 2018;38(10):1386–1390
25 Morse SB, Wu SS, Ma C, Ariet M, Resnick M, Roth J. Racial and gender differences in the viability of extremely low birth weight infants: a population-based study. Pediatrics 2006;117(01): e106–e112
26 Anderson JG, Baer RJ, Partridge JC, et al. Survival and major morbidity of extremely preterm infants: a population-based study. Pediatrics 2016;138(01):e20154434
27 Bowyer RCE, Jackson MA, Le Roy CI, et al. Socioeconomic status and the gut microbiome: a TwinsUK cohort study. Microorganisms 2019;7(01):17
28 Miller GE, Engen PA, Gillevet PM, et al. Lower neighborhood socioeconomic status associated with reduced diversity of the colonic microbiota in healthy adults. PLoS One 2016;11(02):e0148952
29 Levin AM, Sitarik AR, Havstad SL, et al. Joint effects of pregnancy, sociocultural, and environmental factors on early life gut microbiome structure and diversity. Sci Rep 2016;6:31775
30 Gschwendtner S, Kang H, Thiering E, et al. Early life determinants induce sustainable changes in the gut microbiome of six-year-old children. Sci Rep 2019;9(01):12675
31 Xu J, Lawley B, Wong G, et al. Ethnic diversity in infant gut microbiota is apparent before the introduction of complementary diets. Gut Microbes 2020;11(05):1362–1373
32 Lewis CR, Bonham KS, McCann SH, et al. Family SES is associated with the gut microbiome in infants and children. Microorganisms 2021;9(08):1608
33 Mello CS, Carmo-Rodrigues MS, Filho HB, et al. Gut microbiota differences in children from distinct socioeconomic levels living in the same urban area in Brazil. J Pediatr Gastroenterol Nutr 2016;63(05):460–465
34 Neu J, Pammi M. Necrotizing enterocolitis: the intestinal microbiome, metabolome and inflammatory mediators. Semin Fetal Neonatal Med 2018;23(06):400–405
35 Cong X, Xu W, Janton S, et al. Gut microbiome developmental patterns in early life of preterm infants: impacts of feeding and gender. PLoS One 2016;11(04):e0152751
36 Chen J, Li H, Hird SM, et al. Sex differences in gut microbial development of preterm infant twins in early life: a longitudinal analysis. Front Cell Infect Microbiol 2021;11:671074
37 Aguilar-Lopez M, Dinsmoor AM, Ho TTB, Donovan SM. A systematic review of the factors influencing microbial colonization of the preterm infant gut. Gut Microbes 2021;13(01):1–33
38 Zhou Y, Shan G, Sodergren E, Weinstock G, Walker WA, Gregory KE. Longitudinal analysis of the premature infant intestinal microbiome prior to necrotizing enterocolitis: a case-control study. PLoS One 2015;10(03):e0118632
39 Zwittink RD, Renes IB, van Lingen RA, et al. Association between duration of intravenous antibiotic administration and early-life microbiota development in late-preterm infants. Eur J Clin Microbiol Infect Dis 2018;37(03):475–483
40 Chong CYL, Vatanen T, Alexander T, Bloomfield FH, O'Sullivan JM. Factors associated with the microbiome in moderate-late preterm babies: a cohort study from the DIAMOND randomized controlled trial. Front Cell Infect Microbiol 2021;11:595323

41 Marcial GE, Ford AL, Haller MJ, et al. Lactobacillus johnsonii N6.2 modulates the host immune responses: a double-blind, randomized trial in healthy adults. Front Immunol 2017;8:655
42 Caporaso JG, Lauber CL, Walters WA, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 2012;6(08):1621–1624
43 Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 2016;13(07):581–583
44 Callahan BJ, McMurdie PJ, Holmes SP. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME J 2017;11(12):2639–2643
45 McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 2013;8(04):e61217
46 Martin BD, Witten D, Willis AD. Modeling microbial abundances and dysbiosis with beta-binomial regression. Ann Appl Stat 2020; 14(01):94–115
47 Wickham H. ggplot2: Elegant Graphics for Data Analysis. New York, NY: Springer; 2016
48 Gasparrini AJ, Wang B, Sun X, et al. Persistent metagenomic signatures of early-life hospitalization and antibiotic treatment in the infant gut microbiota and resistome. Nat Microbiol 2019;4 (12):2285–2297
49 Devanga Ragupathi NK, Muthuirulandi Sethuvel DP, Inbanathan FY, Veeraraghavan B. Accurate differentiation of *Escherichia coli* and *Shigella* serogroups: challenges and strategies. New Microbes New Infect 2017;21:58–62
50 Hackam DJ, Sodhi CP. Bench to bedside - new insights into the pathogenesis of necrotizing enterocolitis. Nat Rev Gastroenterol Hepatol 2022;19(07):468–479
51 Sodhi CP, Wipf P, Yamaguchi Y, et al. The human milk oligosaccharides 2′-fucosyllactose and 6′-sialyllactose protect against the development of necrotizing enterocolitis by inhibiting toll-like receptor 4 signaling. Pediatr Res 2021;89(01):91–101
52 Korpela K, Blakstad EW, Moltu SJ, et al. Intestinal microbiota development and gestational age in preterm neonates. Sci Rep 2018;8(01):2453
53 Desorcy-Scherer K, Bendixen MM, Parker LA. Determinants of the very low-birth-weight infant's intestinal microbiome: a systematic review. J Perinat Neonatal Nurs 2020;34(03):257–275
54 La Rosa PS, Warner BB, Zhou Y, et al. Patterned progression of bacterial populations in the premature infant gut. Proc Natl Acad Sci U S A 2014;111(34):12522–12527

