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## Authors
Wu, Allison J
Lee, David J
Li, Fan
et al.

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# Impact of clinical factors on the intestinal microbiome in infants with gastroschisis

**Allison J. Wu, MD**[1], **David J. Lee, BS**[1], **Fan Li, PhD**[1], **Nicole H. Tobin, MD**[1], **Grace M. Aldrovandi, MD**[1], **Stephen B. Shew, MD**[2], **Kara L. Calkins, MD, MS**[3]

[1]Department of Pediatrics, David Geffen School of Medicine, University of California Los Angeles, CA

[2]Department of Surgery, Division of Pediatric Surgery, Stanford University School of Medicine, Palo Alto CA

[3]Department of Pediatrics, Neonatal Research Center of the UCLA of Children's Discovery and Innovation Institute, David Geffen School of Medicine UCLA and UCLA Mattel Children's Hospital, Los Angeles CA

## Abstract

**Background:** Infants with gastroschisis require operations and lengthy hospitalizations due to intestinal dysmotility. Dysbiosis may contribute to these problems. Little is known on the microbiome of gastroschisis infants.

**Methods:** The purpose of this study was to investigate the fecal microbiome in gastroschisis infants. Microbiome profiling was performed by sequencing the V4 region of the 16S rRNA gene. The microbiome of gastroschisis infants was compared to the microbiome of healthy controls, and the effects of mode of birth delivery, gestational age, antibiotic duration, and nutrition type on microbial composition and diversity were investigated.

**Results:** The microbiome of gastroschisis infants (n=13) was less diverse (Chao1, $p < 0.001$), lacked *Bifidobacterium* ($p = 0.001$) and had increased *Staphylococcus* ($p = 0.007$) compared to controls (n=83). Mode of delivery ($R^2 = 0.04$, $p = 0.001$), antibiotics duration greater than or equal to seven days ($R^2 = 0.03$, $p = 0.003$), age at sample collection ($R^2 = 0.03$, $p = 0.009$), and gestational age ($R^2 = 0.02$, $p = 0.035$) explained a small portion of microbiome variation. In gastroschisis infants, *Escherichia-Shigella* was the predominate genus, and those delivered via cesarean section had different microbial communities, predominantly *Staphylococcus* and *Streptococcus*, from those delivered vaginally. While antibiotic duration contributed to the variation in microbiome composition, there were no significant differences in taxa distribution or alpha diversity by antibiotic duration or nutrition type.

**Conclusion:** The microbiome of gastroschisis infants is dysbiotic, and mode of birth delivery, antibiotic duration, and gestational age appear to contribute to microbial variation.

Corresponding Author: Kara L. Calkins, 10833, Le Conte Avenue, MDCC-22-402, Los Angeles, CA 90095-1752, PHONE: 310-825-9350, FAX: 310-267-0154; KCalkins@mednet.ucla.edu.

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## Introduction

Gastroschisis is a congenital abdominal wall defect with an increasing incidence in the United States (approximately 1 in 3000 live births) and frequently requires multi-disciplinary, high-acuity medical care from birth. While >90–95% of infants with gastroschisis survive, these infants have high rates of comorbidities and costly hospital stays.[1,2] Infants with gastroschisis are often born premature, have intrauterine growth restriction, and require multiple operations.[3,4] Beginning at birth, most gastroschisis infants receive prophylactic antibiotics, and all gastroschisis infants are made *nil per os* requiring parenteral nutrition (PN) due to intestinal dysmotility of uncertain duration. As a result, these infants develop intestinal dysbiosis or an imbalance of gut microbiota. Dysbiosis has been linked to intestinal dysmotility, sepsis, necrotizing enterocolitis (NEC), and intestinal failure-associated liver disease—common complications faced by infants with gastroschisis.[5,6] Also, children with gastroschisis have been noted to have increased rates of childhood respiratory and gastrointestinal infections, both of which have been associated with altered intestinal microbiome.[7]

Little is known about the microbiome in infants with gastroschisis. To our knowledge, there is only one study on the microbiome of gastroschisis infants. The study was a randomized placebo-controlled trial of probiotic administration and its impact on the microbiome of 24 infants with gastroschisis. This study highlighted the dysbiosis and dearth of commensal microbes such as *Bifidobacteriaceae* in the microbiota of these infants. Families present were mainly *Enterobacteriaceae, Staphlyococcaceae, Streptococcaeae,* and *Enterococcaeae*.[8] Early life factors are well-known to alter the intestinal microbiome and include mode of birth delivery, gestational age, antibiotics, and breast milk. In a group of infants with gastroschisis, we sought to: 1. compare their microbiome to the microbiome of healthy infants, 2. describe the early evolution of the microbiome, and 3. investigate the impact of potentially modifiable clinical factors, namely mode of birth delivery, gestational age, antibiotic duration and nutrition type on the microbiome. This study's goal was to provide a better understanding of the intestinal microbiome in infants with gastroschisis so that strategies to restore gut microbiota can be developed.

## Methods

### Controls

The control group included healthy, full-term infants recruited from Los Angeles, California.[9] Study methods relevant for our control group including sample collection, DNA extraction and sequencing, and statistical analysis have been previously published.[9] Linear mixed effects models were used to assess differences in bacterial diversity and abundance between healthy controls and infants with gastroschisis.

### Infants with gastroschisis

Infants with gastroschisis were recruited from the University of California Los Angeles neonatal intensive care unit at Mattel Children's Hospital between May 2016 to November 2017. The study was approved by the local Institutional Review Board. Parent(s)/legal

guardian(s) provided verbal consent. Inclusion criteria included infants with gastroschisis and <14 days of age. Exclusion criteria were infants unlikely to survive.

### Management of Gastroschisis

At our institution, the management of infants with gastroschisis follows a clinical pathway developed by the University of California Fetal Consortium, which focuses on prompt surgical closure, minimal duration of intubation, avoidance of routine paralysis, and select use of opiates and antibiotic prophylaxis.[10]

### Specimen Collection

Fecal specimens of infants with gastroschisis were collected at specific time points, including the start of the study (enrollment – study day 2), then weekly while on PN, and for four weeks after PN discontinuation if the infant remained in the hospital. Samples were collected from infant diapers using sterile collection kits and stored at −80°C until processing. Because of intestinal dysmotility, infants with gastroschisis have delayed passage of meconium and do not stool frequently in the first couple weeks of life.

### DNA Processing, Extraction and Library Preparation

Samples were transferred to Lysing Matrix E tubes (MP Biomedicals, Burlingame, CA, USA) with RLT lysis buffer (Qiagen, Hilden, Germany) and bead-beated on a TissueLyser (Qiagen) for DNA processing. Following manufacturer protocol, AllPrep DNA/RNA/Protein kit (Qiagen) was used to extract DNA. In addition to negative controls from the DNA extraction and PCR steps used to identify contaminant sequences, independent aliquots of a bacterial mock community were processed together with samples to evaluate extraction, amplification and relative abundance of bacteria included.[11] Contaminant removal and library preparation were performed as previously published.[11,12]

### Microbiome Profiling

Microbiome profiling was performed by sequencing of the V4 (515F/806R) region of the 16S rRNA gene followed by exact sequence inference and chimera removal using DADA2.[13,14] Statistical analyses were performed using the ‘phyloseq’, ‘vegan’, and ‘lmerTest’ packages in the R statistical software (version 3.5.0). Differences in bacterial diversity and relative abundances were assessed using linear mixed effects models of the form $Y \sim X + infant\ age + delivery\ mode + sex + ethnicity + (1 \mid SubjectID)$ where $Y$ is the bacterial diversity or abundance measure, $X$ is the clinical variable, and $(1 \mid SubjectID)$ is a random effect to control for the longitudinal nature of the study design.

## Results

Specimens from infants with gastroschisis were analyzed and compared to control infants. Our gastroschisis group comprised of 41 specimens from 13 infants. For the gastroschisis group, 1 sample was collected in the first week of life; 2 samples were collected in weeks 7, 9, and 10; 3 samples were collected in weeks 5 ,6, and 8; 7 samples were collected in weeks 2 and 3; 11 samples were collected in week 4. Our control group comprised 143 specimens from 83 healthy, full-term infants. In the control group, the median number of specimens

per infant was one (IQR 1–2 samples per infant, range 1–4 samples per infant). All samples were collected within the first 90 days of age (mean age 23.6 days).

Infants from our healthy, control group were predominantly vaginally-delivered ($n$ = 56, 68%) and Hispanic/Latino ($n$ = 66, 80%). There were 39 male infants (47%). The maternal mean (SD) age was 29.1 (5.3) years. 28 infants (34%) received antibiotics at delivery. The infants with gastroschisis were predominantly vaginally-delivered, late preterm, male infants born to Hispanic mothers. All infants underwent a minimum of two gastrointestinal operations. Silo placement followed by abdominal closure was recorded as two operations. One infant underwent four gastrointestinal operations. Two infants with gastroschisis developed fever with late-onset bacteremia, which was defined as a positive blood culture after the first 72 hours of age requiring for antibiotics for at least five days. All infants with gastroschisis survived to discharge with a median length of hospital stay of 30 days (IQR 23–76 days) (Table 1).

### Infants with gastroschisis had a compositionally distinct and less diverse microbiome

The microbiome of infants with gastroschisis was marked by a lack of *Bifidobacterium* ($p$ = 0.001) and an increase in *Staphylococcus* ($p$ = 0.007) compared to the microbiome of healthy infants (Figure 1A). The microbiome of infants with gastroschisis was significantly less diverse (Chao1 metric, $p < 0.001$) (Figure 1B).

### Mode of birth delivery impacted microbiome composition

Overall, there was a predominance of *Escherichia-Shigella* in infants with gastroschisis, particularly in the infants born vaginally. Infants born via cesarean section were mainly colonized by *Staphylococcus* and *Streptococcus* (Figure 2). Permutational multivariate analysis of variance (PERMANOVA) identified mode of birth delivery as a contributor to a small portion of overall variation in the microbiome among the clinical variables investigated ($R^2$ = 0.04, $p$ = 0.001) (Table 2).

### Microbiome Variation Was Affected by Age at Collection, Gestational Age, and Antibiotic duration

Age at sample collection ($R^2$ = 0.03, $p$ = 0.009), gestational age ($R^2$ = 0.02, $p$ = 0.035) and antibiotic duration greater than or equal to seven consecutive days ($R^2$ = 0.03, $p$ = 0.003) explained some degree of microbiome variation (Table 2). Over half of the mothers of the infants in our study received antibiotics prior to delivery. As anticipated, 100% of the infants received antibiotics, with the first dose being administered shortly after birth. The median duration for infant antibiotics was five days (IQR 4–14 days) (Table 1). There was no difference ($p$ = 0.10) in the alpha diversity among infants who were on antibiotics for greater than or equal to seven days compared to infants who were on antibiotics less than seven days. There was no significant difference in bacterial relative abundances at the genus level by antibiotic duration (Figure 3).

### Nutrition type did not appear to affect microbiome composition or alpha diversity

PN duration varied widely with a median of 27 days; the shortest PN course was 12 days and one infant remained PN-dependent at hospital discharge, which was 110 days. Upon

initiation of enteral nutrition, infants received human milk, formula, or a mix of both (Table 1). Being on PN ($R^2$ = 0.01, $p$ = 0.19) and the type of enteral nutrition ($R^2$ = 0.02, $p$ = 0.14) were not significant contributors of overall microbiome variation (Table 2). There was no significant difference in bacterial relative abundances at the genus level by nutrition type (Figure 3). There was no significant difference in alpha diversity ($p$ = 0.98) while on versus off PN and principal coordinates analysis (PCoA) did not show significant compositional differences in microbiome by enteral nutrition type (PERMANOVA $p$ = 0.14, Figure 4).

## Discussion

In this pilot, hypothesis-generating study, a small group of infants with gastroschisis had a dysbiotic microbiome dominated by *Enterobacteriaceae*, and at the genus level, *Escherichia-Shigella*. However, the microbial signatures among infants with gastroschisis born vaginally versus via cesarean section were distinct. Those born via cesarean section harbored microbiomes dominated by *Staphylococcus* and *Streptococcus*. Duration of antibiotics for greater than or equal to seven days, age at sample collection, and gestational age explained a small degree of microbial variation. The microbiome did not appear to be significantly different while infants were on PN versus enteral nutrition, or enteral feeds of breastmilk alone.

The dysbiosis in the fecal microbiota of infants with gastroschisis is highlighted by the lack of commensal *Bifidobacterium* and predominance of *Escherichia-Shigella* and *Enterobacter*, the latter which is often considered more pathogenic. Our healthy control group showed dominance of both *Bifidobacteriaceae* and *Enterobacteriaceae* as well as increased alpha diversity compared to infants with gastroschisis. The accumulation of *Bifidobacterium, Bacteroides,* and *Clostridium* in the maturation of healthy infant gut microbiota has been described.[15] However, our infants with gastroschisis did not follow this pattern of *Bifidobacteriaceae* predominance and accumulation.

Infants with gastroschisis likely have compromised gut barriers, and their intestinal microbiome may contribute to this problem. The mucosal gut barrier plays a critical role in modulating the postnatal immune system.[16] The disruption of homeostasis between commensal and pathogenic bacteria has been shown to alter the host-microbe interaction mediated by specific Toll-like receptors, and thus the mucosal defense barrier may become susceptible to offending pathogens.[17] This imbalance likely predisposes infants with gastroschisis, who are often born premature, to complications such as dysmotility and sepsis, making it difficult to wean PN and increasing their hospital length of stay. However, it is still unclear whether microbial dysbiosis is a precipitating event or sign of intestinal disease.[18]

The difference in the fecal microbiome by mode of birth delivery was the starkest observation in our study. Among infants with gastroschisis born via cesarean section, staphylococcus predominated in the initial sample, and thereafter staphylococci and streptococci persisted. Previous studies have demonstrated that infants born via cesarean section appear to carry similar bacteria to that on skin epithelial layers. Moreover, these infants lack *Bifidobacterium* and have a low *Bacteroides* prevalence and alpha diversity.[19,20] In contrast, vaginally delivered infants typically develop microbiomes approximating their

own mother's vaginal microbiota with a *Bacteroides* predominance.[21,22] Of note, in the fecal microbiome of healthy, vaginally delivered, term infants, roughly half of the microbes belong to species present in the microbiome of their mothers, and the other half may be from other environmental exposures.[23] While *Bacteroides* was present in our gastroschisis infants born vaginally, *Escherichia-Shigella* was the predominant bacteria. *Escherichia-Shigella* and *Enterobacter* belong to the *Enterobacteriaceae* family. The finding in this study is similar to the increased relative abundance of *Enterobacteriaceae* observed in preterm infants with NEC, as well as in children with short bowel syndrome on PN.[24,25]

All infants with gastroschisis were started on antibiotics immediately at birth. All four infants with gastroschisis who were delivered by cesarean section required antibiotics for greater than seven days. In contrast, only two infants delivered vaginally (22%) required antibiotics for greater than seven days. Mode of birth delivery may be a confounder when assessing antibiotics duration, as infants who required cesarean delivery may be "sicker" when compared to infants born vaginally. In our study, two infants developed fever and were found to have bacteremia requiring treatment with prolonged antibiotic courses. One infant, who was delivered vaginally, had *Staphylococcus epidermidis* and *Enterococcus faecalis* bacteremia. Another infant, who was delivered by cesarean section, had *Staphylococcus epidermidis* bacteremia alone. While it is possible that these blood cultures were contaminated by skin flora or that the catheter alone was colonized, bacteria were detected within 24 hours, repeat cultures showed persistent bacteremia, and these infants were febrile.

In the absence of maternal or fetal indications for cesarean section and preterm delivery, vaginal delivery of infants with gastroschisis is considered safe. Studies comparing infants with gastroschisis born via vaginal delivery versus cesarean section revealed no significant differences in mortality, primary or secondary repair, NEC, sepsis, short bowel syndrome, day of initiation of enteral feeds and length of hospitalization.[26,27] Moreover, when compared to term infants with gastroschisis, preterm infants with gastroschisis are at higher risk for death, NEC, re-operations, intestinal failure associated liver disease, prolonged hospital stays, and common complications associated with prematurity including respiratory distress syndrome and apnea.[10,28] Given the health benefits of vaginal delivery and term gestation for both the infant and mother, vaginal delivery and term gestation should be prioritized.

In general, minimizing antibiotic exposure continues to be important and has shown to have long-term impacts on pediatric health. In one metagenomic study of 39 Finnish children aged 2–36 months, children who had received antibiotics as infants and toddlers were found to have microbiota with decreased bacterial diversity at species and strain levels at three years of age. They were also found to have an overall less stable gut microbiome.[21] Prior studies have shown the dysbiotic effect of antibiotics can last for months to years and have a long-term impact on immune and metabolic health.[29,30] While duration of antibiotics greater than or equal to seven days drove some degree of overall microbiome variation, our study did not find significant differences in alpha diversity or taxa composition by antibiotic exposure. This finding may be because our study was limited in sample size and duration. Restoration of the microbiome may require more time after antibiotic exposure. Infant age at

time of sample collection accounted for a small proportion of microbiome variation. By the end of our study, the microbiome of infants with gastroschisis remained dysbiotic compared to healthy infants without gastroschisis of similar ages.[9,15]

While there was no significant difference in the alpha diversity of the microbiome by nutrition type, previous studies have shown varying patterns in the intestinal colonization of infants on breast milk compared to formula. Infants exclusively breastfed show higher counts of *Bifidobacterium* and *Lactobacillus* in contrast to formula-fed infants.[31,32] *Bifidobacterium* have successfully adapted to the breastfed infant gut likely due to their genetic adaptation specializing in human milk oligosaccharide catabolism.[33] There was little to no *Bifidobacterium* colonization in our infants with gastroschisis regardless of nutrition type. We hypothesize this may be partly related to delayed initiation of enteral nutrition among this population. It remains to be determined if infants with gastroschisis who are breastfed re-establish a microbiome comparable to that of healthy infants after hospital discharge. Though our study sample size was too small to realize recommendations of optimal nutrition type, maternal milk should still be recommended based on current literature in the absence of any contraindications to breastfeeding. Literature has shown that infants fed breastmilk have a lower risk of NEC and sepsis and higher cognitive scores than infants fed formula.[34,35] Human milk also possesses its own microbiome, directly transferring organisms to the infant gut that are protective against the development of allergic diseases.[9]

Our study showed consistency in the dominance of *Enterobacteriaceae* with the findings by Powell in 24 infants with gastroschisis.[8] In children with short bowel syndrome, a common complication associated with gastroschisis, *Enterobacteriaceae* dominates the fecal microbiota.[24] Previous animal and human studies of gastroschisis have demonstrated that impaired development of the enteric nervous system, specifically interstitial cells of Cajal, is associated with dysmotility[36,37] The dysmotility in gastroschisis is likely not only attributed to impaired *in utero* development, but further exacerbated by prematurity, prolonged periods of fasting, sepsis, antibiotics, narcotics, and gastrointestinal operations, which altogether contribute to the dysbiosis of their intestinal microbiota. Additional studies suggest that there is a two-way interaction between motility and the microbiome. While dysmotility may predispose to alterations in the gut microbiome, microbial disruptions can also lead to gut motor dysfunction and dysmotility.[38] Dysmotility is strongly associated with severe comorbidities including increased infection rates, delayed introduction of feeds, intestinal failure, and prolonged hospitalization in infants with gastroschisis.

Our study has limitations. This study was conducted at a single site and has a small sample size. It should also be noted while there are not sex differences in the overall prevalence of gastroschisis, our study enrolled two females and eleven males with gastroschisis. Thus, we are unable to interpret if sex is truly a driver of overall microbiome variation ($R^2 = 0.03$, $p = 0.006$). In addition, our study was comprised predominantly of Hispanic infants. Ethnicity did appear to contribute to overall microbiome variation ($R^2 = 0.02$, $p = 0.017$). Our control group included a similar population of predominantly Hispanic infants. Additional studies have demonstrated ethnic, cross-cultural and geographic differences in the healthy infant microbiome.[39,40] The control infants, however, were not hospitalized. Therefore, we are

unable to distinguish between the impact of gastroschisis from that of hospitalization on the microbiome. Last, we only collected samples from infants with gastroschisis during hospitalization, with a median length of hospital stay of 30 days (IQR 23–76 days). As a result, we cannot comment on changes in the microbiome post-hospital discharge.

## Conclusion

In this study, we investigated the microbiome of 13 hospitalized infants with gastroschisis and compared these results to the microbiome of healthy infants. The microbiome of infants with gastroschisis is dysbiotic and its composition is influenced by mode of birth delivery. Moreover, mode of birth delivery, antibiotic duration, and gestational age appear to impact microbial variation. Future investigation on how to promote and maintain a healthy microbiome in infants with gastroschisis is warranted. This type of research may help improve intestinal motility and clinical outcomes for this population.

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## Clinical Relevancy Statement

Infants with gastroschisis have intestinal dysmotility and are at high risk for infections, necrotizing enterocolitis, and feeding intolerance during the neonatal period. Intestinal dysbiosis may contribute to these problems. In this study, the microbiome of infants with gastroschisis was noted to be distinctly dysbiotic at birth, and mode of birth delivery, antibiotic duration, and gestational age explained some degree of microbial variation.

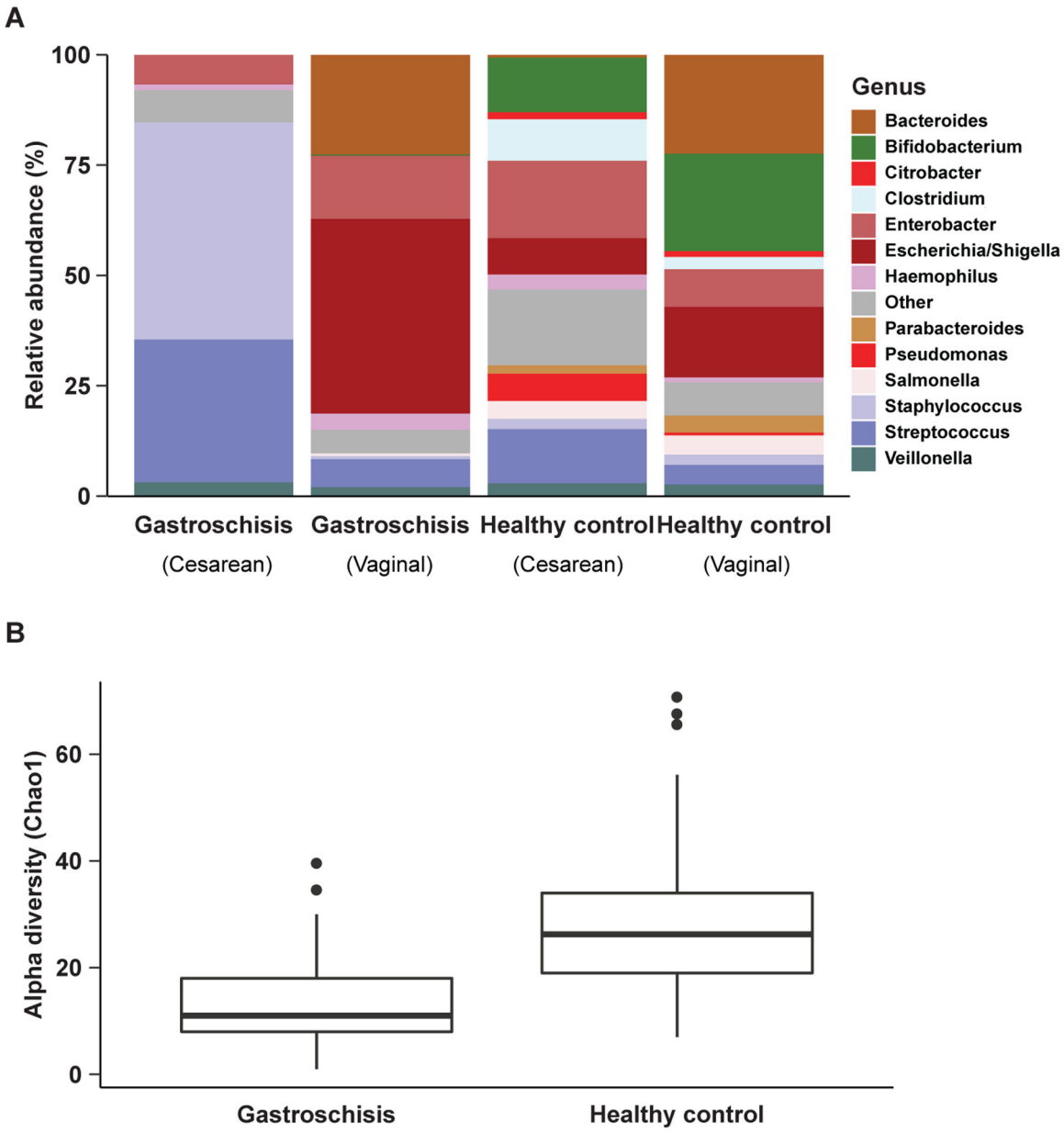


**Figure 1.**

Differences in the microbiome by mode of delivery among all gastroschisis (n=41) and control (n=143) samples by A) genus-level relative abundance (%) and B) boxplot of alpha diversity (Chao1) index. 'Other' includes genera representing <1% of the overall relative composition.

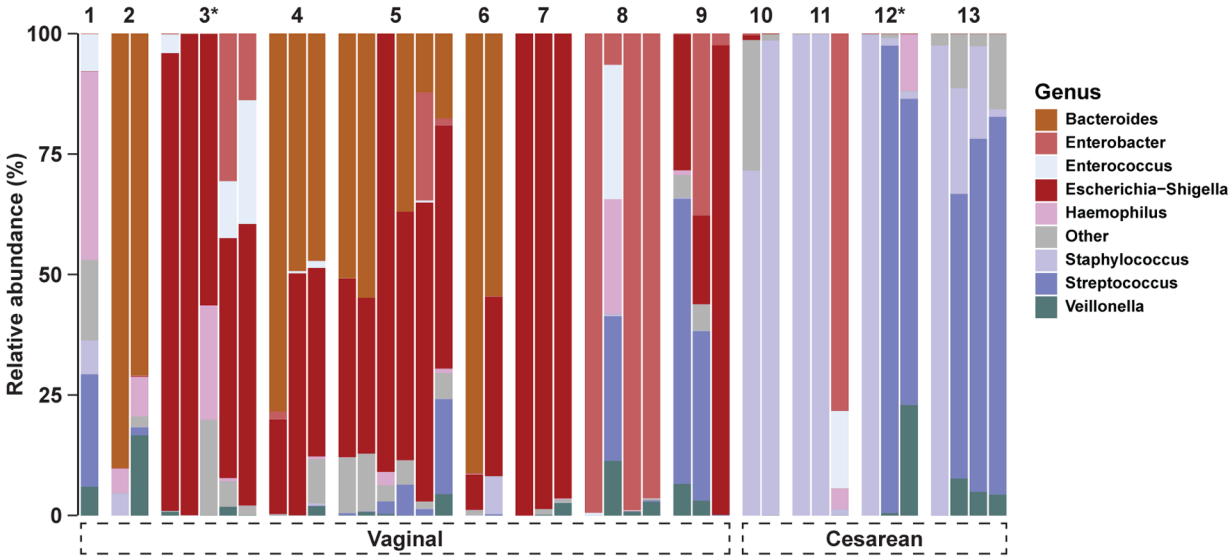


**Figure 2.**

Genus-level relative abundance (%) showed the microbiome of infants with gastroschisis differed by mode of birth delivery. 'Other' includes genera representing <1% of the overall relative composition. *Subjects who developed bacteremia.

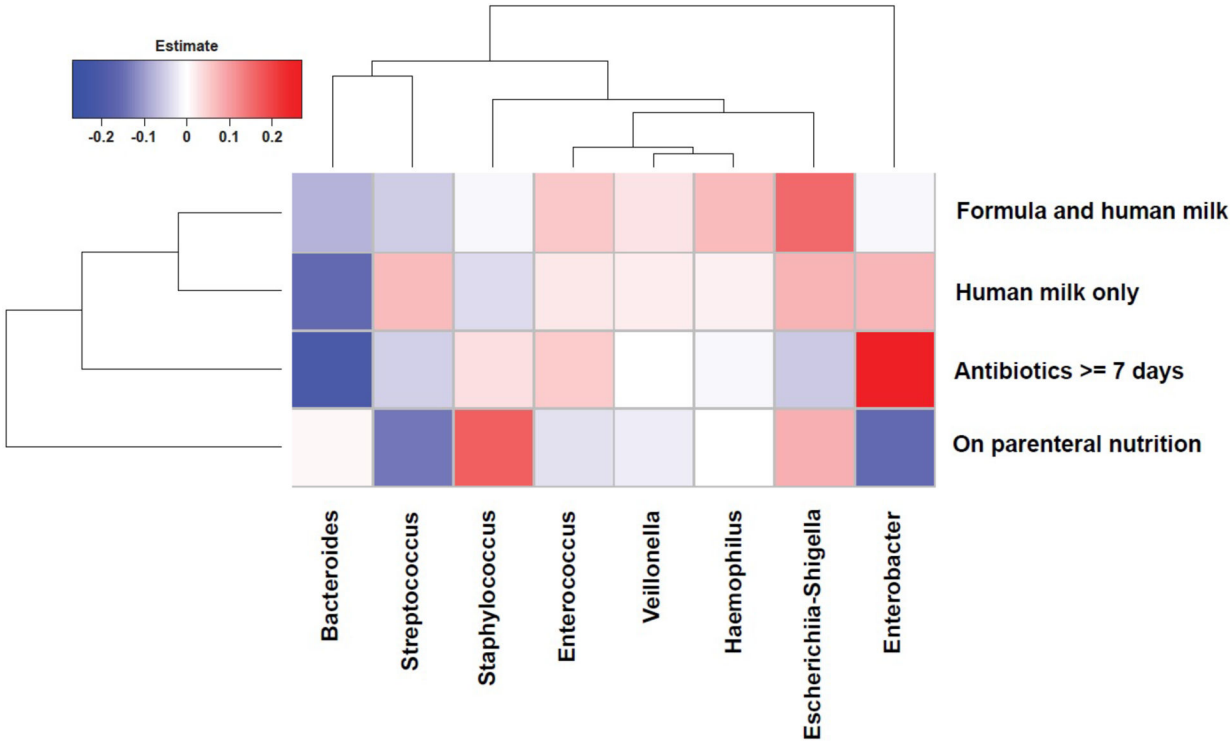


**Figure 3.**

Heat map of associations by clinical variables showed no significant differences in genus-level relative abundance in the microbiome of gastroschisis infants. Estimates are derived from linear mixed models for the indicated variables.

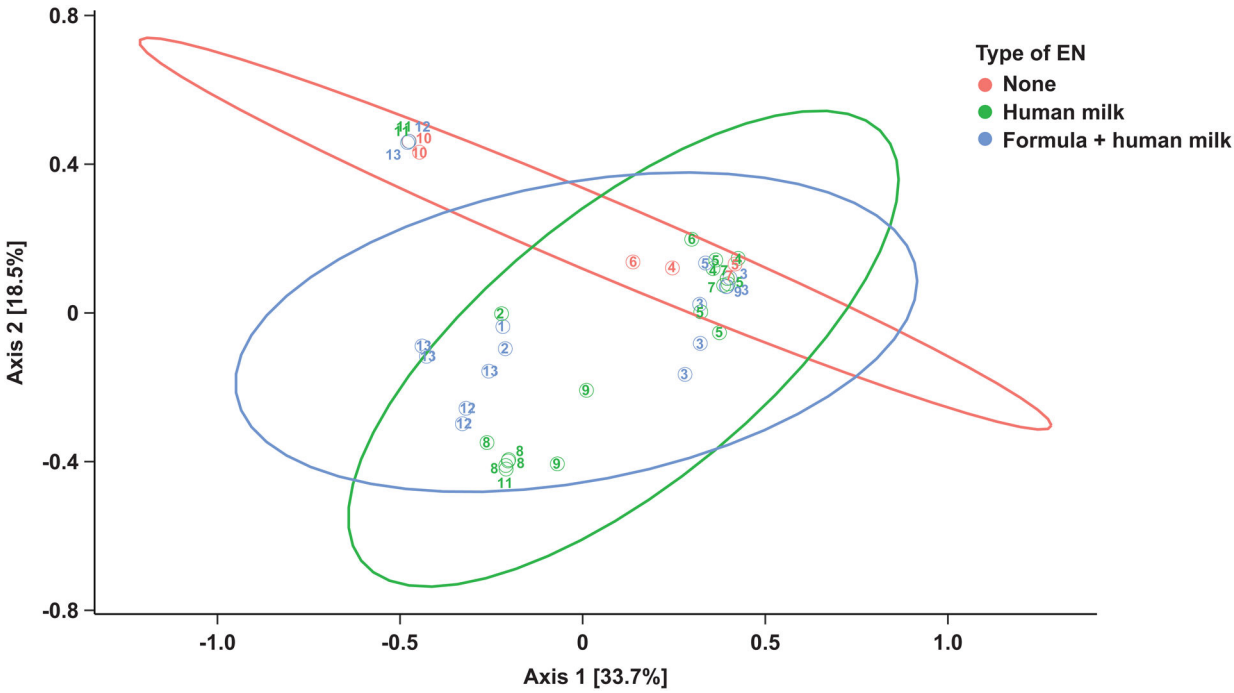


**Figure 4.**

Principal coordinates analysis (PCoA) using Bray-Curtis distances showed no significant differences in the microbiome of infants with gastroschisis by type of enteral nutrition (PERMANOVA $p = 0.14$). Numbers represent subject number.

**Table 1.**

Maternal and infant characteristics in gastroschisis subjects

| | **Gastroschisis n=13** |
|---|---|
| Maternal age (years) | 23 (21–26) |
| Maternal race – White | 12 (92%) |
| Maternal ethnicity – Hispanic | 7 (54%) |
| Maternal antibiotics prior to delivery * | 8 (62%) |
| Vaginal delivery | 9 (69%) |
| Infant sex – Male | 11 (85%) |
| Infant gestational age (weeks) | 36 (35–37) |
| Infant birthweight (kilograms) | 2.7 (2.3–3.0) |
| Infant ethnicity – Hispanic † | 8 (62%) |
| Small for gestational age ‡ | 2 (15%) |
| Apgar score | |
| 1 min | 8 (7–9) |
| 5 min | 9 (8–9) |
| Type of first feed - Breast milk | 11 (85%) |
| Day of life of first feed | 14 (10–18) |
| Days on parenteral nutrition § | 27 (18–58) |
| Days on antibiotics | 5 (4–14) |
| Bacteremia | 2 (15%) |
| Necrotizing enterocolitis | 0 (0%) |
| Number of gastrointestinal surgeries | 2 (2–3) |
| Small bowel resection | 2 (15%) |
| Intestinal failure-associated liver disease | 4 (31%) |
| Length of hospital stay (days) | 30 (23–76) |

* One mother with unknown antibiotic exposure.

† Infants were classified as Hispanic if one parent was Hispanic.

‡ Small for gestational age was defined as birth weight < 10$^{th}$ percentile.

§ One subject remained on parenteral nutrition at time of discharge.

Data presented as median (IQR) or n (%).

**Table 2.**

PERMANOVA using Bray-Curtis distances summarizing drivers of microbiome compositional variance in infants with gastroschisis at all time points for all specimens.

| | R2 | p-value |
|---|---|---|
| Mode of birth delivery | 0.038 | 0.001 |
| Antibiotics >= 7 days | 0.033 | 0.003 |
| Sex | 0.027 | 0.006 |
| Age at sample collection | 0.026 | 0.009 |
| Gestational age | 0.020 | 0.035 |
| Ethnicity | 0.022 | 0.017 |
| Type of enteral nutrition | 0.024 | 0.140 |
| On parenteral nutrition | 0.012 | 0.192 |