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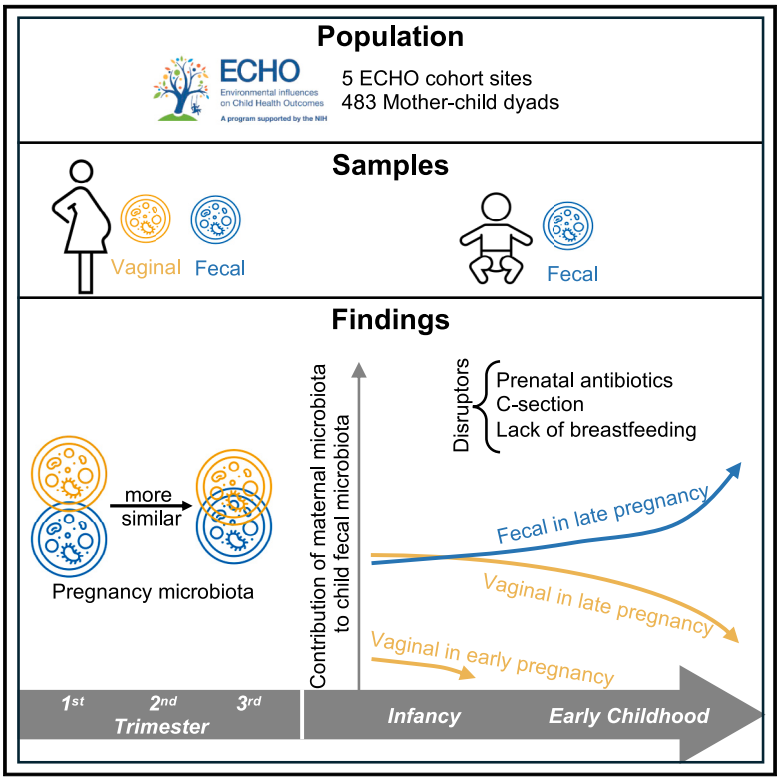
Graphical abstract



Highlights

- As pregnancy progresses, maternal vaginal and fecal microbiota become more similar
- Maternal vaginal microbiota contributes more to sharing if sampled later in pregnancy
- Maternal fecal microbiota contributes more to sharing in older than younger children
- Mother-child microbiota sharing varies by antibiotics, birth mode, and breastfeeding

Authors

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In brief

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Maternal vaginal and fecal microbiota in later pregnancy contribute to child fecal microbiota development in the ECHO cohort

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SUMMARY

There is growing interest in the use of microbial-seeding interventions to mitigate the impacts of prenatal antibiotics, C-section, and lack of breastfeeding on mother-child microbe sharing. However, the relative importance of maternal vaginal vs. fecal microbiota in this process is unclear. Analyzing 16S rRNA sequences from five US birth cohorts, we found that maternal vaginal and fecal microbiota became more similar as pregnancy progressed, and both niches influenced the child's fecal microbiota. The relative contribution of maternal vaginal microbiota increased when vaginal sampling occurred later in gestation. As children aged from birth to 5 years, their fecal microbiota increasingly resembled their mother's fecal microbiota as compared to vaginal microbiota. Patterns of sharing appeared to differ by prenatal antibiotic use, birth mode (C-section vs. vaginal), and breastfeeding. Our findings enhance understanding of niche-specific mother-child microbe sharing and may inform microbial-seeding interventions. Metagenomic studies are needed to identify specific shared strains.

INTRODUCTION

Microbes from the birthing mother serve as both initial colonizers and key influencers of the child's microbiota evolution, growth, and long-term health.[1–4] Even at 5 years of age a child's gut microbiota is more similar to the gut microbiota of their biological mother than a non-related mother.[5] Infant acquisition of microbes from the mother is a selective process, with specific microbial genera like *Bifidobacterium* and *Bacteroides* being more frequently shared than others.[6–12] This sharing process is

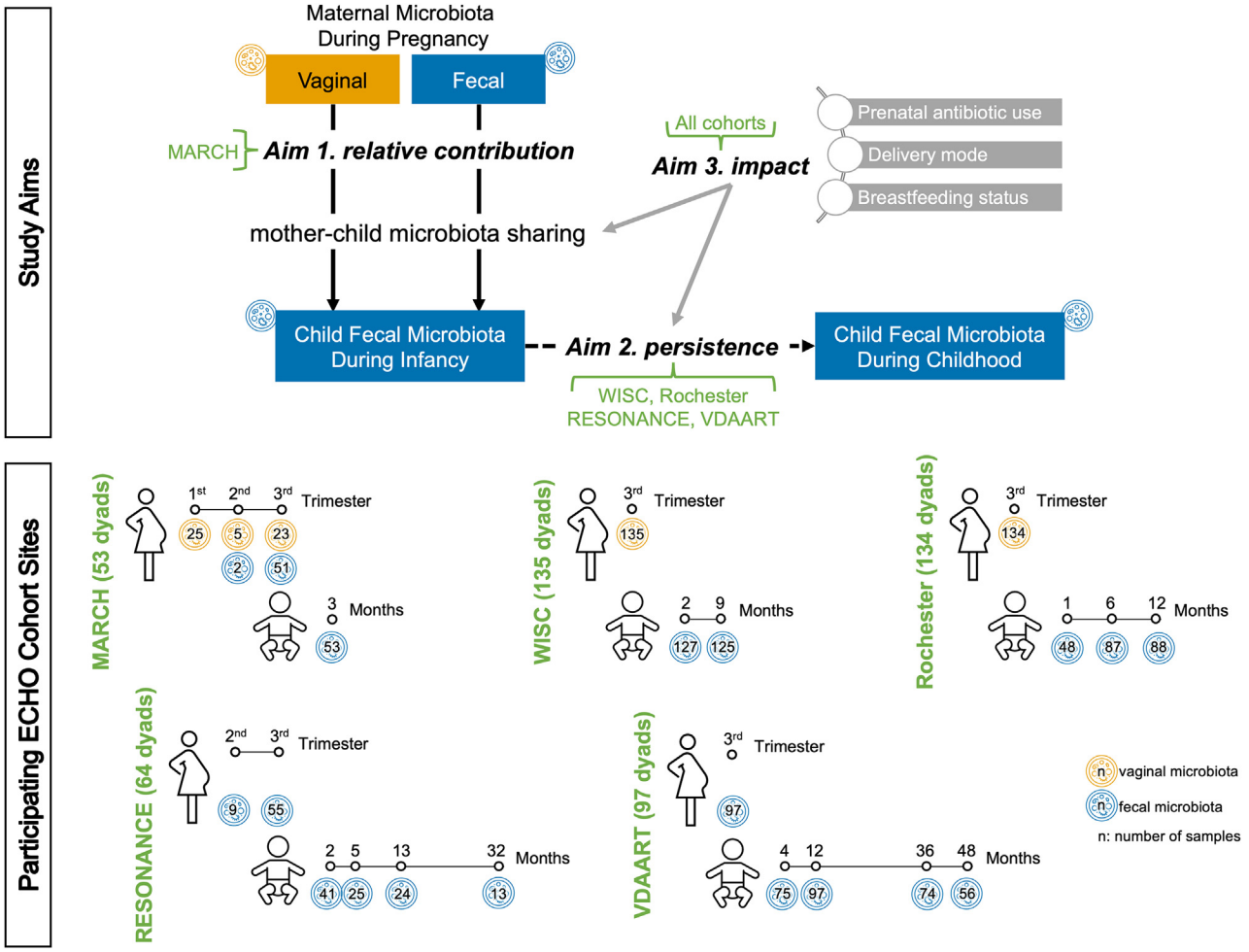

Figure 1. Overview of study aims and participating ECHO cohort sites for this analysis

vulnerable to disruptions from critical factors, such as prenatal antibiotics, C-section, and lack of exposure to human milk, as well as other less influential factors.[1,3,13] These factors that are potentially disruptive to mother-child microbe sharing are highly prevalent worldwide, including in the United States, where 80% of medication prescriptions in pregnancy are antibiotics,[14] over 30% of births are via C-section,[15] and almost half of infants do not receive any human milk at the age of 6 months.[16] Given this evidence, there is now growing interest among parents, clinicians, and researchers related to the potential of microbial-seeding interventions to counteract these disruptions and support healthy microbiota development in children.[17,18]

However, developing effective microbial-seeding interventions is hindered by gaps in our understanding of mother-child microbial sharing. First, most studies on mother-child microbiota sharing have been small in scale,[6,7,9,19–25] and only a few have directly compared the contributions of maternal vaginal microbiota vs. fecal microbiota[19,21,24]—the two primary sources of maternal microbes that have been used in microbial-seeding interventions.[8,10,26–28] Second, it is unknown how long maternal microbes from these sources persist in a child's gut, which is an essential factor in determining the potential effectiveness of these interventions. Lastly, it remains unclear the extent to which niche-specific, mother-child sharing differs by prenatal antibiotic use, birth mode (C-section vs. vaginal), and breastfeeding.

In this study, we aim to address these research gaps through three aims: (1) to characterize the contribution of the biological mother's vaginal microbiota vs. fecal microbiota, collected during pregnancy, to the fecal microbiota of their offspring in early infancy; (2) to assess the persistence of maternal pregnancy vaginal and fecal microbiota, including microbial taxa in the genera *Bifidobacterium* and *Bacteroides*, in children's fecal microbiota throughout the first year of life and into early childhood; and (3) to investigate whether niche-specific, mother-child microbiota sharing differs by prenatal antibiotic use, delivery mode, or breastfeeding.

RESULTS

In total, 483 mother-child dyads from 5 ECHO cohorts contributed to this study (Figures 1 and S1; Table 1). Detailed description of participant characteristics are provided in STAR Methods.

Table 1. Characteristics of mother-child dyads in the five ECHO cohorts participating in the study

	MARCH (*N* = 53)	RESONANCE (*N* = 64)	VDAART (*N* = 97)	Rochester (*N* = 134)	WISC (*N* = 135)	Overall (*N* = 483)
Maternal Characteristics						
Age at delivery, years						
Mean (SD)	32.2 (4.5)	30.9 (5.3)	27.7 (5.8)	30.1 (4.3)	30.7 (4.0)	30.1 (4.9)
Missing	0 (0%)	0 (0%)	0 (0%)	0 (0%)	<5	<5
Race						
White	49 (92.5%)	44 (68.8%)	33 (34.0%)	88 (65.7%)	71 (52.6%)	285 (59.0%)
Black	<5	<10	49 (50.5%)	31 (23.1%)	0 (0%)	90 (18.6%)
Other or multiple races	<5	9 (14.1%)	<15	<10	0 (0%)	33 (6.8%)
Missing	0 (0%)	<5	<5	<10	64 (47.4%)	75 (15.5%)
Ethnicity						
Non-Hispanic	51 (96.2%)	49 (76.6%)	80 (82.5%)	122 (91.0%)	74 (54.8%)	376 (77.8%)
Missing	<5	<5	0 (0%)	0 (0%)	60 (44.4%)	61 (12.6%)
Highest education						
College or above	49 (92.5%)	29 (45.3%)	61 (62.9%)	82 (61.2%)	124 (91.9%)	345 (71.4%)
Missing	<5	29 (45.3%)	<5	18 (13.4%)	0 (0%)	49 (10.1%)
Pre-pregnancy BMI, kg/m^2						
Mean (SD)	25.9 (5.3)	32.2 (6.6)	Not available	28.4 (7.1)	27.8 (6.3)	28.4 (6.7)
Missing	<5	11 (17.2%)		<10	28 (20.7%)	144 (29.8%)
Prenatal antibiotics use						
No	28 (52.8%)	32 (50.0%)	52 (53.6%)	86 (64.2%)	55 (40.7%)	251 (52.0%)
Missing	0 (0%)	17 (26.6%)	<5	0	0 (0%)	<20
Child Characteristics						
Race						
White	48 (90.6%)	44 (68.8%)	29 (29.9%)	82 (61.2%)	132 (97.8%)	335 (69.4%)
Black	<5	7 (10.9%)	50 (51.5%)	31 (23.1%)	<5	91 (18.8%)
Other or multiple races	<5	13 (20.3%)	18 (18.6%)	21 (15.7%)	<5	57 (11.8%)
Ethnicity						
Non-Hispanic	52 (98.1%)	44 (68.8%)	78 (80.4%)	120 (89.6%)	130 (96.3%)	424 (87.8%)
Missing	0	0 (0%)	0 (0%)	0 (0%)	<5	<5
Biological sex						
Male	29 (54.7%)	31 (48.4%)	52 (53.6%)	67 (50.0%)	71 (52.6%)	250 (51.8%)
Delivery mode						
Vaginal delivery	43 (81.1%)	39 (60.9%)	61 (62.9%)	104 (77.6%)	109 (80.7%)	356 (73.7%)
Missing	0	<10	<5	0 (0%)	0 (0%)	7 (1.4%)
Gestational age at birth, weeks						
Mean (SD)	39.0 (1.5)	39.0 (1.4)	38.9 (1.5)	39.6 (1.1)	39.1 (1.2)	39.2 (1.3)
Birth weight, kg						
Mean (SD)	3.5 (0.5)	3.4 (0.4)	3.2 (0.5)	3.4 (0.5)	3.5 (0.5)	3.4 (0.5)
Missing	0 (0%)	<5	<5	0 (0%)	0 (0%)	<5

SD, standard deviation; BMI, body mass index.

Although there were some variations, the predominant genera within the maternal microbiota demonstrated considerable consistency across all cohorts. *Bacteroides*, *Blautia*, *Prevotella 9*, *Faecalibacterium*, and *Bifidobacterium* were among the five most abundant genera in the maternal fecal microbiota (Figure S2A). *Lactobacillus* was the most abundant genus in the maternal vaginal microbiota, ranging from 70% to 75% relative abundance (Figure S2B).

Child's fecal microbiota is more similar to biological mother's fecal microbiota

Across cohorts, child's fecal microbiota, especially in older children, tended to more closely resemble their biological mother's fecal microbiota compared to that of a randomly assigned non-biological mother (Figure S3A). This was indicated by significantly lower Jaccard and/or Bray-Curtis dissimilarity values (thus higher similarity) in biological pairs than random pairs in

children aged 13 months–32 months in RESONANCE and children aged 4 months–48 months in VDAART. Conversely, the dissimilarity between child's fecal microbiota and their biological mother's vaginal microbiota was similar to that between child and a random mother's vaginal microbiota (Figure S3B).

Relative contribution of maternal fecal vs. vaginal microbiota varies across trimester

In the MARCH cohort, the overlap of microbial amplicon sequence variants (ASVs) from the maternal vaginal microbiota with the 3rd trimester maternal fecal microbiota as well as with the infant fecal microbiota increased as vaginal sample collection approached delivery (Figures 2A1–2A3). Collectively, maternal fecal and vaginal microbiota combined accounted for 41.1%, 61%, and 64.2% of the infant fecal microbiota ASVs when maternal vaginal samples were collected in the 1st, 2nd, and 3rd trimester, respectively (ASV sharing analysis; Figures 2B1–2B3). The combined contribution of the 3rd trimester maternal fecal and vaginal microbiota combined surpassed contributions from other microbiota sources (ASV sharing analysis; 64.2% vs. 35.8%, $p < 0.001$; Figure 2B3).

The relative contribution of maternal fecal and vaginal microbiota also varied according to the timing of vaginal sample collection. Based on the ASV sharing analysis, 3rd trimester maternal fecal microbiota alone accounted for a significantly higher proportion of infant fecal microbiota ASVs than 1st trimester maternal vaginal microbiota alone (38.6% vs. 1.5%, $p < 0.001$; Figure 2B1). It was non-significantly higher when compared to 2nd trimester maternal vaginal microbiota alone (31.7% vs. 15.2%; Figure 2B2). When not accounting for sequencing depth, 3rd trimester maternal fecal microbiota contributed significantly less than 3rd trimester maternal vaginal microbiota alone (12.7% vs. 23.1%, $p < 0.05$; Figure 2B3). However, given 3rd trimester vaginal microbiota data had higher sequencing depth, we repeated the analysis after accounting for sequencing depth and found that 3rd trimester maternal fecal microbiota alone accounted for 19.4% of infant fecal microbiota ASV, which was close to that of 3rd trimester maternal vaginal microbiota alone (14.6%; Figure S4B3). The source tracking method, which accounted for sequencing depth by rarefaction, also indicated that 3rd trimester maternal fecal and vaginal microbiota made similar contributions (19.5% vs. 21.0%; Figure 2D). In the sensitivity analysis that considered both biological and random mother as potential sources of infant fecal microbiota, the biological mothers' fecal microbiota tended to be more influential than random mothers' fecal microbiota ($p = 0.056$), as well as more influential than biological mothers' vaginal microbiota ($p < 0.05$, Figure S5).

Mother-child microbiota sharing persists beyond infancy

Maternal pregnancy fecal microbiota made significantly increasing contributions to children's fecal microbiota from around 2 months of age to 3 years in the RESONANCE cohort and from around 4 months to 4 years in the VDAART cohort (Figure 3A0). In RESONANCE, the shared ASVs were approximately 25% ASVs at 2 months, 30% at 5 months, 35% at 13 months ($p < 0.001$), and 45% at 32 months ($p < 0.001$). In VDAART, these numbers were 25% at 4 months, 30% at 12 months ($p < 0.001$), and 35% at 36 and 48 months (both $p < 0.001$). Similar trends of increasing maternal pregnancy microbiota contributions in older children were also observed using the source tracking method, though the proportions appeared to be larger in magnitude compared to the ASV sharing analysis (Figure 3A0).

Regarding maternal pregnancy vaginal microbiota, significantly decreasing contributions to children's fecal microbiota were noted from 2 to around 9 months in the WISC cohort and from 1 to 12 months in the Rochester cohort (Figure 3B0). For example, in WISC, ASV sharing reduced from 25% at 2 months to around 20% at 9 months ($p < 0.001$), and in Rochester, infants at 1 month shared about 15% ASVs with their mothers' pregnancy vaginal microbiota, decreasing to 10% at 12 months ($p < 0.01$), as per ASV sharing analysis. Across cohorts, accounting for sequencing depth did not materially change the results. With the source tracking method, decreases in maternal pregnancy vaginal microbiota contribution to children's fecal microbiota in older children were less apparent.

Prenatal antibiotics, C-section, and lack of breastmilk exposure disrupt microbiota sharing

In the 3-month-old infants from MARCH cohort, more ASVs were shared with their mother's 3rd trimester microbiota from combined fecal and vaginal sources compared to other unmeasured sources only for the dyads without prenatal antibiotics use ($n = 12$), or who were born vaginally ($n = 19$), or who received breastmilk at 3 months ($n = 21$) (Figure 2C). Among these dyads, maternal vaginal microbiota tended to contribute to the infant fecal microbiota more profoundly than maternal fecal microbiota, though the differences lessened when accounting for sequencing depth (Figure S4C). Conversely, in dyads with prenatal antibiotic use, or C-section births, or no breastfeeding, maternal contributions were no longer statistically significantly different than contributions from other unmeasured sources, and maternal pregnancy vaginal contributions became similar to or smaller than maternal fecal contributions. The source tracking analysis yielded consistent results as described above (Figure 2D).

When comparing between groups of prenatal antibiotic use, delivery mode, or breastfeeding status, contribution of either maternal fecal or vaginal microbiota did not differ by these factors among the 3-month-olds in MARCH (Figure S6). However, in the other four cohorts, sharing of maternal pregnancy fecal microbiota (in RESONANCE and VDAART) or vaginal microbiota (in WISC and Rochester) with infant fecal microbiota was more pronounced in vaginally delivered children compared to children born by C-section (Figures 3A2, 3B2) across multiple time points of children's age, whereas impacts by prenatal antibiotics were less discernible (Figures 3A1, 3B1).

Sharing of Bifidobacterium and Bacteroides by prenatal antibiotics use, delivery mode, and breastfeeding

Among the 3-month-olds in the MARCH cohort, of the six *Bifidobacterium* ASVs present in infant fecal microbiota, 66.7% (4 ASVs) were shared with maternal fecal microbiota and 83.3% (5 ASVs) were shared with maternal vaginal microbiota (Figure 4A). Regardless of the maternal microbiota source, in

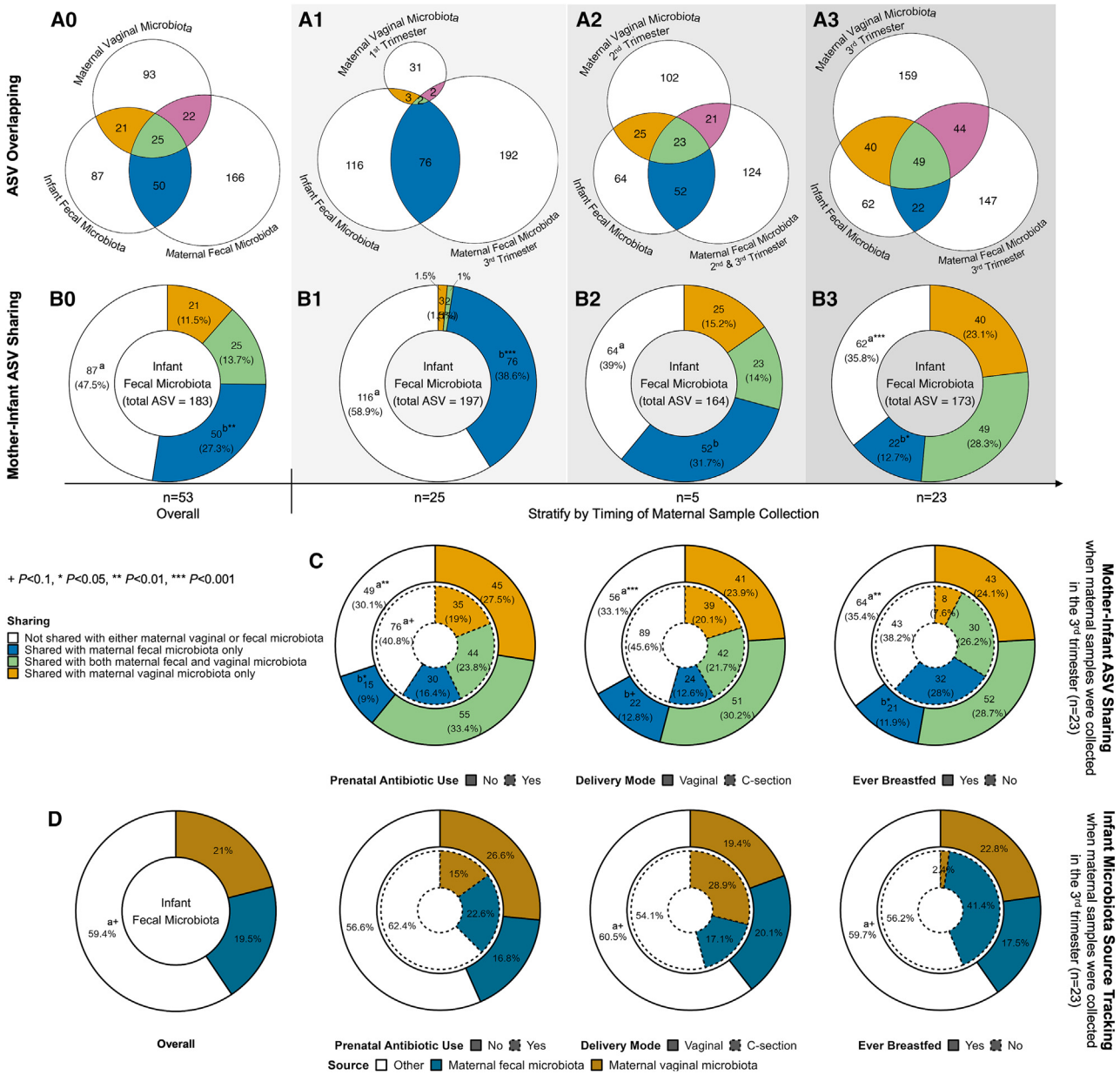

Figure 2. Overview of microbiota sharing between mothers and infants in the MARCH cohort

(A0–A3) illustrates the number of amplicon sequence variants (ASVs) that overlap between maternal and infant samples. (B0–B3) shows the number and proportion of infant fecal microbiota ASVs shared with maternal pregnancy vaginal and/or fecal microbiota. (A0-A3 and B0-B3) present the overall view (A0, B0) and followed by a detailed breakdown based on the timing of maternal sample collection (A1–A3, B1–B3). (C and D) show the maternal microbiota contribution to infant fecal microbiota across groups of prenatal antibiotic use, delivery mode, and breastfeeding status, based on the ASV sharing method and source tracking method via fast expectation maximization source tracking (FEAST) algorithm, respectively. Results in (C and D) are restricted to mother-infant dyads of which maternal samples were collected in the 3rd trimester. In (B, C, and D), "a" denotes comparisons between the combined maternal vaginal and fecal sources vs. other sources; "b" denotes comparisons between maternal vaginal vs. fecal sources; statistical testing is performed using Wilcoxon signed-rank test. ASV sharing analysis is conducted based on non-rarefied count data and without accounting for sequencing depth.

C-section deliveries or when breastfeeding was absent, there were fewer shared *Bifidobacterium* ASVs between mothers and infants (not statistically significant), as well as fewer number of mother-infant dyads sharing many specific *Bifidobacterium* ASVs (not statistically significant; Figure S7). In RESONANCE, VDAART, and WISC, compared to younger children, the proportion of shared *Bifidobacterium* ASVs in older children was higher (statistically significant in the RESONANCE cohort while non-statistically significant in the other two cohorts; Figures S8A0 and S8B0), but prenatal antibiotics and delivery mode's effects

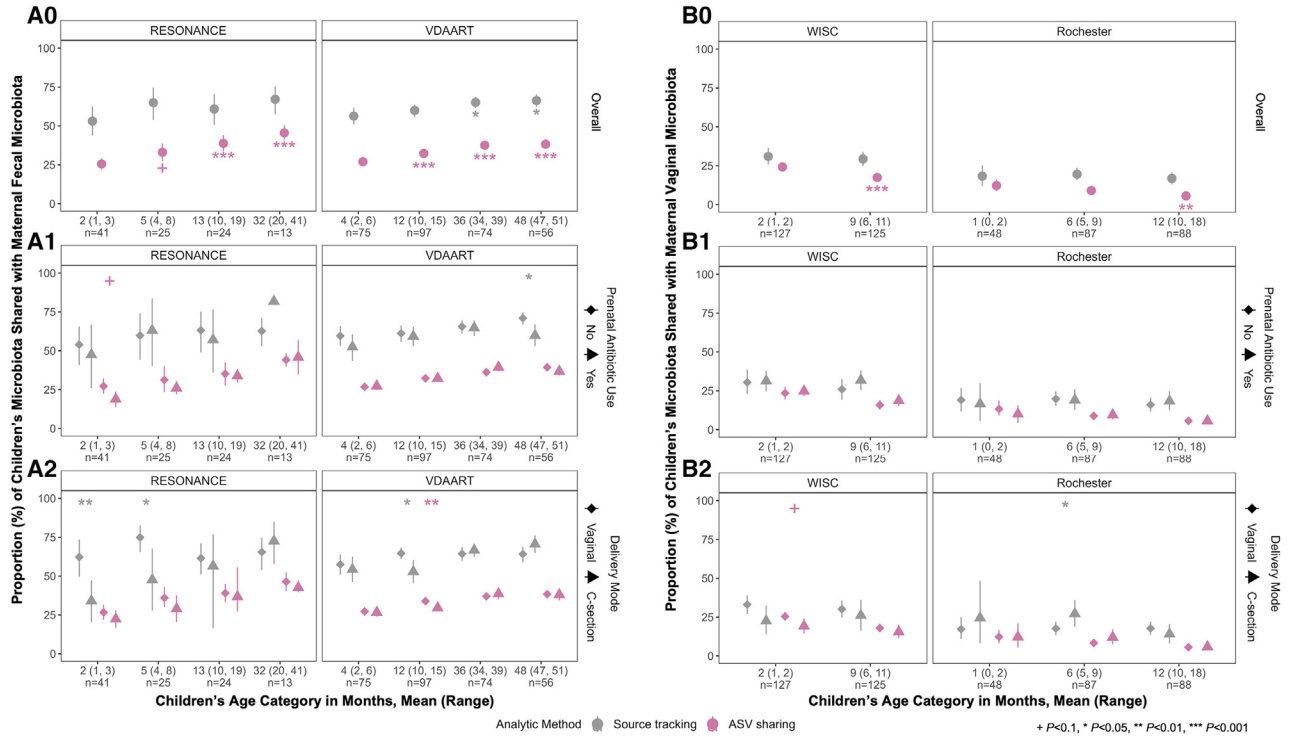

Figure 3. Mother-child microbiota sharing during and beyond infancy in the RESONANCE, VDAART, Rochester, and WISC cohorts

Sharing with maternal fecal (A0–A2) or vaginal (B0–B2) microbiota were estimated through two methods: the amplicon sequence variant (ASV) sharing analysis and source tracking via fast expectation maximization source tracking (FEAST) algorithm. Corresponding 95% confidence intervals were estimated through nonparametric bootstrap. (A0 and B0) show the sharing over children's age category at microbiota measurement without stratification, with symbols indicating statistical tests comparing later age categories against the first. (A1, B1, A2, and B2) present the sharing by prenatal antibiotic use and delivery mode, respectively, with symbols marking statistical tests between groups at each age category. Statistical testing is performed using Wilcoxon rank-sum test. Error bars represent 95% confidence intervals computed using nonparametric bootstrap. The ASV sharing analysis is conducted based on non-rarefied count data and without accounting for sequencing depth.

on *Bifidobacterium* sharing were not definitive (Figures S8A1, A2, B1, B2).

For *Bacteroides*, among the 3-month-olds in the MARCH cohort, 61.5% (n = 24) ASVs were shared between maternal fecal and infant fecal microbiota and 61.5% (24 ASVs) were shared between maternal vaginal and infant fecal microbiota (Figure 4B). Prenatal antibiotics, C-section, and lack of breastmilk were also linked to fewer shared *Bacteroides* ASVs (Figure 4B), and prenatal antibiotics were further associated with fewer mother-infant dyads sharing specific ASVs ($p < 0.05$; Figure S9). An increasing trend in shared *Bacteroides* ASVs was observed comparing older vs. younger children (with maternal fecal microbiota: $p < 0.01$ in the RESONANCE cohort, $p > 0.05$ in the VDAART cohort; with maternal vaginal microbiota: $p > 0.05$ in the WISC cohort; Figures S10A0 and B0). Prenatal antibiotic use was non-significantly associated with fewer shared *Bacteroides* ASVs from maternal fecal source in the RESONANCE cohort (Figure S10A1), and C-section delivery was associated with non-significantly fewer shared *Bacteroides* ASVs (Figures S10A2 and B2). Accounting for sequencing depth yielded consistent sharing results on *Bifidobacterium* and *Bacteroides* with the results described above.

Profile of shared microbes

Among the microbial ASVs shared between mothers and children, there were 237 genera, spanning 77 families, 13 phyla, and 2 domains involved. Detailed information on each shared ASV, including taxonomy, MD5 hash, sequence, and the number of mother-child dyads sharing the ASV, is provided in Tables S1 and S2. The top 20 genera, accounting for the largest proportion of mother-child dyads sharing any ASVs, are presented in Figure S11. The dominantly shared genera was largely consistent across cohorts within the same maternal microbiota source. Infant fecal ASVs from the genera *Bacteroides* and *Bifidobacterium* were among those most commonly shared with both maternal fecal and vaginal microbiota.

Of the 92 potential contaminant genera identified by Salter et al. (2024),[29] only eight were shared between mothers and children in our data (Table S3). These genera were shared by relatively few mother-child dyads across cohorts (e.g., 1–8 dyads per ASV on average), with the exception of *Streptococcus*, which was shared among a higher number of dyads (e.g., 7–17 dyads per ASV on average). However, *Streptococcus* is a well-documented member of the human fecal[30] and vaginal[31] microbiota, suggesting that its presence in our study could reflect biological sharing rather than contamination.

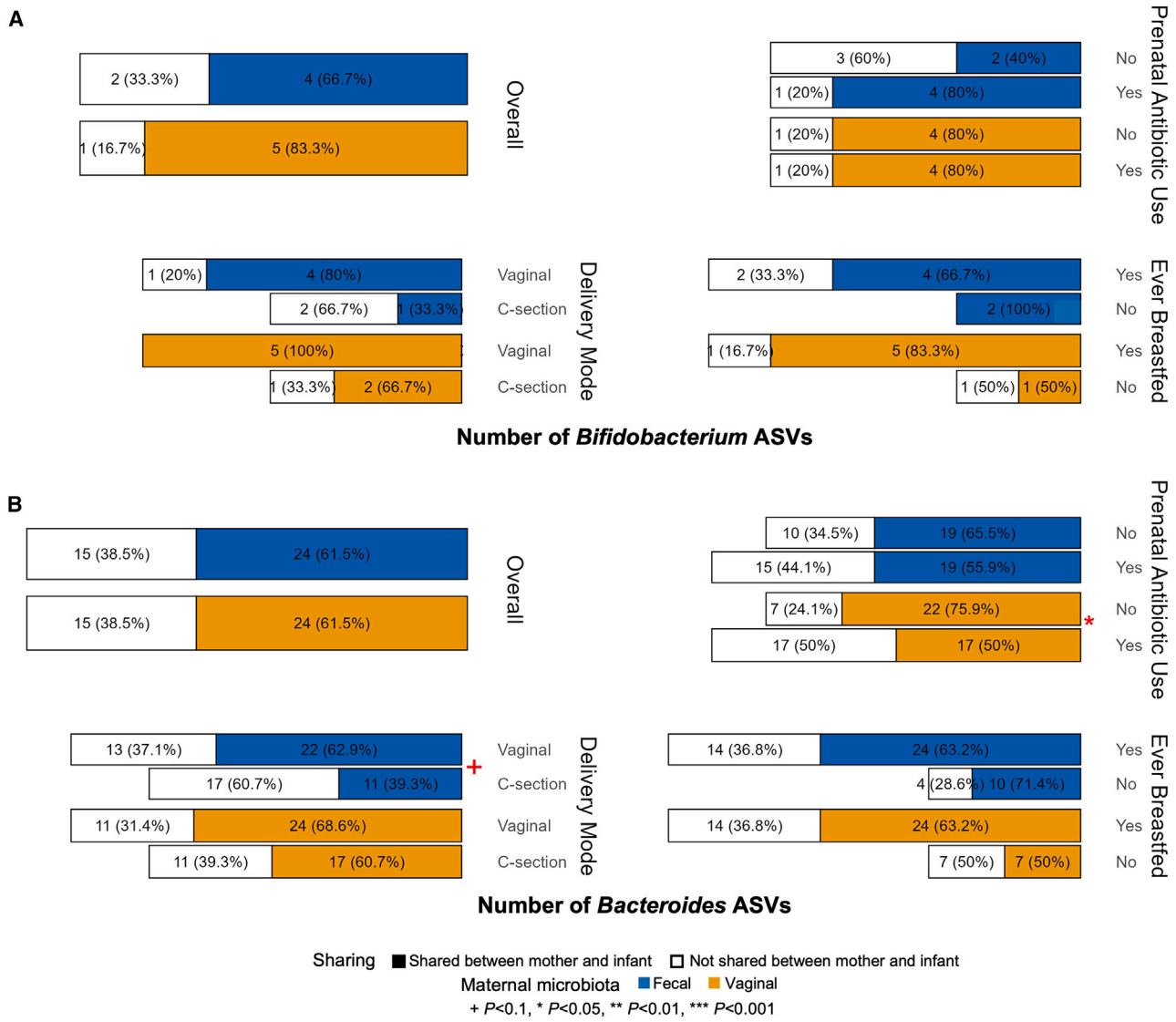

Figure 4. Sharing of Bifidobacterium and Bacteroides between mothers and 3-month-old infants in the MARCH cohort

Sharing of *Bifidobacterium* and *Bacteroides* between mothers and 3-month-old infants in the MARCH cohort. Text in the bar displays the number and proportion of *Bifidobacterium* (A) and *Bacteroides* (B) ASVs that are shared and not shared within mother-infant dyads. Analysis is restricted to mother-infant dyads of which maternal samples were collected in the 3rd trimester (n = 23 dyads). Symbols ($^{+, *, **, ***}$) denote the statistical difference on sharing by prenatal antibiotic use, delivery mode, and breastfeeding status at the time of infant microbiota measurement, using Fisher's tests. ASV, amplicon sequence variant.

DISCUSSION

Analyzing 16S rRNA gene amplicon sequences from five diverse birth cohorts across the United States, our study contributes valuable insights into the contributions of maternal vaginal and fecal microbiota in pregnancy to fecal microbiota of children from infancy to around 5 years of age. We observed that (1) maternal vaginal and fecal microbiota become more similar later in pregnancy, (2) both maternal microbiota sources contributed significantly to child fecal microbiota composition, with the relative contribution of vaginal microbiota increasing when samples were collected later in pregnancy, (3) sharing of both maternal vaginal and fecal microbiota with infant fecal microbiota was impacted by prenatal antibiotic use, delivery mode, and breastfeeding status, and (4) comparing older vs. younger children, the overlap of their fecal microbiota with maternal fecal microbiota became more pronounced and progressively increased up to around 5 years, in contrast to the diminishing overlap with maternal vaginal microbiota. Furthermore, our study also documents a marked and sustained sharing of *Bifidobacterium* and *Bacteroides*—bacterial genera important to infant gut microbiota development—between mothers and their offspring, spanning from infancy through early childhood.

Our study complements the understanding of how vaginal and fecal microbiota from the birthing parent during pregnancy may differentially shape offspring's fecal microbiota composition due to the unique involvement of varied timing in collecting maternal vaginal microbiota samples in the MARCH cohort. This enables a more nuanced comparison of the contributions of maternal pregnancy vaginal vs. fecal microbiota within the same and different trimesters. This stands in contrast to the few prior observational studies that measured both maternal fecal and vaginal microbiota and compared their contribution to infants' fecal microbiota. Ferretti et al. (2018)[21,32] conducted a study in Italy involving 25 pairs of mothers and vaginally delivered infants. They collected maternal vaginal samples shortly before delivery and maternal fecal samples before or shortly after delivery at various time points. Infant fecal samples were collected at 1, 3, 7 days, as well as at 1 and 4 months. They used metagenomic sequencing to assess microbe transmission at the strain level, quantifying transmission based on the number and frequency of transferred strains. Similar to our study, Ferretti and colleagues[21] found ~60% of infant fecal microbiome at 4 months of life were shared with the maternal microbiome. However, they found that the sharing of the maternal fecal microbiome was greater than the sharing of the vaginal microbiome. Bogaert et al. (2023)[24] conducted a study in Netherlands with 120 pairs of mothers and infants, including both vaginally and C-section delivered. They collected maternal vaginal samples at around 35 weeks of gestation and maternal fecal samples 2 weeks after delivery, whereas infant fecal samples were collected at 0 and 1 day, 1 and 2 weeks, and 1 month. They estimated maternal microbiota contributions using the FEAST algorithm based on 16S rRNA sequences and reported higher contribution of maternal fecal than vaginal microbiota. The inconsistencies in the method and timing of sample collection, sample processing, and sample sequencing (e.g., amplicon vs. metagenomic) may have contributed to variations observed between these studies and our own findings. Furthermore, differences across study populations in terms of demographics, lifestyle, and how deliveries are performed may also have contributed to divergent results; e.g., the MARCH cohort, which we used to compare the contribution of maternal vaginal vs. fecal microbiotas, comprised predominantly non-Hispanic white mother-infant dyads from the US, whereas the other two studies comprised dyads from Europe.

Our finding that vaginal and fecal microbiota become more similar later in pregnancy extends previous literature suggesting a gut-vagina axis, possibly due to the proximity of the anus and vulva but also to pregnancy-related factors. The presence of typically gut-associated ASVs among the vaginal microbiota observed in our study is not unexpected, as previous studies, including the Isala citizen-science project,[31] the largest study of vaginal microbiota (n = 3,345), found in healthy women presence of many of the same gut-associated bacterial genera we found, including *Bacteroides* and *Bifidobacterium*, and suggested this reflects a gut-vagina axis due to the proximity of the anus and vulva. Furthermore, Mueller et al. (2024)[29] and Song et al. (2021)[33] found ~30%–60% of vaginal bacteria overlap with fecal bacteria in late pregnancy, with Song et al. (2021)[33] further demonstrating that this gut-vagina bacterial overlap is greater in pregnant women than non-pregnancy women. Together with our results, this evidence suggests a possible pregnancy-related increasing effect on the overlap of maternal vaginal and fecal microbiota.

In our study, the contribution of maternal fecal microbiota was similar to or slightly higher than that of vaginal microbiota among 3-month-old infants exposed to prenatal antibiotics, born by C-section, or who had no exposure to breastfeeding, when both maternal fecal and vaginal samples were collected in 3rd trimester. This is consistent with previous interventional studies that underscored the potential of both maternal fecal[8] and vaginal[26–28] microbes in colonizing the gut of C-section-delivered newborns. For example, Korpela et al. (2020)[8] demonstrated that C-section-born infants receiving a diluted fecal sample from their own mothers collected 3 weeks prior to delivery had fecal microbiota more akin to vaginally born infants. Zhou et al. (2023)[26] reported a markedly higher presence of maternal vaginal microbiota in the fecal samples of C-section-born infants exposed to maternal vaginal fluid gauze collected before delivery, compared to those exposed to a sterile saline gauze.

In contrast, some studies suggest that maternal vaginal microbiota play a less important role in mother-infant microbiota sharing.[8,10,23] For instance, Dos Santos et al. (2023)[23] reported that infant fecal microbiota at either 10 days or 3 months did not cluster together with maternal vaginal microbiota measured at delivery. Although our study also found no greater similarity between offspring's fecal microbiota and biological mother's vaginal microbiota compared to unrelated mother's vaginal microbiota, it is important to exercise caution when relying solely on similarity-based methods to infer mother-infant microbe sharing. Biologically, infants acquire microbes from maternal sources through a selective process focusing on specific microbes. Such selectivity might not always lead to noticeable shifts in microbial community structures that can be captured by clustering analysis. Additionally, the shared microbes might not necessarily have high abundance in the infant's gut, making them elusive to abundance-based clustering analysis.

Our study extends current understanding of mother-child microbiota sharing beyond infancy, revealing that maternal microbiota persistently contributed to children's fecal microbiota, particularly *Bifidobacterium* and *Bacteroides*, from infancy to around 5 years. Previously, Feehily et al. (2023)[11] used both the culture-based and metagenomic approach and identified that *Bifidobacterium* and *Bacteroides* were the genera most frequently transmitted from mother to infant. They also noted a higher likelihood of detecting maternal originated strains in 1-month-old infants' feces than newborns. Our study expands these findings by showing that as children grew from infancy into early childhood, the proportion of these two genera shared with their mothers increased. The higher convergence between maternal pregnancy fecal microbiota and offspring's fecal microbiota later in childhood does not undermine the importance of early infancy as a critical window of acquisition. These observations may reflect a better colonization fitness and evolutionary advantages of microbes acquired from mothers.[21,22] However, they could also result from shared microbial environments due to cohabitation of offspring and mothers,[34] the natural progression of children's fecal microbiota maturation,[13] or site-specific microbiota selection. Nevertheless, the increase in overlap was

not observed for maternal vaginal-child fecal microbiota sharing, suggesting that the overlap at earlier ages is more likely due to peripartum sharing. More detailed taxonomic characterization of microbiota longitudinally using methods such as metagenomic sequencing is necessary to provide greater clarity and deeper insight into the complex mother-child microbiota sharing dynamics. Additionally, it is important to note that in our analysis from infancy and onward, the specific children included at each age category had varying degrees of overlap over time in each cohort due to different collection protocols implemented across cohorts. Therefore, the changes observed over time could also be impacted by the fluctuating participation of children at different ages, especially considering that age is a strong predictor of fecal microbiota composition in children.[35]

Our findings also add to prior research showing that prenatal antibiotic use, C-section delivery, and lack of breastmilk may disrupt mother-child microbiota sharing. These factors are postulated to influence microbiota sharing by altering maternal microbial ecology or interrupting contact with maternal microbial communities.[1] Such disruptions could potentially account for increased risks of adverse health outcomes such as asthma, allergies, and obesity in infants exposed to these factors. In the United States, the overuse of prenatal antibiotics[36] and the high prevalence of medically unnecessary C-section deliveries,[15] as well as insufficient breastfeeding,[16] call for a critical reevaluation of current obstetric practices. Our findings show that for infants that were exposed to prenatal antibiotics, C-section delivery, or non-breastmilk, the 3rd trimester maternal vaginal microbiota contributed equal to or less than the fecal microbiota with respect to the infant fecal microbiota. This raises the possibility that future microbial seeding interventions could consider incorporating both maternal vaginal and fecal sources to counteract these disruptions. Although our study thus offers a foundation for future research, it is essential to have further evidence to support the potential for clinical applications. Due to the limited sample size in MARCH, the sole cohort with complete data on all the three factors, our study cannot conclusively determine which factor exerts the most profound impact on mother-infant microbial sharing. The MARCH cohort also had limited numbers of C-section-delivered infants and infants who were never breastfed, increasing statistical uncertainty in findings in these subgroups and limiting our statistical power in detecting differences by delivery mode and breastfeeding status. This constraint warrants cautious interpretation of the results on the impacts of delivery mode and breastfeeding status on the relative contributions of maternal fecal and vaginal microbiota to infant fecal microbiota. However, it should be noted that the other four cohorts had larger numbers of C-section-delivered infants and infants exposed to prenatal antibiotics, which improved the statistical power of our analysis for examining the impacts of delivery mode and prenatal antibiotics on mother-child microbial sharing. Some cohorts in our study also lacked detailed information on the nature of C-sections (e.g., with or without labor onset), which could offer additional nuances to our findings. Future cohort studies, equipped with larger sample sizes and detailed assessments of these factors, will be better positioned to test the associations observed in our study and inform targeted interventions.

Our study has several strengths. We used a larger, more socioeconomically and demographically diverse study population than prior studies, particularly for our analysis on maternal microbial contribution to children's fecal microbiota beyond infancy, which enhances both the robustness and generalizability of our findings. This aspect is crucial as prior research has demonstrated that geographic, environmental, and lifestyle factors can lead to population-specific differences in microbiota composition.[37,38] In addition, our analysis of maternal vaginal microbiota across three trimesters allowed us to explore trimester-specific contributions of maternal microbiota to infant microbiota. Further setting our work apart from prior literature, we examined contribution of maternal microbiota to children's fecal microbiota at multiple ages from infancy to early childhood, which has not been done in earlier studies. Lastly, the combination of similarity-based analysis, microbial source tracking, and ASV sharing analysis in our methodology provides a more comprehensive comparison between maternal and children's microbial communities.

In summary, our study advances the understanding of contributions of maternal fecal and vaginal microbiota to offspring fecal microbiota beyond the initial months of life and provides evidence suggesting that prenatal antibiotics, C-section, and lack of breastfeeding may modify this crucial microbial exchange. However, given certain limitations of our study, such as the inability to capture lower-resolution level microbial sharing, caution is needed in interpreting our findings. Although our findings may have implications for public health and clinical practice, particularly in guiding microbial-seeding interventions to foster a healthier early life microbial ecosystem and potentially improve long-term health, further research is needed to test and potentially confirm the observations made in our study. Our study sets a direction for future research, emphasizing the need for high-resolution whole-genome microbiome analysis in additional birth cohorts. Such research could validate and extend our discoveries, especially regarding the long-term persistence of microbes from the birthing mother in her child.

Limitations of the study

Our study has several limitations and caveats worthy of acknowledgment. Vaginal samples in MARCH were taken from different mothers at various time points during pregnancy, rather than from the same group of mothers repeatedly over time. We cannot exclude the possibility that this contributed to our trimester-specific findings. Second, although our study focused on child sharing of maternal pregnancy microbiota, the earliest time point available across the participating cohorts was around one month of age. The absence of data from the immediate postpartum period may lead to an underestimation of early maternal microbial contributions. Prior research highlights that early microbial seeding in the first days of life is critical for shaping the infant gut microbiota, and some microbes shared during birth may persist only transiently.[12,39] The dynamic changes in infant microbiota, particularly in the first month, suggest that early colonizers from maternal sources may no longer be detectable by the time we sampled child microbiota. Third, our examination of the disruption effects on mother-child microbiota sharing only focused on the factors that have consistently demonstrated

the most significant impacts: prenatal antibiotic use, delivery mode, and breastfeeding. There may be other factors influencing this process, such as skin-to-skin contact, which we did not explore, primarily due to data unavailability. In addition, the absence of breastfeeding information at the time of child fecal sample collection in cohorts other than MARCH prevented us from assessing the influence of breastfeeding on mother-child microbe sharing beyond infancy. Our study also did not explore the potential complement of mother-child microbe sharing by other routes, such as father-child microbe sharing.[40] Additionally, we did not have data on breast milk microbiota, which may be a source of microbiota, particularly in infants born by C-section.[24] Without breast milk microbiota data, we may overestimate the role of maternal vaginal and fecal microbiota. Future studies should incorporate early postpartum and breast milk microbiota data to provide a more comprehensive understanding of microbial transmission and its impact on infant and childhood microbiota development.

It is also worth noting that we did not pursue more advanced multivariable-regression-based etiologic analyses in parts of our study that could have been influenced by confounding, for example in Aim 3, due to unavailability of some covariate data across cohorts. Those unadjusted findings should thus be interpreted as descriptive results. Nevertheless, descriptive results still allow us to achieve our aims of characterizing the relative contribution of the vaginal vs. fecal microbiota to the infant (Aim 1); how long maternal microbiota are shared with the child (Aim 2); and whether this sharing is consistent by strata of prenatal antibiotic use, delivery mode, and breastfeeding status (Aim 3). Previous studies investigating similar research questions have also used the same analytical method (i.e., Wilcoxon rank sum tests) without adjustment,[7,9,24] thus aligning our approach with prior work. Additionally, it should be noted that confounding is not a concern for Aim 1 because the comparison is between the vaginal vs. fecal microbiota of the same mother. Nevertheless, future studies with more comprehensive data should, when called for, adopt advanced statistical techniques to better account for potential confounders and further validate our findings.

Another important caveat of our study was that we relied on 16S rRNA gene amplicon sequencing, which constrained our ability to characterize microbiota at the species and strain levels and infer mother-child sharing beyond the ASV level. Given that prior studies have suggested mother-child microbial sharing or transmission often occurs at the strain level, the observations in our study may be mixed or incomplete, potentially underestimating the true extent of microbial sharing. We can also not precisely distinguish between ASVs acquired from mothers versus environments. As such, findings in our study can only infer mother-child sharing rather than transmission of microbes. In-depth tracking of microbial sources based on phylogenetic similarity derived from whole-genome sequences are necessary to infer origins of infant microbes and conclude on mother-infant microbe transmission. However, the FEAST algorithm is not affected by sequencing type,[41] making our findings on the relative contributions robust. Furthermore, in terms of comparing the relative contribution of maternal microbes from vaginal vs. fecal source to child fecal microbiota, 16S rRNA sequencing has advantages over shotgun metagenomic sequencing. Unlike 16S rRNA sequencing, shotgun metagenomic sequencing results are strongly impacted by host DNA concentration because it sequences the DNA of everything in the sample. For instance, samples from vaginal swabs can contain >90% human DNA when sequenced, diluting the microbiome sequencing reads and reducing resolution compared to samples with low host DNA interference, such as fecal samples.[42] Differing levels of host DNA thus impact sensitivity of taxonomic profiling, making comparisons of microbiomes with differing levels of host DNA content difficult when using whole-genome sequencing.[43] 16S rRNA sequencing minimizes this bias by amplifying only the 16S rRNA gene region specific to bacteria and facilitating between-sample type bacterial DNA comparisons.[42] Additionally, 16S rRNA sequencing offers better bacterial coverage and generates fewer false positives compared to shotgun metagenomics due to more developed reference databases and error correction protocols.[7,9,24]

It is also worth highlighting that differences in sample collection and sequencing protocols across cohorts likely introduced variance in microbiota sequences. For instance, maternal vaginal samples were obtained using recto-vaginal swabs at group B streptococcus screenings in WISC, whereas cervicovaginal fluid was collected during a study visit in Rochester; children fecal samples were collected using rectal swabs in Rochester, in contrast to direct feces collection in other cohorts. In addition, differences across cohorts in DNA extraction kits, primers, hypervariable regions, and sequencing platforms may have introduced variability in observed microbial sequences.[44] For example, among the MARCH, Rochester, and WISC cohorts that collected maternal vaginal samples during pregnancy, *Bifidobacterium* was less adequately amplified in the Rochester cohort (used 319F-805R primer targeting the V3-V4 hypervariable region) compared to MARCH or WISC (used different primers targeting the V4 hypervariable region). Further, the dissimilarity between maternal vaginal and infant fecal microbiota tended to be smaller in the MARCH cohort compared to the Rochester or WISC cohorts, which could be partly attributed to differences in DNA extraction and sequencing protocols. To address these technical variations, we analyzed the data from each cohort individually and considered cohort-specific variations when drawing our overall conclusions. Nevertheless, the consistency of key findings across cohorts strengthens the reliability of our results. Future multi-site studies should consider standardizing collection and sequencing protocols to reduce variability and enhance comparability.

Lastly, we cannot completely rule out the possibility of sequencing contamination in our study. Only a small number of potential contaminant genera identified by Salter et al. (2014)[29] were shared in our data, and these genera were found in relatively few mother-child dyads. *Streptococcus*, which was shared among a higher number of dyads, is a known member of the human fecal[29] and vaginal[31] microbiota, supporting its biological relevance in our findings. The presence of some gut-associated genera in shared ASVs with maternal vaginal samples is consistent with other studies of the vaginal microbiome, which suggested a gut-vaginal axis,[31] and it is also consistent with microbiota shifts during pregnancy, where vaginal microbiota can

transiently resemble fecal microbiota as pregnancy progresses, as observed in ours and previous studies.[28,33]

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to the lead contact, Dr. Noel T. Mueller (noel.mueller@cuanschutz.edu).

Materials availability

This study did not generate new reagents.

Data and code availability

- Select de-identified data from the ECHO Program are available through NICHD's Data and Specimen Hub (DASH). Information on study data not available on DASH, such as some Indigenous datasets, can be found on the ECHO study DASH webpage.
- The 16S rRNA amplicon gene sequences are publicly available via accession numbers listed in the key resources table.
- All original R scripts used in this study are publicly available at https://github.com/tiangeliu-epi/ECHO-Microbe-Sharing-Analysis-16S.

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AUTHOR CONTRIBUTIONS

Conceptualization, T.L., A.M.K., E.S., S.S.C., K.McK., and N.T.M.; methodology, T.L. and N.T.M.; investigation and formal analysis, T.L. and N.T.M.; visualization, T.L.; bioinformatics, J.D.; funding acquisition, N.T.M., J.E.G., D.K.-M., T.G.O'C., J.B.S., and L.P.J.; data acquisition, J.E.G., S.S.C., T.G.O'C., K.L.-S., A.A.L., D.K.-M., K.McK., and J.B.S.; writing—original draft, T.L., A.K., and N.T.M.; writing—review & editing, all authors. All authors agreed to submit the manuscript, read and approved the final draft.

DECLARATION OF INTERESTS

C.R.-S. serves as a consultant for Amgen, AstraZeneca, and The KOL Connection on a topic not related to this manuscript. K.L.-S. is employed by and owns stock in Vertex Pharmaceuticals. N.T.M. is on the Scientific Advisory Board for Tiny Health LLC. Other authors report no conflict of interest.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Fecal samples of pregnant mothers	This study	N/A
Vaginal samples of pregnant mothers	This study	N/A
Fecal samples of children	This study	N/A
Deposited data		
Participant characteristics in the ECHO Program	This study	NICHD Data and Specimen Hub (https://dash.nichd.nih.gov/explore/study?q=echo&filters=%5b%5d&page=1&sortBy=relevance&asc=true&size=50)
MARCH cohort 16S rRNA sequences	This study	Sequence Read Archive, accession number PRJNA1218274
RESONANCE cohort 16S rRNA sequences	This study	Sequence Read Archive, accession number PRJNA695570
VDAART cohort 16S rRNA sequences	This study	Sequence Read Archive, accession number PRJNA1074705
Rochester cohort 16S rRNA sequences	This study	Sequence Read Archive, accession number PRJNA1099167
WISC cohort 16S rRNA sequences	This study	European Nucleotide Archive, accession number PRJEB46659
Software and algorithms		
QIIME 2 v 2022.2	Bolyen et al.[45]	https://docs.qiime2.org/2022.2/
Cutadapt	Martin[46]	https://cutadapt.readthedocs.io/en/stable/
VSEARCH	Rognes et al.[47]	https://github.com/torognes/vsearch
q2-quality-filter	Bokulich et al.[48]	https://github.com/qiime2/q2-quality-filter
deblur	Amir et al.[49]	https://github.com/biocore/deblur
Greengenes 13-8	McDonald et al.[50]	https://docs.qiime2.org/2021.4/data-resources/
SEPP	Janssen et al.[51]	https://github.com/qiime2/q2-fragment-insertion
Silva 138.1	Quast et al.[52]	https://www.arb-silva.de/
RESCRIPt	Robeson et al.[53]	https://github.com/bokulich-lab/RESCRIPt
FEAST	Shenhav et al.[41]	https://github.com/cozygene/FEAST
digest	Eddelbuettel et al.[54]	
R v 4.2.2	R Core Team	https://www.r-project.org/
Original analytical codes	This study	https://github.com/tiangeliu-epi/ECHO-Microbe-Sharing-Analysis-16S

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Study population

We invited cohorts from the Environmental Influences on Child Health Outcomes (ECHO) cohort to participate in this study based on the data availability of fecal and/or vaginal microbiota from mothers during pregnancy, and fecal microbiota from children during and/or beyond infancy. The ECHO cohort was initiated by the National Institutes of Health in 2016 to understand how the environment from preconception through early childhood influences child health and development.[55] To date, the ECHO cohort study comprises 69 pregnancy and pediatric cohort sites from across the United States and Puerto Rico; although not all cohort sites have collected microbiota samples.[55] Following a standard operation procedure, a total of 15 ECHO cohort sites transferred their microbiota data to the ECHO Data Analysis Center as of December 2022, where raw sequences were re-processed with a standardized bioinformatics pipeline. In this study, we used data from cohorts where vaginal and/or fecal microbiota from the birthing mother were measured at least in the 3rd trimester during pregnancy (hereafter ‘‘maternal microbiota’’) and fecal microbiota from the biological children of the mothers was measured at least once between birth and age five years. This left us with data from five ECHO cohort sites (Figure S1): Michigan Archive for Research in Child Health (MARCH; n=53 mother-child dyads), RESONANCE (n=64 dyads), Vitamin D Antenatal Asthma Reduction Trial (VDAART; n=97 dyads), Rochester (n=134 dyads), and Wisconsin Infant Study Cohort (WISC; n=135 dyads). These cohorts provided a combined total of 483 mother-child dyads.

More than half of mothers and children across 4 of the 5 cohorts in our analysis identified as non-Hispanic White; the exception was the VDAART cohort where over 50% identified as Black. More than 90% of mothers in the MARCH and WISC cohorts held a college degree or above, though this percentage was lower in other cohorts. The mean (SD) pre-pregnancy BMI was 28.4 (6.7) kg/m^2 among all participants with data, ranging from 25.9 (5.3) in the MARCH cohort to 32.2 (6.6) in the RESONANCE cohort. The mean (SD) age at delivery was 30.1 (4.9) years, ranging from 27.7 (5.8) in the VDAART cohort to 32.2 (4.5) in the MARCH cohort. Across cohorts, 52.0% of mothers did not take prenatal antibiotics. Of the children, 51.8% were male and 73.7% were born vaginally (n=7, 1.4% missing). On average, children had a birth weight of 3.4 (0.5) kg and were born at a gestational age of 39.2 (1.3) weeks. For infants in MARCH, 88.7% (n=43) were breastfed at the time of fecal sample collection (i.e., approximately 3 months of age); such information was not complete or available in other cohorts. For detailed characteristics of the study population, please see Table 1.

Local institutional and/or the central ECHO review boards granted approval to all the included cohorts. Participants and parent or guardians provided written informed consent for participation in individual cohorts and data sharing with ECHO.

METHOD DETAILS

Sample collection and processing

The RESONANCE, VDAART and WISC cohorts implemented specific exclusion criteria for participants providing microbiota samples (Table S4). In RESONANCE, children who had taken antibiotics within the last two weeks prior to biospecimen sample collection were not eligible to provide fecal microbiota samples. The VDAART cohort excluded mothers who had used antibiotics within seven days before sample collection. Likewise, WISC excluded mothers with perinatal infections or antibiotic use in the month preceding delivery. The MARCH and Rochester cohorts did not implement such exclusion criteria for their microbiota samples.

Maternal vaginal and fecal samples were either self-collected at home or taken by clinicians during a study or clinical visit (Table S5), which was in the 3rd trimester for most cohorts (Figure 1). In the MARCH cohort, 47.2%, 9.4%, and 43.4% of vaginal samples were collected in the 1st, 2nd, and 3rd trimester, respectively, and 3.8% and 96.2% of fecal samples were collected in the 2nd and 3rd trimester, respectively. In RESONANCE, 14.1% of maternal fecal samples were collected in the 2nd trimester with the rest collected in the 3rd trimester. As for infants and children, their fecal samples were either collected by parents at home or by research staff during a study visit. In the MARCH cohort, infant samples were collected once at around 3 months of age. In contrast, the other four cohorts conducted repeated children sample collections from birth, either at certain ages (VDAART, WISC, and Rochester) or across a range of ages (RESONANCE). To maximize sample size in subsequent analysis, we grouped infants and children based on their age at the time of fecal sample collection into categories as following (shown as mean [range] in months): MARCH: 3 [3, 6]; RESONANCE: 2 [1, 3], 5 [4, 8], 13 [10, 19], 32 [20, 41]; VDAART: 4 [2, 6], 12 [10, 15], 36 [34, 39], 48 [47, 51]; Rochester: 1 [0, 2], 6 [5, 9], 12 [10, 18]; WISC: 2 [1, 2], and 9 [6, 11] (Figure 1).

Samples collected at home were stored in a freezer or refrigerator until transferred to the lab, portioned into sterile tubes, and stored at −80°C. After DNA was extracted from the samples, the hypervariable region (i.e., V3-V4, V4, or V4-V5) of the 16S rRNA gene was amplified using barcoded primers, and the samples were sequenced on Illumina platforms (details provided in Table S5). We used 16S rRNA gene sequencing data to address our study objectives, because it is less influenced by level of host DNA than whole genome metagenomic sequencing. Specifically, level of host DNA impacts sensitivity of microbiome taxonomic profiling, such that it is difficult to compare microbiomes with vastly different levels of host DNA. This could be particularly problematic for our study in which we were comparing stool samples, which comprise less than 10% of human DNA, to vaginal swabs that contain >90% of human DNA. Moreover, 16S rRNA data was the only microbiome data available at the time of our analysis.

Bioinformatics of 16S rRNA sequences

The individual cohort and ECHO Data Analysis Center verified the fidelity of the sequencing data transfer. We imported the sequencing data into QIIME 2 (v 2022.2)[45] using the manifest format and processed them on a cohort-by-cohort basis. We trimmed the primers using Cutadapt (q2-cutadapt), retaining all reads.[46] For paired reads, we joined them using VSEARCH with its default parameters (q2-vsearch)[47]; and subsequently applied a quality filter to all reads through the q2-quality-filter qiime2 plugin.[48] We denoised the sequences to amplicon sequence variants (ASVs) using deblur for each cohort using cohort-specific trimming parameters while retaining default parameters elsewhere.[49] We built a specific phylogenetic tree for each cohort using fragment insertion into the Greengenes 13-8 reference backbone using the SEPP algorithm (q2-fragment-insertion).[50,51] We assigned taxonomy using a naive Baysian classifier, which we trained against the full-length 99% SSU Silva 138.1 reference database accessed via RESCRIPt.[50,52,53] We calculated β diversity metrics that consider presence or absence of microbiota, which was most relevant to analysis of microbiota sharing. Specifically, we included Jaccard[56] and Bray-Curtis[57] (which also takes into account abundance) dissimilarities, calculated using the q2-diversity plugin in QIIME 2 on count data rarefied to 1000 (about the lowest number across samples) sequences per sample. Analyses that did not involve β diversity metrics were conducted based on non-rarefied count data. All samples with fewer than 1000 sequences were excluded from analysis.

Measurement of other variables

The ECHO Data Analysis Center harmonized data on prenatal antibiotic use and delivery mode for all the participating cohorts from sources including medical records and self-reports by parents. We categorized maternal prenatal antibiotic use (during any trimester

and before labor) as yes or no, and birth delivery mode as vaginal or C-section delivery. Information on breastfeeding status at the time of infant fecal sample collection was most complete in the MARCH cohort. We categorized breastfeeding in our analysis as ever or never received breastmilk at the time of infant fecal sample collection based on parental reports. Descriptive characteristics of participants, including age at delivery, race, ethnicity, highest educational achievement, and pre-pregnancy body mass index (BMI) of mothers, and race, ethnicity, biological sex, gestational age, and weight at birth of children, were harmonized by ECHO Data Analysis Center.

QUANTIFICATION AND STATISTICAL ANALYSIS

First, we assessed whether the overall fecal microbiota structure of infants or children, over different child-age categories, were more similar to pregnancy fecal or vaginal microbiota structure of the child's biological mother compared to a non-biological mother in each of the five cohorts. We quantified microbiota structure dissimilarity using Jaccard and Bray-Curtis distances for pairs of a child and their biological mother, as well as for those of a child and a randomly assigned non-biological mother. We assessed the differences in dissimilarity between biological and random pairs using Wilcoxon rank sum tests. We conducted analysis for each group of children's age in each cohort (see details on sample size of each group in Figure S3).

Next, we used data from the MARCH cohort to address Aim 1, which was to quantify the relative contribution of maternal vaginal vs. fecal microbiota to infant fecal microbiota (Figure 1). MARCH was the sole cohort among the five that measured both maternal vaginal and fecal microbiota, making it the only eligible cohort for this analysis. We visualized the overlap of ASVs across maternal fecal, vaginal, and infant fecal samples using proportional Venn diagrams. Considering that maternal microbiota were measured throughout pregnancy in the MARCH cohort, we both pooled data across trimesters (n=53 dyads) and also stratified it based on the timing of maternal sample collection to discern potential trimester-specific differences (n=25 dyads when both vaginal and fecal samples were collected in 1st trimester, 5 when vaginal samples were collected in 2nd trimester and fecal samples were collected in 2nd or 3rd trimester, and 23 when both vaginal and fecal samples were collected in 3rd trimester).

Our estimation of maternal pregnancy microbiota contribution to infant microbiota employed two methods: 1) ASV sharing and 2) source tracking. Both of these approaches were conducted based on non-rarefied count data. The ASV sharing method summarized the number and proportion of infant ASVs that a child shared with either their biological mother's fecal or vaginal microbiota, or both. This analysis was performed both among all dyads and by the timing of maternal sample collection. Due to the minimal evidence of sharing with maternal 1st trimester microbiota and the limited sample size of maternal 2nd trimester samples, our subsequent source tracking and other analyses in MARCH were focused only on the 23 mother-infant dyads of which both maternal vaginal and fecal samples were collected in the 3rd trimester.

For the source tracking method, we used the Fast Expectation-Maximization Microbial Source Tracking (FEAST) algorithm via R package "FEAST".[41] FEAST employs an expectation-maximization algorithm to determine the proportions of sources for a given microbiota community (referred to as the "sink"). In our study, we tagged maternal microbiota as sources and their own infant's microbiota as sinks. FEAST estimated the fraction of infant fecal microbiota that was contributed by their mother's vaginal or fecal microbiota, as well as other unmeasured maternal or environmental sources (hereafter "other sources"). We set the expectation-maximization iterations to 1000 and rarefied ASV count data to the minimal sequencing depth within each mother-infant dyad, adhering to FEAST's default settings. After estimating maternal contributions using each method, we applied Wilcoxon signed-rank tests to compare within dyads whether the combined maternal contributions differed from those of other sources, and whether contributions from maternal fecal microbiota differed from that of vaginal microbiota.

To address Aim 2, which was to examine the persistence of maternal vaginal or fecal microbiota in children's fecal microbiota from infancy into childhood (Figure 1), we performed separate analyses in the RESONANCE, VDAART, Rochester, and WISC cohorts. We conducted cohort-specific analyses to minimize bias due to batch effects in microbiota sequences. Like Aim 1, we used both the ASV sharing and source tracking methods to estimate the contribution of maternal pregnancy fecal microbiota (in RESONANCE and VDAART) or vaginal microbiota (in Rochester and WISC) to their child's fecal microbiota at each age group since birth (described above). In these four cohorts, most maternal microbiota measurements were taken in the 3rd trimester except in RESONANCE where 14.1% of maternal fecal samples were collected in the 2nd trimester (Table S5). We summarized the maternal contribution to children's microbiota at each age group and computed 95% confidence intervals using nonparametric bootstrap methods. We then used Wilcoxon rank sum tests to assess whether the maternal contribution to children's microbiota measured at later age groups statistically differed from that measured at the first age group in early infancy (see details in Figure 3 legends).

For Aim 3, which focused on determining whether mother-child microbiota sharing differed by prenatal antibiotic use, delivery mode, and breastfeeding status, we included all five cohorts in our analyses and conducted analysis separately within each cohort (Figure 1). Within the MARCH cohort, we examined the relative contributions of maternal fecal versus vaginal microbiota by strata of prenatal antibiotic use (yes/no), delivery mode (C-section/vaginal), or breastfeeding status (yes/no) at the time of microbiota measurement, using Wilcoxon signed-rank tests. Then within each cohort, we compared mother-child microbiota sharing between groups of prenatal antibiotic use or delivery mode, and additionally between groups of breastfeeding status in the MARCH cohort. We repeated these analyses for mother-child microbial sharing estimated by the ASV sharing and source tracking methods (which are described above).

In addition, given our *a priori* interest in *Bifidobacterium* and *Bacteroides*, we further analyzed the number of ASVs from these genera shared within biological mother-child dyads in all cohorts other than the Rochester cohort. This exclusion was due to concerns about the inadequate amplification and classification of these ASVs in the Rochester cohort. Unlike other cohorts that focused on the V4 or V4-V5 hypervariable regions, the Rochester cohort used the 319F-805R primer set targeting the V3-V4 hypervariable region for DNA amplification, which could lead to variations in the efficiency of amplification and the accuracy of microbe identification. In the other four cohorts, we calculated the proportion of shared ASVs by dividing the number of shared ASVs by the total number of detectable ASVs in each infant fecal microbiota. We then determined the influences of prenatal antibiotic use, delivery mode, and breastfeeding (in MARCH only) on the sharing at each age group of children's microbiota measurement, using Fisher's tests. Moreover, we conducted a detailed analysis of each shared *Bifidobacterium* and *Bacteroides* ASV from maternal fecal and vaginal sources in the MARCH cohort. For each shared ASV, we calculated the proportion of dyads sharing it from each maternal source and assessed the sharing according to level of prenatal antibiotic use, delivery mode, and breastfeeding. We named ASVs by their name at the lowest available taxonomy level following by the first four characters of an MD5 hash of the sequence via "digest" package[54] in R.

Finally, we conducted several sensitivity analyses to evaluate the robustness of our findings. First, in estimating maternal contribution using the ASV sharing method, we repeated the analysis after accounting for sequencing depth by defining a shared ASV as one whose relative abundance exceeded 0.1% within a mother-child dyad. Second, for the source tracking analysis in the MARCH cohort, we additionally assigned a randomly selected non-biological mother alongside the biological mother as source communities for each infant. We then compared the contributions from random versus biological mothers using Wilcoxon signed-rank tests. Third, we assessed the potential for sequencing contamination by analyzing the sharing profiles of ASVs within the 92 genera previously identified as potential contaminants in DNA extraction kits and laboratory reagents by Salter et al. (2024).[29] Specifically, we calculated the total number of shared ASVs for each genus and the average number of mother-child dyads sharing each ASV within these genera.

Results are given as mean (SD) if not otherwise indicated. We performed all analyses in R software (version 4.2.2). We determined statistical significance based on a two-sided $P < 0.05$.

ADDITIONAL RESOURCES

VDAART randomized clinical trial is registered at ClinicalTrials.gov with identification number NCT00920621.