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Intrapartum care

Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: a prospective cohort study

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Objective Dysbiosis of the infant gut microbiota may have long-term health consequences. This study aimed to determine the impact of maternal intrapartum antibiotic prophylaxis (IAP) on infant gut microbiota, and to explore whether breastfeeding modifies these effects.

Design Prospective pregnancy cohort of Canadian infants born in 2010–2012: the Canadian Healthy Infant Longitudinal Development (CHILD) Study.

Setting General community.

Sample Representative sub-sample of 198 healthy term infants from the CHILD Study.

Methods Maternal IAP exposures and birth method were documented from hospital records and breastfeeding was reported by mothers. Infant gut microbiota was characterised by Illumina 16S rRNA sequencing of faecal samples at 3 and 12 months.

Main outcome measures Infant gut microbiota profiles.

Results In this cohort, 21% of mothers received IAP for Group B *Streptococcus* prophylaxis or pre-labour rupture of membranes; another 23% received IAP for elective or emergency caesarean section (CS). Infant gut microbiota community structures at 3 months differed significantly with all IAP exposures, and differences persisted to 12 months for infants delivered by emergency CS. Taxon-specific composition also differed, with the genera *Bacteroides* and *Parabacteroides* under-represented, and *Enterococcus* and *Clostridium* over-represented at 3 months following maternal IAP. Microbiota differences were especially evident following IAP with emergency CS, with some changes (increased Clostridiales and decreased Bacteroidaceae) persisting to 12 months, particularly among non-breastfed infants.

Conclusions Intrapartum antibiotics in caesarean *and* vaginal delivery are associated with infant gut microbiota dysbiosis, and breastfeeding modifies some of these effects. Further research is warranted to explore the health consequences of these associations.

Keywords Breastfeeding, caesarean section, gut microbiome, gut microbiota, infant, intrapartum antibiotics.

Tweetable abstract Maternal #antibiotics during childbirth alter the infant gut #microbiome.

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Introduction

Up to 40% of newborns are exposed to perinatal antibiotics, either directly with intravenous ampicillin and gentamicin treatment for early-onset sepsis, or indirectly through the administration of maternal intrapartum antibiotic prophylaxis (IAP).[1–3] In Canada and the USA, these treatments patterns adhere to clinical practice guidelines for the prophylaxis of vaginal *Group B Streptococcus* (GBS) and caesarean section (CS) delivery.[4,5] With rising rates of CS delivery and GBS colonisation during pregnancy,[6,7] IAP has become a routine part of the birthing process in North America. However, these practices are not universal. In Norway, women undergoing elective CS do not receive IAP,[8] and Denmark has adopted a non-culture based risk factor approach for GBS that has reduced IAP to 13% of vaginal deliveries.[1] In fact, there appears to be a continental divide, with guidelines from North America following a culture-based approach to GBS prophylaxis, whereas those from the UK and Australia recommend risk-based management.[9]

Antibiotic exposure of newborn infants is not without risk. While effective in preventing early-onset neonatal sepsis, maternal GBS prophylaxis has been linked to amoxicillin-resistant late-onset *Escherichia coli* infections in infants.[10] Longer-term infant antibiotic use has been associated with childhood asthma, allergy and obesity,[11–14] conditions linked to gut microbiota aberrancies during early life.[15,16] Although this evidence originates from studies of postnatal antibiotic use, perinatal exposures that disrupt initial gut colonisation may have greater potential to change patterns of microbiota development. Significant gut microbiota disruption has been observed following neonatal antibiotic treatment at birth,[17,18] including reduced microbiota diversity and altered taxonomic distribution persisting for up to 8 weeks. Recently, Aloisio et al.[19] reported lower bifidobacteria counts 7 days after maternal IAP for GBS. However, no studies have evaluated the impact of IAP on the infant gut microbiota beyond the first few months of life.

Given that IAP regimens for CS and GBS are not universal, and in light of evidence that infant gut microbiota dysbiosis may be detrimental to the developing metabolic and immune systems, further study on the short- and long-term impact of newborn exposure to maternal IAP is needed. As a follow up to our report on infant gut dysbiosis following CS delivery,[20] the objective of our current study was to determine the impact of IAP exposure on the infant gut microbiota. As gut microbiota 'recovery' has been reported following antibiotic treatment in exclusively breastfed infants,[21] a secondary objective was to evaluate the role of breastfeeding in modifying antibiotic-induced dysbiosis of infant gut microbiota.

Methods

Study design

This study of 198 infants represents a subset of the larger Canadian Healthy Infant Longitudinal Development (CHILD) national population-based birth cohort (www.canadianchildstudy.ca). Women in their second trimester, attending regional centres for ultrasound, were enrolled in Winnipeg, Manitoba, Canada, between June 2009 and January 2011. Microbiome analyses were conducted for an unselected subsample comprising the first 198 enrolled infants with available faecal samples and complete perinatal antibiotic exposure data. Supporting Information Table S1 shows demographic characteristics of the full Winnipeg CHILD cohort compared with the subsample assessed here, showing no major differences except for slightly increased maternal age in the current subsample (mean 31.4 versus 30.3 years, $P < 0.05$). Written informed consent was obtained from parents at enrolment. This study was approved by the University of Manitoba Human Research Ethics Board and the Health Information Privacy Committee.

Exposures (IAP, method of birth and breastfeeding)

Mode of delivery (classified as vaginal, elective CS or emergency CS), maternal intrapartum antibiotic prophylaxis (IAP), and hospital-administered infant antibiotics were documented from hospital records. Emergency CS was defined clinically by an obstetrician, and includes CS with and without labour. These data were used to assign each infant to one of four exposure groups: no IAP with vaginal delivery, IAP with vaginal delivery, IAP with elective CS delivery, and IAP with emergency CS delivery. Infant oral antibiotic use was documented from parent report and linked provincial prescription data (the Manitoba Drug Program Information Network). The prescription database was also a source for maternal postpartum antibiotic treatment. Mothers completed standardised questionnaires at 3, 6 and 12 months postpartum, reporting on breastfeeding duration and exclusivity.

Faecal microbiota analysis

Methods of sample collection, DNA extraction and amplification, 16S rRNA sequencing and taxonomic classification have been previously described.[22] In brief, fresh or previously refrigerated faecal samples were collected at a home visit (3 months) or brought to a clinic visit (12 months). Samples were refrigerated during transport and stored at −80°C until analysis. Whole genome DNA was extracted from faeces using the QIAamp DNA Stool Mini Kit (Qiagen, Venlo, Netherlands). The bacterial 16S rRNA gene, hypervariable region V4, was amplified by PCR using

universal bacterial primers specific for use in Illumina MiSeq.[23] The reverse primer was barcoded so that each sample could be uniquely identified post-sequencing. Reactions were performed in triplicate and pooled with a negative control included in each run. Pooled PCR amplicons were subjected to paired-end sequencing by Illumina MiSeq. Using a QIIME (v 1.6.0, Quantitative Insights Into Microbial Ecology, qiime.org) pipeline, forward and reverse reads were assembled, demultiplexed and filtered against the Greengenes bacterial reference database (v 12.10, http://greengenes.secondgenome.com) to remove all sequences with less than 60% similarity. Taxonomic assignment of remaining sequences was achieved using the RDP classifier constrained by Greengenes. Operational taxonomic units (OTUs) with overall relative abundance below 0.0001 were excluded from subsequent analyses. After cleaning and processing, a total of 110 million reads were retained (median 3.1×10^5 per sample, range 8.1×10^4–1.0×10^6), representing 1127 unique OTUs. For subsequent analyses, data were rarefied to 80 000 sequences per sample.

Statistical analysis

Associations of maternal IAP with breastfeeding or other antibiotic exposures were evaluated by chi-square test. Using default settings in QIIME, microbiota OTU relative abundance was summarised at the phylum, order, family and genus levels of taxonomy. Microbiota diversity within samples (alpha diversity) was calculated using two standard metrics: the Chao1 estimator of species richness (which estimates the number of different OTUs present) and the Shannon diversity index (which evaluates both the number of OTUs and the evenness of their distribution).

Overall microbiota community differences between samples (beta diversity) were tested in QIIME by permutational multivariate analysis of variance (adonis) of unweighted UniFrac distance matrices, with 1000 permutations. Mean richness and diversity were compared across IAP and breastfeeding groups by linear regression, with a test for linear trend for breastfeeding exclusivity or duration. Median relative abundances of dominant bacterial taxa were compared using the Kruskal–Wallis test (non-parametric ANOVA) and Spearman rank correlation, with a false discovery rate (FDR) correction for comparisons of multiple genera. We had sufficient sample size to test previously reported microbial changes at the genus level. Assuming 80% power and a two-sided alpha of 5%, 18 infants would be needed per CS type to detect a difference of 31.69 (standard deviation 32.91 in our pilot data) in relative abundance of *Bacteroides* between CS and vaginally delivered infants.

A heat map was constructed to visualise relative differences (ratios of median values) for genus-level comparisons. Stratified analyses by breastfeeding status were conducted to evaluate the potential modifying effect of breastfeeding; the most significant IAP-microbiota associations were tested separately for breastfed and non-breastfed infants, using the Kruskal–Wallis test and Dunn's post-test for multiple comparisons. Sensitivity analyses were pursued to: (1) exclude infants directly receiving antibiotics during the perinatal period and (2) minimise heterogeneity among the 'not breastfed at 3 months' group by excluding infants for whom breastfeeding was never initiated.

Results

Study population and IAP exposures

Faecal samples were collected from 198 healthy full-term infants at the ages of 3 months ($n = 176$, mean age 3.1 ± 0.6 months) and 1 year ($n = 189$, mean age 11.8 ± 0.8 months) (Table S2). Maternal IAP exposure and method of birth were classified in four groups: no IAP with vaginal delivery ($n = 113$; 57%), IAP with vaginal delivery ($n = 42$; 21%), IAP with elective CS delivery ($n = 18$; 9%), and IAP with emergency CS delivery ($n = 25$; 13%). All CS deliveries received antibiotic prophylaxis in accordance with Canadian practice guidelines; no elective CS but most emergency CS (20/25) occurred after the initiation of labour (Supporting Information Table S3). Among vaginal deliveries, IAP was typically administered for GBS prophylaxis (76%) or pre-labour rupture of membranes (PROM, 24%). As previously reported, cefazolin and penicillin were commonly administered for CS and GBS prophylaxis, respectively.[2] Perinatal antibiotics were directly administered to 8 (4%) infants for suspected sepsis within the first 48 hours after birth, and 69 (37%) of infants received postnatal antibiotics before the 1-year stool collection. At 3 months of age, 52% of infants were exclusively breastfed, 30% were partially breastfed (supplemented with formula), and 18% were not breastfed. At 1 year of age, 49% of infants were still receiving breast milk.

The median number and interquartile range for IAP courses were highest for emergency CS and IAP in vaginal delivery (Table S3); 12% of women who underwent emergency CS additionally received IAP for GBS. Maternal postpartum antibiotics (amoxicillin, cephalexin, azithromycin, cloxacillin) and newborn treatment with intravenous ampicillin and gentamicin were more common following emergency CS ($P < 0.10$ and $P < 0.001$, respectively), whereas postnatal infant antibiotic use (after the perinatal period) was similar across all four IAP exposure groups (Table S3). Breastfeeding rates were also similar across the four IAP groups.

Gut microbiota: overall community structure

Infant gut microbiota community structure was influenced by maternal IAP exposure and method of birth, as well as breastfeeding (Table S4). Community differences were

observed following all maternal IAP exposures, with a moderate effect size for IAP with vaginal delivery ($F = 1.45$, $P < 0.05$) and larger effect sizes for IAP with CS delivery ($F = 2.89$, $P = 0.001$ for IAP with emergency CS and $F = 2.77$, $P = 0.001$ for IAP with elective CS). Significant community differences persisted to 1 year of age for IAP with emergency CS ($F = 1.42$, $P < 0.05$). At both 3 months and 1 year, microbiota communities differed significantly according to current breastfeeding status ($F = 3.46$, $P = 0.001$ at 3 months; $F = 2.20$, $P = 0.002$ at 1 year) (Table S4).

Gut microbiota: diversity and richness

Maternal IAP with vaginal delivery was associated with decreased infant gut microbiota richness at 3 months ($P = 0.005$), while IAP with emergency CS was associated with increased microbiota diversity at 1 year ($P < 0.001$) (Table 1). Breastfeeding exclusivity was inversely associated with both microbiota richness ($P = 0.003$) and diversity ($P < 0.001$) at 3 months. In contrast, at 1 year, microbiota diversity was *positively* associated with breastfeeding exclusivity ($P = 0.003$) and duration ($P = 0.02$), and microbiota richness was no longer associated with breastfeeding.

Gut microbiota: taxonomic composition

Average phylum-level profiles by maternal IAP exposure, method of birth and breastfeeding are shown in Figure 1A, B. At 3 months of age, infants exposed to maternal IAP were deficient in Bacteroidetes (Table 2). The median relative abundance of this phylum was 46% among unexposed infants, compared with 24% following IAP with vaginal delivery ($P < 0.05$), and <1% following IAP with elective or emergency CS delivery ($P < 0.001$). IAP with CS delivery was also associated with elevated proportions of Firmicutes ($P < 0.01$), and Proteobacteria ($P < 0.05$ for IAP with emergency CS). These differences persisted to 1 year of age among infants exposed to IAP with emergency CS, with Bacteroidetes remaining lower ($P < 0.001$) and Firmicutes ($P < 0.001$) and Proteobacteria ($P < 0.05$) remaining higher in this group compared to vaginally delivered infants without IAP. In contrast, there were no persistent microbiota differences at 1 year among infants exposed to IAP with elective CS or vaginal delivery. Similar results were obtained in a sensitivity analysis excluding the eight infants receiving direct perinatal antibiotics (Table S5).

Maternal IAP exposure also influenced infant gut microbiota composition at the genus level, especially at

Table 1. Infant gut microbiota richness and diversity at 3 and 12 months according to maternal IAP, method of birth and breastfeeding

	Microbiota at 3 months (n = 176)					Microbiota at 1 year (n = 189)				
	n	Chao1 richness estimator	*P*	Shannon diversity index	*P*	*n*	Chao1 richness estimator	*P*	Shannon diversity index	*P*
IAP and method of birth										
No IAP, Vaginal	96	52.8 (7.1)		2.07 (0.66)		108	66.4 (5.8)		2.72 (0.50)	
IAP, Vaginal	40	49.1 (5.6)	**0.005**	2.00 (0.64)	0.56	41	66.2 (5.6)	0.86	2.66 (0.59)	0.50
IAP, Elective Caesarean	17	55.5 (7.7)	0.13	2.26 (0.57)	0.26	16	66.8 (8.8)	0.80	2.52 (0.62)	0.14
IAP, Emergency Caesarean	23	50.1 (7.3)	0.09	2.16 (0.68)	0.52	24	66.8 (6.9)	0.78	3.16 (0.44)	**<0.001**
Breastfeeding at 3 months										
None	35	55.0 (6.3)	**0.003**	2.63 (0.57)	**<0.001**	33	67.2 (6.3)	0.62	2.60 (0.68)	**0.02**
Partial	53	51.7 (6.8)		2.06 (0.61)		57	65.0 (6.2)		2.67 (0.52)	
Exclusive	88	50.7 (7.3)		1.88 (0.57)		99	67.0 (6.0)		2.84 (0.49)	
Breastfeeding at 6 months										
None						49	66.6 (7.7)	0.93	2.56 (0.65)	**0.003**
Partial						124	66.3 (5.7)		2.80 (0.49)	
Exclusive						12	66.8 (3.4)		2.96 (0.47)	
Breastfeeding duration										
Never						10	67.6 (6.9)	0.87	2.36 (0.62)	**0.02**
<3 months						23	67.0 (6.2)		2.70 (0.70)	
3 to <6 months						16	65.3 (10.2)		2.48 (0.57)	
6 to <12 months						44	65.7 (5.4)		2.86 (0.53)	
≥12 months						96	66.7 (5.5)		2.79 (0.47)	

IAP, intrapartum antibiotic prophylaxis.
Values are presented as mean (SD). Comparisons by univariate linear regression (*P* for IAP and method of birth compares each type of IAP exposure with 'no IAP, vaginal' as the reference group; *P* for breastfeeding variables reflects test for linear trend). Biodiversity metrics calculated at genus level of taxonomy. Significant *P* values are shown in bold.

3 months of age (Figure 1E). The top 10 differentially abundant genera for each IAP exposure group (compared to unexposed infants) are listed in Table 3. Consistent with results at the phylum level, genus *Bacteroides* and *Parabacteroides* were under-represented at 3 months in all IAP categories, with the most severe deficiencies observed in infants delivered by CS. Genus *Enterococcus* was more abundant at 3 months following any IAP exposure. Another member of the Firmicutes phylum, genus *Clostridium*, was more abundant with IAP-associated vaginal birth and emergency CS but not elective CS. Overall, Proteobacteria tended to be elevated following maternal IAP exposure (Table 2) with one exception at the genus level, where relative abundance of *Sutterella* was lower with IAP exposure (Table 3, Figure 1E). All of these taxonomic differences were especially evident at 3 months following emergency CS; the lower relative abundance of *Bacteroides* persisted in this group to 1 year of age.

Gut microbiota composition also differed by breastfeeding exclusivity and duration (Table S6). At both 3 months and 1 year, Proteobacteria and Actinobacteria were enriched with breastfeeding ($P < 0.001$). At 3 months, breastfeeding exclusivity was inversely associated with the proportions of Bacteroidetes and Clostridiales, including the Veillonellaceae, Lachnospiraceae and Ruminococcaceae families (all $P < 0.001$); however, at 1 year, early breastfeeding was associated with *higher* proportions of Clostridiales ($P < 0.05$), especially Veillonellaceae ($P < 0.001$).

Modification of IAP effects by breastfeeding

At 3 months, IAP-exposed infants were deficient in Bacteroidetes, regardless of breastfeeding status (Figure 1A). However, by 1 year, this deficiency persisted mainly among IAP-exposed infants delivered by emergency CS who were not exclusively breastfed at 3 months (Figure 1B). Similarly, Firmicutes were enriched in the microbiota of IAP-exposed infants, and this difference persisted to 1 year among infants delivered by emergency CS who were not exclusively breastfed at 3 months. Within each of these phyla, effect modification by breastfeeding status was observed at a lower taxonomic level (Figure 1C,D). In the absence of exclusive breastfeeding at 3 months, Bacteroidaceae were significantly less and Clostridiales more abundant in 1-year-old infants exposed to maternal IAP during emergency CS than infants with no IAP exposure ($P < 0.01$ for both). These IAP-associated changes were not detected among breastfed infants. The lower Bacteroidaceae and higher Clostridiales abundances were also statistically different relative to exclusively breastfed infants who were vaginally delivered and not exposed to IAP ($P < 0.01$). Effect modification was not observed at 3 months, with one exception: genus *Clostridium* was significantly more abundant in IAP-exposed infants who were exclusively breastfed at 3 months ($P < 0.05$) but levels were not associated with IAP in partially breastfed infants (Figure S1). These differences did not persist to age 1. Effect modification results were unchanged in a sensitivity analysis excluding infants for whom breastfeeding was never initiated (data not shown).

Discussion

Main findings

In this general population birth cohort of 198 full-term infants, we observed maternal IAP-associated changes in infant gut microbiota. At 3 months of age, significant IAP-associated alterations in overall microbiota community structure were detected, as well as a reduction in microbiota richness and a depletion of Bacteroidetes. Greater relative abundance of Firmicutes (genera *Clostridium* and *Enterococcus*) was also observed. Fewer changes were detected at 1 year of age, although some IAP effects persisted among infants who were not exclusively breastfed for at least 3 months.

Strengths and limitations

Our study has several strengths, including the use of high-throughput genetic sequencing to profile infant gut microbiota in a longitudinal, population-representative pregnancy cohort. Observed changes from 3 to 12 months, such as decreasing abundance of Enterobacteriaceae and increasing predominance of Bacteroidaceae, are consistent with observations in other populations.[24,25] Unique to our study was the capture of maternal postpartum antibiotic use and separate reporting of emergency versus elective caesarean section, both under-reported exposures in the literature. Our study also has limitations. Biases inherent to DNA extraction and sequencing methodology may have led to under-detection of organisms such as bifidobacteria. Although IAP status did not vary according to breastfeeding status and introduction of solids, we did not adjust the 1-year microbiota findings for infant diet after weaning from breastfeeding. A larger sample is required for the simultaneous adjustment of confounding factors, and access to meconium samples would enable assessment of microbiota changes closer to the time of antibiotic administration.

Interpretation

In our cohort, IAP was associated with reduced microbiota richness, depletion of Bacteroidetes, and enrichment of Firmicutes at 3 months. Others have reported similar changes within 8 weeks of newborn intravenous beta-lactam combination treatment[17,26] or administration of perinatal maternal antibiotics.[27] In general, we detected fewer IAP-associated microbiota changes at 1 year of age, similar to the Fallani et al.[28] study of post-weaning gut microbiota. However, even with this apparent 'microbiota recovery'

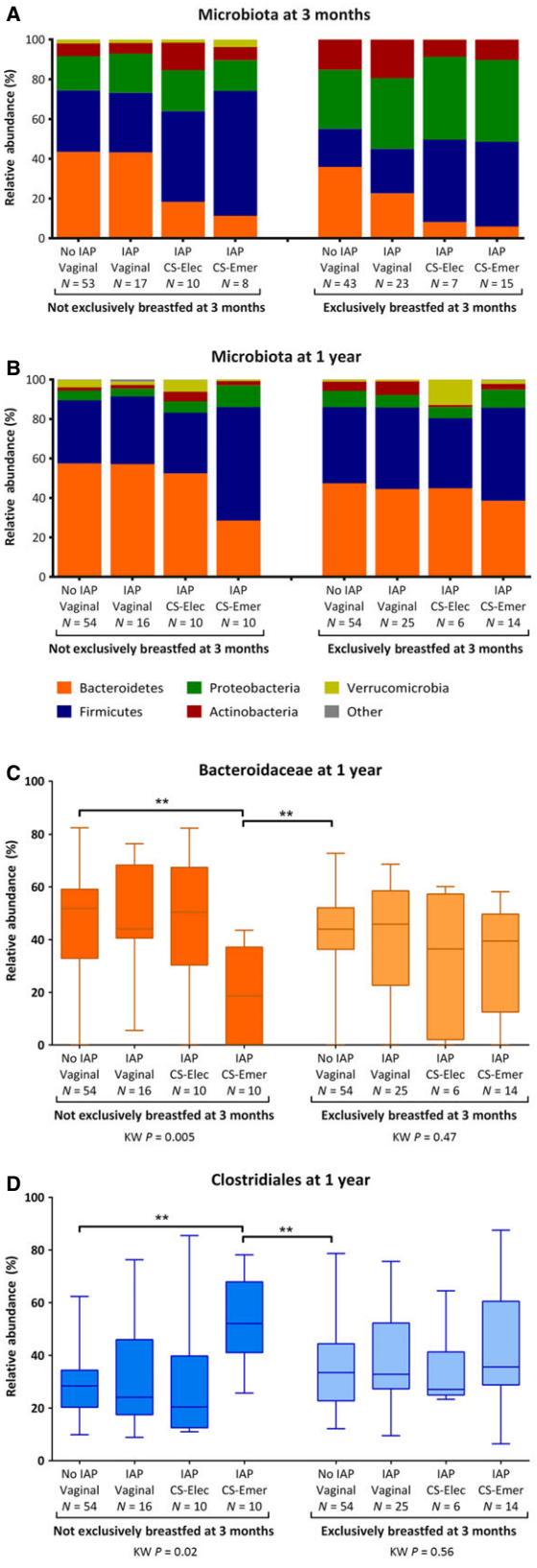

Figure 1. Infant gut microbiota composition at 3 and 12 months by IAP exposure, method of birth and breastfeeding. (A, B) Mean phylum-level composition. (C, D) Modification of IAP effects by breastfeeding for Bacteroidaceae and Clostridiales at 1 year: relative abundance was compared across IAP exposure groups using the Kruskal–Wallis test (non-parametric ANOVA), with Dunn's post-test for multiple comparisons; $*P < 0.05$, $**P < 0.01$. Boxes indicate interquartile range, lines indicate medians, and whiskers indicate range. (E) Difference in median relative abundance of dominant genera according to IAP group (versus no IAP, Vaginal): comparisons using Kruskal–Wallis test with FDR correction for multiple testing. '–' indicates not estimable due to median of zero in reference group. In all, 54 genera with overall median relative abundance >0 are included. CS, caesarean section; Elec, elective; Emer, emergency; FDR, false discovery rate; IAP, intrapartum antibiotic prophylaxis; KW, Kruskal–Wallis; uncl, unclassified. 'Breastfed' refers to any breast milk. Mean relative abundances of dominant phyla are shown.

Table 2. Median relative abundance of dominant† taxa in faecal microbiota of infants at 3 months and 1 year, according to maternal IAP

Dominant taxa	Microbiota at 3 months (*n* = 176)				Microbiota at 1 year (*n* = 189)			
	no IAP Vaginal (82% BF) *n* = 96	IAP Vaginal (83% BF) *n* = 40	IAP Elective CS (78% BF) *n* = 17	IAP Emergency CS (80% BF) *n* = 23	no IAP Vaginal (50% BF) *n* = 108	IAP Vaginal (60% BF) *n* = 41	IAP Elective CS (39% BF) *n* = 16	IAP Emergency CS (38% BF) *n* = 24
Bacteroidetes	46.2	24.3*	0.4***	0.2***	55.0	54.2	52.1	38.1***
Bacteroidaceae	34.4	13.0*	0.3**	0.2***	46.9	44.8	45.6	33.1***
Firmicutes	20.1	16.8	42.6**	52.1***	32.5	36.0	27.6	48.0***
Clostridiales	12.8	12.7	27.6*	49.3***	30.2	32.8	25.7	43.4***
Clostridiaceae	0.08	0.96*	0.79	1.55**	0.10	0.24*	0.30	0.20*
Veillonellaceae	3.6	3.4	2.3	24.1***	5.0	4.4	2.3	3.6
Lachnospiraceae	1.8	0.9	5.9	0.2	13.1	13.5	13.0	22.4*
Ruminococcaceae	0.1	0.1	0.9~	0.1	6.3	8.9	8.1	10.8*
Proteobacteria	15.5	21.9~	25.9	30.0*	4.6	4.2	4.6	7.5*
Enterobacteriaceae	13.0	20.2~	25.9	29.9*	1.0	1.0	0.7	1.7
Actinobacteria	5.4	4.8	8.0	4.0	1.6	2.4	1.3	1.4
Bifidobacteriaceae	5.3	4.7	7.8	3.9	1.6	2.4	1.3	1.4
Verrucomicrobia	0.01	0.00	0.00	0.00	0.01	0.01	0.10~	0.03

BF, any breastfeeding at sample collection (3 months or 1 year); CS, caesarean section; IAP, intrapartum antibiotic prophylaxis.
†Dominant taxa have overall median relative abundance >1% at 3 months and/or 1 year.
Comparisons by non-parametric Kruskal–Wallis test (versus no IAP, Vaginal). $***P < 0.001$, $**P < 0.01$, $*P < 0.05$, $~P < 0.10$.
Bacteroidetes Firmicutes Proteobacteria Actinobacteria Verrucomicrobia Other.
Colours distinguish bacterial phyla and correspond to those used in Figure 1 and Table 3.

after IAP exposure, long-term health effects are possible. This was recently demonstrated by Cox et al.[29] in a rodent model, where transient antibiotic-induced perturbation of the neonatal gut microbiota caused permanent aberrations in metabolism and immunity.

Notably, the maternal IAP associations in our study were independent of a 'caesarean section effect', since reduced microbiota richness and altered composition were also seen following IAP with vaginal delivery. These findings are consistent with a recent report that fewer bifidobacterial species are detected in the infant gut following IAP during vaginal delivery.[19] Analogous to the 'dose effect' for neonatal antibiotics reported by Fouhy et al.,[17] we found stronger and more frequent taxon-specific associations with emergency CS delivery, which often resulted in higher cumulative exposure from multiple courses of IAP plus newborn antibiotic treatment. Further, alterations in taxon relative abundance were consistent with expectations based on antibiotic spectrum and emergence of resistant organisms after surgical prophylaxis.[10,30–32] For example, genus *Bacteroides*, which is sensitive to beta-lactams,[33] was substantially depleted after any maternal IAP exposure. Genus *Clostridium* and *Enterococcus*, which have intrinsic or acquired resistance to cefazolin and ampicillin[30,31] were significantly elevated 3 months after emergency CS. In addition, streptococci were more abundant after CS delivery; they are becoming more resistant to penicillin therapy and are increasingly detected in late-onset neonatal infections.[10,32]

We observed microbial community changes (including lower abundance of Bacteroidaceae) similar to those seen at 1–3 months following elective CS with no IAP before delivery.[25,34] Yet compared with IAP during vaginal delivery, where changes were limited to the Bacteroidaceae and Enterobacteriaceae families, and genus *Clostridium*, gut microbiota dysbiosis was extensive in CS with IAP, occurring at the phylum level (Bacteroidetes, Firmicutes

Table 3. Top 10 differentially abundant genera for each IAP group: median % relative abundance (% prevalence)

	Microbiota at 3 months						Microbiota at 1 year					
IAP, Vaginal (versus no IAP)	**No IAP *n* = 96**		**IAP, Vaginal *n* = 40**		***P***	**FDR *P***		**No IAP *n* = 108**		**IAP, Vaginal *n* = 41**		***P***	**FDR *P***
Clostridium	0.023	(100)	0.392	(100)	0.007	0.18	*Clostridiaceae* uncl.	0.023	(98)	0.073	(100)	0.02	0.36
Parabacteroides	0.028	(100)	0.013	(100)	0.009	0.18	*Veillonella*	1.009	(100)	3.614	(100)	0.02	0.36
Dorea	0.002	(90)	0.001	(85)	0.01	0.18	*Collinsella*	0.005	(97)	0.002	(93)	0.02	0.36
Rothia	0.010	(96)	0.026	(95)	0.02	0.19	*Acinetobacter*	0.001	(67)	0.000	(46)	0.03	0.39
Enterococcus	0.011	(98)	0.031	(100)	0.02	0.19	*Clostridium*	0.018	(94)	0.063	(98)	0.05	0.51
Sutterella	0.002	(89)	0.001	(80)	0.02	0.19	*Rothia*	0.001	(72)	0.002	(76)	0.06	0.52
Ruminococcus	0.303	(100)	0.024	(100)	0.02	0.19	*Lachnospiraceae* uncl.	0.850	(100)	0.718	(100)	0.08	0.53
Collinsella	0.002	(90)	0.001	(95)	0.03	0.19	*Ruminococcaceae* uncl.	0.164	(99)	0.029	(98)	0.08	0.53
Bacteroides	34.402	(100)	12.958	(100)	0.04	0.25	*Oscillospira*	1.468	(100)	0.876	(100)	0.09	0.53
Prevotella	0.005	(85)	0.002	(78)	0.05	0.25	*Atopobium*	0.000	(49)	0.000	(59)	0.11	0.55
IAP, Elective CS (versus no IAP)	**No IAP *n* = 96**		**IAP, Elective CS *n* = 17**		***P***	**FDR *P***		**No IAP *n* = 108**		**IAP, Elective CS *n* = 16**		***P***	**FDR *P***
Parabacteroides	0.028	(100)	0.012	(94)	<0.001	0.01*	*Fusobacterium*	0.002	(79)	0.012	(94)	0.003	0.18
Peptostreptococcaceae uncl.	0.003	(28)	0.253	(47)	0.001	0.03*	*Phascolarctobacterium*	0.002	(90)	0.001	(75)	0.02	0.44
Bacteroides	34.402	(100)	0.295	(100)	0.001	0.03*	*Clostridium*	0.018	(94)	0.129	(400)	0.03	0.44
Streptococcaceae uncl.	0.001	(79)	0.003	(94)	0.007	0.09~	*Megamonas*	0.002	(75)	0.000	(63)	0.03	0.44
Faecalibacterium	0.000	(66)	0.002	(82)	0.01	0.11	*Megasphaera*	0.007	(97)	0.003	(100)	0.06	0.60
Clostridiales uncl.	0.000	(52)	0.003	(71)	0.01	0.11	*Akkermansia*	0.010	(99)	0.098	(100)	0.08	0.60
Gemellaceae uncl.	0.003	(90)	0.009	(94)	0.02	0.14	*Parabacteroides*	0.291	(100)	0.010	(100)	0.09	0.60
Enterococcus	0.011	(98)	0.051	(100)	0.02	0.14	*Lactobacillales* uncl.	0.007	(98)	0.012	(94)	0.09	0.60
Sutterella	0.002	(89)	0.001	(71)	0.02	0.14	*Clostridia* uncl.	0.023	(97)	0.012	(100)	0.14	0.86
Megamonas	0.000	(52)	0.000	(24)	0.03	0.14	*Campylobacter*	0.001	(59)	0.000	(44)	0.16	0.88
IAP, Emergency CS (versus no IAP)	**No IAP *n* = 96**		**IAP, Emergency CS *n* = 23**		***P***	**FDR *P***		**No IAP *n* = 108**		**IAP, Emergency CS *n* = 24**		***P***	**FDR *P***
Bacteroides	34.402	(100)	0.146	(100)	<0.001	<0.001***	*Bacteroides*	46.888	(100)	33.142	(100)	<0.001	0.02*
Enterococcus	0.011	(97)	0.059	(100)	<0.001	<0.001***	*Prevotella*	0.017	(97)	0.029	(100)	0.009	0.24
Veillonella	2.437	(100)	24.120	(100)	<0.001	<0.001***	*Ruminococcus*	1.347	(100)	2.423	(100)	0.02	0.40
Clostridium	0.023	(100)	0.244	(100)	0.003	0.04*	*Clostridiaceae* uncl.	0.023	(98)	0.099	(96)	0.04	0.40
Atopobium	0.001	(67)	0.024	(91)	0.003	0.04*	*Lactobacillales* uncl.	0.007	(98)	0.012	(100)	0.04	0.40
Parabacteroides	0.028	(100)	0.012	(100)	0.007	0.06~	*Ruminococcaceae* uncl.	0.164	(99)	0.362	(100)	0.05	0.40
Streptococcus	0.579	(100)	1.506	(100)	0.02	0.13	*Pasteurellaceae* uncl.	0.005	(85)	0.013	(92)	0.06	0.40
Clostridiales uncl.	0.000	(52)	0.001	(74)	0.02	0.14	*Blautia*	1.025	(100)	1.986	(100)	0.06	0.40
Collinsella	0.002	(90)	0.001	(83)	0.03	0.17	*Streptococcus*	0.207	(100)	0.428	(100)	0.07	0.40
Streptococcaceae uncl.	0.001	(79)	0.002	(87)	0.04	0.23	*Lachnospiraceae* uncl.	3.072	(100)	4.434	(100)	0.08	0.41

CS, caesarean section; IAP, intrapartum antibiotic prophylaxis; uncl, unclassified.
54 genera with overall median relative abundance >0 were included in this analysis.
Comparison of median relative abundance by nonparametric Kruskal–Wallis test with false discovery rate (FDR) correction for multiple testing:
***FDR $P < 0.001$, **<0.01, *<0.05, ~<0.10. Bacteroidetes Firmicutes Proteobacteria Actinobacteria Verrucomicrobia Other.

and Proteobacteria) and affecting several genera (such as *Clostridium* and *Enterococcus*). Further, the effect size for beta diversity, which quantifies whole microbiota community differences, was greater with IAP and CS than with IAP alone. Maternal postpartum antibiotic treatment and transfer of antibiotics via breast milk may also have contributed to greater perturbations of infant gut microbiota in the CS group. Indeed, lower breast milk levels of bifidobacteria and lactobacilli, and increased colonisation of the infant gut by *C. difficile* and *Enterococcus* have been reported during maternal postnatal antibiotic treatment.[18,35,36] In our study population, 43% of mothers delivering by emergency CS received antibiotics during the first 3 months postpartum and their infants had a higher abundance of *Clostridium* species if they were exclusively breastfed. *Clostridium* was also elevated in exclusively breastfed infants following vaginal delivery with IAP.

Additional discussion on emergency CS is warranted due to the multiplicity of exposures with this intervention, including relatively frequent newborn treatment with intravenous ampicillin, which is eliminated into the gut after secretion into bile.[37] In contrast to the other two IAP groups, these infants had greater microbiota abundance of

Enterobacteriaceae, *Streptococcus* and *Veillonell*a at 3 months. In a new publication from the ALADDIN birth cohort, Enterobacteriaceae and *Veillonell*a were more abundant in gut microbiota of acute and elective CS-delivered infants at 2 months of age.[38] Fouhy et al. also found elevated abundance of Enterobacteriaceae and *Streptococcus* in the infant gut at 2 months following intravenous antibiotic treatment.[17] Of interest, streptococci are more common in breast milk microbiota after emergency (but not elective) CS.[39] Finally, microbiota diversity was significantly elevated following emergency CS. As reported by Pender's et al.,[40] this higher diversity could be a function of prolonged hospitalisation of these infants.

Early breastfeeding can influence microbiota composition later in infancy, even post weaning.[28] As others have reported,[38,41] the gut microbiota of breastfed infants at 3 months in our study was characterised by reduced species richness and diversity, enrichment of bifidobacteria, and lower abundance of Bacteroidetes and Clostridiales compared with non-breastfed infants. Similar to infants in other European countries,[28,38,42] the post-weaning microbiota of these early breastfed infants continued to have increased levels of bifidobacteria, and reduced levels of Bacteroidetes. Interestingly, early breastfeeding predicted *higher* microbiota diversity at 1 year of age; this association was dose-dependent according to the duration and exclusivity of breastfeeding, and was independent of birth mode.

Finally, our findings provide new evidence that breastfeeding can modify IAP-induced microbiota changes. Among infants who were not exclusively breastfed for at least 3 months, IAP for emergency CS was associated with a persistent reduction in Bacteroidaceae and elevation in Clostridiales abundance at 1 year; however, no such differences were detected among breastfed infants. Savino et al.[21] reported 'recovery' of gut microbiota among 5-month-old breastfed infants treated with intravenous antibiotics, but antibiotic exposure in our study occurred before the initiation of breastfeeding. Thus, our results demonstrate the benefit of continued breastfeeding after emergency CS in promoting a post-weaning gut microbiota profile comparable to vaginally born infants without IAP exposure. Variation in breastfeeding modification effects across IAP groups could be related to differences in milk microbiota composition, which have been reported to differ by mode of delivery.[39]

Conclusion

Intrapartum antibiotic prophylaxis is increasingly common, owing to rising CS delivery rates and the growing prevalence of risk factors such as maternal overweight during pregnancy.[2,43] Our results highlight infant gut microbiota dysbiosis as a previously under-recognised consequence of IAP. Further research is warranted to replicate these findings in other populations and determine the impact on infant health. It is also important to develop improved methods for optimising IAP,[44] and to further explore how breastfeeding can minimise the adverse effects of IAP when it cannot be avoided.

Disclosure of interests

Full disclosure of interests available to view online as supporting information.

Contribution to authorship

The CHILD Study Director (MRS) and site leaders (PJK, SET, PS and ABB) conceived the cohort design, managed study recruitment and oversaw clinical assessments of study participants. JAS and ALK conceived the Synergy in Microbiota (SyMBIOTA) research program to study infant gut microbiota within the CHILD cohort. TK performed DNA extractions and PCR amplifications; DSG oversaw 16S rRNA sequencing; JAS performed initial bioinformatics. RRP reviewed hospital charts to extract intrapartum and perinatal antibiotic exposure data. MBA conceived and conducted analyses of microbiota profile data. RSC and CJF provided expertise in obstetrics and infant nutrition, respectively, and interpreted results. MBA and ALK drafted the manuscript; all co-authors provided feedback and approved the final version.

Details of ethics approval

This study was approved by the University of Manitoba Human Research Ethics Board (10 December 2008; H2007:255) and the Health Information Privacy Committee (18 November 2011; 2009/2010-55).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Modification of IAP effects by breastfeeding (*Clostridium*). Relative abundance of genus *Clostridium* at 3 months was compared across IAP exposure groups using the Kruskal–Wallis test (non-parametric ANOVA), with Dunn's post-test for multiple comparisons. Boxes indicate interquartile range, lines indicate medians, and whiskers indicate range.

Table S1. Population characteristics according to selection for the current analysis.

Table S2. Characteristics of 198 study subjects: demographics, maternal IAP exposures, method of birth and breastfeeding.

Table S3. IAP courses and indications, other antibiotic exposures, labour characteristics, and breastfeeding by IAP group.

Table S4. Comparison of gut microbiota community structures (beta diversity) at 3 and 12 months, according to maternal IAP, method of birth, and breastfeeding.

Table S5. Sensitivity analysis: median relative abundance of dominant taxa in faecal microbiota of infants at 3 months and 1 year, according to IAP group (excluding eight infants who received direct perinatal antibiotics).

Table S6. Median relative abundance of dominant taxa in faecal microbiota of infants at 3 months and 1 year, according to breastfeeding. ■

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