Impact of Early Exposure to Cefuroxime on the Composition of the Gut Microbiota in Infants Following Cesarean Delivery

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Objectives To assess in mothers giving birth by cesarean delivery if prophylactic antibiotics administered either before skin incision or immediately after cutting the umbilical cord influences gut microbiota colonization and antibiotic susceptibility of the gut bacteria in the newborn.

Study design Forty-two pregnant women scheduled for elective cesarean delivery were recruited at Odense University Hospital, Denmark, and randomly assigned to receive cefuroxime either before skin incision or immediately after the umbilical cord was cut. Fecal samples were collected from all infants at age 10 days and 9 months. Composition of the gut microbiota was determined by 16S ribosomal RNA gene amplicon high-throughput sequencing. Gram-positive cocci and Enterobacteriaceae were isolated and identified before antimicrobial susceptibility tests were performed by disk diffusion.

Results No clear difference in the composition of the gut microbiota was observed between infants whose mothers received cefuroxime before or after cesarean delivery at neither time point, though surprisingly at 9 months of age, but not at 10 days of age, the number of observed species was higher in infants where mothers received cefuroxime after cord clamping. No differences in antimicrobial susceptibility of Enterobacteriaceae, *Enterococcus* spp, and *Staphylococcus* spp were seen at 10 days.

Conclusions Timing of cefuroxime administration to mothers undergoing cesarean delivery does not have a major effect on the gut microbiota and bacterial antibiotic resistance traits in infants. *(J Pediatr 2019;210:99-105)*.

Trial registration Clinicaltrials.gov: NCT02072798.

Birth by cesarean delivery is common, with cesarean delivery rates exceeding 50% in some countries.[1] In Denmark, approximately 20% of all newborns are born by cesarean delivery.[2] Women undergoing cesarean delivery compared with vaginal delivery demonstrate an increased risk of postpartum infections with endometritis, urinary tract infection, and wound infection being the most common.[3] Prophylactic administration of antibiotics to the mother lowers the risk of surgical site infection. It is, thus, standard cesarean delivery procedure to administer to the mother a single prophylactic dose of cefuroxime (1500 mg) or other broad spectrum antibiotics either shortly before skin incision or immediately after umbilical cord clamping.[4] Although several studies have shown that administering the antibiotics to the mother 30-60 minutes before skin incision is optimal for lowering surgical site infection risk,[5-9] this also exposes the fetus to the antibiotic via placental transfer.[10] To avoid unintended fetal exposure to antibiotics during cesarean delivery, antibiotics at many hospitals are administered to the mother immediately after the cord is clamped.[11]

At birth, the neonatal gastrointestinal tract is rapidly colonized by microorganisms.[12] Initially, the microbial community composition is relatively simple.[13] During the first year of life, the gut microbiota, influenced by environmental factors (breast vs formula feeding; introduction of weaning food; exposure to antibiotics etc), gradually develops into a more adult-like, complex microbiota.[14-17] Early life gut colonization plays pivotal roles in development of intestinal functions and postnatal immune maturation, which might influence long-term health and risk of development of disorders (eg, autoimmune diseases).[18-21]

High exposure to antibiotics during early life has been shown to influence long-term structure of the gut microbiota and might be a risk factor for the development of certain diseases, such as asthma and obesity.[21-26] Furthermore, because early life antibiotic exposure increases the occurrence of antibiotic resistant gut bacteria, especially *Enterobacteriaceae,* which can have serious health implications,[27] it is highly recommended to limit exposure to antibiotics in infants as much as possible. However, short- and long-term effect on the developing gut microbiome of very early and brief (ie, at or around birth) exposure to antibiotics is unclear.

Cefuroxime administered intravenously to the mother shortly before cesarean delivery is cleared from infants' blood plasma approximately 3 times

OTU	Operational taxonomic unit
PERMANOVA	Permutational multivariate ANOVA
rRNA	Ribosomal RNA

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more slowly than in adults, but nevertheless, a single maternal dose of 1500 mg cefuroxime is cleared from the infant within 24 hours.[10] In the present study, we aim to investigate whether maternal exposure to antibiotics shortly before or immediately after parturition influences the gut microbiota of the infant short and moderately long term, and whether use of antibiotics in this window of time increases the prevalence of antibiotic resistant gut bacteria.

Methods

This study was performed at the Department of Gynecology and Obstetrics, Odense University Hospital, Denmark from February 2014 to July 2014 and is registered on ClinicalTrials.gov as "Antibiotics and Microbiota among newborn Infants," NCT02072798. Inclusion criteria were planned cesarean delivery, age above 18 years, a pregestational body mass index <30. Forty-two women were randomly assigned to receive a single dose of intravenous cefuroxime (1500 mg), either administered 15-60 minutes prior to surgical incision (pre, n = 22) or immediately after umbilical cord clamping (post, n = 20). Details on cesarean delivery procedure, recruitment procedure, and medical ethics have been reported elsewhere.[10] A restricted block randomization sequence was created with a 1:1 allocation using a fixed block size of 4. The block size was unknown to the investigators, study nurse, and participants during the trial. No stratification was used. A data manager with no clinical involvement in the trial prepared the randomization sequence using computer-generated random numbers. Infants whose mother received cefuroxime before skin excision (pre) had blood sampled to determine cefuroxime clearance from the blood.[10] It was deemed unethical to also sample blood from infants not exposed to the antibiotic (post), and the study consequently not blinded. Study infants were healthy, did not receive any antibiotics postnatally, and were discharged between 2 and 5 days after birth. Infant health conditions after hospital discharge were monitored by a trained nurse and early feeding practices (exclusive breast feeding, mix breast feeding and infant formula, or exclusive infant formula) were recorded.

During the first 10 days of life, 15 infants were exclusively breast fed, 4 were exclusively fed formula milk, and 25 infants had received both formula and breast feeding. No diet information was recorded at age of 9 months. Fecal samples were collected from the infants during the nurse/midwife's visit 10 days after birth and 9 months after birth. Fecal samples were obtained from 44 infants at day 10 and from 42 infants at 9 months (1 set of twins withdrew from the study). The fecal samples were transferred into sterile tubes and stored at −60°C or colder until further analyses.

Ethics

The trial was approved by the Danish Health and Medicines Authority (EudraCT 2012-002068-29), the Committee on Biomedical Research Ethics (S-20130117), Danish Data Protection Agency (2008-58-0035) and was monitored by the local Good Clinical Research Unit (NCT02072798). All mothers gave written consent prior to cesarean delivery and prior to collecting any samples.

DNA Extraction

Approximately 200 mg of fecal sample was weighted into a PowerSoil Bead Tube and total DNA extracted using the MoBio PowerSoil DNA isolation kit (MOBIO Laboratories, Carlsbad, California), following manufacturer's instructions, except that an initial bead beating step was included to increase cell lysis. Prior to DNA extraction, samples were placed into the PowerBead tubes and heat treated at 65°C for 10 minutes and then at 95°C for 10 minutes. Subsequently, solution C1 was added and bead-beating performed in a FastPrep (MP Biomedicals, Santa Ana, California) using 3 cycles of 15 seconds each, at a speed of 6.5 m s^{-1}. The DNA concentration was measured using Nanodrop (ND-100; NanoDrop Technologies, Waltham, Massachusetts). Extracted DNA was stored at −60°C until further analysis.

16S rRNA Gene Amplicon Sequencing

The fecal composition of the bacterial microbiota was determined using tag-encoded 16S ribosomal RNA (rRNA) gene MiSeq-based (Illumina, San Diego, California) high throughput sequencing. The V3-V4 region of the 16S rRNA gene was amplified using primers compatible with the Nextera Index Kit (Illumina; adapters in bold): NXt_341_F: 5′-**TCGTCGGCA GCGTCAGATG TGTATAA GAG ACAG**CCTAYG GGRBGCASCAG-3′ and NXt_806_R: 5′-**GTCTCGTGGG CTCGGAGATG TGTAAAGAGA CAG** GGACTAC NNGGGTATC TAAT-3'; library construction and sequencing was carried out as previously described.[28]

Amplicon Sequencing Data Analysis

The raw dataset containing pair-ended reads was merged, trimmed, filtered from chimeric reads with the USEARCH pipeline,[29] and subjected to zero radius operational taxonomic units (OTUs) construction using UNOISE.[30] Taxonomy classification was conducted using the SINTAX algorithm[31] based on the Ribosomal Database Project (release 11, update 5, 2016).[32] USEARCH, UNOISE and SINTAX are all available via Robert Edgar (https://www.drive5.com). The Quantitative Insight Into Microbial Ecology (QIIME) open source software package (v 1.8.0) was used for subsequent analysis steps.[32,33]

Weighted, unweighted, and generalized UniFrac distance metrics were calculated based on subsampled OTU-tables (14 000 reads per sample) and projected with principal coordinate analysis plots. Permutational multivariate ANOVA (PERMANOVA) (Vegan R package, https://cran.r-project.org/web/packages/vegan/vegan.pdf) was used to evaluate group differences based on weighted, unweighted, and generalized UniFrac distance matrices. The differences in taxa abundance and prevalence between categories were estimated with a statistic framework: analysis of composition of microbes based on non-normalized OTU-table summarized

to the species level (all *P* values false discovery rate corrected).[33]

Alpha diversity expressed as observed species and Shannon indexes were computed for rarefied OTU tables (14 000 reads per sample) using the alpha rarefaction workflow. Alpha diversity differences were tested by *t*-test using the nonparametric (Monte Carlo) method (999 permutations) that is implemented in the compare alpha diversity workflow.

The full amplicon sequencing data set including metadata has been uploaded to the European Nucleotide Archive under the accession number PRJEB25411.

Bacterial Isolation and Identification

The isolation and identification of bacteria from the fecal samples was carried out as described previously.[13] Briefly, fecal samples from day 10 (~200 mg) were emulsified and transferred in sterile saline (1 mL) and mixed thoroughly before being inoculation onto nonselective and selective agar (SSI Diagnostica, Copenhagen, Denmark). Samples were streaked onto blood agar plates (5% horse blood), blue agar plates (modified Drigalski), and chocolate agar plates and incubated aerobically at 37°C for 24 hours (48 hours for chocolate agar plates). Colonies were selected based on morphology and subcultured to get pure isolates. Bacterial isolates were then identified by matrix-assisted laser desorption ionization-time of flight.[34] Identified isolates were stored in Luria-Bertani broth containing 20% glycerol at −80°C.

Antimicrobial Susceptibility Testing

The antibiotic susceptibility of the fecal bacterial isolates was determined by Kirby-Bauer disk diffusion method according to Clinical Laboratory Standards Institute recommendation[35] using disks containing ampicillin (10 μg), ciprofloxacin (5 μg), cefuroxime (30 μg), gentamicin (10 μg), or piperacillin/tazobactam (100 μg/10 μg). The inhibition zone diameter was measured after anaerobic incubation at 37°C within 18-24 hours, and results were categorized as resistant, intermediate, or susceptible also according to Clinical Laboratory Standards Institute.[35]

Results

Composition of the Gut Microbiota

A total of 86 fecal samples were obtained from 44 babies who donated fecal samples at 10 days (pre, n = 24; post n = 20) and 9 months of age (pre, n = 22; post, n = 20) with 2 twin set of babies participating at 10 days and only 1 set of twin babies at 9 months (**Figure 1**; available at www.jpeds.com).

High throughput 16S rRNA gene amplicon sequencing was used to determine composition of the gut microbiota. No differences in alpha-diversity measures between the groups were observed at day 10 as determined by observed species (pre = 148 ± 49, post = 151 ± 59, *P* = .76; **Figure 2** [available at www.jpeds.com]) and Shannon diversity indices (pre: 3.7 ± 1, post: 4.0 ± 1, *P* = .60; not shown). At 9 months of age, the number of Observed Species was elevated in the post group (pre = 361 ± 141, post = 496 ± 151, *P* = .012; **Figure 2**) and the Shannon diversity index did not differ significantly between the groups (pre = 5.2 ± 1.1, post = 6.0 ± 1.3, *P* = .062).

Unweighted, weighted, and generalized UniFrac distance metrics showed that very early life antibiotic exposure via the umbilical cord does not influence gut microbiota composition neither short (10 days) nor longer (9 months) term (**Figure 3**). When analyzing across the entire cohort, diet had no significant effect on gut microbiota composition after 10 days (**Figure 4**; available at www.jpeds.com). It should be noted that only 4 of the 44 investigated infants were exclusively formula fed with another 25 infants being both formula and breast fed, and 15 infants being exclusively breastfed at day 10.

At the compositional level, no taxa were found to differ significantly between pre and post groups either at 10 days or 9 months of age (analysis of composition, q > 0.05 in all cases). At day 10, the gut microbiota composition of both groups were dominated by OTUs belonging to family Enterobacteriaceae and *Veillonella dispar*, and also *Bifidobacterium* spp and *Clostridium* spp were present in noticeable numbers (**Figure 5**). At 9 months, the gut microbiota of the infants was dominated by obligate anaerobes, mainly *Faecalibacterium prausnitzii*, *Blautia* and other Clostridales, Lachnospiraceae, *Bifidobacterium breve*, *Bacteriodes,* and *Akkermansia muciniphila* (**Figure 5**).

A total of 153 facultative anaerobic and aerobic bacteria were isolated from fecal samples collected at 10 days of age. Most isolates were *Enterococcus* spp., (mainly *Enterococcus faecalis*), *Staphylococcus* spp. (*Staphylococcus epidermidis* and *S aureus*), and Enterobacteriaceae (mainly *Enterobacter cloacae* and *Klebsiella pneumoniae*) (**Table I**; available at www.jpeds.com). The antibiotic susceptibility of the gram-positive isolates was tested against ciprofloxacin, ampicillin, and gentamicin (**Table II**; available at www.jpeds.com). No clear differences in antibiotic susceptibility was observed between isolates obtained from pre and post group infants, with only 1 (pre) and 2 (post) gram-positive isolates being resistant to any 1 of 3 tested antibiotics. Gram-negative isolates were tested against cefuroxime, ciprofloxacin, ampicillin, gentamicin and pipercillin-tazobactam. Only resistance against ampicillin was widespread among gram-negative isolates from both the pre and post group isolates (**Table III**) with no differences observed between groups.

Discussion

When undergoing cesarean delivery, the mother is administered prophylactic antibiotics to prevent postsurgery complications such as surgical site infection, endometritis, and urinary tract infection.[3] According to the 2012 Danish National Obstetric Guideline on antibiotics and cesarean deliveries,[4] women giving birth by cesarean delivery are given a single prophylactic intravenous dose (1500 mg) of cefuroxime

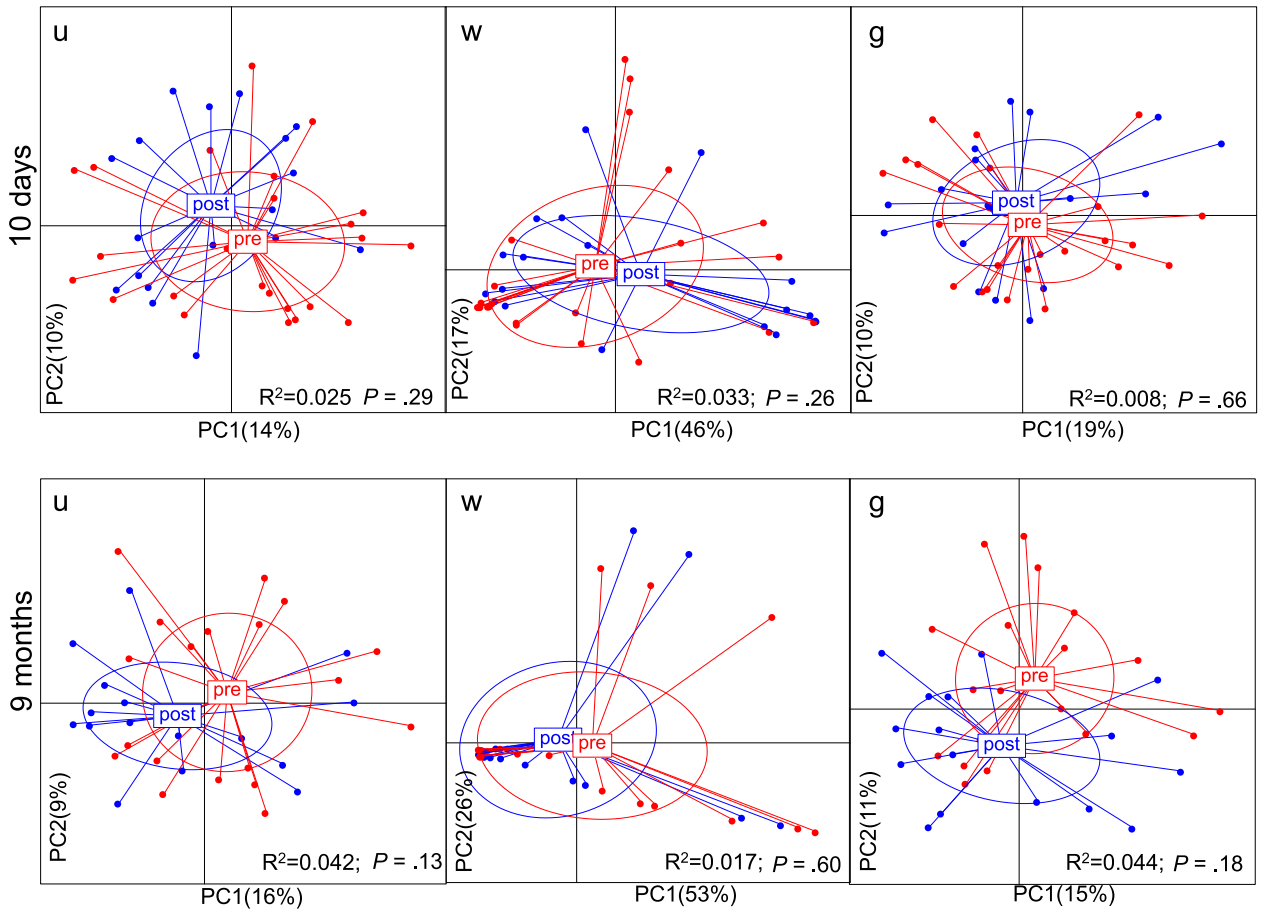

Figure 3. Gut microbiota compositional similarities between the pre and post groups depicted as principle coordinate analysis plots based on unweighted (u), weighted (w), and generalized (g) UniFrac distance metrics showing no clear differences between the gut microbiota of infants born by cesarean delivery whose mothers received cefuroxime before (red, pre) or after (blue, post) skin incision at 10 days and 9 months of age. PERMANOVA values shown each figure. Gut microbiota composition determined by 16S rRNA gene amplicon sequencing. *PC* (x and y-axis), principle coordinate.

15-60 minutes before skin incision. However, hospital practices still differ. At the time of this intervention, the usual practice at Odense University Hospital was to administer a single-dose of cefuroxime immediately after umbilical cord clamping to avoid placental transfer of the antibiotic to the fetus.[10] Several studies found that administration of prophylactic antibiotics pre-incision reduce maternal wound infections.[7-9] Lowering the risk wound infection of the mother is important not only for the mother, but also for the newborn—and from a societal perspective it may also help in reducing the cost of extended stays in the hospital.

We have previously showed that cefuroxime does pass the placental barrier and that a dose of 1500 mg administered to the mother before skin incision is cleared from the blood of the newborn within 24 hours,[10] but whether this very early exposure to antibiotics nevertheless influences gut microbiota composition and antibiotic susceptibility of the gut bacteria in the newborn has previously not been investigated. To assess the effect of maternal antibiotic exposure on the infant, we investigated gut microbiota composition 10 days and 9 months after birth. Ten days was chosen, as an early time point, where the gut microbiota had started to settle after the very dynamic phase immediately birth, when the gut is first colonized. Nine months was chosen as a more long-term time point representing the period after introduction of weaning food and the gradual transition toward a more adult-like gut microbiota.[16]

At day 10, the gut microbiota composition of both groups were dominated by to the family Enterobacteriaceae and genus *Veillonella*, while *Bifidobacterium* spp. and *Clostridium* spp. were present in noticeable numbers, too (**Figure 5**). The dominance of Enterobacteriaceae early in life is in agreement with previous findings,[16] although the relative abundance of especially *Bifidobacterium* spp. and *Bacteroides* tends to be higher in infants born vaginally[16] compared with the infants born by cesarean delivery in the present study. At 9 months, the gut microbiota of the infants was dominated by obligate anaerobes, mainly *Faecalibacterium* and *Blautia* and other Clostridales, Lachnospiraceae, *Bifidobacterium*, *Bacteriodes*, and *Akkermansia*, which is also in agreement with previous investigations of infant gut microbiota around 1 year of age (**Figure 5**).[16]

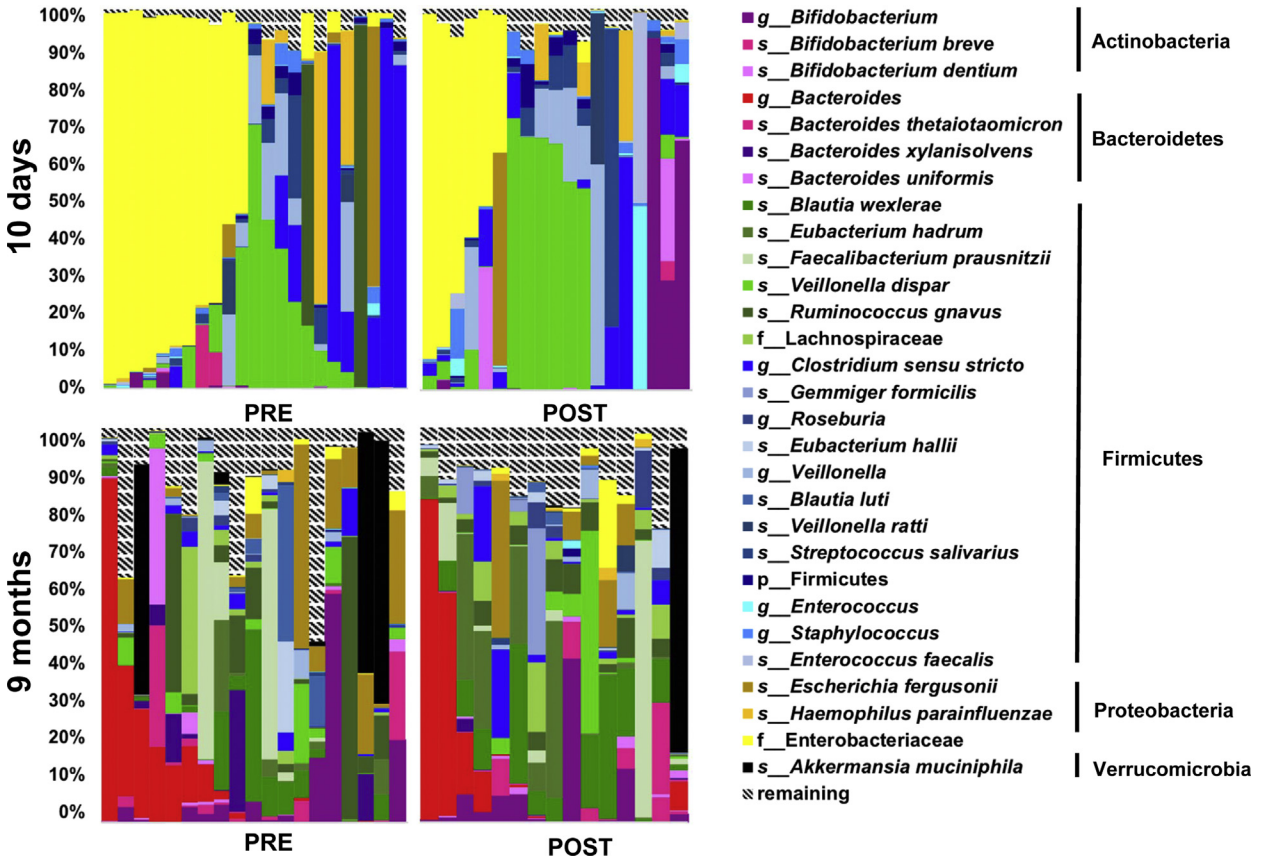

Figure 5. The relative distribution of the most abundant taxa. Relative distribution of the top 20 most abundant taxa at **A,** 10 days and **B,** 9 months of age determined using 16S rRNA gene amplicon sequencing.

In terms of observed species and Shannon diversity index, we did not observe any differences between pre and post parturition antibiotic administration at 10 days of age, but at 9 months of age, we observed a higher number of observed species in the post group (Figure 1). Neither unweighted, weighted, nor generalized Unifrac distance metrics indicated gut microbiota compositional differences between pre and post groups at 10 days and 9 months of age (Figure 3). It is important to note that in the present study, we investigated only the effect of very early-life

Table III. Antibiotic susceptibility of gram-negative (Enterobacteriaceae) bacteria isolated from feces 10 days after birth from babies born by cesarean delivery for which the mother was administered a single dose of cefuroxime either before (pre) or after (post) umbilical cord clamping

		Pre			Post		
Bacterium	**Antibiotics**	**R**	**I**	**S**	**R**	**I**	**S**
Klebsiella pneumoniae (pre, n = 9; post, n = 6)	Cefuroxime	0/9 (0.0)	1/9 (11.1)	8/9 (88.9)	0/6 (0.0)	0/6 (0.0)	6/6 (100.0)
	Ciprofloxacin	0/9 (0.0)	1/9 (11.1)	8/9 (88.9)	0/6 (0.0)	0/6 (0.0)	6/6 (100.0)
	Ampicillin	5/9 (55.6)	3/9 (33.3)	1/9 (11.1)	3/6 (50.0)	3/6 (50.0)	0/6 (0.0)
	Gentamicin	0/9 (0.0)	0/9 (0.0)	9/9 (100.0)	0/6 (0.0)	0/6 (0.0)	6/6 (100.0)
	Pipercillin-tazobactam	0/9 (0.0)	1/9 (11.1)	8/9 (88.9)	0/6 (0.0)	0/6 (0.0)	6/6 (100.0)
Enterobacter cloacea (pre, n = 16; post, n = 8)	Cefuroxime	1/16 (6.3)	13/16 (81.3)	2/16 (12.5)	2/8 (25.0)	2/8 (25.0)	4/8 (50.0)
	Ciprofloxacin	0/16 (0.0)	0/16 (0.0)	16/16 (100.0)	0/8 (0.0)	0/8 (0.0)	8/8 (100.0)
	Ampicillin	7/16 (43.8)	4/16 (25.0)	5/16 (31.3)	5/8 (62.5)	2/8 (25.0)	1/8 (12.5)
	Gentamicin	0/16 (0.0)	0/16 (0.0)	16/16 (100.0)	1/8 (12.5)	0/8 (0.0)	7/8 (87.5)
	Pipercillin-tazobactam	1/16 (6.3)	2/16 (12.5)	13/16 (81.3)	0/8 (0.0)	3/8 (37.5)	5/8 (62.5)
Other *Enterobacteriaceae* (pre, n = 14; post, n = 3)	Cefuroxime	0/14 (0.0)	0/14 (0.0)	14/14 (100.0)	0/3 (0.0)	0/3 (0.0)	3/3 (100.0)
	Ciprofloxacin	0/14 (0.0)	0/14 (0.0)	14/14 (100.0)	0/3 (0.0)	0/3 (0.0)	3/3 (100.0)
	Ampicillin	1/14 (7.1)	1/14 (7.1)	12/14 (85.7)	1/3 (33.3)	1/3 (33.3)	1/3 (33.3)
	Gentamicin	0/14 (0.0)	0/14 (0.0)	14/14 (100.0)	0/3 (0.0)	0/3 (0.0)	3/3 (100.0)
	Pipercillin-tazobactam	0/14 (0.0)	0/14 (0.0)	14/14 (100.0)	0/3 (0.0)	0/3 (0.0)	3/3 (100.0)

I, intermediate; *NA*, not applicable; *R*, resistant; *S*, susceptible.
I, R, and S as determined by the Kirkby-Bauer test.

exposure to cefuroxime on gut microbiota composition. Maternal exposure to cefuroxime around the time of birth could also influence the maternal milk microbiome.

The elevated number of observed species in the post group at 9 months of age could indicate an altered succession of bacteria among infants exposed to the antibiotic, but this effect would be expected to be more pronounced at 10 days of age. As no other indices indicate an effect of very early-life exposure to cefuroxime on the gut microbiota, it seems more likely that the observed difference in observed species between pre and post groups at 9 months of age is a result of the substantial reorganization that infant gut microbiota undergoes in the period after weaning,[16,36] but potential long-term effects of very early-life exposure to antibiotics cannot be completely excluded.

Early life antibiotic exposure has previously been reported to increase the frequency of antibiotic resistant bacterial gut microbiota members, especially Enterobacteriaceae.[27] However, in the present study, we observed no differences in antibiotic susceptibility profiles of gram-positive cocci and Enterobacteriaceae isolated from pre and post fecal samples (10 days). The observation that a relatively high fraction of the investigated *Klebsiella pneumoniae* and *Enterobacter cloacae* isolates were resistant to ampicillin is not surprising, as ampicillin has been widely used to treat bacterial infections for decades. ■

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Data Statement

Data sharing statement available at www.jpeds.com.

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50 Years Ago in *The Journal of Pediatrics*

Type 3 Poliovirus Vaccines

Pagano JS. J Pediatr 1969;75:162-3.

Despite the success of Salk inactivated polio vaccine, the efforts to develop other polio vaccines continued. Several oral candidate vaccines were tried, but the Sabin oral polio vaccine (OPV) was considered to be superior and was licensed in 1961 as a monovalent OPV containing type 1 polio virus. The type 3 poliovirus component was added later.[1] Pagano highlighted an association of paralytic polio with the type 3 component of some of the live-attenuated, non-Sabin, OPV candidates, noted in few European countries, through this editorial in *The Journal*. Fifty years later, through efficient use of Sabin-OPV, poliomyelitis is on the verge of being eradicated. Despite its many advantages, OPV carries certain inherent risks, the most important being the vaccine-associated paralytic poliomyelitis and circulating vaccine-derived poliovirus (cVDPV).[2] Recognition of cVDPV has significantly influenced the progress of the Global Polio Eradication Initiative, leading to the conceptualization of an end game, through which universal cessation of each Sabin virus is planned under cover of inactivated polio vaccine.[3] Owing to differences in transmissibility and attenuation of each vaccine virus in OPV, the type 3 strain has been more often associated with vaccine-associated paralytic poliomyelitis and type 2 with cVDPV. The retrospective analysis with molecular methods conclusively proved that the large outbreak in Poland (1968) was in fact an instance of type 3 cVDPV after a field trial of an experimental type 3 OPV strain, USOL-D-bac.[4] Admittedly, the genesis of cVDPV has made the attainment of global polio eradication a much more arduous process. Since November 2012, WPV type 3 has not been detected anywhere in the world, and in all probability it has been eradicated.[5] With this, 1 more hurdle is crossed toward the attainment of global polio eradication—one of the greatest achievements of science in this century!

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Table I. Distribution of bacterial isolated from fecal samples from 10-day-old infants born by cesarean delivery for which mother was administered a single dose of cefuroxime either before (pre) or after (post) umbilical cord clamping

Bacterial isolates	Number tested (%) Pre	Post
Gram positive bacteria	n = 54	n = 31
Enterococcus spp	27	16
Enterococcus faecalis	20 (21.3)	16 (32.7)
Other *Enterococcus* spp.*	7 (7.4)	0 (0.0)
Staphylococcus spp	27	13
Staphylococcus epidermidis	16 (17.0)	8 (16.3)
Staphylococcus aureus	9 (9.6)	2 (4.1)
Other *Staphylococcus* spp.†	2 (2.1)	3 (6.1)
Other gram-positive bacteria‡	0 (0.0)	2 (4.1)
Gram negative bacteria	n = 40	n = 18
Enterobacteriaceae	39	17
Klebsiella pneumoniae	9 (9.6)	6 (12.2)
Enterobacter cloacae	16 (17.0)	8 (16.3)
Other Enterobacteriaceae§	14 (14.9)	3 (6.1)
Other gram-negative bacteria¶	1 (1.1)	1 (2.0)
Total N = 153	94 (100)	49 (100)

Isolates were identified by matrix-assisted laser desorption/ionization-time-of-flight.
**Enterococcus avium, Enterococcus maldoratus, Enterococcus faecium.*
†Staphylococcus hominis, Staphylococcus lugdunensis.
‡Aerococcus viridans, Micrococcus lutues.
§Enterobacter aerogenes, Enterobacter asburie, Klebsiella oxytoca, Citrobacter braakii, Citrobacter fruendii, Escherichia coli, Leclercia adecarboxylata.
¶Acinetobacter junii, Stenotrophomonas maltophilia.

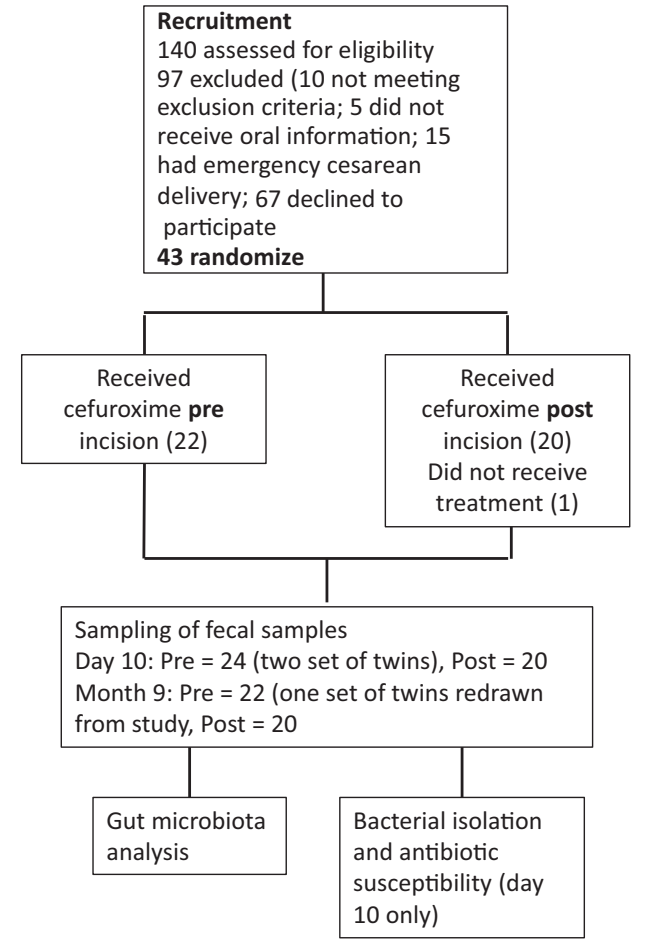

Figure 1. Study design.

Table II. Antibiotic susceptibility of Gram-positive bacteria isolated from feces 10 days after birth from babies born by cesarean delivery for which the mother was administered a single dose of cefuroxime either before (pre) or after (post) umbilical cord clamping

Bacterium	Antibiotics	Pre R	Pre I	Pre S	Post R	Post I	Post S
Enterococcus faecalis (pre, n = 20; post, n = 16)	Ciprofloxacin	0/20 (0.0)	10/20 (50.0)	10/20 (50.0)	0/16 (0.0)	10/16 (62.5)	6/16 (37.5)
	Ampicillin	0/20 (0.0)	0/20 (0.0)	20/20 (100.0)	0/16 (0.0)	0/16 (0.0)	16/16 (100.0)
Other *Enterococcus* spp. (pre, n = 7; post, n = 0)	Ciprofloxacin	0/7 (0.0)	2/7 (28.6)	5/7 (71.4)	NA	NA	NA
	Ampicillin	0/7 (0.0)	0/7 (0.0)	7/7 (100.0)	NA	NA	NA
Staphylococcus epidermidis (pre, n = 16; post, n = 8)	Ciprofloxacin	0/16 (0.0)	0/16 (0.0)	16/16 (100.0)	1/8 (12.5)	0/8 (0.0)	7/8 (87.5)
	Gentamicin	0/16 (0.0)	0/16 (0.0)	16/16 (100.0)	0/8 (0.0)	0/8 (0.0)	8/8 (100.0)
Staphylococcus aureus (pre, n = 9; post, n = 2)	Ciprofloxacin	0/9 (0.0)	2/9 (22.2)	7/9 (77.8)	0/2 (0.0)	0/2 (0.0)	2/2 (100.0)
	Gentamicin	1/9 (11.1)	0/9 (0.0)	8/9 (88.9)	0/2 (0.0)	1/2 (50.0)	1/2 (50.0)
Other *Staphylococcus* spp. (pre, n = 2; post, n = 3)	Ciprofloxacin	0/2 (0.0)	0/2 (0.0)	2/2 (100.0)	0/3 (0.0)	1/3 (33.3)	2/3 (66.7)
	Gentamicin	0/2 (0.0)	1/2 (50.0)	1/2 (50.0)	1/3 (33.3)	0/3 (0.0)	2/3 (66.7)

I, intermediate; *NA*, not applicable; *R*, resistant; *S*, susceptible.
I, R, and S as determined by the Kirkby-Bauer test.

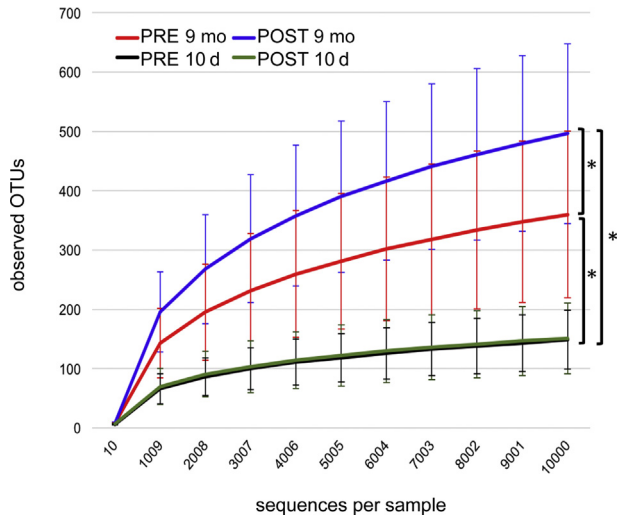

Figure 2. Rarefaction curves based on observed species index (16S rRNA gene amplicon sequencing based). Significant ($P < .05$) differences indicated by *asterisk*.

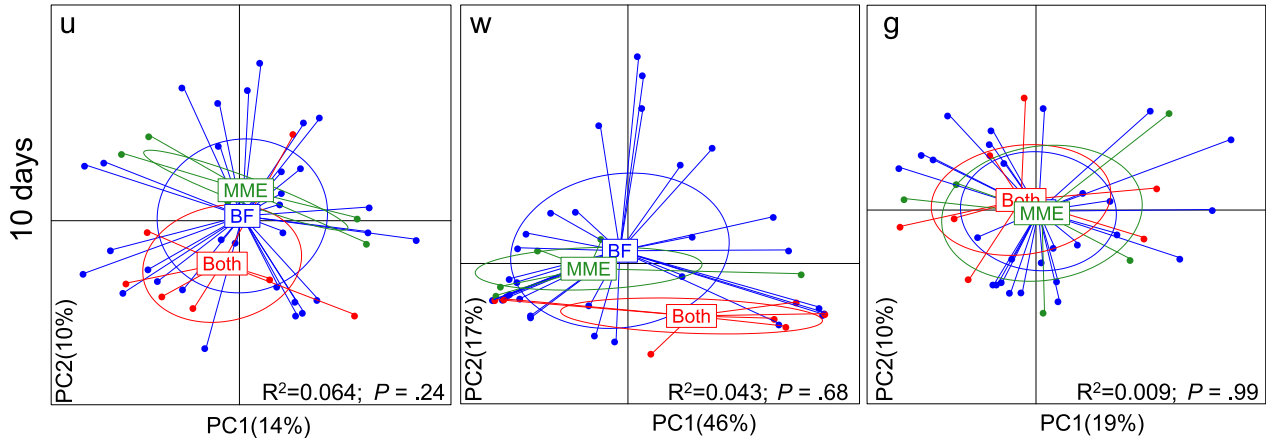

Figure 4. The influence of diet (BF, exclusive breast feeding; MME, formula feeding, Both, mixed breast and formula feeding) on gut microbial compositional similarities depicted as principle coordinate analysis plots based on unweighted (u), weighted (w), and generalized (g) UniFrac distance metrics at 10 days. PERMANOVA results are given in each figure. Gut microbiota composition determined by 16S rRNA gene amplicon sequencing. *PC* (x and y-axis), principle coordinate.