Cesarean section changes neonatal gut colonization

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Background: Delivery by means of cesarean section has been associated with increased risk of childhood immune-mediated diseases, suggesting a role of early bacterial colonization patterns for immune maturation.

Objective: We sought to describe the influence of delivery method on gut and airway colonization patterns in the first year of life in the Copenhagen Prospective Studies on Asthma in Childhood$_{2010}$ (COPSAC$_{2010}$) birth cohort.

Methods: Seven hundred children from the COPSAC$_{2010}$ birth cohort participated in this analysis. Fecal samples were collected at age 1 week, 1 month, and 1 year, and hypopharyngeal aspirates were collected at age 1 week, 1 month, and 3 months and cultured for bacteria. Detailed information on delivery method, intrapartum antibiotics, and lifestyle factors was obtained by personal interviews.

Results: Seventy-eight percent of the children were born by means of natural delivery, 12% by means of emergency cesarean section, and 9% by means of elective cesarean section. Birth by means of cesarean section was significantly associated with colonization of the intestinal tract by *Citrobacter freundii*, *Clostridium* species, *Enterobacter cloacae*, *Enterococcus faecalis*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* at age 1 week, whereas colonization by *Escherichia coli* was associated with natural birth. At age 1 month, these differences were less prominent, and at age 1 year, they were not apparent, which was confirmed by means of multivariate data-driven partial least squares analyses. The initial airway microbiota was unaffected by birth method.

Conclusion: Delivery by means of cesarean section was associated with early colonization patterns of the neonatal gut but not of the airways. The differences normalized within the first year of life. We speculate that microbial derangements, as indicated in our study, can demonstrate a possible link between delivery by means of cesarean section and immune-mediated disease. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: *Cesarean section, microbiota, bacteria, parturition immune diseases*

We recently reported delivery by means of cesarean section as a shared risk factor for several immune-mediated diseases in the child, suggesting the early bacterial colonization pattern as an important environmental factor for health and disease.[1]

The diversity of the human microbiome is established in early life,[2] and it has been hypothesized that the composition of the gut might have the ability to affect immune maturation of the host and that inappropriate exposure might lead to asthma and allergy and other immune disorders.[3-7] Similarly, early-life bacterial composition in the respiratory tract has been associated with asthma development, perhaps because of a local epithelial modulation leading to chronic inflammation.[8,9]

The healthy embryo is believed to be sterile, obtaining the first bacteria from the maternal birth canal and intestinal tract during natural birth. Early colonization patterns can be affected by mode of delivery and even within types of cesarean section because the patterns can differ according to delivery by means of elective or emergency cesarean section.[10] Furthermore, it is common practice to use intrapartum antibiotics during the procedure, which might also lead to long-term microbial derangements.[11]

The objective of this study was to analyze the effects of delivery mode on the colonization patterns of both the intestinal tract and airways during the first year of life of the children from the Copenhagen Prospective Studies on Asthma in Childhood$_{2010}$ (COPSAC$_{2010}$) birth cohort. We hypothesized that colonization patterns differ with delivery type and also within subtypes of cesarean sections because the first encounter with the external environment varies among these.

METHODS

Ethics

The study was performed according to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Copenhagen (H-B-2008-093) and the Danish Data Protection Agency (2008-41-2599). We are aware of and comply with recognized codes of good research practice, including the Danish Code of Conduct for Research Integrity. We comply with national and international rules on the safety and rights of patients and healthy subjects, including Good Clinical Practice, as defined in the European Union's Directive on Good Clinical Practice, the International Conference on Harmonisation's good clinical practice guidelines, and the Helsinki Declaration. We follow national and international rules on the processing of personal data, including the Danish Act on Processing of Personal Data and the practice of the Danish Data Inspectorate.

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COPSAC is funded by private and public research funds all listed on www.copsac.com. The Lundbeck Foundation, the Ministry of Health, the Danish Council for Strategic Research, and the Capital Region Research Foundation have provided core support for COPSAC.

Disclosure of potential conflict of interest: H. Bisgaard has received grants from the Danish Ministry of Health, the Lundbeck Foundation, the Danish Strategic Research Foundation, and the Danish Advanced Technology Foundation, and the National Institutes of Health and has consultant arrangements with Chiesi. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication September 8, 2015; revised December 18, 2015; accepted for publication January 6, 2016.

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0091-6749/$36.00

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http://dx.doi.org/10.1016/j.jaci.2016.01.028

Abbreviations used
aOR: Adjusted odds ratio
COPSAC: Copenhagen Prospective Studies on Asthma in Childhood
LV: Latent variable
PLS: Partial least squares

Study population

The novel $COPSAC_{2010}$ cohort is an ongoing Danish cohort study of 738 unselected pregnant women and their 700 children followed from pregnancy week 24 in a protocol previously described in detail[12] based on the $COPSAC_{2000}$ birth cohort study.[8,13,14] Data validation and quality control follow the guidelines for good clinical practice. Data were collected during visits to the clinical research unit and stored in a dedicated online database. The data were double-checked against source data and subsequently locked.

Delivery method

Information on delivery method was obtained at the COPSAC clinic 1 week after birth and validated against data from the Danish Medical Birth Registry. Children were categorized according to the type of delivery as natural delivery, emergency cesarean section, or elective cesarean section. Emergency cesarean section was defined as a spontaneous birth turning into a cesarean section and elective cesarean section was defined as induced birth without rupture of the membranes. The delivery method was analyzed both as a dichotomized variable (natural birth vs cesarean section) and categorized as natural birth, emergency cesarean section, and elective cesarean section.

Samples for bacterial culture

Fecal samples were collected 1 week, 1 month, and 1 year after birth either at the research clinic or by the parents at home by using detailed instructions. Hypopharyngeal aspirates from children were collected at the research clinic 1 week, 1 month, and 3 months after birth, with a soft-suction catheter passed through the nose into the hypopharynx, as previously described in detail.[8] All samples were cultured within 24 hours at Statens Serum Institut by using standard methods on nonselective and selective media (SSI Diagnostica, Hillerød, Denmark). Blood agar plates (5% horse blood) and chocolate agar plates (including lysed blood cells) were used for general culturing. These were incubated aerobically at 37°C for 18 to 20 hours, whereas the blood agar and chocolate agar plates were incubated under microaerophilic conditions (5% CO_2, 3% H_2, 5% O_2, and 87% N_2) at 37°C for 48 hours. Subsequently, microbial identification was performed according to growth on selective media, characteristics of colonies, and cellular morphology. All bacteria identifications were confirmed biochemically by using VITEK 2 (BioMérieux, Marcy-L'Étoile, France). No bacterial quantification was performed. The isolates were all characterized at the species level. We used a cutoff value of greater than 2% of children colonized at a given time point for a species to be represented in the analyses.

Environmental factors

Information on maternal age at birth, asthma, and smoking during pregnancy; education; household income; antibiotics in pregnancy; pre-eclampsia; gestational diabetes; parity; antibiotics given to the child after birth; hospitalization of the child after birth; household pets; number of older children in the home; and duration of exclusive breast-feeding was obtained during the scheduled visits to the COPSAC clinic.

Intrapartum antibiotics

Information on intrapartum antibiotics was obtained by means of interview 1 week after birth and validated against birth records from hospitals.

Statistical analysis

ANOVA or the χ^2 test was used for analyzing simple associations in the cohort's baseline characteristics regarding differences in birth method and prenatal, perinatal, social, and lifestyle-related variables. With conventional statistics, the associations between cesarean section and bacterial species at different time points were analyzed by using logistic regression. Estimates were expressed as odds ratios with corresponding 95% confidence limits, with the children born naturally as the reference group. A significance level of .05 was used in all types of analysis.

The complementary data-driven pattern analysis was conducted by using partial least squares (PLS) regression based on colonization of the 57 most common bacterial species with at least 5 colonized children during the 3 sample time points. Score plots of the latent variables (LVs) were used to investigate differences in overall bacterial colonization patterns between the children with or without cesarean section. A single 3-way PLS model described the bacterial colonization patterns over time by using children, bacterial species, and sample time points as the 3 dimensions. Only children with complete data at all 3 sample time points were included in the analysis (n = 597). In a separate PLS model to discriminate between children born by means of natural delivery or by means of elective or emergency cesarean section, 1-week fecal colonization was used. Associations between PLS components and method of delivery were quantified by using the area under the receiver operating characteristic curve.

Data processing was conducted with SAS version 9.4 for Windows (SAS Institute, Cary, NC) and MATLAB R2013a v. 8.1.0.604.

RESULTS

Demographics

In the $COPSAC_{2010}$ birth cohort of 700 children, 78% (549) were born by means of natural delivery, 12% (85) by means of emergency cesarean section, and 9% (66) by means of elective cesarean section. A lower number of children born by means of emergency cesarean section attended the 1-week visit compared with those born by means of the other delivery types, which resulted in a lower prevalence of completed fecal and tracheal samples in this group. Fecal samples were obtained and cultured from 92% (645) of all children at 1 week, 96% (672) at 1 month, and 93% (652) at 1 year of age. Tracheal samples were obtained and cultured from 90% (633) of all children at 1 week, 96% (673) at 1 month, and 95% (664) at 3 months of age.

All mothers giving birth by means of cesarean section received intrapartum antibiotics versus only 13% (n = 72) of mothers having a natural birth. We observed a higher gestational age among the children born naturally (mean, 40.0 weeks) compared with children born by means of emergency cesarean section (mean, 39.3 weeks) and elective cesarean section (mean, 39.0 weeks). Mothers of children born by means of emergency cesarean section were more often primiparous (76%) compared with mothers of children born by means of natural delivery (44%) and elective cesarean section (24%). Twenty-seven percent of children born by means of emergency cesarean section were hospitalized after birth compared with 11% of those born by means of elective cesarean section and 9% after natural delivery. The duration of exclusive breast-feeding also tended to differ though only borderline significant; natural delivery (mean, 105.4 days), emergency cesarean section (mean, 88.4 days), and elective cesarean section (mean, 103.5 days; Table I). Therefore all analyses were adjusted for gestational age, parity, hospitalization, and duration of exclusive breast-feeding.

TABLE I. Demographic characteristics for the entire cohort and grouped according to delivery method

	All	Delivery method			*P* value
		Natural	Emergency CS	Elective CS	
All	700	78% (549)	12% (85)	9% (66)	—
Birth					
Intrapartum antibiotics (no.)	33% (223)	13% (72)	100% (85)	100% (66)	**<.0001**
Gestational age (wk), mean (SD)	39.9 (1.7)	40.0 (1.5)	39.3 (2.7)	39.0 (0.8)	**<.0001**
Antibiotics given to the child (no.)	3% (18)	3% (15)	4% (3)	0% (0)	.348
Male sex (no.)	51% (360)	50% (276)	61% (52)	48% (32)	.153
Child hospitalized after birth (no.)	11% (80)	9% (50)	27% (23)	11% (7)	**<.0001**
Breast-feeding duration (exclusive [d]), mean (SD)	103.1 (59.8)	105.4 (57.7)	88.4 (67.0)	102.5 (65.6)	.051
Prenatal Exposure					
Smoking (no.)	8% (54)	8% (42)	9% (8)	6% (4)	.741
Cat or dog in pregnancy (no.)	35% (244)	34% (187)	39% (33)	36% (24)	.668
Antibiotics in pregnancy (no.)	37% (256)	36% (198)	35% (30)	42% (28)	.578
Preeclampsia (no.)	4% (31)	4% (20)	9% (8)	5% (3)	.057
Gestational diabetes (no.)	2% (16)	2% (13)	2% (2)	2% (1)	.905
Socioeconomics					
Maternal age at birth (y), mean (SD)	32.3 (4.4)	32.1 (4.2)	33.0 (4.9)	33.0 (4.5)	.065
White (no.)	96% (669)	95% (523)	96% (82)	97% (64)	.745
Asthma history (no.)*	26% (184)	25% (136)	31% (26)	34% (22)	.189
Primiparity (no.)	46% (323)	44% (242)	76% (65)	24% (16)	**<.0001**
Maternal educational level					.539
Low†	11% (75)	11% (59)	13% (11)	8% (5)	
Medium†	58% (406)	57% (311)	62% (53)	64% (42)	
High†	31% (215)	32% (175)	25% (21)	29% (19)	
Household annual income					.696
Low‡	11% (75)	11% (60)	12% (10)	8% (5)	
Medium‡	51% (355)	50% (273)	56% (48)	52% (234)	
High‡	38% (262)	39% (209)	32% (27)	40% (26)	
Samples					
Fecal, 1 wk	92% (645)	94% (516)	78% (66)	95% (63)	**<.0001**
Fecal, 1 mo	96% (672)	96% (527)	95% (81)	97% (64)	.873
Fecal, 1 y	93% (652)	93% (511)	94% (80)	92% (61)	.912
Hypopharyngeal, 1 wk	90% (633)	92% (505)	75% (64)	97% (64)	**<.0001**
Hypopharyngeal, 1 mo	96% (673)	96% (527)	94% (80)	100% (66)	.164
Hypopharyngeal, 3 mo	95% (664)	95% (521)	96% (82)	92% (61)	.534

Values in boldface are statistically significant.
CS, Cesarean section.
*History of doctor-diagnosed asthma.
†Low = primary school, secondary school or college graduate, medium = tradesman or bachelor degree, and high = masters degree.
‡Low = <€50,000, medium = €50,000-€110,000, and high = >€110,000.

Cesarean section and fecal colonization patterns

At 1 week of age, we found associations between birth by means of cesarean section and *Citrobacter freundii* (adjusted odds ratio [aOR], 2.78; 95% CI, 1.00-7.69; $P = .050$), *Clostridium* species (aOR, 1.82; 95% CI, 1.05-3.15; $P = .033$), *Enterobacter cloacae* (aOR, 2.03; 95% CI, 1.14-3.62; $P = .018$), *Enterococcus faecalis* (aOR, 3.37; 95% CI, 2.19-5.20; $P < .001$), *Klebsiella oxytoca* (aOR, 3.00; 95% CI, 1.43-6.30; $P = .004$), *Klebsiella pneumoniae* (aOR, 2.44; 95% CI, 1.32-4.51; $P = .004$), and *Staphylococcus aureus* (aOR, 1.75; 95% CI, 1.09-2.79; $P = .019$), whereas colonization by *Escherichia coli* was negatively associated with birth by means of cesarean section (aOR, 0.18; 95% CI, 0.10-0.33; $P < .001$; Fig 1, *A*).

At 1 month of age, cesarean section was significantly associated with colonization by *Clostridium* species, *K oxytoca*, and *K pneumoniae*, whereas colonization by *E coli* was negatively associated with birth by means of cesarean section (Fig 1, *B*).

At 1 year of age, we found minor associations with the mode of delivery. Birth by means of cesarean section was only significantly associated with increased *Citrobacter braakii*. All other species were equally represented (Fig 1, *C*).

Intrapartum antibiotics. A subanalysis on fecal colonization at age 1 week stratified by use of intrapartum antibiotics showed similar results with delivery by means of cesarean section being significantly associated with *E faecalis* (aOR, 2.82; 95% CI, 1.40-5.71; $P = .004$) and *S aureus* (aOR, 2.80; 95% CI, 1.20-6.57; $P = .018$), whereas colonization by *E coli* was negatively associated with birth by means of cesarean section (aOR, 0.20; 95% CI, 0.09-0.43; $P < .001$; see Fig E1 in this article's Online Repository at www.jacionline.org).

In children born naturally, treatment with intrapartum antibiotics was associated with colonization by *K oxytoca* (aOR, 5.54; 95% CI, 2.03-15.10; $P < .001$) and *Staphylococcus haemolyticus* (aOR, 4.70; 95% CI, 1.24-17.90; $P = .023$ at age 1 week; see Fig E2 in this article's Online Repository at www.jacionline.org).

Type of cesarean section. We observed further differences in bacterial distribution, depending on the child being born by means of emergency or elective cesarean section (Fig 2). Here differences were seen in early colonization with *Clostridium* species, *E cloacae*, and *E faecalis*. Children born by means of emergency

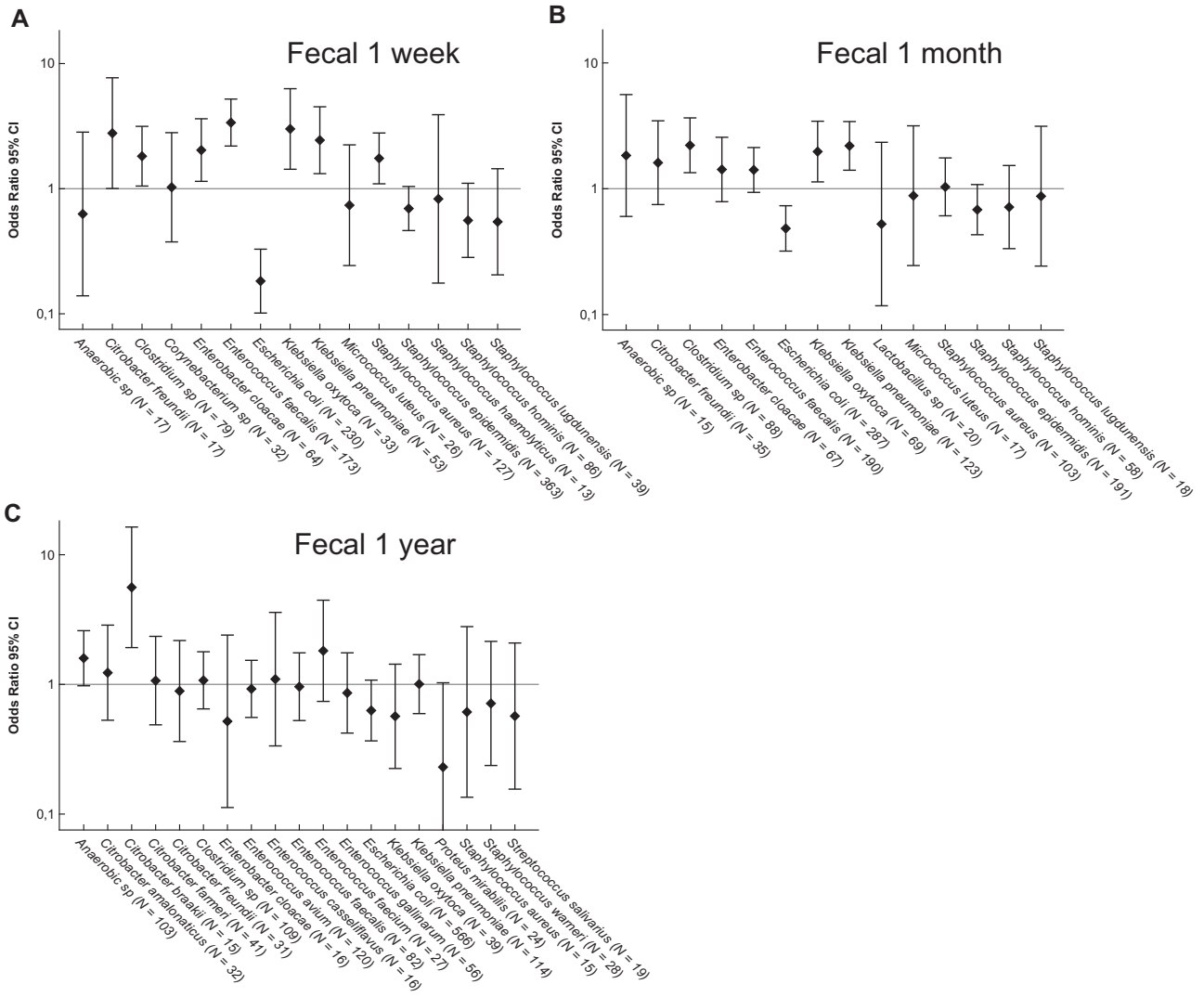

FIG 1. Associations between delivery by means of cesarean section and fecal colonization at age 1 week **(A)**, 1 month **(B)**, and 1 year **(C)**. A cutoff value of greater than 2% of children colonized was used for a species to be represented in the analyses. All analyses were adjusted for gestational age, parity, hospitalization, and duration of exclusive breast-feeding.

cesarean section differed in colonization by *C freundii*, *S aureus*, and *S epidermidis*, in which children born by means of elective cesarean section and natural delivery were similar. No differences were observed between types of cesarean section regarding *E coli*, *K oxytoca*, and *K pneumoniae*, whereas both differed from natural birth.

Cesarean section and hypopharyngeal colonization patterns

We did not observe any effects of birth method on the 1-week colonization patterns of the airways, but at 1 month of age, cesarean section was positively associated with colonization by *S pneumoniae* (aOR, 2.80; 95% CI, 1.46-5.35; $P = .002$) and negatively associated with *E coli* (aOR, 0.23; 95% CI, 0.07-0.80; $P = .020$), and at 3 months of age, cesarean section was positively associated with colonization by *E faecalis* (aOR, 3.31; 95% CI, 1.21-9.03; $P = .020$; Fig 3).

Data-driven approach

A 3-way PLS model was applied to describe the fecal colonization patterns over time. A score plot of the LVs confirmed the significant effects on fecal bacterial composition related to birth by means of cesarean section (Fig 4, *A*). The 2 components, LV1 and LV2, explained 1.4% and 2.6% of the total variation across 57 bacterial species over time, respectively. The bacterial distribution described by the 2-component PLS model was significantly associated with birth by means of cesarean section ($P < .0001$), but cross-validation showed that only the first component contributed to an increased association. Fig 4, *B*, describes the pattern mean score of LV1 over time for children born by means of cesarean section and natural delivery. The pattern mean score differed at age 1 week and age 1 month but equalized at age 1 year.

In a subanalysis we designed a PLS model to describe fecal colonization patterns at 1 week based on delivery type: (1) natural delivery, (2) emergency cesarean section, and (3) elective

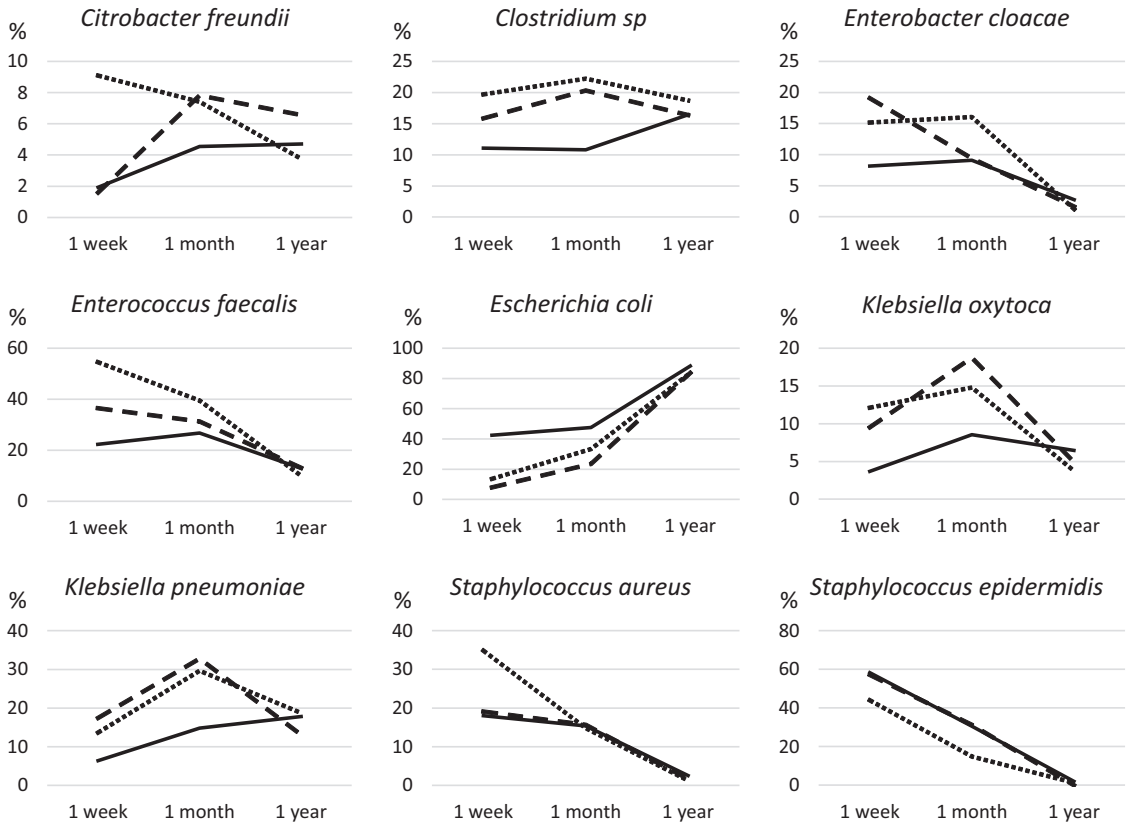

FIG 2. Cultured fecal species compared by delivery method and development over time. Percentages of colonized children at age 1 week, 1 month, and 1 year are shown and grouped by delivery method: natural *(solid lines)*, emergency cesarean section *(dotted lines)*, and elective cesarean section *(dashed lines)*.

cesarean section. LV1 separated the colonization patterns from natural delivery and cesarean section, and LV2 separated the colonization patterns in different types of cesarean section (Fig 5).

DISCUSSION

Principal findings

Delivery method was associated with different gut bacterial colonization patterns in earliest childhood, normalizing during the first year of life. Furthermore, elective versus emergency cesarean section was associated with distinctly different colonization patterns. We found no association between mode of delivery and the earliest airway colonization.

Strengths and limitations

The main strength of this study is the design of the $COPSAC_{2010}$ birth cohort, which is a replication and extension of the $COPSAC_{2000}$ cohort design. Pregnant women were recruited and closely monitored at the dedicated COPSAC clinical research center from pregnancy week 24. Fecal and hypopharyngeal samples were collected longitudinally from birth, allowing for description of colonization development over time in this large data set. All baseline data were collected by means of personal interviews at several time points and scrutinized for consistency in reporting. Children were followed regularly from birth by the dedicated staff in the COPSAC clinic, who performed microbial sampling according to predefined standard operating procedures.

The high reliability of the data obtained on delivery method is a further strength of the study. This information was obtained through personal interviews and validated against recordings from the Danish Medical Birth Registry. This allowed for further stratification into types of cesarean section, which represent different settings that can affect the vertical microbial transfer.

Bacterial culturing was performed by specialized personnel, yet it has a natural methodological limitation, which in itself is a biased test with regard to the composition of the microbiota *in vivo*. Only a percentage of bacterial species can be successfully identified.[15] Bacteriologic culture methods are vulnerable to the transfer time from sampling to analysis, as well as bacterial growth conditions. This poses a limitation when assessing the total composition of the commensal microbiota. In this study anaerobic bacterial species seem to be underestimated, which is a limitation when examining the intestinal microbiota. Furthermore, culture-based methods did not provide information on microbial abundance, which would have allowed analyses of microbial diversity. However, the culturing method allows us to compare our results with previous studies on the subject. Importantly, inefficiency in culturing is not a bias in relation to birth by means of cesarean section; hence it cannot explain the significant differences demonstrated in this study, but our conclusions will be constrained to the observed species.

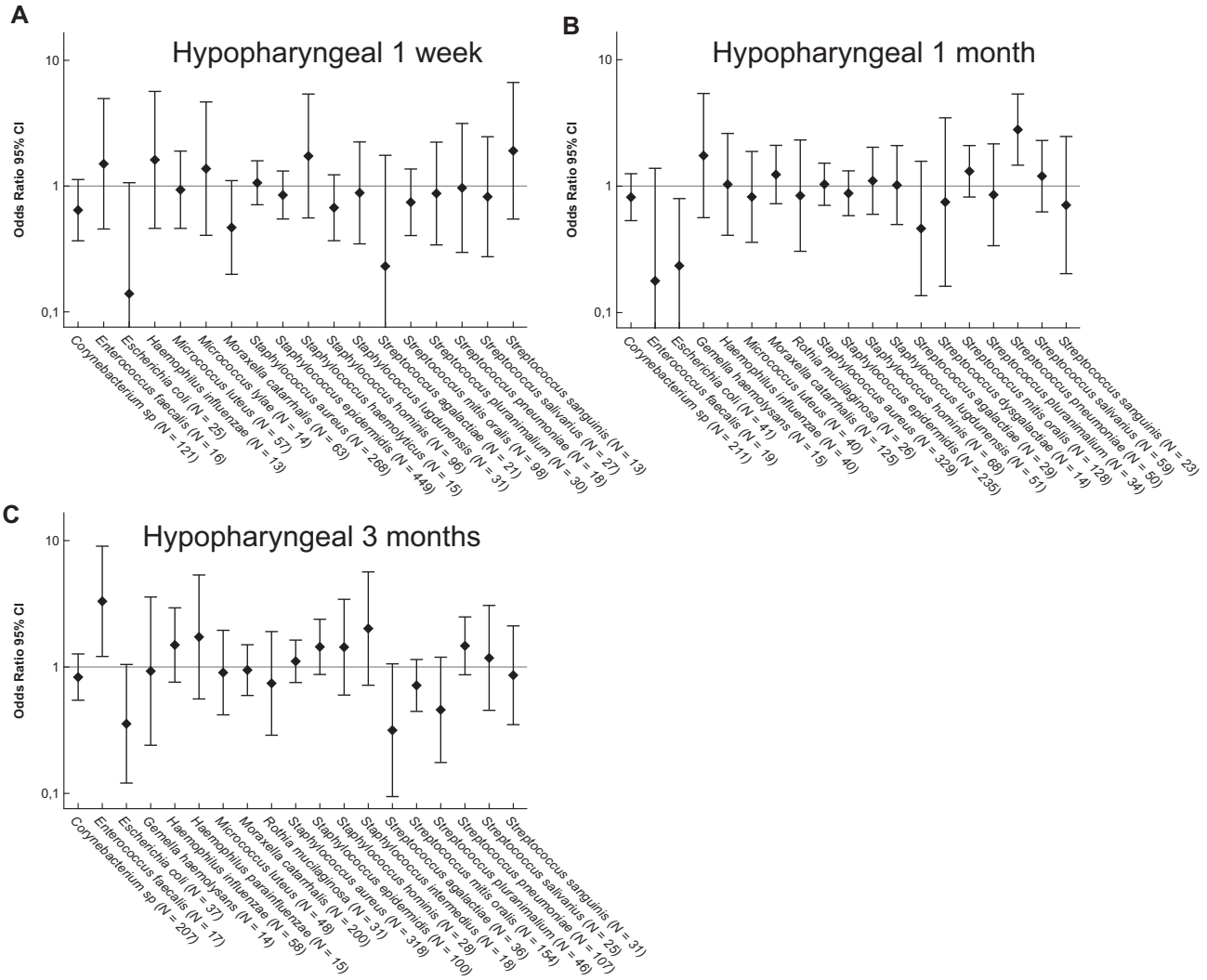

FIG 3. Associations between delivery by means of cesarean section and hypopharyngeal colonization at age 1 week **(A)**, 1 month **(B)**, and 3 months **(C)**. A cutoff value of greater than 2% of children colonized was used for a species to be represented in the analyses. All analyses were adjusted for gestational age, parity, hospitalization, and duration of exclusive breast-feeding.

Interpretation

We have demonstrated significant differences in the commensal intestinal microbiota in neonates born by means of cesarean section compared with those born by means of natural birth. The effects were most obvious at 1 week of age and subsequently equalized until age 1 year. We interpret these changes as consequences of interrupted vertical transmission of bacteria from mother to child. After cesarean section, the initial colonization is mainly obtained from the early environmental contacts and maternal skin rather than the birth canal and anogenital region of the mother.[16]

E coli was associated with natural delivery. Children delivered naturally mainly acquire *E coli* through transmission from their mother, whereas the primary *E coli* source for children born by means of cesarean section is hospital strains.[17] Children delivered naturally tend to have shorter stays in the hospital, and our study demonstrates that especially children born by means of emergency cesarean section were more often admitted to the neonatal ward. *Citrobacter*, *Klebsiella*, *Enterobacter*, and *Enterococcus* species were more often identified among children born by means of cesarean section. These bacteria are often resistant to several antibiotics, and a selection is seen in hospitals.[18] *Enterococcus* and *Clostridium* species are both very resistant to hygienic measures,[19,20] and our higher colonization rate among cesarean section–born children might be explained by a thriving of these species in the otherwise highly hygienic hospital wards.

As presented in Fig 2, we observed equal *Staphylococcus* species colonization rates in the fecal samples of natural-born and elective cesarean section–delivered children, whereas children born by mean of emergency cesarean section had higher rates of *S aureus* and lower rates of *Staphylococcus epidermidis* colonization early in life. This could be a consequence of the first extrauterine environmental encounter and hospital stay. *Staphylococcus* (especially *S epidermidis*) was the most abundant genus in vaginal samples from mothers during pregnancy,[21,22] and thereby colonization in naturally delivered children could be a result of vertical transmission during birth. We observed a high

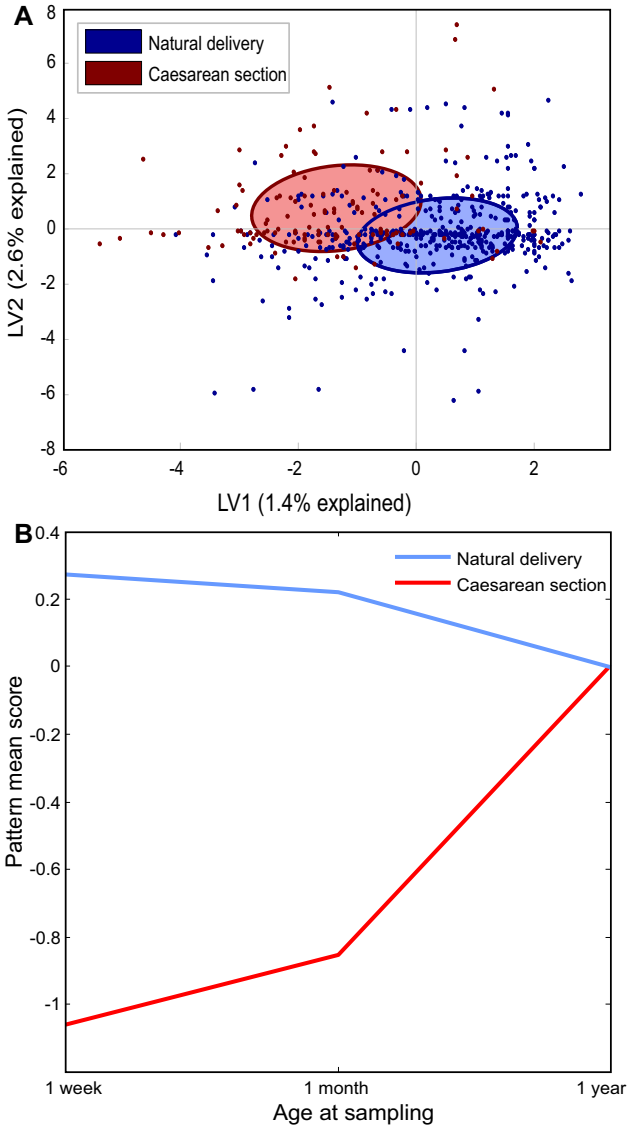

FIG 4. Three-way PLS model describing fecal colonization patterns and time differences for all participants. **A,** Score plot: each point corresponds to 1 child, and colors indicate type of delivery method. **B,** Pattern mean score of LV1 over time for children born by means of cesarean section and natural delivery.

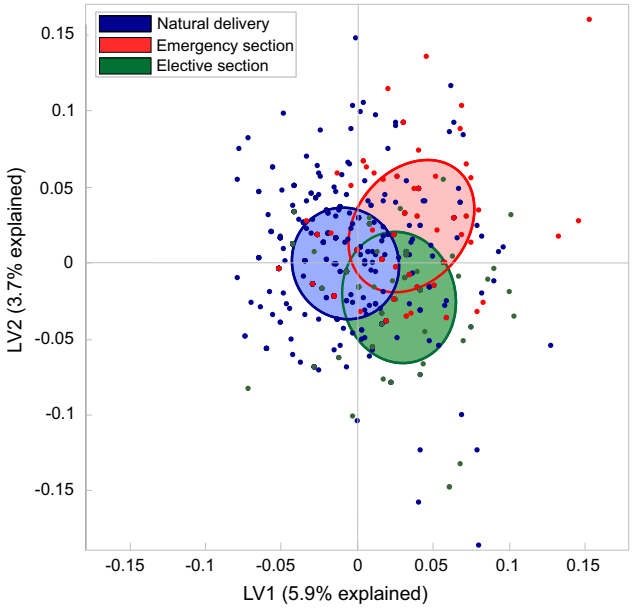

FIG 5. Score plot from PLS model on fecal colonization at age 1 week. Each point corresponds to 1 child, and colors indicate type of delivery method. The distribution of the specific delivery method is shown as ellipses with coverage of 1 SD in 2 dimensions. LV1 explains 5.9% and LV2 explains 3.7% of total variation in bacterial colonization.

initial rate of *Staphylococcus* species colonization, with a tendency toward a decrease over time to age 1 year, which is in line with previous observations.[23] A shift toward earlier colonization by facultative anaerobic *Staphylococcus* species has been observed in recent decades,[24,25] which in part could be due to increased breast-feeding of infants[26] or lack of competition from typical anaerobic gut microbiota.

Delivery by means of cesarean section differs from natural delivery with respect to several factors, including prophylactic intrapartum antibiotics administered to the mother, physiologic effects on the child and hospital stay, and environment after birth.[27] We adjusted all results for gestational age, parity, hospitalization, and duration of exclusive breast-feeding to account for these differences. Furthermore, we performed an analysis stratified for intrapartum antibiotics to study whether the observed effects were actually caused by the cesarean section itself or by prophylactic intrapartum antibiotics, a treatment recommended in all births by means of cesarean section. The number of children born naturally in this stratum was limited (13%); however, we still found cesarean section positively associated with *E faecalis* and *S aureus* and negatively associated with *E coli*. The trends for the other species were much like in the primary analysis, although not significant, which could indicate a lack of statistical power. However, increased *K oxytoca* and *S haemolyticus* colonization was found after treatment with intrapartum antibiotics when stratifying for natural delivery, suggesting the antibiotics as causal for part of the observed effects.

We did not find significant associations between delivery method and hypopharyngeal cultures at 1 week; however, there were differences from 1 month of age. *E coli* was positively associated with vaginal birth at 1 month but only borderline significant at 1 week. However, this could be a true correlation limited by power because *E coli* was identified in much fewer samples from the airways than from the gut, and the results match findings from the fecal samples. The lack of associations between cesarean section and the airway microbiota might indicate that opposite to the gut, the typical airway colonizers mainly derive from other sources than the birth setting or perhaps because very early differences have already disappeared at age 1 week.

Finally, it was surprising that only a few species were positively associated with natural birth because lack of microbial transmission during cesarean section has been speculated to be causative for the increased prevalence of asthma and allergy,[1,28-30] as well as other immune-mediated diseases,[1] in cesarean section–born children, although some studies suggest mechanisms other than microbial to be causative.[31] If a causal

relation exists between cesarean section, early-life microbial derangements, and later disease, our study suggests that the effects happen within the first year of life, because we found no colonization differences at age 1 year. However, if a causal effect does not exist, we might simply observe the colonization reaching an equilibrium over time independent of disease effects. Most anaerobic bacteria cannot be cultured. In particular, taxa from the phylum Bacteroidetes, which we did not identify in cultures, are among the most dominant in the mature gut composition, and it has been shown that cesarean section and intrapartum antibiotics might affect these up to age 1 year.[11] We speculate that our results, to some extent, could be caused by a hidden richer anaerobic abundance in naturally delivered children, protecting these children against opportunistic colonizers. This might represent a pathway affecting disease because microbial composition of the gut has been suggested to be a mediator of chronic low-grade inflammation.[32,33] Only a few studies have associated bacterial gut composition with disease risk; however, these have focused on effects of overall diversity[7] or single species (eg, increased risk of asthma, allergy, and eczema after *Clostridium* colonization),[34] which might be overly simplified models for complex mechanisms. Furthermore, we can speculate that cesarean section also affects the composition of early-life fungal and viral composition in the gut and airways, which future studies can address.

Conclusion

Delivery by means of cesarean section was associated with affected colonization patterns of the neonatal gut but not of the airways. The differences in colonization patterns were most pronounced in the first month of life and influenced by type of cesarean section. We speculate that microbial derangements, as indicated in our study, might demonstrate a possible link between delivery by means of cesarean section and immune-mediated disease.

We thank the children and parents participating in the $COPSAC_{2010}$ cohort, as well as the COPSAC study team, and technician Berit Jensen of the Statens Serum Institut for technical assistance.

Key Messages

- **Caesarean section is a shared risk factor for several immune-mediated diseases in the child, and in this setting the initial colonization mainly originates from the early environmental contacts and maternal skin rather than the birth canal and anogenital region of the mother.**
- **We demonstrate microbial colonization differences between naturally delivered and cesarean section–delivered children and furthermore between children delivered by means of different types of cesarean section, which might demonstrate a possible link between delivery by means of cesarean section and immune-mediated disease.**
- **If causal mechanisms between microbial derangements after delivery by means of cesarean section and immune-mediated disease are established, the findings might lay the groundwork for future microbial manipulation in early life.**

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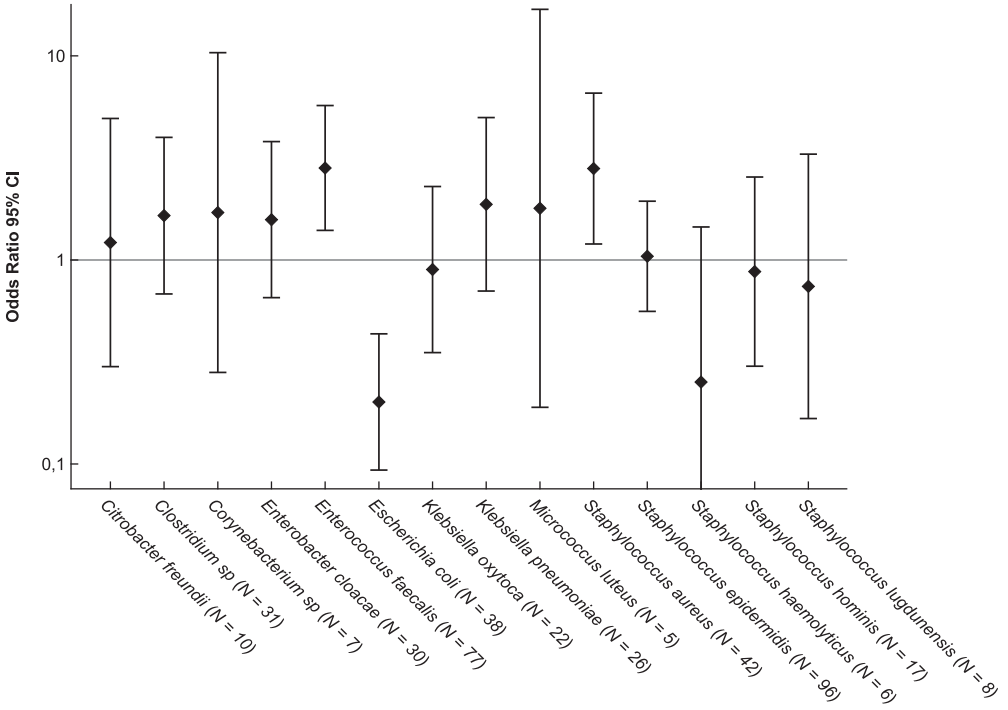

FIG E1. Associations between delivery by means of cesarean section and fecal colonization at age 1 week stratified for intrapartum antibiotics. A cutoff value of greater than 2% of children colonized was used for a species to be represented in the analyses. All analyses were adjusted for gestational age, parity, hospitalization, and duration of exclusive breast-feeding.

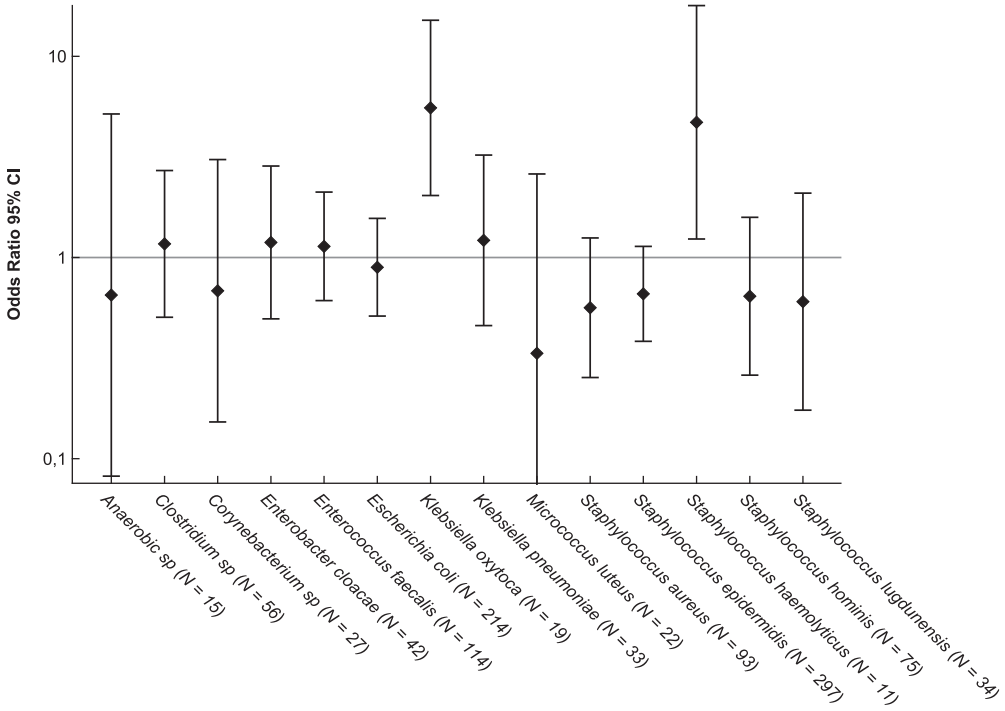

FIG E2. Associations between intrapartum antibiotics and fecal colonization at age 1 week stratified for natural delivery. A cutoff value of greater than 2% of children colonized was used for a species to be represented in the analyses. All analyses were adjusted for gestational age, parity, hospitalization, and duration of exclusive breast-feeding.