Cesarean Delivery May Affect the Early Biodiversity of Intestinal Bacteria\textsuperscript{1,2}

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Abstract

The gastrointestinal tract of neonates becomes colonized immediately after birth with environmental microorganisms, mainly from the mother; strong evidence suggests that the early composition of the microbiota of neonates plays an important role for the postnatal development of the immune system. The present study was designed to evaluate by means of a molecular biology approach the relation between the intestinal ecosystem of the newborn and the mode of delivery. The intestinal bacterial composition on d 3 of life was investigated in 23 infants born by vaginal delivery and in 23 infants delivered by cesarean section. PCR-denaturing gradient gel electrophoresis and PCR-temperature gradient gel electrophoresis have been utilized, together with the specific amplifications for 10 Bifidobacterium species, 3 Ruminococcus species, and Bacteroides. The intestinal microbiota of neonates delivered by cesarean delivery appears to be less diverse, in terms of bacteria species, than the microbiota of vaginally delivered infants. The intestinal microbiota after cesarean delivery is characterized by an absence of Bifidobacteria species. Vaginally delivered neonates, even if they showed individual microbial profiles, were characterized by predominant groups such as B. longum and B. catenulatum. Our data demonstrate that the mode of delivery has a deep impact on the composition of the intestinal microbiota at the very beginning of human life. This study opens the path to further investigations to confirm the link between microbiota composition and immune system development and to identify tools for the modulation of the intestinal microbiota of cesarean-delivered neonates. Additionally, we underline the importance of adequate microbiological tools used to support clinically relevant trials, if intestinal microbiota is considered as a study outcome. J. Nutr. 138: 1796S–1800S, 2008.

Introduction

The intestinal microbiota composition of the neonate has not been clearly defined yet, as many bacterial species living in the gut are unculturable under laboratory conditions. An alternative approach for microbial identification is provided by culture-independent techniques such as the exploitation, by means of molecular biology techniques, of polymorphisms of genes encoding for bacterial 16S rRNA\textsuperscript{(1)}. These techniques have been applied to evaluate the gut microbiota composition of adults and infants, but a lack of knowledge still exists on the comparison of gut microbiota of neonates delivered by cesarean section to the microbiota of naturally delivered babies.

The gastrointestinal tract of newborns is sterile, but it becomes colonized immediately after birth with organisms from the environment, mainly from the mother. During vaginal delivery, the contact with the vaginal and intestinal flora is an important source for the start of the infant's colonization\textsuperscript{(2)}. During cesarean delivery, direct contact of the mouth of the newborn with the vaginal and intestinal microbiota is absent, and environmental bacteria play an important role for infants' intestinal colonization. Some authors have suggested that the composition of the very first human microbiota could have long-lasting effects, up to months\textsuperscript{(3)} or even years\textsuperscript{(4)}.

There is accumulating evidence that intestinal bacteria play an important role in the postnatal development of the immune system\textsuperscript{(5,6)}. Thus, if the intestinal flora develops differently depending on the mode of delivery, the postnatal development of the immune system might also be different. Available epidemiological data show that atopic diseases appear more often in infants after cesarean delivery than after vaginal delivery\textsuperscript{(7–10)}. The composition of enteric microbiota in early days of life seems therefore to be a very important factor for achieving and maintaining good health in the years to come. It follows that it is fundamental to identify more thoroughly the intestinal ecosystem of the newborn.

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Subjects, Materials, and Methods

Forty-six term infants born in October 2003 at the Guglielmo da Saliceto Hospital, Piacenza, Italy were eligible for the study (Table 1).

DNA analysis

Bacterial DNA was extracted using PSP Spin Stool DNA kit (Invitek, Berlin).

The differentiation of the amplicons obtained through the amplification of bacterial DNA was achieved by DGGE and TGGE techniques.

**DGGE and TGGE analysis with PCR amplification.** To amplify V6-V8 regions on bacterial DNA, we utilized U968-GC-f and L1401-r primers (11). To amplify specific regions for *Bifidobacterium* spp., *Ruminococcus* spp., and *Bacteroides* adolescentis, all playing a very relevant physiological role in the intestinal ecosystem of the newborn (11).

**DGGE analysis of amplified samples.** The fragments obtained by PCR as reported were separated through DGGE (Biorad) as described by Favier et al. (11), using a 40–50% gradient for separating fragments obtained by amplification of regions V6-V8 and a 45–55% gradient for fragments obtained with primers for *Bifidobacterium*. The electroph-
retic run was carried out for 16 h at 85 V. DNA fragments, separated by DGGE, were extracted from the electrophoretic gel and identified by sequencing and successive alignment with sequences of the GenBank (www.ncbi.nlm.nih.gov/BLAST/) database (13).

**TGGE analysis of amplified samples.** The fragments coding for regions V6-V8 on bacterial DNA obtained by PCR have been separated by TGGE as described by Satokari et al. (14), with a 37°C to 60°C temperature gradient, 0.5 ramp, and an 18-h run at 85 V.

**PCR amplification with species-specific primers.** Some microbial species considered to be very important in the neonatal intestinal microbiota have been investigated by PCR with species-specific primers (Table 2) (14–16).

The presence of bacteria belonging to the genus *Bifidobacterium*, with the exception of *B. catenulatum* and *B. lactis*, was also investigated by species-specific multiplex PCR. Species-specific amplification of *B. lactis* has been carried out according to Ventura et al. (17). *Bacteroides fragilis* group, *R. obeum* group, *R. callidus* and *R. bromii* PCR-based detection was carried out according to Yamashita et al. (15) and Wang et al. (16).

**Results and Discussion**

DGGE profiles obtained by means of universal primers on fecal samples of newborns delivered either by cesarean section or vaginally were usually characterized by few bands, most of which were in common with all the other subjects in each of the two groups considered (Fig. 1, Fig. 2). In particular, bands corresponding to *Klebsiella oxytoca* and *Bifidobacterium pseudolongum* seem to be present in all the lines obtained from the feces of vaginally delivered infants.

Slight differences have been found, on the other hand, with respect to *E. coli* (Fig. 2). This microbial group, in fact, was found in 9 of 23 (39.1%) spontaneously delivered newborns, whereas overlapping bands were founds only in 2 of 23 (8.7%) cesarean-delivered newborns (Fig. 1).

DGGE analysis, carried out with *Bifidobacterium*-specific primers, revealed the presence of this genus in 13 of 23 (56.5%) samples derived from vaginally delivered newborns but in none of the samples obtained from newborns delivered by cesarean section.

TGGE analysis, carried out with universal primers on fecal samples of both groups of subjects, showed greater inter- and intragroup profile variations, particularly in the group of babies born by vaginal delivery (Fig. 3). In contrast, those born by cesarean section displayed more constant TGGE profiles (Fig. 4).

PCR analysis with *Bifidobacterium* species-specific primers showed that naturally delivered infants had a large number of bifidobacterial species, whereas in cesarean-delivered babies, only 2 samples (8.7%) gave positive results, 1 for *B. longum* and the other for *B. gallicum*.

With regard to the qualitative pattern, spontaneously delivered babies show greater differences concerning investigated *Bifidobacterium* species; the most represented are *Bifidobacterium catenulatum* group and *Bifidobacterium longum*; *B. breve* was detected in 52.2% of samples, *B. bifidum* in 39.1%; *B. infantis*, *B. gallicum*, and *B. adolescentis* species were more scarce (17.4, 4.3, and 21.7%, respectively). In all babies enrolled, microorganisms belonging to *Ruminococcus* species are absent, and *Bacteroides* has been found in 8.7% of spontaneously delivered babies only.

Species-specific PCR analysis shows the presence of *B. catenulatum* and *B. longum* in 43.5% of vaginally delivered babies, sometimes accompanied by the presence of *B. breve*, *B. bifidum*, or *B. adolescentis*.
Specifically, in this group of newborns, 17.4% are colonized at the same time by *B. breve*, *B. bifidum*, the *B. catenulatum* group, and *B. longum*, whereas *B. breve*, the *B. catenulatum* group, and *B. longum* are present in 8.7% of babies.

Although some studies (11) demonstrate that bifidobacteria appear after d 2 or 3 of life and usually dominate after the first 2 wk of life, because of feeding-related differences in the colonization time, little is known concerning the first 3 d after birth. Our study demonstrates that the newborn’s intestinal microbiota, with respect to presence of bacteria, is strongly influenced, within 3 d of life, by the mode of delivery. From a qualitative point of view, the intestinal flora of cesarean- and vaginally delivered infants appears to be very different. The intestinal flora of infants by cesarean delivery is characterized by a substantial absence of *Bifidobacteria* species. Infants by vaginal delivery show subject-specific microbial profiles, although predominant groups such as *B. longum* and *B. catenulatum* group could be identified.

If the intestinal colonization could have health effects, the clear difference in the intestinal flora between vaginally delivered and cesarean-delivered infants would be of clinical relevance. Because only 2 infants born by cesarean delivery had not yet received a detectable amount of breast milk within 3 d of life, at least at this early age, the kind of nutrition seems to have a less crucial impact in modulating intestinal microbiota composition. In addition, we point out the great importance of the methodological aspects for determining intestinal microbiota in clinical trials, if intestinal microbiota composition is to be considered a measure of postnatal adaptation.

In summary, our data indicate that the early stage of the bacterial intestinal colonization in infants born by cesarean delivery is altered from that of infants born vaginally, with no or little influence of the type of feeding. Additionally, our results emphasize the importance of the methodological aspects for determining intestinal microbiota composition in clinical trials, if intestinal microbiota composition is to be considered a measure of postnatal adaptation.

Other articles in this supplement include references (25–34).

**Literature Cited**


