

EARLY COLONIZATION IN CAESAREAN CHILDREN

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GUT MICROBIOTA

Many diseases are increasing among children and young adults in the western world and changes in definition and increased detection rate cannot explain the increases in full. Environmental factors must be behind the increase as changes in genes evolve very slowly. Changes in human gut microbiota may be one of several environmental factors in part responsible.

A complex society of microbes exists in the human gut, and the collective genome of this microbiota greatly exceeds the number of genes in the human genome (Backhed et al., 2005) and we have come to understand its crucial role in host biology and human health. It may be considered as a separate organ with complex interplays with the immune system and the brain in particular.

The composition and functionality of gut microbiota in early life is of special interest due to several reasons. Firstly as a determinant for the subsequent adult-like microbiota, since the colonization of the gut is a dynamic process in which the selection of microbes will in part depend on the processes that have already taken place (Midtvedt et al., 1988; Midtvedt and Midtvedt, 1992; Hooper et al., 1999; Thompson et al., 2008; Eggesbø et al., 2011). In a study on 85 Norwegian new-borns multiple associations were observed between the presence of

certain microbes or microbial groups at four days and the concentrations of microbes at four months, evaluated by the signalling by specific probes developed for that study (Eggesbø et al., 2011). Interestingly, the 23 microbes and/or microbial groups studied had very varying effect on the future colonization process, as evaluated by their associations to gut microbiota at four months (Eggesbø et al., 2011). Only rarely were the associations between the same microbes or microbial groups, with one exception: infants with initially high concentrations of *Bifidobacterium* were still characterized by higher concentrations of *Bifidobacterium* at four months of age. Also interesting was the finding that four days after birth the probe labelled *Lachnospiracea incertae sedis*, which detected species belonging to *Ruminococcus*, showed the highest number of associations with later microbiota (at four months).

Secondly, the composition of infant microbiota is important in its own right due to the presence of developmental windows in young age. Experimental animal studies in germ-free environments have demonstrated time-dependent windows that rely on microbial stimuli from the gut, with persistent dysfunction when microbial exposure is delayed beyond a certain age (Desbonnet et al., 2008; Forsythe et al.,

2010; Neufeld et al., 2011). Reducing microbial load in early life with antibiotics also induces lasting phenotypic differences in mice (Cox et al., 2014). However, very few studies in new-

borns yet exist. One study from our group aimed at identifying critical windows for exposure, by developing a novel method for longitudinal data (White et al., 2013).

ALTERATIONS IN INTESTINAL MICROBIOTA IN MODERN TIMES

There is increasing evidence to support that the composition of early microbial colonization communities have indeed changed during the last 50-100 years in western societies. The flora reported in western babies at the beginning of the 19th century, had amongst others a high count of *Bifidobacteria* and *E. coli* (Escherich, 1885; Gareau et al., 1959; Nelson and Mata, 1970; Simhon et al., 1982). While *E. coli* previously was found in all children by day 3 after birth, and a high turnover is still observed in infants from developing countries (Gareau et al., 1959; Mata et al., 1972; Adlerberth et al., 1998), it can no longer be detected in an increasing proportion of Swedish children (Kühn et al., 1986; Adlerberth et al., 1991; Nowrouzian et al., 2003; Adlerberth et al., 2006). In contrast, a variety of hospital acquired organisms, in particular *Staphylococcus sp.* and *Streptococcus sp.* seems to have partially replaced these organisms in western babies (Lindberg et al., 2000). *Staphylococcus sp.* were seldom encountered in Western infants up to the 1970s (McAllister et al., 1974; Bullen et al., 1976; Stark and Lee; 1982). In the 1980s, *S. epidermidis* was isolated from 30 to 70% of one-week-old Swedish infants (Lundequist et al., 1985; Bennet et al., 1991).

Streptococcus sp. colonization has been associated with lack of colonization by *Bifidobacteria* genera (Eggesbø et al., 2011). In contrast, the presence of Enterobacteriaceae 1, and *Bacteroides fragilis* after birth was significantly associated with a reduction in

opportunistic microbes at four months, specifically *Staphylococcus sp.*, indicating that the early presence of specific microbes may be important for development towards a more natural gut microbiota. If they are lacking, an initial colonization by opportunistic bacteria will occur and may also be maintained.

One of the limitations of old studies is that they had to rely on culture dependent methods, which limited the detection of anaerobic species in particular, and our knowledge of the natural composition of gut microbiota a century ago. Recent studies, on the other hand, do characterize gut microbiota by means of culture independent methods, however commonly fail to take into account factors that may greatly alter the composition of gut microbiota. Therefore the data are not representative of a normal gut microbiota in infants. One study from our group (Eggesbø et al., 2011) restricted the study population to:

- babies delivered vaginally by term;
- exclusively breastfed for at least one month;
- partially breastfed up to four months;
- no intensive care unit treatment;
- mothers who have not used antibiotics the month preceding delivery nor while breastfeeding (e.g. no antibiotic during the four months after birth) to limit microbiota disrupting factors to the highest degree possible.

This study, based on the NoMIC cohort (Eggesbø et al., 2011), used 23 specific

probes and confirmed that, even in infants with minimal medical interventions, the most common microbe colonizing the new born gut is *Staphylococcus sp.* This indicates that the changes in gut microbiota in the last 40 years may have occurred in the whole population. Exposures which affect gut microbiota, such as conservatives in food, traces of antibiotics in food and an ever-increasing sterile milieu, are ubiquitously present and may be hard to avoid in the western world.

In contrast, *Bifidobacteria* genera were common as expected and dominated in these breastfed infants at four months of age (Eggesbø et al., 2011). Moreover, the detection rate of *E. coli* was higher in this study (70% at day four) than among unselected populations of Scandinavian children, and in-

creasing towards four months.

Interestingly, lack of *E. coli* was associated with rapid growth, a risk factor for later obesity, using the same 23 probes (White et al., 2013). This study reported on a novel method for identifying exposure windows. The continued presence or absence of *E. coli*, at 3 age points during the first month of life, was identified as being associated with infant growth pattern. In contrast presence or absence of *E. coli* later on was not associated with infant growth, indicating a critical window for exposure. This finding is supported by more recent studies indicating that *E. coli* plays a key role in regulating food intake (Breton et al., 2016) However, *E. coli* may serve as a marker for a less disrupted early gut microbiota.

CAESAREAN SECTION

Many factors tied to our modern lifestyle, such as hygienic measures, hospital delivery, use of antibiotics in the perinatal period and use of neonatal intensive care units, likely play a role in the shift observed in infant gut microbiota and neonatal intensive care. However, the single most important factor may be the increase in caesarean section (C-section) deliveries.

A sharp increase in C-section has occurred in most western countries. In the US a ten-fold increase in C-section rates was reported in the period from 1937 to 2005 and 30% are now delivered by a C-section (Ecker and Frigoletto, 2007). In Norway, there has been a seven-fold increase in C-section rates between the 1970s and 2001 (from 2 to 15%) (Häger et al., 2006). The reasons for this increase are complex and involve changes in recommendations, for instance for breech deliveries, as well as increase in the prevalence of high-

risk pregnancies, such as obesity and multiple gestations. However, also changes in willingness to accept pain, is changing and many C-section are unnecessary from a strict medical point of view (Ecker and Frigoletto, 2007).

The increase in C-section rates has obvious medical consequences. Focus so far has mainly been on the immediate operative risks of a C-section as well as on the increased risk of adverse outcomes in subsequent pregnancies (Duncan and Doyle, 1937; Liu et al., 2007). However, C-section may have long-term consequences of a more subtle nature, tied to differences in the endocrine milieu during delivery, or to lack of input of microbes of maternal origin and these factors have so far been less acknowledged (Steer and Modi, 2009; Hyde et al., 2010).

There is increasing support for multiple long-term effects in C-section delivered children (Cardwell et al.,

2008; *Koplin et al., 2008; Thavagnanam et al., 2008; Amiri et al., 2012; Keag et al., 2014*). A recent systematic review concluded that children delivered by C-section have an increased risk of asthma, wheeze and obesity during childhood (*Keag et al., 2014*). Many papers, a meta-analysis and a review, support an association between C-section and food allergy (*Bager et al., 2008; Koplin et al., 2008*) where the finding of a stronger effect size among the infants with family risk supports a causal association (*Eggesbø et al., 2003, 2005*). Another meta-analysis finds support for an association between C-section and diabetes Type 1 (*Cardwell et al., 2008*). Associations have also been reported between C-section and childhood leukaemia (*Kaye et al., 1991; Cnattingius et al., 1995*), ADHD (*Amiri et al., 2012*), while a meta-analysis found no support for an association between testicular cancer and C-section (*Cook et al., 2009*). The underlying reason for the observed associations is not yet fully understood, and because C-section deliveries are associated with multiple pathological

conditions in mother and child, the causes may be multifactorial. Studies indicate that early gut microbiota plays a key role in the regulation of the immune system and that babies delivered by C-section have less robust regulatory T-cell suppressive functions (*Ly et al., 2006*). Regulatory T-cells play a key function in the regulation of the immune system, and less robust regulatory T-cell function could result in immune-related diseases such as allergies or autoimmune diseases. Also, exposure to microbial components during birth has been shown to play a role for developing gut-epithelial tolerance to microbes, thus facilitating the subsequent colonization process (*Lotz et al., 2006*). Yet, there are other birth related processes, such as the natural peak in stress hormones and the compression of the brain in the birth canal, which are missed in a C-section, which also may play a role for the differential fate of C-section children. The hypothesis currently probably gaining most support is that it may be due to long-term effects of an altered early gut microbiota (GM).

THE EFFECT OF CAESAREAN SECTION ON GUT MICROBIOTA

Mammals have co-evolved with the microbial world throughout evolution. The foetus is to some degree exposed to maternal microbes already in the womb (*Aagaard et al., 2014*). Then a natural birth ensures massive transfer of commensals microbes from the mother to the child.

In C-section, exposure to bacteria in the maternal birth canal is bypassed, resulting in a delayed and highly disrupted infant GM, for instance resembling human skin rather than the mother's vaginal microbiota (*Dominquez-Bello et al., 2010*). This has been documented in a number of studies go-

ing back a century, using culture dependent methods as well as the more recent culture independent methods based on microbial DNA. They show that babies delivered by a caesarean section have a delayed and different colonization, with lower colonization rates of *Bifidobacterium* and *Bacteroides*, while rates of Clostridia and *Staphylococcus* are higher among caesarean delivered children (*Bennet and Nord, 1987; Neut et al., 1987; Grönlund et al., 1999a, 1999b; Salminen et al., 2004; Penders et al., 2006; Biasucci et al., 2008, 2010; Dominquez-Bello et al., 2010*).

Also a differential pattern of Enterobacteriaceae is found: *E. coli*, the obligate colonizer in historical data, is less common in C-section infants, while other Enterobacteriaceae are more commonly found (Adlerberth et al., 2006). A recent systematic review of seven relevant papers concluded that C-section babies had lower abundance and diversity of the phyla Actinobacteria and Bacteroidetes, and higher abundance and diversity of the phylum Firmicutes from birth to 3 months. *Bifidobacterium*, and *Bacteroides* genera were significantly more frequent in vaginally delivered infants compared with C-section delivered (Rutayisire et al., 2016). Also noteworthy, the gut microbiota of infants delivered by C-section showed significantly less resemblance to their mothers (Bäckhed et al., 2015). This mismatch may be of importance with regard to the foeto-maternal immune cross talk.

With time the C-section babies gut microbiota normalizes and resembles that of the natural delivered babies. The systematic review concluded that by the age of six months the C-section ba-

bies had a similar gut microbiota composition as the vaginal delivered babies (Rutayisire et al., 2016). However, one study reported that *Bacteroides* colonization was delayed by up to 1 year in caesarean section-delivered compared with vaginally delivered infants (Adlerberth et al., 2006), and another study suggests that the differential composition in babies delivered by C-section may be of long-lasting character and still evident at 7 years of age (Salminen et al., 2004). The discrepancy may be due to other factors associated with C-section but still varying across studies, such as gestational age, concurrent use of antibiotics and lack of breastfeeding. Yet, it is important to have a better understanding of the duration of the disruptions caused by C-section itself. Even if the gut microbiota is normalized with time, early disruptions may have long-term effects due to the presence of developmental windows that rely on microbial stimulus from the gut, which were mentioned above. If the disruptions are of short durations on the other hand, they may have no long-term effects.

GUT MICROBIOTA IN TERM, BREASTFED, C-SECTION INFANTS, NOT EXPOSED TO ANTIBIOTICS

It is thus unknown to what degree the observed associations between C-section and gut microbiota composition is influenced by concurrent antibiotic use, prematurity, and lack of breastfeeding, since no studies to our knowledge have categorized C-section deliveries according to these factors and studied them separately. For the purpose of the present paper we used the NoMic cohort in Norway, which consists of 524 new-borns and their mothers. Details of this study have previously been published (Eggesbø et al., 2011; White et al., 2013). We identified term babies,

with no concurrent infant use of antibiotics, exclusively breastfed first month and still partially breastfed at 4 months of age, based on maternal reports that were further validated with information in the Norwegian Medical Birth Registry. Altogether 147 infants were identified who fulfilled these criteria. They were further divided into two groups; C-section or normally delivered composition examined. Microbial species and groups have been identified at 4, 10, 30 and 120 days after birth in this study by 23 probes targeting the gene encoding ribosomal RNA

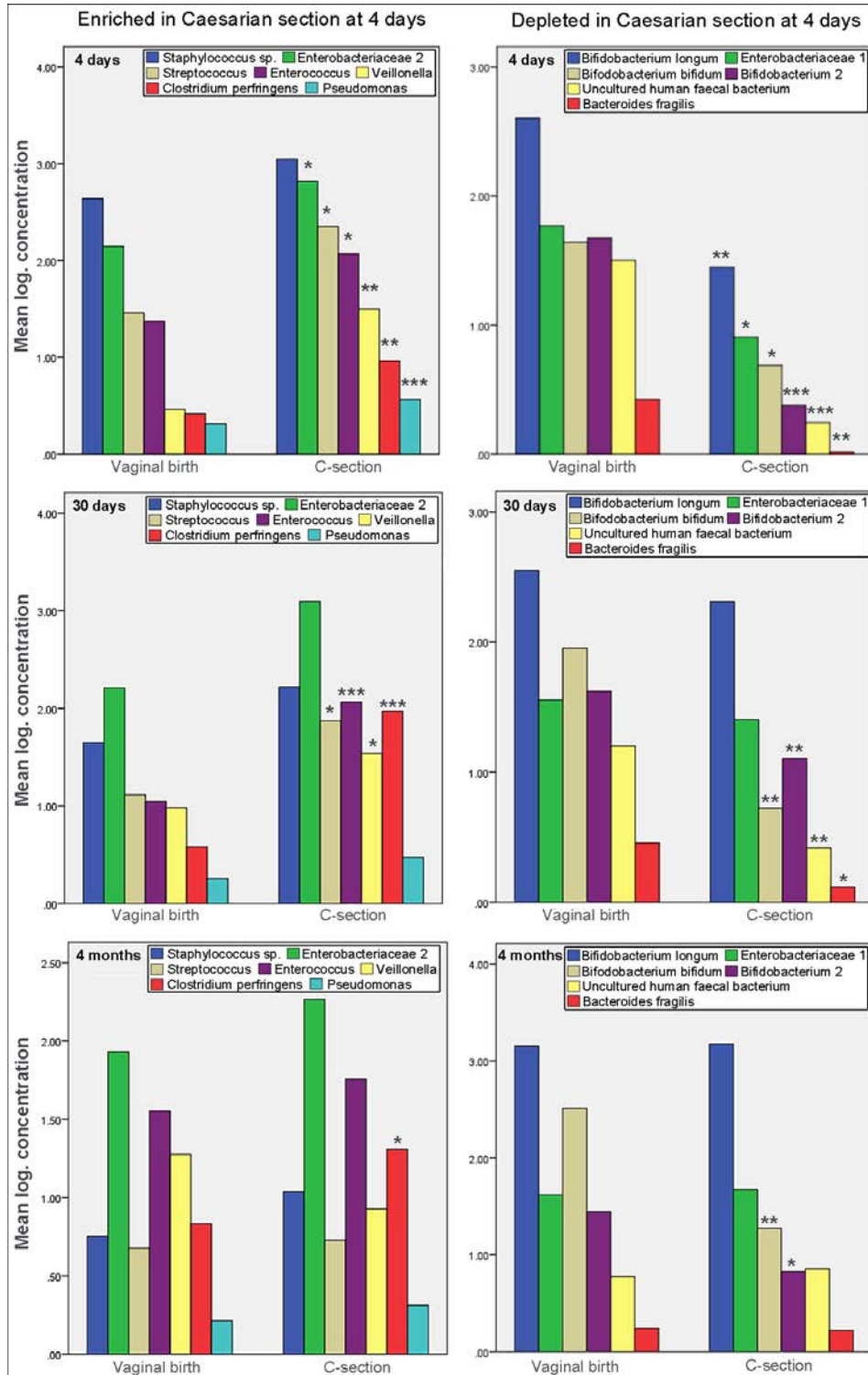


Figure 1: Differences in gut microbiota composition according to mode of delivery, as characterized by means of 22 specific groups (see Table 1) whereby the ones that were significant at day 4 are shown at all time points. *: $p < 0.05$, **: $p < 0.01$, and ***: $p < 0.001$.

(16S rRNA) which were developed based on faecal samples from ten children randomly selected among those who had been delivered by caesarean section, and ten children among those who had had a normal delivery in the NoMIC cohort, as previously described (Rudi et al., 2007) (Table 1). We observed marked differences in 13 out of the 23 probes signalling various species, with regard to mode of delivery, as shown in Figure 1. Note that signalling cannot be compared across microbial groups (e.g. cannot infer that there is more of microbe A than microbe B), but only across delivery mode and age (e.g. there is more of microbe A in C-section infants compared to vaginal delivery and this microbe increases with age). At 4 months the probes *Lachno_Inc_b120lnc* and

Lachnospira_a120lnc were additionally significantly different across groups. The differences were most marked at day 4, but still marked at one month. C-section delivered infants had significantly higher concentrations of *Streptococcus*, *Enterococcus*, *Veillonella*, *Clostridium perfringens* and *Pseudomonas*, while they had lower concentrations of *Bifidobacterium longum*, *Bifidobacterium bifidum* and *Bifidobacterium 2*. *Bacteroides fragilis* was barely detected in C-section infants and also uncultured human faecal bacterium, which represents bacteroides species, was marked lower. At 4 months the differences were less marked, but C-section infants still had more *Clostridium*, and significantly less *Bifidobacteria*.

CONCLUSION

In conclusion, epidemiological studies support an association between C-section and adverse health outcomes, and the underlying mechanism may be due to an altered gut microbiota in C-section delivered babies. Studies indicate that the altered gut microbiota goes beyond the first year of life, thus may be of consequence for developmental windows in early life depending on microbial input.

One of the limitations in current literature is that C-section children have not been characterized with regard to concomitant risk factors. Thus comparing C-section infants across studies is complicated by the fact that the infants may be characterized by highly varying associated risk factors; concomitant disease in mother and/or child, antibiotic use, and varying gestational ages, all factors known to affect

gut microbiota composition. We show in this paper that gut microbiota composition is affected by caesarean delivery independent of these factors. We did this by comparing children delivered naturally or by C-section, who were otherwise equal in terms of being breastfed, having received no antibiotics and all being term babies. We see marked differences up to one month of age, and less marked but still significant differences up to four months of age.

Yet, we still do not know which specific properties of the gut microbiota that may play a role in development of disease, nor the timing of the critical windows. Longitudinal cohort studies describing the functions of gut microbiota in children are needed to give further insight into this field.

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LITERATURE

- Aagaard, K., Ma, J., Antony, K.M., Ganu, R., Petrosino, J., and Versalovic, J.: The placenta harbors a unique microbiome. *Sci. Transl. Med.* 6, 237ra65 (2014).
- Adlerberth, I., Carlsson, B., de Man, P., Jalil, F., Khan, S.R., Larsson, P., Mellander, L., Svanborg, C., Wold, A.E., Hanson, L.A.: Intestinal colonization with Enterobacteriaceae in Pakistani and Swedish hospital-delivered infants. *Acta Paediatr. Scand.* 80, 602-610 (1991).
- Adlerberth, I., Jalil, F., Carlsson, B., Mellander, L., Hanson, L.A., Larsson, P., Khalil K, and Wold A.E.: High turnover rate of Escherichia coli strains in the intestinal flora of infants in Pakistan. *Epidemiol. Infect.* 121, 587-598 (1998).
- Adlerberth, I., Lindberg, E., Aberg, N., Hesselmar, B., Saalman, R., Strannegard, I.L., and Wold, A.E.: Reduced enterobacterial and increased staphylococcal colonization of the infantile bowel: an effect of hygienic lifestyle? *Pediatr. Res.* 59, 96-101 (2006).
- Amiri, S., Malek, A., Sadegfard, M., and Abdi, S.: Pregnancy-related maternal risk factors of attention-deficit hyperactivity disorder: a case-control study. *ISRN Pediatr.* 2012, 458064 (2012).
- Bäckhed, F., Ley, R.E., Sonnenburg, J.L., Peterson, D.A., AND Gordon, J.I.: Host-bacterial mutualism in the human intestine. *Science* 307, 1915-1920 (2005).
- Bäckhed, F., Roswall, J., Peng, Y., Feng, Q., Jia, H., Kovatcheva-Datchary, P., Li, Y., Xia, Y., Xie, H., Zhong, H., Khan, M.T., Zhang, J., Li, J., Xiao, L., Al-Aama, J., Zhang, D., Lee, Y.S., Kotowska, D., Colding, C., Tremaroli, V., Yin, Y., Bergman, S., Xu, X., Madsen, L., Kristiansen, K., Dahlgren, J., and Wang, J.: Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life. *Cell Host Microbe* 17, 690-703 (2015).
- Bager, P., Wohlfahrt, J., and Westergaard, T.: Caesarean delivery and risk of atopy and allergic disease: meta-analyses. *Clin. Exp. Allergy* 38, 634-642 (2008).
- Bennet, R. and Nord, C.E.: Development of the faecal anaerobic microflora after caesarean section and treatment with antibiotics in newborn infants. *Infection* 15, 332-336 (1987).
- Bennet, R., Eriksson, M.F., Tafari, N.F., and Nord, C.E.: Intestinal bacteria of newborn Ethiopian infants in relation to antibiotic treatment and colonisation by potentially pathogenic gram-negative bacteria. *Scand. J. Infect. Dis.* 23, 63-69 (1991).
- Biasucci, G., Benenati, B.F., Morelli, L.F., Bessi, E., and Boehm, G.: Cesarean delivery may affect the early biodiversity of intestinal bacteria. *J. Nutr.* 138, 1796S-1800S (2008).
- Biasucci, G., Rubini, M., Riboni, S., Morelli, L., Bessi, E., and Retetangos, C.: Mode of delivery affects the bacterial community in the newborn gut. *Early Hum. Dev.* 86, Suppl 1, 13-15 (2010).
- Breton, J., Tennoune, N., Lucas, N., Francois, M., Legrand, R., Jacquemot, J., Goichon, A., Guérin, C., Peltier, J., Pestel-Caron, M., Chan, P., Vaudry, D., do Rego, J.C., Liénard, F., Pénicaud, L., Fioramonti, X., Ebenezer, I.S., Hökfelt, T., Déchelotte, P., and Fetissov, S.O.: Gut Commensal E. coli Proteins Activate Host Satiety Pathways following Nutrient-Induced Bacterial Growth. *Cell Metab.* 23, 324-334 (2016).
- Bullen, C.L., Tearle, P.V., and Willis, A.T.: Bifidobacteria in the intestinal tract of infants: an in-vivo study. *J. Med. Microbiol.* 9, 325-333 (1976).

Table 1: Probe number, name, labelling sequence and target bacteria according to search in the Ribosomal database project (RDP) database is shown. The table also shows the number of hits in the Palmer clone library (3845 clone sequences).

Probe nr.	Probe name (bacteria)	Target bacteria ^a	Hits in the Palmer clone library (3845 clone sequences)	Labelling probes
1	<i>Enterococcus</i>	<i>Enterococcus</i> sp. (e.g. <i>E. faecium</i>)	44	TCA TCCCTTGACGGTATCTAA
2.1	<i>Lactobacillus</i> 1	<i>Lactobacillus</i> sp. (<i>L. gassery</i> , <i>L. helveticus</i> , <i>L. acidophilus</i> , <i>L. johnsonii</i> etc.)	50	GTCAAATAAAGGCCAGTTACTA
3.2	<i>Lactobacillus</i> 2a	<i>Lactobacillus</i> sp. (<i>L. casei</i> , <i>L. rhammosus</i> , <i>L. renaquilify</i> , <i>L. paracasei</i>)	1	CAGTTACTCTGCCGACCAIT
4.1	<i>Staphylococcus</i> sp.	<i>Staphylococcus</i> sp.	11	ACACATATGTTCTCCCTAAATA
5	<i>Streptococcus</i>	<i>Streptococcus</i> sp.	149	AGTGAGAGGTGGAAGTCA
6	<i>Clostridium perfringens</i>	<i>Clostridium</i> sp. (<i>C. perfringens</i>)	95	TCAAATTGGGTGCTGCATTC
7.5	<i>Veillonella</i> 1c	<i>Veillonella</i> sp.	49	GATTGGCAGTTTCCATCCCAT
8	<i>Lachnospiraceae</i> 1	<i>Lachnospiraceae</i> sp. (<i>Dorea</i> , <i>L. Incertea sedis</i>)	2	AGCTAGAGTGTCCGAGAGG
10	<i>Lachnospiraceae incertae sedis</i> 2	<i>Lachnospiraceae</i> sp. (<i>L. Incertea sedis</i>)	7	TATCAGCAGGAAGATAGTA
11	<i>Lachnospiraceae</i> 2	<i>Lachnospiraceae</i> sp. (<i>Dorea</i> , <i>L. Incertea sedis</i>)	1	AGTCAGGTACCGTCATTTCT
12.3	<i>Lachnospiraceae incertae sedis</i> 3	<i>Lachnospiraceae</i> sp. (<i>L. Incertea sedis</i>)	1	ACTGCTTTGGAAACTGCAGAT
15.1	<i>Pseudomonas</i> 1a	<i>Pseudomonas</i> sp.	0	GTCAAAACAGCAAAGTATTAATTTA
15.2	<i>Pseudomonas</i> 1b	<i>Pseudomonas</i> sp.	0	GTAGGGTGGTGGAAATTC
16.1	<i>Enterobacteriaceae</i> 1	<i>Enterobacteriaceae</i> (<i>Escherichia coli</i> , <i>Shigella</i>)	221	GAGCAAAGGTATTAACITTAATC
17	<i>Enterobacteriaceae</i> 2	<i>Enterobacteriaceae</i> (<i>Enterobacter</i> sp. (<i>Pantoea</i> sp), <i>Klebsilla</i> , <i>Erwina</i> , <i>Citrobacter</i> , <i>Bulliauxella</i>)	696	CGAAAACCTGGCAGGCTAGAGT
18	Gamma (γ)-Proteobacteria	(γ)-Proteobacteria (<i>Alteromonadales</i> , <i>Vibrionales</i> , <i>Aeromonadales</i> , <i>Enterobacteriales</i>)	1399	CCTGGACAAAAGACTGACGCT
19.1	<i>Varibaculum</i>	<i>Varibaculum</i> sp. (<i>V. cambriense</i>)	3	TTGAGTGTAGGGGTTGATTAG
22	<i>Bifidobacterium longum</i>	<i>Bifidobacterium</i> (<i>B. longum</i>)	114	GAGCAAAGGTGAGTAAAGTTTA
23	<i>Bifidobacterium bifidum</i>	<i>Bifidobacterium</i> (<i>B. bifidum</i>)	12	CCGAAAGGCTTGCTCCCAAA
24.3	<i>Bifidobacterium</i> 1	<i>Bifidobacterium</i> (<i>B. breve</i>)	38	CACTCAACACAAAAGTGCCTTG
25	<i>Bifidobacterium</i> 2	<i>Bifidobacterium</i> (<i>B. thermophilum</i> , <i>B. adolescentis</i>)	7	GCATTATTCGAAAAGGTACACTACCCCCGAAGGG
26	<i>Bacteroides fragilis</i>	<i>Bacteroides</i> sp. (<i>B. fragilis</i>)	1	GGCGCTAGCCTAACCCAG
27.2	Uncultured human fecal bacterium	<i>Bacteroides</i> sp.	179	ATGCA TACCCCGTTTGCA TGTA
16S universal	16S rDNA	Bacteria	3710 (96.5%)	CGTATTACCCGGCTGTGGCA

^aProbe Match search (using the labelling probe+ C) in the RDP database (<http://rdp.cme.msu.edu/problemmatch/search.jsp>). * One error allowed in search sequence.

- Cardwell, C.R., Stene, L.C., Joner, G., Cinek, O., Svensson, J., Goldacre, M.J., Parslow, R.C., Pozzilli, P., Brigis, G., Stoyanov, D., Urbonaite, B., Sipetić, S., Schober, E., Ionescu-Tirgoviste, C., Devoti, G., de Beaufort, C.E., Buschard, K., and Patterson, C.C.: Cesarean section is associated with an increased risk of childhood-onset type 1 diabetes mellitus: a meta-analysis of observational studies. *Diabetologia* 51, 726-735 (2008).
- Cnattingius, S., Zack, M.F., Ekblom, A.F., Gunnarskog, J.F., Linet, M.F., and Adami, H.O.: Prenatal and neonatal risk factors for childhood myeloid leukemia. *Cancer Epidemiol. Biomarkers Prev.* 4, 441-445 (1995).
- Cook, M.B., Akre, O., Forman, D., Madigan, M.P., Richiardi, L., and McGlynn, K.A.: A systematic review and meta-analysis of perinatal variables in relation to the risk of testicular cancer--experiences of the mother. *Int. J. Epidemiol.* 38, 1532-1542 (2009).
- Cox, L.M., Yamanishi, S., Sohn, J., Alekseyenko, A.V., Leung, J.M., Cho, I., Kim, S.G., Li, H., Gao, Z., Mahana, D., Zárate Rodriguez, J.G., Rogers, A.B., Robine, N., Loke, P., and Blaser, M.J.: Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. *Cell* 158, 705-721 (2014).
- Desbonnet, L., Garrett, L., Clarke, G., Bienenstock, J., and Dinan, T.G.: The probiotic *Bifidobacteria infantis*: An assessment of potential antidepressant properties in the rat. *J. Psychiatr. Res.* 43, 164-174 (2008).
- Dominguez-Bello, M.G., Costello, E.K., Contreras, M., Magris, M., Hidalgo, G., Fierer, N., and Knight, R.: Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc. Natl. Acad. Sci. USA* 107, 11971-11975 (2010).
- Duncan, C.J. and Doyle, J.B.: Cesarean section – A ten-year study of 703 cases at the Boston City Hospital. *N. Engl. J. Med.* 216, 1-5 (1937).
- Ecker, J.L. and Frigoletto, F.D. Jr.: Cesarean delivery and the risk-benefit calculus. *N. Engl. J. Med.* 356, 885-888 (2007).
- Eggesbø, M., Botten, G., Stigum, H., Nafstad, P., and Magnus, P.: Is delivery by cesarean section a risk factor for food allergy? *J. Allergy Clin. Immunol.* 112, 420-426 (2003).
- Eggesbø, M., Botten, G., Stigum, H., Samuelssen, S.O., Brunekreef, B., Magnus, P.: Cesarean delivery and cow milk allergy/intolerance. *Allergy* 60, 1172-1173 (2005).
- Eggesbø, M., Moen, B., Peddada, S., Baird, D., Rugtveit, J., Midtvedt, T., Bushel, P.R., Sekelja, M., and Rudi, K.: Development of gut microbiota in infants not exposed to medical interventions. *APMIS* 119, 17-35 (2011).
- Escherich, T.: Die darmbakterien des neugeborenen und säuglings. *Fortschr. Med.* 3, 515-522 and 547-554 (1885).
- Forsythe, P., Sudo, N., Dinan, T., Taylor, V.H., and Bienenstock, J.: Mood and gut feelings. *Brain Behav. Immun.* 24, 9-16 (2010).
- Gareau, F.E., Mackel, D.C., Boring, J.R., Payne, F.J., Hammett, F.L.: The acquisition of fecal flora by infants from their mothers during birth. *J. Pediatr.* 54, 313-318 (1959).
- Grönlund, M.M., Lehtonen, O.P., Eerola, E., and Kero, P.: Fecal microflora in healthy infants born by different methods of delivery: permanent changes in intestinal flora after cesarean delivery. *J. Pediatr. Gastroenterol. Nutr.* 28, 19-25 (1999a).
- Grönlund, M.M., Salminen, S., Mykkänen, H., Kero, P., and Lehtonen, O.P.: Development of intestinal bacterial enzymes in infants--relationship to mode of delivery and type of feeding. *APMIS* 107, 655-660 (1999b).
- Häger, R., Øian, P., Nilsen, S.T., Holm, H.A., and Berg, A.B.: The breakthrough series on Cesarean section. *Tidsskr. Nor. Laegeforen* 126, 173-175 (2006).
- Hooper, L.V., Xu, J., Falk, P.G., Midtvedt, T., and Gordon, J.I.: A molecular sensor that allows a gut commensal to control its nutrient foundation in a competitive ecosystem. *Proc. Natl. Acad. Sci. USA* 96, 9833-9838 (1999).

- Hyde, M.J., Griffin, J.L., Herrera, E., Byrne, C.D., Clarke, L., and Kemp, P.R.: Delivery by Caesarean section, rather than vaginal delivery, promotes hepatic steatosis in piglets. *Clin. Sci. (Lond.)* 118, 47-59 (2010).
- Kaye, S.A., Robison, L.L., Smithson, W.A., Gunderson, P.F., King, F.L., and Neglia, J.P.: Maternal reproductive history and birth characteristics in childhood acute lymphoblastic leukemia. *Cancer* 68, 1351-1355 (1991).
- Keag, O., Stock, S., and Norman, J.: Long-term childhood outcomes following caesarean section: Systematic review and meta-analysis. *Arch. Dis. Child. Fetal Neonatal Ed.* 99, Suppl 1, A8-A9 (2014).
- Koplin, J., Allen, K., Gurrin, L., Osborne, N., Tang, M.L., and Dharmage, S.: Is caesarean delivery associated with sensitization to food allergens and IgE-mediated food allergy: a systematic review. *Pediatr. Allergy Immunol.* 19, 682-687 (2008).
- Kühn I, Tullus K, Möllby R.: Colonization and persistence of *Escherichia coli* phenotypes in the intestines of children aged 0 to 18 months. *Infection* 14, 7-12 (1986).
- Lindberg, E., Nowrouzian, F., Adlerberth, I., and Wold, A.E.: Long-time persistence of superantigen-producing *Staphylococcus aureus* strains in the intestinal microflora of healthy infants. *Pediatr. Res.* 48, 741-747 (2000).
- Liu, S., Liston, R.M., Joseph, K.S., Heaman, M., Sauve, R., and Kramer, M.S.: Maternal Health Study Group of the Canadian Perinatal Surveillance System: Maternal mortality and severe morbidity associated with low-risk planned cesarean delivery versus planned vaginal delivery at term. *CMAJ* 176, 455-460 (2007).
- Lotz, M., Gütle, D., Walther, S., Ménard, S., Bogdan, C., and Hornef, M.W.: Postnatal acquisition of endotoxin tolerance in intestinal epithelial cells. *J. Exp. Med.* 203, 973-984 (2006).
- Lundequist, B., Nord, C.E., and Winberg, J.: The composition of the faecal microflora in breastfed and bottle fed infants from birth to eight weeks. *Acta Paediatr. Scand.* 74, 45-51 (1985).
- Ly, N.P., Ruiz-Perez, B., Onderdonk, A.B., Tzianabos, A.O., Litonjua, A.A., Liang, C., Laskey, D., Delaney, M.L., DuBois, A.M., Levy, H., Gold, D.R., Ryan, L.M., Weiss, S.T., and Celedón, J.C.: Mode of delivery and cord blood cytokines: a birth cohort study. *Clin. Mol. Allergy* 4, 13 (2006).
- Mata, L.J., Mejicanos, M.L., Jimenez, F.: Studies on the indigenous gastrointestinal flora of Guatemalan children. *Am. J. Clin. Nutr.* 25, 1380-1390 (1972).
- McAllister, T.A., Givan, J.F., Black, A.F., Turner, M.J., Kerr, M.M., Hutchison, J.H.: The natural history of bacterial colonization of the newborn in a maternity hospital. *I. Scott. Med. J.* 19, 119-124 (1974).
- Midtvedt, A.C. and Midtvedt, T.: Production of short chain fatty acids by the intestinal microflora during the first 2 years of human life. *J. Pediatr. Gastroenterol. Nutr.* 15, 395-403 (1992).
- Midtvedt, A.C., Carlstedt-Duke, B.F., Norin, K.E. FAU, Saxerholt HF, and Midtvedt T.: Development of five metabolic activities associated with the intestinal microflora of healthy infants. *J. Pediatr. Gastroenterol. Nutr.* 7, 559-567 (1988).
- Nelson, D.P. and Mata, L.J.: Bacterial flora associated with the human gastrointestinal mucosa. *Gastroenterology* 58, 56-61 (1970).
- Neufeld, K.A., Kang, N., Bienenstock, J., and Foster, J.A.: Effects of intestinal microbiota on anxiety-like behavior. *Commun. Integr. Biol.* 4, 492-494 (2011).
- Neut, C., Bezirtzoglou, E., Romond, C., Beerens, H., Delcroix, M., and Noel, A.M.: Bacterial colonization of the large intestine in newborns delivered by cesarean section. *Zentralbl. Bakteriologie Mikrobiologie Hygiene* 266, 330-337 (1987).
- Nowrouzian, F., Hesselmar, B., Saalman, R., Strannegard, I.L., Aberg, N., Wold, A.E., and Adlerberth, I.: *Escherichia coli* in infants' intestinal microflora: colonization rate, strain turnover, and virulence gene carriage. *Pediatr. Res.* 54, 8-14 (2003).
- Penders, J., Thijs, C., Vink, C., Stelma, F.F., Snijders, B., Kummeling, I., van den

- Brandt, P.A., Stobberingh, E.E.: Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics* 118, 511-521 (2006).
- Rudi, K., Zimonja, M., Kvenshagen, B., Rugtveit, J., Midtvedt, T., and Eggesbø, M.: Alignment-independent comparisons of human gastrointestinal tract microbial communities in a multidimensional 16S rRNA gene evolutionary space. *Appl. Environ. Microbiol.* 73, 2727-2734 (2007).
- Rutayisire, E., Huang, K., Liu, Y., and Tao, F.: The mode of delivery affects the diversity and colonization pattern of the gut microbiota during the first year of infants' life: a systematic review. *BMC Gastroenterol.* 16, 86 (2016).
- Salminen, S., Gibson, G.R., McCartney, A.L., and Isolauri, E.: Influence of mode of delivery on gut microbiota composition in seven year old children. *Gut* 53, 1388-1389 (2004).
- Simhon, A., Douglas, J.R., Drasar, B.S., and Soothill, J.F.: Effect of feeding on infants' faecal flora. *Arch. Dis. Child.* 57, 54-58 (1982).
- Stark, P.L. and Lee, A.: The microbial ecology of the large bowel of breast-fed and formula-fed infants during the first year of life. *J. Med. Microbiol.* 15, 189-203 (1982).
- Steer, P.J. and Modi, N.: Elective caesarean sections--risks to the infant. *Lancet* 374, 675-676 (2009).
- Thavagnanam, S., Fleming, J., Bromley, A., Shields, M.D., and Cardwell, C.R.: A meta-analysis of the association between Caesarean section and childhood asthma. *Clin. Exp. Allergy* 38, 629-633 (2008).
- Thompson, C.L., Wang, B., and Holmes, A.J.: The immediate environment during post-natal development has long-term impact on gut community structure in pigs. *ISME J.* 2, 739-748 (2008).
- White, R.A., Bjornholt, J.V., Baird, D.D., Midtvedt, T., Harris, J.R., Pagano, M., Hide, W., Rudi, K., Moen, B., Iszatt, N., Peddada, S.D., and Eggesbø, M.: Novel developmental analyses identify longitudinal patterns of early gut microbiota that affect infant growth. *PLoS Comput. Biol.* 9, e1003042 (2013).