Chapter 4
The impact of birth and postnatal medical interventions on infant gut microbiota

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Abstract

Established during infancy, the initial colonisation and development of the complex gut microbial community of our gastrointestinal tract can be shaped by common medical interventions, such as caesarean section and antibiotic use. This chapter provides evidence on the gut microbial impact of four medical interventions: (1) caesarean delivery, (2) maternal intrapartum antibiotic prophylaxis (IAP), (3) hospitalisation post birth, and (4) postnatal infant antibiotic treatment. Reductions in bifidobacteria and members of the *Bacteroidaceae* family (e.g. *Bacteroides fragilis*) are by far the most common perturbations in microbial composition following each of these interventions, especially after elective or emergency caesarean section. On the other hand, genus *Clostridium* and the *Enterbacteriaceae* (e.g. *Klebsiella, Escherichia coli*) are likely to become more abundant in infants delivered by caesarean, exposed to maternal antibiotics, hospitalised post birth and treated with antibiotics. Often, the enterococci and staphylococci also become more abundant. Differential impact on gut microbiota is observed by type of caesarean delivery and antibiotic administered to the mother or infant. IAP with penicillin or cefazolin, or newborn treatment with intravenous penicillin (plus gentamicin) is associated with higher abundance of *Enterococcus* and *Staphylococcus aureus*. *Klebsiella* emerge after newborn intravenous ampicillin (plus gentamicin) treatment. The *Veillonella* become more abundant in the infant gut after emergency (but not elective caesarean), whereas they are found to be depleted two months after newborn treatment with oral cephalexin. Of note, dysbiosis from perinatal medical interventions also occurs in the early breastfed infant and is enhanced by prematurity.

Keywords: gut dysbiosis, caesarean delivery, intrapartum antibiotic prophylaxis, hospitalisation post birth, postnatal antibiotic treatment

P.D. Browne, E. Claassen and M.D. Cabana (eds.) **Microbiota in health and disease: from pregnancy to childhood**
DOI 10.3920/978-90-8686-839-1_4, © Wageningen Academic Publishers 2017

4.1 Introduction

Women who undergo caesarean section delivery uniformly report a greater number of medical interventions, less mother-infant contact after birth and suboptimal breastfeeding practices (Chalmers *et al.*, 2009). As unsatisfying as they are for women and their families, they also derail the normal development of the gut microbiome during infancy. This development starts with pioneer microbes, the enterobacteria, which prepare the gut for anaerobic bacteria like bifidobacteria, *Clostridia* and other *Firmicutes*, and members of the *Bacteroidetes*. For a concise and comprehensive overview of all factors that affect this process, see Van Best *et al.* (2015). The focus of this chapter is on medical interventions during birth and the postnatal period, and how they affect the infant gut microbiome.

Four medical interventions will be reviewed: (1) caesarean section delivery (CS); (2) maternal intrapartum antibiotic prophylaxis (IAP); (3) hospitalisation post birth (home and hospital birth); and (4) postnatal infant antibiotic treatment. Appendix 4.1 provides details on most of the studies cited in this chapter, grouped according to taxon perturbation within each of the medical interventions. By way of background, high-throughput sequenced-based studies typically report on taxon-specific microbial abundance relative to the total abundance of other microbes present in the biological sample. Culture and qPCR (polymerase chain reaction assay) studies enumerate absolute quantities of microbes, which are not reported in relative terms since quantification of all microbial species present in a sample is rarely possible with these methods. On the other hand, culture and qPCR methods enable greater reporting of microbes at the species level. All methods enable determinations of the percent colonisation with a microbe.

4.2 Caesarean section delivery

The WHO recommendation that population rates of CS not exceed 15% has been in effect for almost 30 years (WHO, 1985). This benchmark remains appropriate to protect the woman and foetus from life-threatening complications, and also to prevent excess maternal and neonatal morbidity and mortality (Althabe and Belizan, 2006; Karlstrom *et al.*, 2013). Currently in North America and Europe, 24 and 19% of births, respectively, are by CS; at 29%, Latin America has the highest global rates (Betran *et al.*, 2016). CS rates are rising despite a lack of evidence for increasing prevalence of obstetric emergencies or medical risk (Bailit *et al.*, 2004; MacDorman *et al.*, 2008). The convenience of scheduling birth and fear of pain are contributing factors (Holmgren *et al.*, 2012; Miesnik and Reale, 2007). Also, contrary to WHO guidelines, less than 30% of infants in middle-income countries are exclusively breastfed for the first 5 months of life (Victora *et al.*, 2016), and less so if they are born by CS (Al-Sahab *et al.*, 2010). The consequences of CS birth should not be underestimated. Women undergoing CS are at greater risk for haemorrhage and uterine rupture (Armson, 2007; Liu *et al.*, 2007), and their newborns have higher rates of respiratory distress and infection (Karlstrom *et al., 2013;* Magnus *et al.,* 2011*)*. Beyond maternal and neonatal risks, a number of risks to child health have been linked to CS birth, including food allergy (Sanchez-Valverde *et al.*, 2009), asthma (Huang *et al.*, 2015) and overweight (Li *et al.*, 2013).

Impact on infant gut microbiota: summary of evidence

No less than 7 cohort studies around the world have documented reduced faecal abundance of genus *Bacteroides* or reduced diversity of the phylum *Bacteroidetes* in infants up to 4 months following caesarean delivery. This evidence comes from 5 European (n=1,853) and 2 North American cohorts (n=300) (Backhed *et al.*, 2015; Hesla *et al.*, 2014; Jakobsson *et al.*, 2014; Madan *et al.*, 2016; Penders *et al.*, 2006, 2013). Bifidobacteria are also less abundant and less diverse in the newborn gut within one week after CS (Backhed *et al.*, 2015; Dogra *et al.*, 2015; Penders *et al.*, 2006), while the staphylococci are more plentiful (Backhed *et al.*, 2015; Madan *et al.*, 2016; Stokholm *et al.*, 2016). Maternal skin has been identified as the source of staphylococci at birth in caesarean-delivered infants (Dominguez-Bello *et al.*, 2010). Finally, *Clostridium difficile* has also been found to a greater extent in the stools of caesarean versus vaginally-delivered infants within one month after birth (Penders *et al.*, 2006). Colonisation of the infant gut with *C. difficile* is becoming increasingly more prevalent than it was in the 1980s (Adlerberth and Wold, 2009), especially following CS (Adlerberth *et al.*, 2014). While microbial changes have been detected up to 7 years following CS (Salminen *et al.,* 2004), *C. difficile* colonisation as soon as 1 month after birth has been found to mediate the association between caesarean-induced gut dysbiosis and atopic disease (Penders *et al.*, 2013; Van Nimwegen *et al.*, 2011).

Differences between elective and emergency caesarean

Emergency and elective caesarean differ by indication, with foetal distress a common feature in the former. As well, many emergency caesareans are performed after a trial of labour, exposing newborns to maternal vaginal microbes. Unfortunately, most published studies do not separately report on elective versus emergency caesarean or even the percentage contribution for each to the study population. Among the few publications to date (Azad *et al.*, 2016; Stokholm *et al.*, 2016), gut microbiota profiles before three months of age in infants delivered by emergency CS were found to differ from those following elective CS; in the Canadian Healthy Infant Longitudinal Development (CHILD) cohort, this difference was independent of breastfeeding status (Azad *et al.*, 2016). Based on culture methods, a higher percentage of neonates were colonised with *Citrobacter freundii* and *S. aureus* one week after emergency than elective CS (or vaginal birth) in the $COPSAC_{2010}$ cohort; by one month of age, *Citrobacter* colonisation rates were equivalent between elective and emergency caesarean (Stokholm *et al.*, 2016). Relative to vaginal birth in two Swedish cohorts, the faecal abundance of *Veillonella* species was higher at age 1-3 weeks in mixed samples of infants delivered by elective and emergency CS (Hesla *et al.*, 2014; Jakobsson *et al.*, 2014). Among infants in the CHILD cohort, the *Veillonella* were more abundant in faecal samples at three months following emergency CS than vaginally delivery; these differences were not seen for elective CS (Azad *et al.*, 2016).

Common findings for elective and emergency caesarean

Certainly, varying indications for CS across studies may contribute to conflicting findings. In addition, culture-based studies are less effective in detecting strict anaerobes, such as the *Bacteroides* species (Stokholm *et al.*, 2016). Notwithstanding these differences, the relative abundance of infant faecal *Bacteroidetes* (from the CHILD sequence-based study) was substantially reduced at 3 months of infant age following elective and emergency CS (Azad *et al.,* 2016). Faecal enterobacteria were also more abundant in both types of this surgical intervention (Azad *et al.*, 2016; Backhed *et al.*, 2015; Stokholm *et al.*, 2016). Interestingly, Stokholm *et al.* (2016) observed opposing results for individual species of cultured enterobacteria that were common to emergency and elective CS; a higher proportion of infants were colonised with *Klebsiella* within one month after delivery, while *Escherichia coli* were detected less often in faecal samples (Stokholm *et al.*, 2016). *Klebsiella* species were also found to be more abundant in the newborn gut following CS in the GUSTO cohort (Dogra *et al.*, 2015). Finally, *Enterococcus* becomes more abundant in the infant gut at one or three months following emergency and elective CS (Azad *et al.*, 2016; Stokholm *et al.*, 2016); Stokholm *et al.* (2016) also reported greater colonisation of the infant hypopharyngeal microbiome with *Enterococcus faecalis* three months after CS delivery.

Conclusions

Against a backdrop of climbing rates of CS, evidence is accumulating on the impact of CS on the infant gut microbiome. While the mechanism for how CS increases the risk of disease is not well understood, perturbations in infant gut microbial ecology, such as lowered abundance of the genus *Bacteroides*, likely play a role. Beyond its direct impact on microbial exposure, CS normally requires antibiotic prophylaxis, may follow microbial colonisation from prelabour rupture of membranes (DiGiulio, 2012) and can delay onset of breastfeeding (Brown and Jordan, 2013; McDonald *et al.*, 2012). Noteworthy is that before three months of age, the impact of CS on gut microbial composition has been found to be stronger than breastfeeding (Madan *et al.*, 2016) and to be independent of breastfeeding status (Azad *et al.*, 2016).

4.3 Maternal intrapartum antibiotics

Less well-studied is the impact of maternal intrapartum antibiotic prophylaxis (IAP) on infant gut microbiota, despite the fact that almost 40% of newborn infants are indirectly exposed to antibiotics administered during vaginal delivery (Fairlie *et al.*, 2013; Persaud *et al.*, 2015; Stokholm *et al.*, 2013). In North America, IAP constitutes a standard of care to prevent early-onset neonatal *Group B Streptococcus* (GBS) sepsis and maternal infection post CS (Van Schalkwyk, 2010). It is also becoming a routine part of the birthing process subsequent to climbing rates of CS delivery and GBS colonisation in pregnancy (Johri *et al.*, 2006). However, IAP is not routinely practised across the globe. While North American guidelines recommend IAP following universal vaginal screening for GBS, those from the UK and Australia advocate for risk-based management approaches (Homer *et al.*, 2014). The adoption of a non-culture risk factor approach for GBS in Denmark has reduced IAP to 13% of vaginal deliveries (Stokholm *et al.*, 2013). In contrast, 27% of vaginal deliveries or more than 85% of GBS-culture positive pregnancies result in IAP in Canada and the US (Fairlie *et al.*, 2013; Persaud *et al.*, 2015).

Consequences for offspring health

Effective in preventing early-onset neonatal sepsis, IAP for GBS has been linked to amoxicillin-resistant late-onset *E. coli* infections in hospitalised infants up to 90 days after birth (Didier *et al.*, 2012). In the long-term, infant antibiotic treatment is associated with childhood asthma and obesity (Azad *et al.*, 2014; Penders *et al.*, 2011), conditions also linked to gut dysbiosis in early life (Penders *et al.*, 2007; Vael *et al.*, 2011). While this evidence originates from studies of postnatal antibiotic use, IAP disruption of initial gut colonisation of the newborn has greater potential to alter the natural succession of microbiota throughout infancy.

Impact on infant gut microbiota: summary of evidence

Findings on IAP in vaginal birth provide the main evidence for IAP independence from CS since, with few exceptions, for example in Norway (Opoien *et al.*, 2007), all women undergoing

elective CS receive IAP. In a study of 84 full-term Italian newborns born vaginally, Aloisio *et al.* (2014) found lower faecal bifidobacterial counts seven days after maternal IAP with intravenous ampicillin for GBS than in its absence. In a follow-up study of these infants, bifidobacteria were no longer reduced in number at 30 days post IAP. No differences were seen in faecal concentrations of lactobacilli or *Bacteroides fragilis* at seven or 30 days (Corvaglia *et al.*, 2016). In a smaller scale comparison by Arboleya *et al.* (2015), exclusively-breastfed full-term infants exposed to IAP (single dose of ampicillin) and hospitalised for three days exhibited an IAP effect between two and 90 days after birth, namely reduced abundance of *Bifidobacteriaceae* and *Bacteroidaceae* relative to controls.

Recently published from the CHILD cohort at three months of age, Azad *et al.* (2016) reported lowered abundance of *Bacteroidaceae* in faecal samples of full-term infants delivered vaginally after maternal IAP with penicillin (n=40) versus no IAP (n=96). Statistical significance was also found at the *Bacteroides* or *Parabacteroides* species level, but it did not survive correction for multiple testing. The family *Clostridiaceae* but not individual *Clostridium* species were more abundant following vaginal IAP. Compared to the infant gut dysbiosis seen following elective and emergency caesarean, there were fewer taxon differences at the genus level with IAP. Stokholm *et al.* (2016) also observed fewer changes with vaginal birth IAP than CS in their term infants at age one month, manifested as greater colonisation with *Klebsiella* and *Staphylococcus* species.

Interactive effects with gestational age and breastfeeding status

While studies of full-term infants indicate that IAP has a less profound effect on gut microbes than CS delivery, the impact of IAP may vary by gestational maturity. In the preterm context (mean gestational age of 30 weeks, primarily CS birth and hospitalisation for 50 days), Arboleya *et al.* (2015) observed several changes to gut microbial composition following maternal IAP. To begin with, all preterm infants had a much lower abundance of *Bacteroidetes* than term infants throughout the 3-month study. A large IAP effect was not evident at two days after birth, when only *Leuconostaceae* were more abundant in preterm infants not exposed to either IAP or postnatal antibiotics (e.g. ampicillin/gentamicin). Rather it emerged 30 days later, when significant clustering by IAP status was observed; infants exposed to IAP exhibited a lower relative abundance of *Bifidobacteriaceae*, *Lactobacillales* and *Streptococcaceae,* and a higher abundance of *Enterobacteriaceae* relative to non-exposed infants. At this postnatal age, no differences were observed between IAP-exposed and non-exposed infant with respect to gestational age, birth weight or length of hospital stay. The IAP impact was greater than direct administration of antibiotics to the infant.

As with CS, the impact of IAP in vaginal delivery on gut microbiota is also evident in the early breastfed infant. Azad *et al.* (2016) found genus *Clostridium* to be more abundant in IAP-exposed than non-exposed vaginally-born term infants who were exclusively breastfed at three months; the *Clostridia* did not vary by IAP status in partially breastfed infants. Findings from Corvaglia *et al.* (2016) show that IAP of vaginal birth mitigates faecal enrichment with bifidobacteria that

is normally seen with exclusive breastfeeding at 7 days after birth; increases to lactobacilli with breastfeeding were unaffected at seven or 30 days after birth in their study.

4.5 Hospitalisation post birth

Anywhere between 11% and 27% of infants, born by emergency and elective CS respectively, are hospitalised beyond one day in Western Europe (Penders *et al.*, 2006). In Canada (and the US), almost 100% of these infants stay for at least two days (Tun, 2016). Up to 62% of newborns born vaginally remain in hospital for longer than one day.

The isolation of pathogenic bacteria from the hands and clothing of hospital staff suggests that patients, as well as hospital staff, are in close proximity to antibiotic-resistant strains of bacteria namely: methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), *Pseudomonas, Acinetobacter* and *Clostridium* (Weber *et al.*, 2013). While many have published on hospital-based antibiotic resistance, impact on the gut microbial composition of the newborn is a new field of inquiry. Furthermore, unlike in the adult, pathogens like *C. difficile* have higher colonisation rates in infants in the absence of obvious gastrointestinal adverse effects (Adlerberth *et al.*, 2014); a variety of maternal and household factors have promoted colonisation with this organism, such as birth mode, extent of breastfeeding, maternal parity and household pets (Azad *et al.*, 2013; Bridgman *et al.*, 2016).

Home birth: impact on infant gut microbiota

Vaginal birth at home relative to the hospital setting has been shown to strikingly reduce the risk of asthma at age seven and to food sensitisation at age one in offspring of parents with allergic disease (Van Nimwegen *et al.*, 2011). In the same study from the KOALA cohort, the presence of *C. difficile* in the gut one month after birth was found to mediate the association between birth site (home versus hospital) and childhood asthma. In fact, findings from the Swedish ALADDIN cohort indicate that relative to a home birth, being born vaginally in a hospital alters the composition of the whole gut microbial community six months later (Hesla *et al.*, 2014).

Hospital birth: impact on infant gut microbiota

Extended hospitalisation post birth to two days or longer further alters gut microbial composition of infants. In the KOALA cohort, the two largest changes to one-month microbial composition after extended hospitalisation were increases to the colonisation rates and microbial counts for *C. difficile*, and reductions in the colonisation and counts for *Bifidobacteriaceae* (Penders *et al.*, 2006). Among infants hospitalised for 2-3 days versus same day discharge, *E. coli* were more common in faecal samples one month later. When hospital length-of-stay was longer at 4-6 days, the *B. fragilis* were reduced in number. Duration of hospitalisation remained a significant determinant for detecting *C. difficile* in infant faecal samples, independent of maternal antibiotic use, birth mode, infant feeding and other home environment factors. Each additional day of

hospitalisation after birth increased the likelihood of *C. difficile* colonisation by 1.13 (95% CI: 1.01-1.25) (Penders *et al.*, 2006).

More recently, in the CHILD cohort study we reported depletion of specific gut microbiota at three months according hospitalisation post birth and this depended on birth mode (Tun, 2016). The relative abundance of *Bacteriodetes* was inversely proportional to the duration of hospital stay for each birth type. Only following elective caesarean delivery was the correlation between abundance of *Bacteroidaceae* or *Bacteroides* and hospital length-of-stay statistically significant (r=-0.27, $P<0.01$). Put together, these observations suggest a role for hospital-acquired microbes such as *C. difficile,* and reduced colonisation resistance, in modifying community gut composition (Rousseau *et al.*, 2011).

4.6 Infant postnatal antibiotics

After breast milk and other dietary supplements, antibiotics are the next most commonly ingested substances by infants. Antibiotics affect colonisation of the intestine by suppressing commensal bacteria and causing the emergence of resistant pathogens. Despite a plethora of published articles relating infant antibiotic treatment to development of allergic disease (Penders *et al.*, 2011), and more recently overweight in children (Azad *et al.*, 2014), evidence for the impact of infant antibiotic treatment on gut microbial composition is less plentiful.

Antibiotic treatment of infant: impact on gut microbiota

Oral amoxicillin is the drug of choice for acute otitis media and other respiratory infections in infants. When given to older infants between the ages of 1-2 years, Mangin *et al.* (2010) found a reduction in the number of different bifidobacterial species in faecal samples immediately at the end of the antibiotic course compared to baseline. Total bifidobacterial concentrations were unchanged and other gut microbiota were not assessed. In contrast, intravenous (IV) treatment of 6-month old infants for five days with ceftriaxone (an antibiotic excreted in bile) for pneumonia (Savino *et al.*, 2011), resulted in a significant reduction in total faecal bacterial counts at the end of treatment. Faecal concentrations of the *Enterobacteriaceae* and enterococci in these full-term infants were also reduced compared to baseline. After five days of ceftriaxone therapy, lactobacilli were no longer detected in infant faecal samples (Savino *et al.*, 2011).

Among young infants at 1-3 months of age, most oral antibiotics (penicillins, cephalosporins, macrolides) cause gut microbial changes 3-5 days after the cessation of treatment (Bennet *et al.*, 2002). These changes are evident in vaginally-born, breastfed infants; reductions to bifidobacteria, lactobacilli and *Bacteroides* are the most profound. Similarly, in the KOALA cohort of predominantly vaginally-born and exclusively breastfed infants at age one month, Penders *et al.* (2006) observed lower faecal counts of bifidobacteria and *B. fragilis* as determined by qPCR in those previously treated with oral antibiotics. Within a European consortium of birth cohorts, of which three-quarters of infants were delivered vaginally but only 50% had been

exclusively breastfed, Fallani *et al.* (2010) reported greater abundance of faecal enterobacteria among infants receiving antibiotics by six weeks of age. No gut dysbiosis was observed in these infants in the period after breastfeeding cessation.

Intravenous antibiotic treatment of the neonate for suspected sepsis immediately after birth can significantly and persistently alter gut microbial composition. Using sequenced-based methods, Fouhy *et al.* (2012) reported that a 48 hour course of IV ampicillin and gentamicin in full-term newborns significantly elevated the abundance of the *Proteobacteria* phylum compared to controls, and lowered proportions of genus *Bifidobacterium* and *Lactobacillus* four weeks after the cessation of treatment. *Bacteroidetes* were also detected less often in antibiotic-treated infants. By week eight, the faecal abundance of *Bifidobacterium* and *Lactobacillus* returned to levels comparable to control infants. Yet, levels of Proteobacteria remained significantly higher and the number of different *Bifidobacterium* species remained low in antibiotic-treated infants. A reduction in gut microbial diversity was also observed in the Tanaka *et al.* (2009) study of Japanese full-term newborns hospitalised for one week and supplemented with formula. Unlike the typical IV regimen, neonates received oral cephalexin for four days after birth. Gut microbial diversity was reduced on the third day of treatment and remained lowered two months later. These community changes were accompanied by the appearance of *Enterococcus faecium,* and reductions in genus *Bifidobacterium* and *Veillonella,* and in members of the *Bacteroidaceae* family; the latter two microbial taxa were not detected at two months of age.

Using a cross-over design of rectal cultures, Parm *et al.* (2010) nicely demonstrated that antibiotic type determines the extent of colonisation by individual species. Treatment of preterm newborns with an IV penicillin G and gentamicin regimen resulted in colonisation of the gut with *S. aureus* and *Enterococcus* species 5-7 days after treatment, while combined treatment with ampicillin and gentamicin favoured colonisation by other staphylococci. Moreover, the ampicillin regimen resulted in a greater duration of colonisation with *Klebsiella pneumonia* and ampicillin-resistant Serratia (both members of the *Proteobacteria*). Similarly, Lindberg *et al.* (2011) reported a negative association between antibiotic treatment (with broad spectrum beta-lactams) and *S. aureus* colonisation rate in six month old Italian infants. This correlation was not seen in Swedish infants who were mainly treated with the narrow spectrum penicillin V, without activity against *S. aureus.*

Indirect exposure from breast milk

Maternal postnatal antibiotics are an understudied source of antibiotic exposure to infants. Although most beta-lactams are considered safe during breastfeeding, the presence of even small quantities of antibiotics in breast milk can potentially alter infant gut microbiota *(Soto et al.*, 2014). In the CHILD cohort, where postnatal antibiotics were mostly administered to women after an emergency CS, a higher abundance of genus *Clostridium* at three months of age after emergency CS was observed in exclusively breastfed but not among infants supplemented with formula (Azad *et al.*, 2016).

4.7 Conclusions

Clearly, emergency CS is medically indicated, antibiotics are needed to treat and prevent infection, and breastfeeding has a multitude of benefits. In this review, we presented evidence that gut dysbiosis in the developing infant occurs after common medical interventions in the perinatal time period. Reductions in bifidobacteria and members of the *Bacteroidaceae* family (e.g. *B. fragilis*) are by far the most common perturbations in microbial composition following exposure to IAP, hospitalisation post birth and treatment with antibiotics, and especially after elective or emergency caesarean delivery. On the other hand, genus *Clostridium* and *Enterbacteriaceae* (e.g. *Klebsiella, E. coli*) are likely to become more abundant in infants following these exposures. Often, the enterococci and staphylococci also become more abundant. Differential impact on gut microbiota is observed by type of caesarean delivery and antibiotic administered to the mother or infant. IAP with penicillin or cefazolin, or newborn treatment with IV penicillin (plus gentamicin) is associated with higher abundance of *Enterococcus* and *S. aureus*. *Klebsiella* emerge after newborn IV ampicillin (plus gentamicin) treatment. The *Veillonella* become more abundant after emergency but not elective caesarean, whereas they are found to be depleted two months after newborn treatment with oral cephalexin. Of note, dysbiosis from perinatal medical interventions also occurs in the early breastfed infant and is enhanced by prematurity.

While findings from the CHILD cohort suggest that early breastfeeding may modify IAP and CS-associated dysbiosis of the gut microbiome later in infancy (Azad *et al.*, 2016), not all IAP-induced dysbiosis can be 'restored' with breastfeeding (Corvaglia *et al.*, 2016). Reported reversals to dysbiosis, for example to *Enterobacteriaceae,* after the discontinuation of antibiotic treatment (Jakobsson *et al.*, 2014), may also be subsequent to emerging resistant strains and not continued breastfeeding. Indeed, higher abundance of these gram-negative microbiota at six months of age has been associated with higher adiposity among toddlers (Dogra *et al.*, 2015). Higher ratios in the abundance of the *Enterobacteriaceae* to *Bacteroidaceae* have also been reported to predict food sensitisation in infants (Azad *et al.*, 2015). Finally, readers should bear in mind that gut microbial alterations, which occur during critical windows in the development of the immune system, even minor perturbations, may have long-term consequences (Cox *et al.*, 2014).

Conflict of interest

The authors confirm that there are no conflicts of interest.

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Appendix 4.1
Study details of infant gut microbial dysbiosis by medical intervention type and taxon category.

Microbial group Citation	Study details, methods and findings
Caesarean section (CS)	
Bacteroidetes (phylum), *Bacteroidaceae* (family), *Bacteroides* (genus)	
Jakobsson *et al.,* 2014	
Observed microbial change at infant age	• ↓ *Bacteroidetes* diversity and % *Bacteroides* colonisation at 1 week, 3 and 12 months in all CS (n=9) vs all vaginal birth (n=15)
Study groups and number of infants	• Vaginal (n=15) no IAP (87%), elective (n=6)/emergency CS (n=3)
Infant population	• Placebo arm of probiotics trial, full term Swedish; 83% exclusively breastfed until 3 months; CS antibiotics given after cord clamping, no postnatal antibiotics
Microbial profiling method	• Roche 454 Genome Sequencer at V3/V4 • FDR correction
Azad *et al.,* 2016 (a); Azad *et al.,* 2013 (b)	
Observed microbial change at infant age	• ↓ *Bacteroidetes*, *Bacteroidaceae*, *Bacteroides* and *Parabacteroides* abundance at 3 months in each CS type (or combined) vs vaginal birth no IAP (vaginal overall)
Study groups and number of infants	• Vaginal no IAP (n=96), elective CS (n=17), emergency CS (n=23)[a] • Vaginal (n=18), elective/emergency CS (n=6)[b]
Infant population	• Cohort, full term, Canadian; 52% exclusively breastfed until 3 months; same results if exclusive or no exclusive breastfeeding; postnatal antibiotics in 11%; IV cefazolin IAP for CS
Microbial profiling method	• Illumina MiSeq at V4[a] • SiSeq at V5-V7[b] • FDR correction
Hesla *et al.,* 2014	
Observed microbial change at infant age	• ↓ *Bacteroides* abundance at 1 week, 3 weeks, 2 months in CS (n=18) vs vaginal birth (n=95)
Study groups and number of infants	• Vaginal ± IAP (n=95) including home birth (n=24), elective/emergency CS (n=18); 55% of CS were emergency in the original cohort
Infant population	• Cohort, full term Swedish with anthroposophic sample, IAP in 10% of all births; 83% exclusively breastfed until 2 months; postnatal antibiotics in 3% of original cohort
Microbial profiling method	• Roche 454 Genome sequencer at V3/V4 • FDR correction

Microbial group Citation	Study details, methods and findings
Backhed *et al.,* 2015	
Observed microbial change at infant age	• ↓ *Bacteroides* and *Parabacteroides* abundance at birth and 4 months of age in emergency CS versus vaginal birth
Study groups and number of infants	• Vaginal (n=98) no IAP (90%), emergency CS (n=15)
Infant population	• Cohort, full term, Swedish, antibiotic prophylaxis for GBS before delivery not intrapartum; 67% CS antibiotics given after cord clamp; 69% exclusively breastfed until 4 months; postnatal antibiotics in 5% of vaginal births
Microbial profiling method	• HiSeq of genome contigs
Penders *et al.,* 2006	
Observed microbial change at infant age	• ↓ *Bacteroides fragilis* counts and % colonisation at 1 month in CS vs vaginal birth at home
Study groups and number of infants	• Vaginal birth at home (n=480) vs CS (n=108)
Infant population	• Cohort, full term, Dutch with anthroposophic sample; 68% exclusively breastfed until 1 month; postnatal antibiotics in 3%
Microbial profiling method	• qPCR assay for bifidobacteria, *Escherichia coli, Clostridium difficile, B. fragilis,* lactobacilli of 16S rDNA gene sequences • FDR correction
Penders *et al.,* 2013	
Observed microbial change at infant age	• ↓ % *B. fragilis* colonisation (and most counts) at 5 weeks, 13 weeks, 1 month in CS vs spontaneous vaginal birth
Study groups and number of infants	• Vaginal (n=391), elective/emergency CS (n=144); assisted vaginal births not in comparison
Infant population	• Bacterial lysate randomised trial of high (atopic) risk infants, full term, Dutch; 89% breastfed until 1 month; no postnatal antibiotics
Microbial profiling method	• qPCR assay for bifidobacteria, *E. coli, C. difficile, B. fragilis,* lactobacilli of 16S rDNA gene sequences
Madan *et al.,* 2016	
Observed microbial change at infant age	• ↓ *Bacteroides* abundance at 6 weeks in CS vs vaginal birth, independent of breastfeeding status
Study groups and number of infants	• Vaginal ± IAP (n=70), elective/emergency CS (n=32)
Infant population	• Cohort, full term, US, 69% exclusively breastfed at 6 weeks; no postnatal antibiotics
Microbial profiling method	• Illumina MiSeq at V4/V5 • FDR correction

Microbial group Citation	Study details, methods and findings
Bifidobacteria	
Dogra *et al.*, 2015	
Observed microbial change at infant age	• ↓ *Bifidobacterium* abundance on day 3 after birth in CS vs vaginal birth
Study groups and number of infants	• Vaginal (n=57) no IAP (67%), CS (n=18)
Infant population	• Cohort, late preterm and full-term, Singapore, 20% exclusively breastfed on day 3
Microbial profiling method	• V1-3 and V5-7 Illumina sequencing • FDR correction
Backhed *et al.*, 2015	
Observed microbial change at infant age	• ↓ *Bifidobacterium* species richness at birth and 4 months in CS vs vaginal birth
Study groups and number of infants	• Vaginal (n=98) no IAP (90%), emergency CS (n=15)
Infant population	• Cohort, full term, Swedish, antibiotic prophylaxis for GBS before delivery not intrapartum; 67% CS antibiotics given after cord clamp; 69% exclusively breastfed until 4 months; postnatal antibiotics in 5% of vaginal births
Microbial profiling method	• HiSeq of genome contigs
Penders *at al*. 2006	
Observed microbial change at infant age	• ↓ bifidobacterial counts and % colonisation at 1 month in CS vs vaginal birth at home
Study groups and number of infants	• Vaginal birth at home (n=480) vs CS (n=108)
Infant population	• Cohort, full term, Dutch with anthroposophic sample; 68% exclusively breastfed until 1 month; postnatal antibiotics in 3%
Microbial profiling method	• qPCR assay for bifidobacteria, *E. coli, C. difficile, B. fragilis,* lactobacilli of 16S rDNA gene sequences • FDR correction
Staphylococcus	
Dominguez-Bello *et al.*, 2010	
Observed microbial change at infant age	• ↑ proportion of *Staphylococcus* in rectum at birth in CS vs vaginal birth
Study groups and number of infants	• Vaginal no IAP (n=4), elective CS (n=5)
Infant population	• Cohort, full term, Venezuela
Microbial profiling method	• Roche 454 Genome Sequencer at V2

Microbial group Citation	Study details, methods and findings
Backhed *et al.,* 2015	
	• ↑ *Staphylococcus* abundance at birth in CS vs vaginal birth • Vaginal (n=98) no IAP (90%), emergency CS (n=15) • Cohort, full term, Swedish, antibiotic prophylaxis for GBS before delivery not intrapartum; 67% CS antibiotics given after cord clamp; 69% exclusively breastfed until 4 months; postnatal antibiotics in 5% of vaginal births • HiSeq of genome contigs
Madan *et al.,* 2016	
Observed microbial change at infant age	• ↑ *Staphylococcus* abundance at 6 weeks in CS vs vaginal birth, independent of breastfeeding status
Study groups and number of infants	• Vaginal +/- IAP (n=70), elective/emergency CS (n=32)
Infant population	• Cohort, full term, US, 69% exclusively breastfed at 6 weeks; no postnatal antibiotics
Microbial profiling method	• Illumina MiSeq at V4/V5 • FDR correction
Stokholm *et al.,* 2016	
Observed microbial change at infant age	• ↑ *Staphylococcus aureus* at 1 week in emergency and elective CS vs vaginal birth, independent of breastfeeding status
Study groups and number of infants	• Vaginal (n=549) no IAP (87%), emergency CS (n=85), elective CS (n=66)
Infant population	• Cohort, full term, Danish; exclusive breastfeeding for 3.4 months on average; postnatal antibiotics in 3%
Microbial profiling method	• Bacterial culture on selected media; unable to culture *Bacteroides*
Clostridiales*, *Clostridium* and *C. difficile	
Penders *et al.,* 2006	
Observed microbial change at infant age	• ↑ *C. difficile* counts and % colonisation at 1 month in CS vs vaginal birth at home
Study groups and number of infants	• Vaginal birth at home (n=480) vs CS (n=108)
Infant population	• Cohort, full term, Dutch with anthroposophic sample; 68% exclusively breastfed until 1 month; postnatal antibiotics in 3%
Microbial profiling method	• qPCR assay for bifidobacteria, *E. coli, C. difficile, B. fragilis,* lactobacilli of 16S rDNA gene sequences • FDR correction

Microbial group Citation	Study details, methods and findings
Azad *et al.*, 2016	
Observed microbial change at infant age	• ↑ *Clostridiales* abundance at 3 months in each CS type vs vaginal no IAP; ↑ *Clostridium* in emergency CS
Study groups and number of infants	• Vaginal no IAP (n=96), elective CS (n=17), emergency CS (n=23)
Infant population	• Cohort, full term, Canadian, 52% exclusively breastfed until 3 months; same results if exclusive or no exclusive breastfeeding; postnatal antibiotics in 11%; IV cefazolin IAP for CS
Microbial profiling method	• Illumina MiSeq at V4 • FDR correction
***Enterococcus* species**	
Azad *et al.*, 2016	
Observed microbial change at infant age	• ↑ *Enterococcus* abundance at 3 months in emergency CS vs vaginal no IAP; higher abundance in elective CS did not survive FDR correction
Study groups and number of infants	• Vaginal no IAP (n=96), elective CS (n=17), emergency CS (n=23)
Infant population	• Cohort, full term, Canadian, 52% exclusively breastfed until 3 months; same results if exclusive or no exclusive breastfeeding; postnatal antibiotics in 11%; IV cefazolin IAP for CS
Microbial profiling method	• Illumina MiSeq at V4 • FDR correction
Stokholm *et al.*, 2016	
Observed microbial change at infant age	• ↑ *Enterococcus faecalis* colonisation at 1 week in CS (n=151) vs vaginal birth
Study groups and number of infants	• Vaginal (n=549) no IAP (87%), elective CS (n=66), emergency CS (n=85)
Infant population	• Cohort, full term, Danish; exclusive breastfeeding for 3.4 months on average; postnatal antibiotics in 3%
Microbial profiling method	• Bacterial culture on selected media; unable to culture *Bacteroides*
Proteobacteria*, *Enterobacteriaceae*, *Klebsiella	
Stokholm *et al.*, 2016	
Observed microbial change at infant age	• ↑ *Klebsiella* species colonisation at 1 week, 1 month in CS (n=151) vs vaginal birth
Study groups and number of infants	• Vaginal (n=549) no IAP (87%), elective CS (n=66), emergency CS (n=85)
Infant population	• Cohort, full term, Danish; exclusive breastfeeding for 3.4 months on average; postnatal antibiotics in 3%
Microbial profiling method	• Bacterial culture on selected media; unable to culture *Bacteroides*

Microbial group Citation	Study details, methods and findings
Backhed *et al.*, 2015	
Observed microbial change at infant age	• ↑ *Enterobacter* abundance at 4 months in emergency CS vs vaginal birth
Study groups and number of infants	• Vaginal (n=98) no IAP (90%), emergency CS (n=15)
Infant population	• Cohort, full term, Swedish, antibiotic prophylaxis for GBS before delivery not intrapartum; 67% CS antibiotics given after cord clamp; 69% exclusively breastfed until 4 months; postnatal antibiotics in 5% of vaginal births
Microbial profiling method	• HiSeq of genome contigs
Azad *et al.*, 2016	
Observed microbial change at infant age	• ↑ *Enterobacteriaceae* abundance at 3 months in emergency CS vs vaginal no IAP
Study groups and number of infants	• Vaginal no IAP (n=96), elective CS (n=17), emergency CS (n=23)
Infant population	• Cohort, full term, Canadian, 52% exclusively breastfed until 3 months; same results if exclusive or no exclusive breastfeeding; postnatal antibiotics in 11%; IV cefazolin IAP for CS
Microbial profiling method	• Illumina MiSeq at V4 • FDR correction
Intrapartum antibiotic prophylaxis (IAP) for GBS	
Bacteroidetes and genus *Bacteroides*	
Corvaglia *et al.*, 2016	
Observed microbial change at infant age	• ↔ counts of *B. fragilis* counts at 7 or 30 days relative to controls
Study groups and number of infants	• Vaginal no IAP (n=35), vaginal IAP with IV ampicillin (n=49)
Infant population	• Full term Italian infants born vaginally to mothers +/- GBS, exclusively breastfed
Microbial profiling method	• Ion Torrent Sequencer at V2-8
Azad *et al.*, 2016	
Observed microbial change at infant age	• ↓ *Bacteroidetes* abundance at 3 months relative to controls; trend at genus level
Study groups and number of infants	• Vaginal no IAP (n=96), vaginal IAP (n=40)
Infant population	• Cohort, full term, Canadian, 52% exclusively breastfed until 3 months; same results if exclusive or no exclusive breastfeeding; postnatal antibiotics in 11%; IV penicillin G for IAP or clindamycin if penicillin allergy
Microbial profiling method	• Illumina MiSeq at V4 • FDR correction

Microbial group Citation	Study details, methods and findings
Bifidobacteria	
Aloisio *et al.*, 2016 (a); Corvaglia *et al.*, 2016 (b)	
Observed microbial change at infant age	• ↓ bifidobacterial diversity at 7 days • ↔ bifidobacterial counts at 30 days relative to controls
Study groups and number of infants	• Vaginal no IAP (n=10), vaginal IAP with IV ampicillin (n=10)[a] • Vaginal no IAP (n=35), vaginal IAP with IV ampicillin (n=49)[b]
Infant population	• Full term Italian infants born vaginally to mothers +/- GBS, exclusively breastfed
Microbial profiling method	• Ion Torrent Sequencer at V2-8
Arboleya *et al.*, 2015	
Observed microbial change at infant age	• ↓ *Bifidobacteriaceae* and *Bacteroidaceae* abundance between 2-90 days after birth relative to controls
Study groups and number of infants	• Vaginal no IAP (n=10), vaginal IAP with single dose of ampicillin (n=3)
Infant population	• Full-term infants exclusively-breastfed and hospitalised for 3 days
Microbial profiling method	• Ion Torrent Sequencer • FDR correction
Clostridium, Enterococcus, Staphylococcus, Klebsiella	
Azad *et al.*, 2016	
Observed microbial change at infant age	• ↑ *Clostridium* ($P<0.01$) and *Enterococcus* ($P=0.02$) abundance at 3 months relative to controls; FDR $P>0.05$
Study groups and number of infants	• Vaginal no IAP (n=96), vaginal IAP (n=40)
Infant population	• Cohort, full term, Canadian, 52% exclusively breastfed until 3 months; same results if exclusive or no exclusive breastfeeding; postnatal antibiotics in 11%; IV penicillin G for IAP or clindamycin if penicillin allergy
Microbial profiling method	• Illumina MiSeq at V4 • FDR correction
Stokholm *et al.*, 2016	
Observed microbial change at infant age	• ↑ % colonisation by *Klebsiella*, *Staphylococcus haemolyticus* at 7 days relative to controls
Study groups and number of infants	• Vaginal no IAP (n=477), vaginal IAP (n=72)
Infant population	• Cohort, full term, Danish; exclusive breastfeeding for 3.4 months on average; postnatal antibiotics in 3%
Microbial profiling method	• Bacterial culture on selected media; unable to culture *Bacteroides*

Microbial group Citation	Study details, methods and findings
Hospitalisation beyond 1 day after birth	
Bacteroidetes and *B. fragilis*	
Tun, 2016	
Observed microbial change at infant age	• Inverse correlation between hospital length-of-stay (LOS) and *Bacteroidetes* abundance (phylum, family, genus level) at 3 months; statistically significant in elective CS only
Study groups and number of infants	• Vaginal no IAP (n=379), vaginal IAP (n=194), elective CS (83), emergency CS (121)
Infant population	• Cohort, full term, Canadian, 52% exclusively breastfed until 3 months
Microbial profiling method	• Illumina MiSeq at V4 • FDR correction
Penders *et al.*, 2006	
Observed microbial change at infant age	• ↓ *B. fragilis* counts and % colonisation at 1 month if LOS 4-6 days vs none; no longer significant after adjustment for covariates
Study groups and number of infants	• No hospitalisation (737), LOS 4-6 days (73)
Infant population	• Cohort, full term, Dutch with anthroposophic sample; 68% exclusively breastfed until 1 month; postnatal antibiotics in 3%
Microbial profiling method	• qPCR assay for bifidobacteria, *E. coli, C. difficile, B. fragilis,* lactobacilli of 16S rDNA gene sequences • FDR correction
Bifidobacteria	
Penders *et al.*, 2006	
Observed microbial change at infant age	• ↓ counts and % colonisation at 1 month if LOS >3 days vs none; no longer significant after adjustment for covariates
Study groups and number of infants	• No hospitalisation (737), LOS >3 days (97)
Infant population	• Cohort, full term, Dutch with anthroposophic sample; 68% exclusively breastfed until 1 month; postnatal antibiotics in 3%
Microbial profiling method	• qPCR assay for bifidobacteria, *E. coli, C. difficile, B. fragilis,* lactobacilli of 16S rDNA gene sequences • FDR correction

Microbial group Citation	Study details, methods and findings
C. difficile	
Penders *et al.*, 2006	
Observed microbial change at infant age	• ↑ *C. difficile* counts and % colonisation at 1 month if LOS >1 day. Risk of colonisation at 1 month of 1.13 (95%CI: 1.01-1.25) for each additional hospitalisation day, independent of covariates
Study groups and number of infants	• No hospitalisation (737), LOS >1 day (183)
Infant population	• Cohort, full term, Dutch with anthroposophic sample; 68% exclusively breastfed until 1 month; postnatal antibiotics in 3%
Microbial profiling method	• qPCR assay for bifidobacteria, *E. coli*, *C. difficile*, *B. fragilis*, lactobacilli of 16S rDNA gene sequences • FDR correction
Postnatal infant antibiotics at birth or during infancy	
Bifidobacteria, lactobacilli, phylum *Bacteroidetes*, family *Bacteroidaceae* and genus *Bacteroides*	
Bennet *et al.*, 2002	
Observed microbial change at infant age	• ↓ counts of bifidobacteria and *B. fragilis* 1 week after treatment
Study groups and number of infants	• Oral cefaclor or cefadroxil (n=7), no antibiotics (n=18)
Infant population	• Post antibiotics with controls, all Swedish infants vaginally born and breastfed at 1-3 months of age
Microbial profiling method	• Bacterial culture on specific media
Penders *et al.*, 2006	
Observed microbial change at infant age	• ↓ counts of bifidobacteria and *B. fragilis* at 1 month independent of covariates
Study groups and number of infants	• Oral antibiotics before 1 month (n=28), no antibiotics (n=98)
Infant population	• Cohort, full term, Dutch with anthroposophic sample; 68% exclusively breastfed until 1 month; postnatal antibiotics in 3%
Microbial profiling method	• qPCR assay for bifidobacteria, *E. coli*, *C. difficile*, *B. fragilis*, lactobacilli of 16S rDNA gene sequences • FDR correction
Fouhy *et al.*, 2012	
Observed microbial change at infant age	• ↓ abundance of bifidobacteria, lactobacilli and *Bacteroidetes*/*Bacteroidaceae*/*Bacteroides* at 4 but not 8 weeks after treatment; no change in counts of bifidobacteria
Study groups and number of infants	• At birth, course of IV ampicillin/gentamicin (n=9); no antibiotics (n=9)
Infant population	• Post antibiotics with controls, full term, 72% vaginal birth; 44% breastfed
Microbial profiling method	• 16S rRNA and rpoB-specific primers; quantitative PCR

Microbial group Citation	Study details, methods and findings
Tanaka *et al.,* 2009	
Observed microbial change at infant age	• ↓ % colonisation by bifidobacteria and *Bacteroidetes* at 1 month after treatment
Study groups and number of infants	• At birth, oral cephalexin for 4 days (n=5); no antibiotics (n=18)
Infant population	• Post antibiotics with controls, full term infants vaginally born and partially breastfed
Microbial profiling method	• V2 by DGGE
Enterococcus and *Clostridium*	
Fouhy *et al.,* 2012	
Observed microbial change at infant age	• ↑ *Enterococcus* and *Clostridium* abundance at 4 weeks post treatment; only *Clostridium* was higher at 8 weeks
Study groups and number of infants	• At birth, course of IV ampicillin/gentamicin (n=9); no antibiotics (n=9)
Infant population	• Post antibiotics with controls, full term, 72% vaginal birth; 44% breastfed
Microbial profiling method	• 16S rRNA and rpoB-specific primers; quantitative PCR
Tanaka *et al.,* 2009	
Observed microbial change at infant age	• ↑ % colonisation by *Enterococcus* at end of treatment
Study groups and number of infants	• At birth, oral cephalexin for 4 days (n=5); no antibiotics (n=18)
Infant population	• Post antibiotics with controls, full term infants vaginally born and partially breastfed
Microbial profiling method	• V2 by DGGE
Parm *et al.,* 2010	
Observed microbial change at infant age	• ↑ % colonisation by *Enterococcus* 5-7 days after treatment in penicillin vs ampicillin regimen
Study groups and number of infants	• At birth, course of IV penicillin/gentamicin (n=71); ampicillin/gentamicin (n=68)
Infant population	• Cross over design, preterm infants, Estonia
Microbial profiling method	• Bacterial culture on specific media

Microbial group Citation	Study details, methods and findings
Staphylococcus	
Tanaka *et al.,* 2009	
Observed microbial change at infant age	• ↑ % colonisation by *Staphylococcus* at end of treatment
Study groups and number of infants	• At birth, oral cephalexin for 4 days (n=5); no antibiotics (n=18)
Infant population	• Post antibiotics with controls, full term infants vaginally born and partially breastfed
Microbial profiling method	• V2 by DGGE
Parm *et al.,* 2010	
Observed microbial change at infant age	• ↑ % colonisation *Staphylococcus aureus* 5-7 days after treatment in penicillin vs ampicillin regimen
Study groups and number of infants	• At birth, course of IV penicillin/gentamicin (n=71); ampicillin/gentamicin (n=68)
Infant population	• Cross-over design, preterm infants, Estonia
Microbial profiling method	• Bacterial culture on specific media
Proteobacteria (phylum), *Enterobacteriaceae* (family) and *Klebsiella* (genus)	
Fouhy *et al.,* 2012	
Observed microbial change at infant age	• ↑ *Proteobacteria*, *Enterobacteriaceae* at 4 and 8 weeks post treatment
Study groups and number of infants	• At birth, course of IV ampicillin/gentamicin (n=9); no antibiotics (n=9)
Infant population	• Post antibiotics with controls, full term, 72% vaginal birth; 44% breastfed
Microbial profiling method	• 16S rRNA and rpoB-specific primers; quantitative PCR
Tanaka *et al.,* 2009	
Observed microbial change at infant age	• ↑ % colonisation by *Enterobacteria* 3 weeks after treatment
Study groups and number of infants	• At birth, oral cephalexin for 4 days (n=5); no antibiotics (n=18)
Infant population	• Post antibiotics with controls, full term infants vaginally born and partially breastfed
Microbial profiling method	• V2 by DGGE
Bennet *et al.,* 2002	
Observed microbial change at infant age	• ↑ counts of *Klebsiella* 3-5 days after treatment
Study groups and number of infants	• Oral amoxil (n=9), no antibiotics (n=18)
Infant population	• Post antibiotics with controls, all Swedish infants vaginally born and breastfed at 1-3 months of age
Microbial profiling method	• Bacterial culture on specific media

Microbial group Citation	Study details, methods and findings
Parm *et al.*, 2010	
Observed microbial change at infant age	• ↑ % colonisation *Klebsiella* 5-7 days after treatment in ampicillin vs penicillin regimen
Study groups and number of infants	• At birth, course of IV ampicillin/gentamicin (n=68); penicillin/ gentamicin (n=71)
Infant population	• Cross-over design, preterm infants, Estonia
Microbial profiling method	• Bacterial culture on specific media

[1] DGGE = denaturing gradient gel electrophoresis; FDR = false discovery rate; IAP = intrapartum antibiotic prophylaxis; LOS = length of stay.