Early life colonization of the human gut: microbes matter everywhere

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Microbes colonising the infant intestine, especially bacteria, are considered important for metabolic and immunological programming in early life, potentially affecting the susceptibility of the host to disease. We combined published data to provide a global view of microbiota development in early life. The results support the concept that the microbiota develops with age in an orchestrated manner, showing common patterns across populations. Furthermore, infants are colonised at birth by specific, selected maternal faecal bacteria and likely their bacteriophages. Therefore, infants are adapted to receiving specific bacterial signals, partly derived from the maternal microbiota, at successive immunological time windows during early development. Birth by caesarean section compromises the initial vertical transmission of microbes whereas antibiotic use shifts the microbiota away from the normal developmental pattern. These disruptions alter the microbial signals that the host receives, potentially affecting child development.

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Introduction

Infants are colonised by microbes at birth, and over the first years of life the microbes form stable communities, termed microbiota, on all body surfaces. The microbiota consists of bacteria, archaea, viruses and fungi, different species living in different parts of the body depending on the ecological requirements of the microbial species. The bacterial component of the microbiota has been extensively investigated, while considerably less is currently known on the other microbes colonising infants. The intestine is the largest body surface with a very high density of microbes, mainly anaerobic bacteria. The microbes in the gut are in constant contact and interaction with host cells [1]. The reciprocal interaction of bacteria and immune cells plays an important role during the time when the immune system is developing: signals from the gut microbiota are thought to be important for proper immune maturation and the development of tolerance towards benign microbes [1]. Furthermore, the gut microbiota has strong effects on host energy metabolism, especially in early life [2,3,4,5], via degradation of non-digestible food components [2,3], production of short-chain fatty acids that the host cells use as an energy source [4], metabolism of hormone-like bile acids [6], production of inflammatory and anti-inflammatory signals to the host [1], and by influencing hormone levels [7], gene expression in the liver [8], and overall energy use and storage in adipose and muscle tissue [9]. Thus, the microbiota is considered important for metabolic and immunological programming in early life, potentially affecting both current growth [2,3,5], and the long-term susceptibility of the host to diet-induced metabolic disease [10].

Materials and methods

Several dozens of recent studies have addressed the development of infant bacterial microbiota since birth (Supplementary Table 1). In order to effectively summarise the existing data, we collected data on the average relative abundance of the five most abundant bacterial classes from the early life microbiota studies ($N = 34$) where this information was available and accessible. In most studies it was possible to obtain the data separately for the different birth modes and antibiotic-treated and non-treated groups, but data from formula-fed and breastfed infants were usually not presented separately. When possible, we collected information on the proportion of breastfed (exclusively or partially) infants in the cohort. Information on the proportion of breastfed infants was available for 30 of the 34 studies.

Significance of associations between the relative abundance of selected bacterial taxa and age, proportion breastfed, birth mode, antibiotic use, and location were assessed using linear regression or analysis of variance models, weighted by the cohort size. The associations with age, breastfeeding and location were tested among the vaginally born, non-antibiotic-treated cohorts (termed 'normal' cohorts).

Temporal development of the gut microbiota

Already during birth, infants are exposed to a large diversity of maternal and environmental microbes, some of which find a suitable habitat on the infant body.

Immediately after birth, the bacterial communities that the infant has been exposed to, such as maternal vaginal or skin bacteria, can be detected on the infant [11]. However, these communities do not colonise the infant as such [12••]: only the microbes that are adapted to the conditions present in the different body sites of the infant can survive: the environment thus selects which microbes colonise which body surface.

After the initial colonisation, considerable temporal fluctuation of the gut microbiota composition takes place during the first years of life. In spite of different sample processing procedures, phylogenetic approaches, and geographic location of the cohorts, it is of interest to note that a common general picture emerges when data from different studies are combined (Figure 1). Typically during the first days of life, aerobic and facultative bacteria, such as streptococci and enterobacteria dominate the infant gut community (Figure 1a, b). It is assumed that the gut environment becomes increasingly anoxic with age, although actual luminal oxygen levels have not been determined in infants [13]. Presumably the reduction in oxygen levels enables anaerobic bacteria to grow, as bifidobacteria commonly become the most abundant member during the first weeks of life (Figure 1c). In some infants, *Bacteroides* spp. form a large fraction of the microbiota from early on (Figure 1d), while in others, these remain a minority and *Bifidobacterium* spp. dominate. Unlike the other taxa, *Bacteroides* spp. do not appear to follow an age-associated developmental pattern, but may be abundant at any age. Several *Bifidobacterium* and

Figure 1
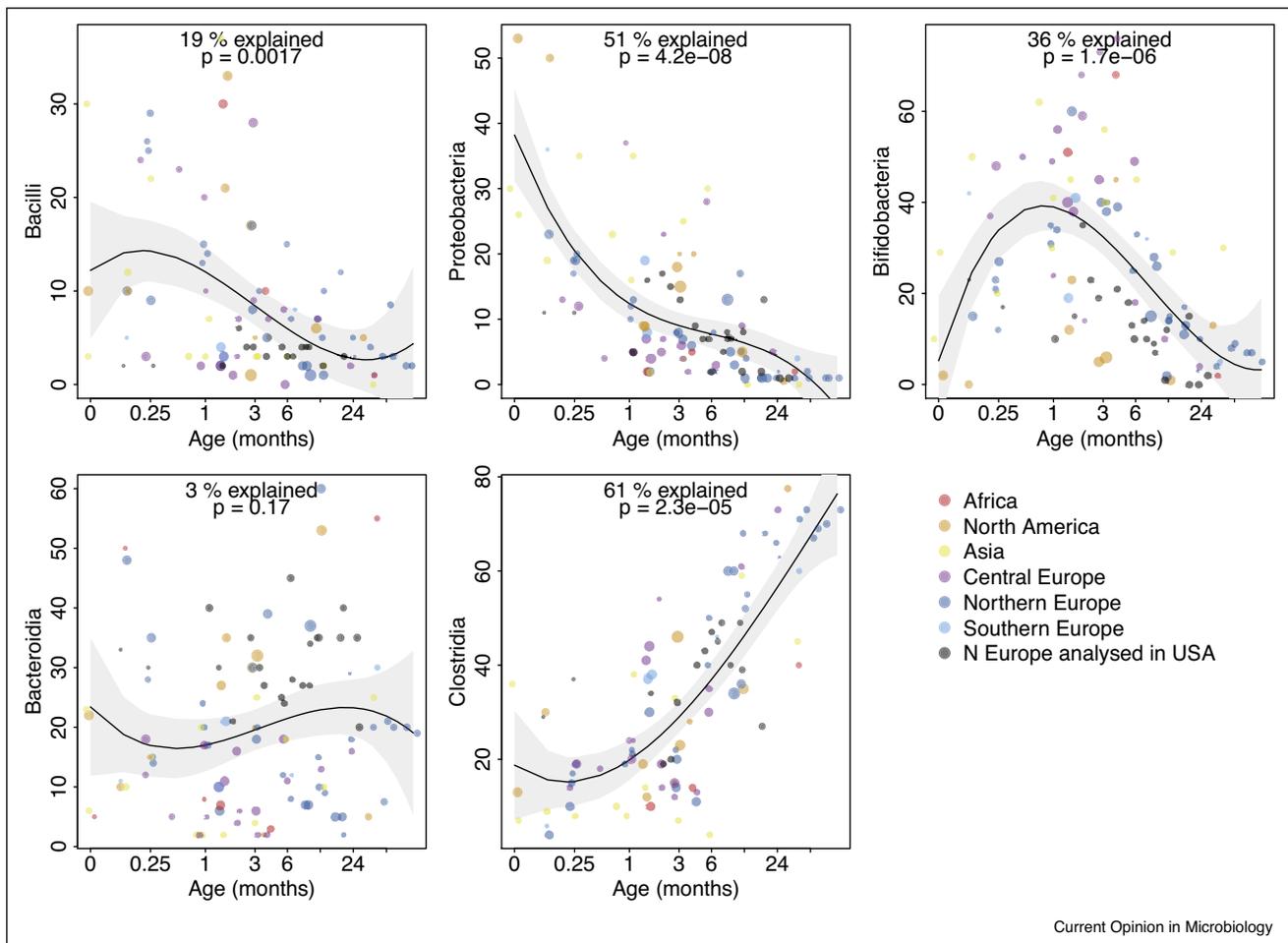
Gut microbiota succession in early life. Data on the average relative abundance of the five most abundant bacterial taxa in vaginally born healthy children from different cohorts were collected from available literature (see Supplementary Table 1, references [3•,14,20,29•,34•,32•,36••,38–60]). The cohorts are coloured by continent, with Europe further geographically divided. Two studies [15,16•] contained samples from Northern Europe (N Europe), but were analysed in the US. As we found several similarities between the American data and these two cohorts, we chose to highlight them (black). The size of the symbols indicates the size of the cohort. The trend line, 95% confidence interval, proportion of variation explained, and the p-value are obtained from a regression model with the third degree polynomial of log-transformed age, weighted by the size of the cohort.

Bacteroides spp. are adapted to the milk-based diet of infants, being able to utilise human milk oligosaccharides (HMOs) as well as similar structures in the host-produced mucus. The dominance of HMO-utilizing bacteria prevails normally as long as the infant is breastfed [14]. Once breast milk is no longer available and the infant's diet consists of solid foods, which provide a very different type of fermentation substrates to the bacteria, a major change occurs in the infant gut. The abundance of bifidobacteria decreases as they are replaced by clostridia, a diverse group of mainly anaerobic bacteria specialised in plant polysaccharide degradation (Figure 1e). As recently shown [14], weaning is an important milestone in the infant gut microbiota development, marking the transition from infant-type microbiota to child-type microbiota and initiating the long maturation process towards adult-like microbiota.

Indeed, part of the observed temporal development appears to be explained by changes in diet, as the proportion of breastfed infants in the cohort, even when adjusting for age, was significantly associated with the abundance of Bacilli ($p = 0.036$), bifidobacteria ($p = 0.027$), and clostridia ($p < 0.0001$) (see unadjusted trends in Supplementary Figure 1). However, age remained a significant variable in a multiple regression model adjusting for the proportion of breastfed infants in the cohort (Bacilli, $p = 0.0086$; Proteobacteria, $p < 0.0001$; bifidobacteria, $p = 0.0008$; clostridia, $p = 0.0084$), indicating that maturation as such plays a role independent of diet.

These patterns appear fairly universal in the modern world, being observed in children from all continents, which suggests that the timing of the microbiota succession may be biologically determined, although undoubtedly affected by local culture, for example via feeding practices. Thus, infants are likely adapted to receiving specific bacterial signals at certain immunological time windows during early development. We tested for geographical differences in microbiota composition after adjusting for age (Figure 2). The most apparent geographical difference in microbiota development is the lack of clear bifidobacterium dominance in the American cohorts (Figure 1c, Figure 2c); instead, American children appear to have a relatively high abundance of *Bacteroides* spp. (Figure 1d, Figure 2d) and enterobacteria (Figure 1b, Figure 2b) during the time window (1–6 months) when bifidobacteria dominate in other continents. Conversely, African, Asian and Central European cohorts have high abundances of bifidobacteria (Figure 1c), and low abundances of clostridia (Figure 1e) during the first year, implying that their microbiota follows a relatively slow maturation pattern. The geographical differences are even more pronounced when adjusting for the proportion of breastfed infants in the cohort, but due to missing breastfeeding data for several cohorts (see Supplementary Table 1), we chose to present the more robust age-adjusted data. The association between the proportion breastfed and the microbiota in the different geographical

locations is shown in Supplementary Figure 1. Overall, the lowest breastfeeding proportions among the <12 month old cohorts were in the Southern (median 63%), Central (median 70%) and Northern European cohorts (median 76%), while the other cohorts had mostly 100% breastfeeding rates. This suggests that at least not all of the observed differences are driven by local breastfeeding practices. However, we found an indication that technical bias may explain some of the differences. Two Finnish cohorts [15,16•] showed a resemblance to the American cohorts in terms of low *Bifidobacterium* and high *Bacteroides* abundance. These two cohorts were analysed in the US, which is suggestive that some of the observed differences may be technical, rather than biological, as has been suggested previously [17], but this needs further investigation.

While the bacterial microbiota is relatively well defined in infants across geographic settings, the intestinal virome and virus-bacterium interactions in infants are just starting to be elucidated. Bacteriophages, especially, are increasingly being recognized as modulators of the intestinal microbiota in health and disease [18]. A longitudinal study of 4 twin pairs in a developed country setting showed that neonates harbour a high diversity of bacteriophages, most likely acquired at birth, while eukaryotic RNA and DNA viruses were mostly acquired postnatally [19]. The diversity of bacteriophages decreased over the first two years of life as *Microviridae* became dominant [19]. Whether these patterns are general and what drives these changes in gut virome is currently unclear, but recent data from two developing countries support the delayed appearance of eukaryotic viruses [20].

While fungi and other micro-eukarya are found in the human intestinal tract, their diversity is usually low and only few dozen species have been described [21,22]. Limited information exist on the early gut mycobiota but a recent study with close to 300 mothers and their offspring showed fungi to be detectable as early as a few days after birth and reported a strong correlation between the detection of fecal fungal DNA in mothers and their infants, suggestive of vertical transmission [23]. Similarly, archaea were detected on a low level in infants from the very first time points after birth and were more abundant and prevalent than the fungi. The majority were methanogens, which were more abundant in vaginally born than in C-section delivered infants [24]. These studies indicate the need to complement studies on bacterial community development with those addressing viral, archaeal and microeukaryotic dynamics.

How long the development continues and when the child microbiota becomes mature, is currently not known. Some longitudinal studies have shown the development to proceed beyond 5 years [25•] and the age at which the microbiota becomes fully mature is yet to be identified.

Figure 2
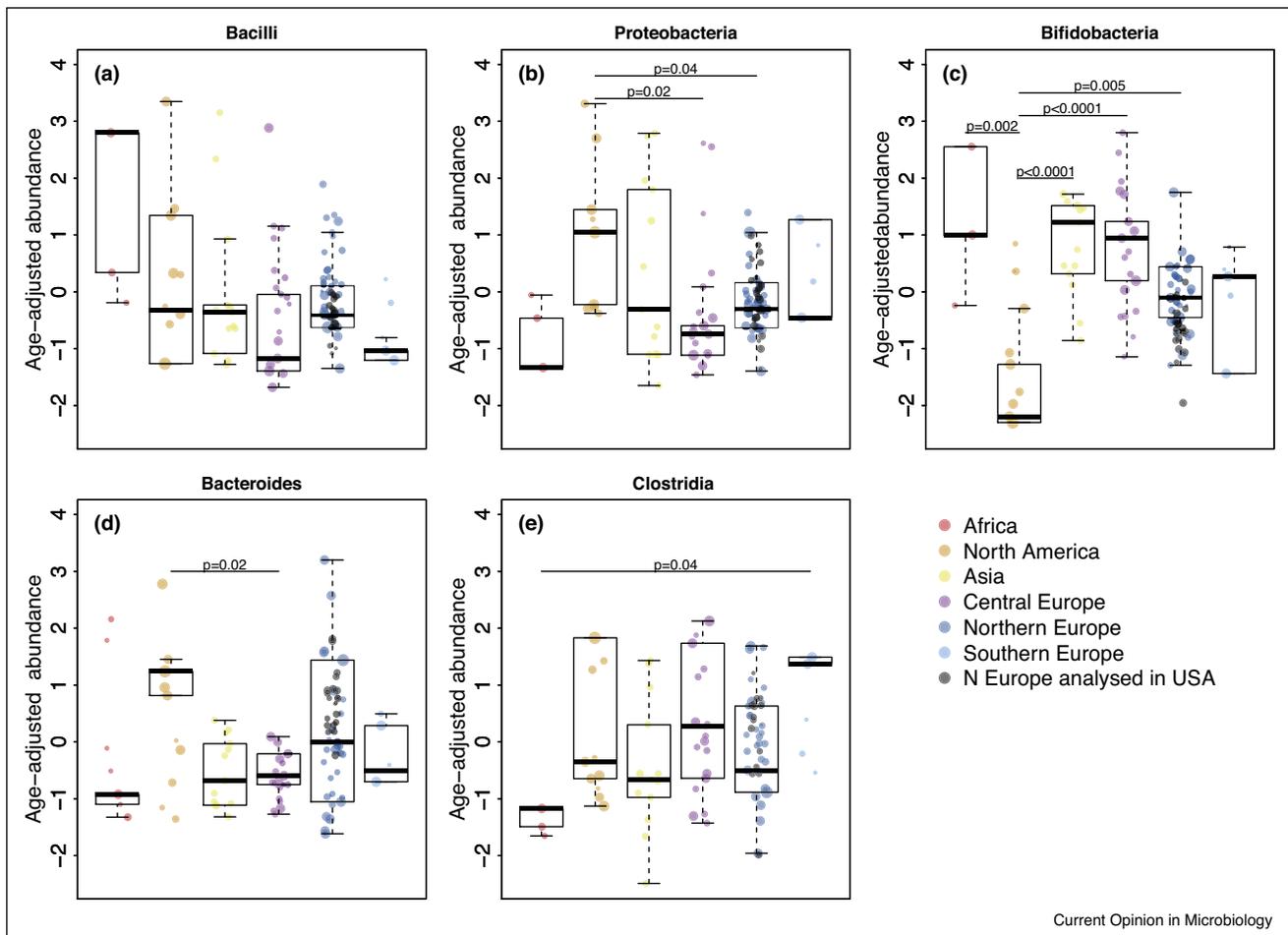
Geographical differences in microbiota composition. Age-adjusted relative abundance, obtained from the model presented in Figure 1, was used to test for geographical differences in an analysis of variance model, weighted by the size of the cohort. Tukey HDS test was used to assess the significance of the pairwise differences. The samples marked with black were omitted from the test. Data collected from available literature [3•,14,20,29•,32•,34•,36••,38–60].

Where do the microbes come from?

It has long been assumed that infants are colonised by maternal microbes during birth from the birth canal, and by microbes present in breast milk. However, it is important to note that the ecological conditions in maternal vagina and breast are different from those in the infant gut. Even if the same classes of bacteria are observed in different body sites, the species and strains are likely highly adapted to the specific body site and thus may be unable to successfully transfer from one environment to another. A recent study matched bifidobacterial strains in the infant to those in maternal breast milk [26•], suggesting that such vertical transmission may occur. This is yet to be verified in large cohorts and likely applies only to specific taxa. Furthermore, it has been suggested that the origin of breast milk bacteria is in fact the maternal gut, allowing gut bacteria to be transmitted via breast milk [27]. On the contrary, it was recently shown that the

bacterial taxa present in infant faecal samples are rarely seen in maternal vaginal samples [12••]. The authors concluded that vaginal bacteria are not transmitted to the infant. The only adult body site that consistently contains all the taxa commonly observed in the infant gut is, not surprisingly, the gut itself. Several studies have observed the same species or strains present in faecal samples of infants and their mothers, implying maternal transmission of faecal bacteria [14,28••,29•]. It is thus highly likely that the maternal gut is the main source of microbes colonising the infant gut.

Establishing a microbial transmission event requires stringent scientific methods [30]. Many microbial species and strains are prevalent in the population, and observing the same species, OTU, or strain in two individuals is not sufficient evidence of transmission. Deep metagenomic sequencing coupled with single nucleotide variant (SNV)

analysis allows sufficiently high genomic resolution to identify truly rare strains that are very unlikely to be derived from the environment [28[••],29[•],31[••]]. Furthermore, it is possible to follow the strains of all abundant species simultaneously, extending the analysis from selected species to large communities of microbes.

Using this approach, various groups, including ours, have recently verified that maternal faecal bacteria are transmitted to the infant gut and that they colonise persistently [28[••],29[•],31[••]]. However, our data show that not all maternal gut inhabitants are transmitted: only specific maternal taxa, notably bifidobacteria and bacteroides, colonise the infant permanently and in a rather dominant way [31[••]]. Although clostridia are the most abundant group of bacteria in the maternal gut, these were not found to colonise the infant, at least not in detectable abundance [31[••]]. Interestingly, in a recent study, bacteriophages of bifodobacteria, termed bifidophages, were followed in infants and found to be transmitted vertically, like their bacterial hosts [26[•]]. This result and the discovery of a diverse bacteriophage community in neonates [19] suggest that bacteriophages may be transmitted vertically at birth, while this may not apply to eukaryotic viruses [19,32[•]].

The differences in colonisation ability between bacterial taxa are likely attributable to the early life milk-based diet, including the human milk oligosaccharides, which determines which bacteria are able to grow in the infant gut. According to our recent results, most of the clostridia that colonise children are not of maternal origin [31[••]]; where they come from is currently not known. In some cases the origin may be the father or siblings [31[••]], but their role has not been fully addressed. It is tempting to speculate that while bifidobacteria and bacteroides appear to be directly vertically transmitted at birth, clostridia, which commonly form spores and thus survive periods outside of their habitat, would rely mainly on non-direct transmission between hosts, being transmitted via the environment. This hypothesis has received support from the finding that many strict anaerobes of the class Clostridia survive extended exposure to air and ethanol due to spore formation, while *Bacteroides* and *Bifidobacterium* spp. do not [33[•]]. Furthermore, we have found that clostridial strains are less stable within individuals than strains of bifidobacteria and bacteroides [31[••]], again suggesting that their life history strategy may be profoundly different.

Impact of disturbances

The most common disturbances to infant colonisation are perinatal antibiotics and caesarean delivery, which often co-occur. Several studies have shown clear and long-term differences in the microbiota composition between vaginally born and caesarean born infants, usually increased abundance of bacilli (Figure 3a) and enterobacteria (Figure 3b), reduced abundance of bifidobacteria (Figure 3c), and especially a clear and long-term lack of *Bacteroides* in the caesarean-born infants (Figure 3e). Generally, the major differences seem to disappear by the age of 12 months (Figure 3), but even transient differences in the pattern of microbiota succession may have long-term effects on the immunological and metabolic development of the host [1,34[•]].

In a recent study it was suggested that body site rather than delivery mode affected the infant microbiota [32[•]]. However, careful re-analysis of these data indicates that the birth mode effect on the gut microbiota is strong even after 6 weeks, confirming the general trend (see Supplementary Table 1 and [31[••]]).

As the two taxa that are normally transmitted at birth from the mother, bifidobacteria and bacteroides, are often reduced in caesarean born infants, it can be expected that caesarean birth interferes with vertical transmission of the gut microbiota. This is exactly what we discovered in a recent metagenomic study [31[••]]. Infants born by caesarean section showed a complete lack of maternal strains during the first weeks of life. Over several months, they did acquire maternal strains, but there is reason to believe that this late postnatal colonisation may distort the timing of the microbial successional phases. It is not yet known how important the identity of the strains is and whether replacing the missing maternal strains by e.g. probiotic bifidobacteria is sufficient to completely normalise the caesarean-born infant microbiota and consequently infant development. The lack of vertical transmission in caesarean-born infants supports the hypothesis that microbial colonisation normally begins at birth, not *in utero*. However, the perinatal use of antibiotics should be addressed as to eliminate this potentially confounding factor. Although bacteria are observed in meconium samples, there is currently no evidence in human to support *in utero* colonisation, as concluded in a recent review [35[•]].

Another very common disturbance to infant microbiota is antibiotic use (Figure 3). Antibiotics given in early life usually deplete especially the bifidobacterial community (Figure 3c), increasing the abundance of clostridia (Figure 3d). This means that infants that are given antibiotics during the time when bifidobacteria normally dominate, will be shifted away from the normal developmental pattern. While the microbiota does recover within 6–12 months, depending on the antibiotic used [36[••]], the recovery will occur after the time window for bifidobacterial dominance has passed. Disruption of the normal microbiota succession may influence infant development [5[••],34[•]]. Indeed early life antibiotic use has been associated with increased risk of overweight [37[••]], countering the protective effects of breastfeeding that are likely mediated by the microbiota [37[••]]. In a recent study, we found an increased level of antibiotic resistance genes as a function of the number of antibiotic courses given to a

Figure 3
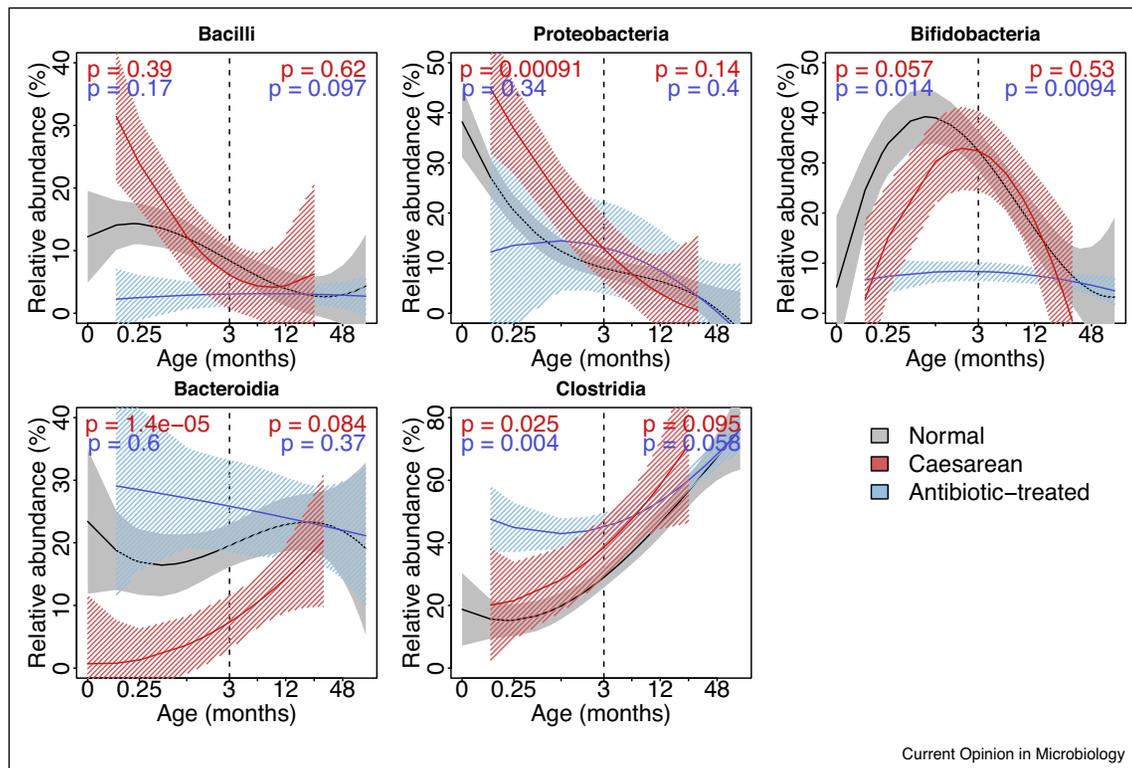
Comparison of the microbiota development in the caesarean born and in the antibiotic-treated children to the normal development. Data collected from available literature [3•,14,20,29•,32•,34•,36••,38–60]. The *p*-values in the top left corner refer to the significance of the difference before or at age 3 months, and the *p*-values in the top right corner after age 3 months. The *p*-values were obtained linear regression models adjusting for age.

child [36••], indicating that the observed associations may be causal. A causal relation is further supported by studies in mice that upon repeated treatment with antibiotics, developed obesity and other metabolic defects [10].

Conclusion

The intimate interactions between host development and intestinal microbiota have been documented, but only now insight is emerging on how finely tuned the colonisation process is and what impact the disturbances in early life may have on later life health. Our analysis of global data has revealed common patterns in the way the child microbiota is disrupted by caesarean birth and antibiotic use. Since these large-scale disturbances affect a significant proportion of the human population, targeted treatment approaches are needed. Knowledge on the development of infant microbiota provides opportunities to normalize the impact of disturbances. Development of specific probiotic (live bacteria), prebiotic (oligosaccharides, fibres) and postbiotic (products of bacterial metabolism such as short-chain fatty acids and secondary bile acids) products, aimed to support a natural microbiota at different ages and targeting specific defects will be an important next step to take the microbiome field from description to treatment.

Conflict of interest statement

Nothing declared.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.mib.2018.06.003.

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