**Factors Influencing the Establishment of the Intestinal Microbiota in Infancy**

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**Abstract**

The establishment of the intestinal microbiota commences at birth and new bacteria establish in succession during the first years of life until an adult-type highly complex microbiota has been achieved. The first bacteria to establish in the neonatal gut are usually aerobic or facultatively anaerobic bacteria, like enterobacteria, enterococci and staphylococci. During their growth they consume oxygen and change the intestinal milieu making it suitable for the proliferation of anaerobic bacteria. *Bifidobacterium*, *Clostridium* and *Bacteroides* are among the first anaerobes establishing in the microbiota. As more oxygen-sensitive species establish and the complexity of the microbiota increases, the population sizes of aerobic and facultative bacteria decline. This phenomenon is thought to result from oxygen depletion, substrate competition and the accumulation of toxic metabolites. A wide range of factors influence the intestinal microbiota and its establishment, including delivery and feeding mode, antibiotic treatment, and contacts with parents, siblings, and hospital staff. Differences in colonization pattern can be observed between vaginally and section-delivered infants, and between infants in industrialized and developing countries, reflecting the importance of maternal microbiota and the environment as sources of colonizing bacteria. This article describes the intestinal colonization pattern in human infants, and reviews factors affecting this process.

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**Sequential Establishment of the Intestinal Microbiota**

As the neonate leaves the sterile environment of the womb and encounters a world full of microbes, colonization of the skin and mucosal membranes begins immediately. With time, diversified bacterial ecosystems establish at these sites, of which the intestinal microbiota is the most complex.

The first bacteria to establish in the gut are usually aerobic or facultative anaerobic bacteria, since the neonatal gut is rich in oxygen. Such bacteria
include *Escherichia coli* and other enterobacteria, enterococci and staphylo-
cocci [1–7]. As these early colonizers do not have to compete with other bac-
teria, they may reach population levels of $10^9–10^{11}$ colony forming units (CFU)/g 
feces early in life, which is roughly 1,000 times higher than their population 
levels in adults [6–11].

We have recently reported the longitudinal colonization pattern in more 
than 100 Swedish infants [7]. The colonization by facultative bacteria is 
shown in figure 1. Coagulase-negative staphylococci are the first bacteria to 
establish in the infantile gut, colonizing practically 100% of the infants from 3 
days of age. Enterococci also rapidly colonize most infants. *E. coli* colonizes 
half of the infants within the first week of life, but the colonization frequency 
increases slowly, and *Staphylococcus aureus* is, actually, equally or more 
common than *E. coli* in Swedish infants between 1 week and 2 months of age 
(fig. 1). Members of the *Enterobacteriaceae* family other than *E. coli*, e.g. *Klebsiella*, *Enterobacter* and *Proteus*, do not colonize the infants at birth, 
but become increasingly common in the microbiota over the first 6 months of 
life (fig. 1). The colonization frequency of coagulase-negative staphylococci 
decline after some weeks and colonization by *S. aureus* and enterobacteria 
other than *E. coli* decline after the 6th month of life. This phenomenon can 
be regarded as a reflection of their relatively poor adaptation to the more 
complex intestinal ecosystem which develops over the first year of life.

The population counts of the facultative bacteria are shown in figure 2. *E. coli*, 
a species well adapted to the human gut, display high population numbers ini-
tially which decrease only moderately over the first year of life. In contrast,
the population levels of staphylococci decline substantially after the first weeks of life (fig. 2), indicating the poor fitness of staphylococci in an increasingly complex microbiota.

As facultative bacteria consume oxygen the intestinal milieu becomes more suitable for strictly anaerobic bacteria [8, 9]. *Bifidobacterium*, *Clostridium* and *Bacteroides* are examples of anaerobic bacteria which colonize young infants in population levels reaching $10^9–11$ CFU/g feces [6–9, 12]. The same groups dominate the early microbiota in recent studies using non-culture-dependent molecular methods for analyses of the intestinal microbiota [13–17].

The colonization pattern with anaerobic bacteria in Swedish infants is shown in figure 3. Bifidobacteria are the earliest anaerobes, colonizing two thirds of the 1-week-old infants. Clostridia are initially less common, but rapidly catch up with bifidobacteria. In contrast, *Bacteroides* appears much later, colonizing only one third of Swedish 2-month-old infants (fig. 3). The mean population counts of different anaerobic bacteria in colonized infants are shown in figure 4.

Among bifidobacterial species, *B. breve*, *B. bifidum* and *B. infantis* seem to be especially apt to colonize the infantile intestine (table 1). Clostridia are a poorly defined accumulation of Gram-positive anaerobic sporeformers,
**Fig. 3.** Frequency of colonization by various anaerobic bacteria in Swedish vaginally delivered infants at different time points after birth. Adapted from references Adlerberth et al. [7] and Ahrne et al. [18].

**Fig. 4.** Population levels of bifidobacteria, *Bacteroides* and lactobacilli in culture-positive Swedish infants at different time points after birth. Vegetative clostridial bacterial levels were not determined. Clostridial spores amounted to $10^5$–$10^6$ CFU/g feces. Adapted from references Adlerberth et al. [7] and Ahrne et al. [18].
many of which are not genetically related to one another. *C. perfringens* and *C. difficile* belong to group I that is common in the infantile microbiota (table 1). *C. difficile* is found in less than 4% of healthy adult individuals. Among *Bacteroides* species, the *B. fragilis* group are common inhabitants of the gut microbiota throughout life (table 1).

Lactobacilli are quite infrequent colonizers of the infant gut and their population numbers are low compared to many other anaerobes [6, 9, 18] (fig. 3, 4). Approximately one third of Swedish infants acquire lactobacilli in the first month (fig. 3), but persistent colonization by lactobacilli is quite uncommon [18]. Furthermore, lactobacilli show much lower population counts than, e.g., *Bacteroides* and bifidobacteria (fig. 4). The species most commonly isolated in the first 6 months of life are *L. rhamnosus* and *L. gasseri*, which are later replaced by *L. paracasei, L. plantarum, L. acidophilus* and *L. delbruckeii* [18].

*Eubacterium, Veillonella, Fusobacterium, Peptostreptococcus* and *Ruminococcus* are examples of anaerobes that are frequent in the intestinal microbiota of adults [19], but less commonly isolated from infants. This could be related either to a lack of exposure to such bacteria in early life, or to poor fitness of such bacteria in the intestinal milieu during the first period of life. *Eubacterium* species are isolated from less than half of infants in the first year of life [3, 9, 20]. Peptostreptococci appear when solid foods are introduced [9, 21], and most infants harbor these bacteria at 12 months of age [3, 9]. *Ruminococcus* and *Fusobacterium* species are rarely isolated from infants in the first year of life [3, 21, 22]. In contrast, *Veillonella* may be isolated from up to 50% of 1-month-old infants [4, 5, 21, 23] and when present they often reach high population counts [9, 24].

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**Table 1.** Species of bifidobacteria, clostridia and *Bacteroides* colonizing infants and adults

<table>
<thead>
<tr>
<th>Infants</th>
<th>References</th>
<th>Adults</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bifidobacterium</td>
<td>4, 16</td>
<td><em>B. catenulatum</em></td>
<td>4, 16</td>
</tr>
<tr>
<td>B. breve</td>
<td></td>
<td><em>B. adolescens</em></td>
<td></td>
</tr>
<tr>
<td>B. bifidum</td>
<td></td>
<td><em>B. longum</em></td>
<td></td>
</tr>
<tr>
<td>B. infantis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. longum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium</td>
<td>5, 21</td>
<td><em>C. perfringens</em></td>
<td>19</td>
</tr>
<tr>
<td>C. perfringens</td>
<td></td>
<td><em>C. ramosum</em></td>
<td></td>
</tr>
<tr>
<td>C. difficile</td>
<td></td>
<td><em>C. biferrmentans</em></td>
<td></td>
</tr>
<tr>
<td>C. paraputrificum</td>
<td></td>
<td><em>C. inocuum</em></td>
<td></td>
</tr>
<tr>
<td>C. tertium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteroides</td>
<td>21, 100</td>
<td><em>B. vulgatus</em></td>
<td>19</td>
</tr>
<tr>
<td>B. vulgatus</td>
<td></td>
<td><em>B. thetaiomaticron</em></td>
<td></td>
</tr>
<tr>
<td>B. thetaiomaticron</td>
<td></td>
<td><em>B. distasonis</em></td>
<td></td>
</tr>
<tr>
<td>B. distasonis</td>
<td></td>
<td><em>B. fragilis</em></td>
<td></td>
</tr>
<tr>
<td>B. fragilis</td>
<td></td>
<td><em>B. ovatus</em></td>
<td></td>
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<tr>
<td>B. ovatus</td>
<td></td>
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</tbody>
</table>

Intestinal Colonization of Newborn Infants
Many of the above-mentioned bacteria are extremely sensitive to oxygen and are therefore difficult to culture, and this might have underestimated their prevalence in the infantile microbiota. Recent studies using non-culture-dependent DNA-based methods have indicated that *Eubacterium* and *Ruminococcus* are present quite early in the microbiota [25–27]. In addition, many of the bacterial DNA sequences identified especially in fecal samples obtained after some months of age show little homology with known bacteria, indicating the presence of bacteria not detectable by culture [25].

Anaerobic bacteria establish successively until a highly diverse ‘adult’ type of microbiota has developed at a few years of age [24, 28]. The increasingly complex anaerobic microbiota provides selective pressure on the facultative bacteria and suppresses their growth. The mean ratio of strict to facultative anaerobes is approximately 1:10 at one week, 4:1 at one month and 60:1 at one year of age using culture-based identification [6]. In the adult microbiota the strict anaerobes are hundred- or thousand-fold more numerous than the facultative bacteria.

The ability of the microbiota to suppress the growth of the facultative bacteria, such as *E. coli*, enterococci and staphylococci, or certain anaerobes, e.g. *C. difficile*, is termed ‘colonization resistance’. It also implies resistance to the implantation of new bacterial strains into the ecosystem. The exact mechanisms leading to colonization resistance are not defined, but are likely to include competition for nutrients and binding sites, and production of toxic metabolites [29, 30].

### Source of Bacteria Colonizing Infants

Staphylococci, which are the first colonizers of the infant gut, are ubiquitously present on the skin and mucosal membranes of most individuals. Strains colonizing the neonatal intestine may be acquired from any individual in close contact with the baby, e.g. parents or hospital staff. The parental skin flora is the most important source of *S. aureus* strains colonizing the neonatal gut [31]. Bacteria may be transferred during general care, but breastfeeding may be another important source as the nipples are frequently colonized by staphylococci, and staphylococci are isolated from breast milk in large numbers [32].

Most infants acquire enterococci within the first week of life. Enterococci, especially *E. faecalis* and *E. faecium*, are typical fecal bacteria, but they are also sturdy bacteria that resist most hygienic measures, which makes them spread easily in, e.g., the hospital milieu [33]. Infants may acquire enterococci from their mother’s fecal microbiota during delivery (vertical transfer) or from environmental sources (horizontal transfer), although this has not been specifically studied. As both infants delivered vaginally and infants delivered by cesarean section acquire enterococci very early [7], it is evident that contact with fecal material is not necessary for acquisition of these bacteria.
E. coli was previously the first colonizer of the infant gut [34, 35]. E. coli is present in the gut of almost all adult individuals, and approximately one third of babies acquire E. coli from the mother’s fecal microbiota during delivery [35–39]. The remainder of infants previously became colonized by the spread of E. coli strains between infants in the maternity wards by the hospital staff [35, 37]. ‘Rooming in’, short hospital stays and strict hygiene have reduced such exposure, and today it takes 6 months before 80% of the infants have acquired E. coli [6, 7], indicating a very limited spread of E. coli not only in the hospital, but also in families and homes in modern Western societies.

Enterobacteria other than E. coli, e.g. Klebsiella and Enterobacter, that are common colonizers of the infantile gut [1, 2, 7, 40] rarely derive from the mother [36, 41], because they are uncommon in the adult fecal microbiota [19]. Instead they may spread in maternity and neonatal wards between infants carried by the staff [36, 41]. As these bacteria are present in soil and other natural environments they may also be acquired from foods [39]. We recently showed that early introduction of solid foods was associated with colonization by Klebsiella species [6].

The origin of the anaerobic bacteria colonizing neonates has been little studied. Transfer of bifidobacteria from mother to infant may occur during a vaginal delivery [42] but horizontal transfer is probably also common. Infants’ intestinal carriage of different bifidobacterial species or subgroups varies between different maternity wards, indicating transfer between infants carried by the staff [36, 41]. As these bacteria are present in soil and other natural environments they may also be acquired from foods [39]. We recently showed that early introduction of solid foods was associated with colonization by Klebsiella species [6].

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Clostridial spores resist disinfectants and are ubiquitous in the hospital milieu and other environments [45], and these bacteria are therefore quite easily acquired by the neonate [12, 46–49]. The gut microbiota of the mother is probably less important as a source of colonizing strains. Thus, strains of C. difficile colonizing neonates are rarely of maternal origin [49].

Lactobacilli of the species L. crispatus, L. gasseri, L. iners and L. jensenii dominate the vaginal microbiota of healthy women [50], and maternal vaginal lactobacilli may transiently colonize the intestine of the baby [51]. In accordance, we found that L. gasseri was the commonest Lactobacillus species in the first month of life in Swedish infants [18]. Another source of lactobacilli may be breast milk, as lactobacilli may be found in human milk [52]. Furthermore, as lactobacilli are part of the oral microbiota, these bacteria may be transferred via saliva from parent to infant. L. rhamnosus is a common oral
colonizer and the dominant *Lactobacillus* species in the infantile gut in the first 6 months of life [18]. *Lactobacillus* species that appear later in the microbiota, like *L. paracasei, L. plantarum, L. acidophilus* and *L. delbruckeii*, may be picked up from foods [18].

The origin of the wide range of other anaerobic bacteria successively establishing in the infantile gut has not been determined. Possibly, many of these bacteria are acquired from the maternal gut microbiota during normal vaginal delivery, although their proliferation is restricted during the first period after birth. Alternatively, they are acquired from other yet not identified sources at later time points.

### Influence of Delivery Mode on Intestinal Colonization Pattern

An infant born by cesarean section is not exposed to maternal intestinal and vaginal microbiota during delivery and, thus, the first bacteria establishing in the gut are derived exclusively from other sources. The acquisition of several of the common early colonizers is delayed, especially colonization by *Bacteroides*, but also bifidobacteria and *E. coli* [6, 7, 15, 47, 53–55]. As seen in figure 5, infants delivered by cesarean section do not catch up within the first year of life with respect to colonization by *Bacteroides* and *E. coli*, an illustration of the low exposure to these fecal bacteria in the society [7]. Furthermore, infants delivered by cesarean section have a lower ratio of anaerobic to facultative bacteria at 1 year of age, which might be regarded as a sign of a poorly developed anaerobic microbiota, unable to suppress the growth of facultative bacteria [6].

Infants born by cesarean section commonly show increased colonization with enterobacteria other than *E. coli*, e.g. *Klebsiella* and *Enterobacter* species [6, 7, 56, 57], suggesting that these bacteria may take the place of *E. coli*, whose colonization is reduced due to lack of contact with fecal bacteria. Furthermore, the lack of competition from anaerobic bacteria probably enables their establishment and persistence, a sign of reduced colonization resistance in infants delivered by cesarean section [58].

Clostridia, including *C. perfringens* and *C. difficile* are also more common in the gut microbiota of infants born by cesarean section than in vaginally delivered infants [6, 7, 15, 47]. Clostridial spores are ubiquitous in the environment and might easily expand in the poorly developed anaerobic microbiota of neonates delivered by cesarean section.

### Neonatal Intensive Care and Treatment with Antibiotics

Infants cared for at neonatal intensive care units (NICUs) have few bacterial species in their gut microbiota [59–62]. Coagulase-negative staphylococci,
intestinal colonization of newborn infants

enterobacteria and enterococci are the bacteria most frequently isolated from these infants and anaerobes are almost completely absent [59–61]. Parenteral nutrition and use of broad-spectrum antibiotics selecting for coagulase-negative staphylococci, enterococci and non-\textit{E. coli} enterobacteria, which frequently have resistance mechanisms, may be responsible for this quite 'unnatural' colonization pattern.

Breastfeeding and Intestinal Colonization Pattern

It is often stated that breastfeeding has a profound influence on the gut microbiota, but many studies from the 1980s and onwards report only minor differences in the colonization pattern of breast- and formula-fed infants (table 2). Bifidobacteria are mostly found equally often and in similar counts

\textbf{Fig. 5.} Frequency of colonization by (\textit{a}) \textit{E. coli} and (\textit{b}) \textit{Bacteroides} at different time points after birth in Swedish infants delivered vaginally or by cesarean section. Adapted from reference Adlerberth et al. [7].
in breast- and formula-fed infants using conventional culture-[2, 9, 21, 23, 40, 63–66] or non-culture-dependent methods [15, 67–71]. Previous reports of marked differences in the colonization frequency of bifidobacteria between breast- and formula-fed infants may be explained by the use of older types of infant formulas with higher protein and phosphate content.

*Bacteroides* are isolated less frequently from breastfed than from formula-fed infants in many studies [2, 15, 21, 72, 73], but others find no differences regarding colonization by *Bacteroides* [14, 23, 40, 65, 66, 74]. Clostridia, including *C. difficile*, are usually more prevalent in formula-fed infants [2, 9, 15, 21, 23, 69, 72, 73, 75].

Among facultative bacteria, some studies report higher counts of staphylococci in breastfed than bottle-fed infants, especially during the first weeks of life [2, 40, 65, 66, 76]. As mentioned earlier, these bacteria might be acquired from the mother during breastfeeding. In contrast, breastfed infants may have lower counts of enterococci [2, 9, 21, 72, 73, 77] and enterobacteria [9, 15, 21, 69, 73] than formula-fed infants.

It is commonly stated that breastfeeding promotes the expansion of lactobacilli in the neonatal gut. This might relate to the fact that bifidobacteria were included earlier in the genus *Lactobacillus*, as *L. bifidus*, and not until 1986 were they transferred to the genus *Bifidobacterium* in Bergey’s Manual of Systematic Bacteriology [78]. Clearly, most studies find no differences regarding *Lactobacillus* colonization between breast- and formula-fed infants, or even more lactobacilli in formula-fed infants [15, 21, 23, 72]. However, we recently reported that *L. rhamnosus* was more common in 6-month-old Swedish infants if they still received breast milk than if they had been weaned by that age [18], indicating that certain species of lactobacilli may be favored by breastfeeding.

### Table 2. Results of studies performed from 1980 and onwards comparing the intestinal microbiota of breastfed and formula-fed infants

<table>
<thead>
<tr>
<th>Bacterial group</th>
<th>Number of studies</th>
<th>↑ in breastfed</th>
<th>no clear difference</th>
<th>↓ in breastfed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bifidobacteria</td>
<td>7/27 20/27</td>
<td>2/19</td>
<td>9/19 8/19</td>
<td>4/17 13/17</td>
</tr>
<tr>
<td><em>Bacteroides</em></td>
<td>2/19 9/19</td>
<td>4/17 13/17</td>
<td>10/16 4/16</td>
<td>5/16 11/16</td>
</tr>
<tr>
<td>Clostridia</td>
<td>–</td>
<td>1/21</td>
<td>5/16 11/16</td>
<td>7/12 –</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>2/16 10/16</td>
<td>1/21</td>
<td>5/16 11/16</td>
<td>–</td>
</tr>
<tr>
<td>Enterobacteria</td>
<td>1/21 9/21</td>
<td>5/16 11/16</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Enterococci</td>
<td>–</td>
<td>5/16 11/16</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Data from references [12, 15, 67–71, 81–85].
Infant formulas have been adapted in several ways in order to induce a colonization pattern similar to breastfed infants. For example, addition of lactoferrin or nucleotides to infant formulas has been tested, but does not seem to change the microbiota of bottle-fed infants towards the ‘breastfed’ pattern [63, 74, 79, 80]. Supplementation with galactooligosaccharides and fructooligosaccharides may increase bifidobacterial counts [68, 81–85], although it is doubtful if this would represent a ‘breastfed’ pattern as high bifidobacterial counts are common in both formula-fed and breastfed infants. Most studies find no effect of supplementation with galacto- and fructooligosaccharides on colonization by Bacteroides, clostridia, enterococci, E. coli or other Enterobacteriaceae [81, 82, 84, 86, 87], although one study reported reduced colonization by facultative bacteria [85] and another reduced counts of clostridia [88] in infants receiving supplemented formula. Intake of a formula supplemented with fructo- but not galactooligosaccharides led to decreased counts of E. coli and enterococci but increased counts of Bacteroides in one study [89], and to increased counts of clostridia and Bacteroides in another [90]. In summary, the effects of oligosaccharides on intestinal bacteria other than bifidobacteria are inconclusive and probably minor.

**Effects of Lifestyle on the Intestinal Microbiota**

The effect of family, home environment and lifestyle on the establishment of the intestinal microbiota has been little studied. When examining the colonization pattern of 300 Swedish, Italian and British infants over the first year of life, we noted an effect of lifestyle on some colonization parameters [6]. Infants with older siblings were less often colonized by non-E. coli enterobacteria and clostridia than single children and had a higher ratio of anaerobic over facultative bacteria at 12 months of age, suggesting a more mature colonization pattern. The colonization pattern of single children, thus, showed some resemblance to that of infants born by cesarean section, although the observed effects were weaker. Thus, both delivery by cesarean section and being first-born may result in an anaerobic microbiota of low complexity, unable to suppress the growth of facultatives [6]. In accordance, another study found that infants with older siblings tended to have higher counts of bifidobacteria in the gut at 1 month of age compared to firstborn infants [15]. Whether this results from a spread of bacteria between siblings, or results from differences in transfer of bacteria during first and subsequent deliveries is not known. Usually, second and third deliveries are more rapid, and it is possible that contamination of the baby with fecal bacteria occurs more often compared with first deliveries.

Having pets in the home does not seem to influence the colonization pattern of neonates [6, 15]. However, in a recent study including more than 200 Swedish and Italian neonates, having pets was associated with decreased colonization by S. aureus early in life [Lindberg et al., in preparation].
Penders et al. [15] also examined the effect of farm residence on the intestinal microbiota, i.e. colonization by bifidobacteria, *Bacteroides*, *E. coli*, lactobacilli and *C. difficile* by 1 month of age. No statistically significant associations were found, but there was a tendency towards lower counts of *C. difficile* in infants in farming families [15], which could indicate a generally more complex microbiota in these infants. Clearly, further studies are needed to address the effects of various lifestyle factors on intestinal colonization pattern in infancy.

### Colonization Pattern of Infants in Non-Western Societies

Whereas Western infants are born in hospitals with high hygienic standards and grow up in households with excellent sanitary conditions, infants in developing countries are often delivered and raised under poor and crowded conditions. It is not surprising that these conditions influence the early colonization pattern. Although few direct comparative studies have been performed, it is clear that infants in developing countries are colonized earlier by *E. coli* and other enterobacteria, enterococci and lactobacilli and have a more varied microbiota early in life than infants in Western societies [1, 3, 39, 91, 92]. Furthermore, in developing countries, also infants delivered by cesarean section acquire gut bacteria, including the anaerobes bifidobacteria and *Bacteroides*, very early [1, 92], a testimony to the circulation of fecal bacteria in the hospital.

The heavy exposure to a wide variety of bacteria in developing countries leads to a rapid turnover of strains in the microbiota. Pakistani infants harbor 8–9 different *E. coli* strains in their microbiota over the first 6 months of life [39], compared with two strains in Swedish infants followed over the first year of life [11].

‘Opportunistic’ colonizers are less common in the gut microbiota of infants in developing countries than in Western societies, as a sign of a much earlier acquisition of a complex microbiota. Skin bacteria like coagulase-negative staphylococci are common in the gut flora of European infants but uncommon in Ethiopian infants [6, 91]. *S. aureus* is less common in the gut microbiota of infants in developing as compared to Western countries, as evident when comparing isolation rates in different studies [3, 10]. Although Pakistani infants frequently become colonized by enterobacteria other than *E. coli* in the first week of life, they disappear from the microbiota already after some weeks, probably in response to overwhelming competition [1, 39]. In contrast, Swedish infants display increasing colonization by enterobacteria other than *E. coli* over the first 6 months of life [7].

Differences in colonization pattern have also been observed between infants in Western societies and infants in the former socialist countries of Eastern Europe, the latter being colonized earlier with lactobacilli and eubacteria [20, 93]. At 1 year of age Estonian children less frequently harbor *C. difficile* than Swedish infants [20]. Interestingly, colonization by *S. aureus* is more common
in Swedish than in Italian infants in the first year of life [Lindberg et al., in preparation], possibly indicating an earlier maturation of the intestinal microbiota in Italian infants.

**Possible Consequences to Health of the ‘Non-Western’ and ‘Western’ Colonization Patterns**

In developing countries, the early and diverse gut microbiota containing, e.g., various enterobacterial strains may be an important source of bacteria causing septicemia in the neonatal period, as bacteria may reach the bloodstream by direct translocation over the intestinal epithelial barrier when reaching high population counts in the gut [94]. The incidence of neonatal septicemia is much higher in developing as compared to industrialized societies (20/1,000 vs. 4/1,000) [12], and a majority of these infections is caused by intestinal bacteria such as *E. coli*, *Klebsiella* and other enterobacteria, enterococci and *Pseudomonas* [12, 95, 96].

The consequences for health of the ‘Western’ colonization pattern are not known. It is interesting to note that staphylococci are today the most common cause of neonatal septicemia in Sweden and many other Western societies [97], and the intestinal microbiota may well be a source of infecting strains [59].

Intestinal commensal bacteria are a major stimulus for the gut immune system [98], and a late acquisition of typical fecal bacteria or a delay in the establishment of a complex and diverse intestinal microbiota may have profound effects on the developing immune system. In accordance, Pakistani infants show a strong salivary IgA response against a pool of *E. coli* O antigens at 2 months of age, while similar levels are not reached by Swedish infants until 1–2 years of age [99].

Whether the rising incidence of immunoregulatory disorders such as allergies, Crohn’s disease and type-1 diabetes in Western countries depends on a paucity of immune stimulation, e.g. by gut bacteria, remains to be investigated.

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Intestinal Colonization of Newborn Infants

Intestinal Colonization of Newborn Infants


Discussion

Dr. Isolauri: You mentioned two points of confusion: one was that not constantly breastfeeding seems to promote bifidobacteria in the infant. I think that is not very confusing if one accepts that breast milk is not a standard product, also in terms of
prebiotics as well as bifidobacteria; breast milk is a reflection of the mother. You also mentioned another point that the addition of oligosaccharides increases bifidobacteria and sometimes it doesn’t. At the same time you said that not all infants are colonized with bifidobacteria early on. I don’t see a confusion here either because bifidogenic agents probably do not promote bifidobacteria if the baby is not colonized early with bifidobacteria. Somebody needs to be stimulated; so the early days appear to be very important. Do you agree?

Dr. Adlerberth: I agree with you. Clearly, prebiotics need to have some bacteria present from the start to be able to support their growth. The data concerning promotion of bifidobacterial growth by those oligosaccharides are quite convincing, but the results are less convincing regarding lactobacilli [1–7]. As you said, this could well have to do with a low colonization frequency with lactobacilli from the beginning.

Dr. Björkstén: I would like to have your comments on microbial diversity. Most studies, including most of our studies and those of our chairperson, have looked at single species rather than using a holistic approach. You used a different approach comparing variations in *Escherichia coli* strains in Pakistani and Swedish children, showing that the Pakistani children had a constant change in strains while in Sweden the same strains remained for long periods of time in the individual child. We have seen the same thing looking for lactobacilli in Estonia as compared to Sweden, all species are present in both countries but the Estonians often change strains while the diversity is less in Swedish children. I think it is a major difference from an immunological point of view if one is continuously exposed to hundreds of strains or keeps the same strains over long periods of time. Have you looked at diversity in your recent studies?

Dr. Adlerberth: Yes, for example we have done strain typing of all *E. coli* stains from Swedish infants in the Allergyflora Study, and it is clear that infants in Sweden encounter very few *E. coli* stains and commonly keep a single strain over prolonged periods. Thus, there is little immune stimulation provided by *E. coli*, for instance, since the immune system is activated by each new strain that colonizes, but this activation ceases as soon as a secretory IgA response against the strain has developed, as S-IgA prevents translocation of the strain and further interactions with the immune system [8]. But if one is constantly exposed to new strains they will have constant stimulation of the immune system.

Dr. Björkstén: Benn et al. [9] in Copenhagen showed that the presence of staphylococci in the vaginal flora in mid-pregnancy is associated with a more than twofold increased risk of asthma medication at age 5 years, so it seems to be a very early event indeed.

Dr. Walker: As I look at the literature there are some questions that come up that I find difficult to explain in the context of bacterial flora and clinical disease. For example, there are number of studies suggesting that in inflammatory bowel disease there is a difference in the composition of flora compared to non-inflammatory bowel disease patients and the same thing has been reported for allergic patients. What I don’t quite understand is if one is dealing with a billion organisms in the gut, if some strains are reduced by a couple of logs, how does that potentially influence the pathophysiology the diseases or is it just a marker of the disease?

Dr. Adlerberth: There are great variations between gut bacteria, for instance regarding their ability to evoke an inflammatory response. To start with, gram-positive and gram-negative bacteria differ with regard to the cytokine patterns and proinflammatory mediators they induce, so one could expect differences between a flora dominated by gram-positive and one dominated by gram-negative bacteria [10]. Furthermore, at the species level, there may be great variations between different strains in their immunotogenic and immunogenic properties. For example, it has
been very nicely shown for E. coli that certain strains are very potent in evoking inflammatory responses depending on their expression of toxins and other virulence factors [11]. However, we know very little about the properties of most bacteria residing in the gut. I believe that the composition of the microflora could be very important for the emergence of a range of diseases, but we know very little about it yet.

Dr. Walker: Is there a corollary to that observation, e.g., can we extrapolate from culturing stools to what is going on at the surface of the colon and small intestine with regard to disease? Is there an association?

Dr. Adlerberth: There is clearly an association, but in the mucosa there are proportionally more aerobic bacteria than in the gut lumen and in the fecal sample. So very much the same species are present but in different proportions, and there are also bacteria in the gut which are not in association with the mucosa and which feed mostly from dietary substances, although most bacteria are likely to feed from the mucous layer.

Dr. Salminen: I found your presentation quite intriguing especially when you compared the older and the newer studies which I sometimes think are not really comparable. To the best of my knowledge the only published studies for bifidobacteria are from Benno et al. [12] who have followed the Japanese bifidobacterial numbers and species composition in infants since 1970. They also used a similar culturing method which was upgraded along the line to the neomolecular methods. I think their results in Japanese infants actually point out that there is no change in the concentration over the decades, but there is a significant change in the species composition, and therefore the metabolic activity of bifidobacteria in breastfed infants indicates that it is either environmental or dietary due to the mother's diet. So it is quite difficult to actually interpret whether there is a change. But to actually put them into the perspective of the method used, I think the traces are perhaps not as significant within the gut.

Dr. Adlerberth: Of course, methodological differences between studies make it difficult to compare the results, but still we can get indications pointing towards changes in the microbiota over the last decades. Regarding bifidobacteria, colonization today is as frequent as in earlier studies, but it would be very interesting to look at the species variation in the bifidobacterial flora of Western infants to see if it is as diverse today as it has been, and if the same species predominate.

Dr. Salminen: That poses an additional question because not very many differences were seen; the country differences haven't been shown yet. As Dr. Björkstén also pointed out, if we look at our studies no differences are seen with the use of the traditional culture method, and I think the culture method is just not sensitive enough. When molecular methods are used, and when you go to species composition and start measuring the metabolic activity of the different species, then significant differences are actually seen: differences between different birth methods and, most significantly, early differences in microbiota between the children who later develop the atopic disease. I understand that you are continuing on the molecular methods with your recent cohort, is that right?

Dr. Adlerberth: Yes we are. I agree with you, we need to go over these molecular methods but still today I think that both culturing and molecular methods are needed, since most molecular methods detect only the dominant bacterial populations. For instance, with T-RLFP you can only detect bacteria which are present in populations above $10^6$ or $10^7$/g, whereas culture on selective media may detect much smaller bacterial populations. With time, however, I think that the molecular methods will take over completely.

Dr. Salminen: I completely agree with you, they complement each other, but when we take modern molecular methods coupled with metabolic activity and bioavailability measurement, that is perhaps the best outcome so far.
Dr. Saavedra: This was a great summary with regard to what we are seeing from the point of view of colonization in the distal bowel and colon. One thing we always neglect to consider is that there are different populations in the gut depending on where we look in the gut: close to the mucosa or closer to the lumen, or the proximal gut versus the distal gut. When we examine stool we are of course looking at the very distal end of things. If we compare what the differences today might be with the differences yesterday, it is likely that bigger differences are happening in the small bowel. Today most of the intervention studies suggest that anything from $10^6$, $10^8$, or higher colonies be given orally as an intervention to correct what might be the difference between today's flora in the proximal gut rather than the distal gut. If an endoscopy is done today in an average child to obtain a duodenal aspirate and $10^6$ colony-forming units of bacteria are found, it would be called abnormal bacterial overgrowth, and that is really what we are giving orally in all these intervention studies. So aren't we looking at the wrong thing from the point of view of really trying to understand what the immunologic potential is that dietary microbes might have?

Dr. Adlerberth: I agree with you in that what happens in the small intestine is of prior importance for stimulation of the immune system. This is where most of the antigen uptake is going on. However, also in the colon there are lymphoid patches and also translocation and induction of immune responses, although the events in the small intestine are probably most important. It is not so easy, however, to study the microbiota of the small intestine.

Dr. Hernell: I think it is true that when you start to introduce complementary food the microbiota becomes more diversified as the diet becomes more diversified. When we now postpone the introduction of complementary foods to 6 months, how would that affect the flora and the stimulation of the immune system? Actually, that hasn't been discussed much.

Dr. Adlerberth: Many classical studies have clearly shown that at weaning there is a major change in the microbiota, for example the studies by Mata and Urratia [13] and Stark and Lee [14] from Guatemala and Australia. However, in a recent study in which molecular methods were applied to study infantile microbiota, there were actually very little changes detected in the flora in connection to weaning [15]. So perhaps today weaning does not have a great impact on the microbiota.

Dr. Corthésy: Among all the factors that can influence early gut colonization you didn't mention bacterial translocation. There is evidence that bacteria can translocate from the gut of mothers via the milk to the children. What are your views on the impact that this can have on early colonization?

Dr. Björkstén: It has been known for more than 30 years that gut bacteria are present in human milk, but there is still no evidence that they are translocated. We don't know how they got there, though. One of the currently marketed probiotic strains (Lactobacillus reuteri) was actually isolated from the breast milk of a Peruvian woman.

Dr. Isolauri: Currently we agree that breast milk is not sterile like infant formulas.

References


