Colonization of the gastrointestinal tract

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Introduction

The colonization of the gastrointestinal tract is a complex and multifactorial process characterized by the dynamic interaction of forces exerted by environmental, dietary, microbially associated and host-related factors. Colonization can be defined as the persistence of microorganisms in a particular bodily site without causing disease in healthy hosts under normal circumstances. This is in contrast to infection whereby the establishment and persistence of a microbial population in the body has the potential to cause disease [1].

Generally, the various body surfaces and gastrointestinal tracts of humans and animals are colonized by vast numbers of prokaryotic and eukaryotic microbial cells whose total numbers have been estimated in humans, to be around $10^{14}$ cells. As mammalian cells of the adult human are approximately $10^{13}$, they are outnumbered by a factor of around 10 by colonizing microbial cells [2]. Therefore, a profound influence of the latter on the metabolic, nutritional, immunological and physiological processes of the host can be postulated.

The intestine represents a dynamic (open) and stable microbial ecosystem in a continually changing environment. The gastrointestinal tract is the most heavily colonized area of the human body. The terms “intestinal microflora” or “indigenous flora” usually are applied to describe the collection of microorganisms that normally inhabit various gastrointestinal sites and form stable climax (i.e. reaching a maximal density) communities that are always present within the intestines of normal adults. Of the various anatomical regions of the gastrointestinal tract, extending from the oral cavity to the anus, the large intestine (i.e. the colon) is the most diversely populated region of the intestine. The colon contains bacteria from well over 400 different species and in concentrations ranging from $10^{10}$ to $10^{11}$ microbial cells per g of intestinal contents [3].

The role of intestinal microflora in health and disease is becoming increasingly recognized. There is evidence that the composition and activities of the gastrointestinal microflora can have beneficial and pathogenic outcomes for the host. Gibson and Roberfroid [4] generally have split predominant human faecal bacteria into 3 groups, those that can exert harmful or beneficial effects on the host or both.

Beneficial health-promoting effects could arise from: i) energy salvage from the fermentation of dietary carbohydrates and proteins reaching the colon [5]; ii) synthesis of vitamins, primarily of the B and K groups, although the
metabolic relevance of this in the hindgut is likely to be low [3, 6]; iii) production of short-chain fatty acids (SCFA) as bacterial metabolic end products that can exert antipathogenic effects by lowering the pH of the intestinal lumen thereby facilitating water absorption by the colon [4, 6]; iv) production of antimicrobial compounds [7-9]; and v) enhancement of gut barrier functions by competing with pathogens for adhesion receptors on the intestinal mucosa, competition for nutrients and stimulation of the host immunity [3, 10-12].

However, the intestinal microflora also can have detrimental effects upon the host’s health. Such pathogenic effects could arise from the conversion of non-carcinogens or pre-carcinogens to carcinogens and the production of toxic end products of protein metabolism (e.g. ammonia, phenolic compounds and amines), microbial overgrowth following disruption of the gastrointestinal microecology by oral antibiotics, shock or other conditions, and opportunistic infections by the passage of bacteria across the mucosal barrier to the mesenteric lymph nodes and other extra intestinal sites (known as bacterial translocation), especially in immuno-compromized or traumatized hosts [3-5].

There is currently a great deal of scientific, medical and commercial interest in improving the understanding of the role and function of intestinal microflora to generate appropriate modulation strategies beneficial to the host. For example, there is accumulating evidence that dietary modulation using functional foods (e.g. probiotics and prebiotics) could be beneficial for the host by effecting a health-promoting modification of the composition and activities of the intestinal microbiota [13]. In particular, one critical period of gut colonization during the sensitive stage of infancy has been receiving attention as it is thought to have subsequent implications in later life.

The aim of this review is to provide a basic understanding of the gut colonization process by examining major factors that may influence it, and describe the composition and metabolic activities of the gut microflora in human infants. Colonization of the neonatal intestine is a complex process that depends upon forces exerted by external environmental and dietary, microbially associated and host-related factors. Thus, differences in the gut microflora composition and its metabolic activities between breast-fed and formula-fed infants are pointed out and discussed.

Factors affecting the gut colonization process

Environmental and dietary factors

Shortly following birth, the previously sterile infant gut begins to be colonized by an array of bacteria that are facultative anaerobes and strict anaerobes. The newborn first comes in contact with bacteria from the birth canal and its surroundings. Factors such as microbial flora of the female genital tract [14-16], sanitary conditions [17, 18], obstetric techniques [19], vaginal or Caesarean mode of delivery [10, 20, 21], geographical distribution of bacterial species [18, 20, 22] can all affect the level and frequency of various species colonizing the infant gut.

Gross differences exist in the composition of the intestinal microflora and the gastrointestinal disease incidence as exemplified by breast- and formula-feeding. These differences could be due to human milk components that currently cannot be replicated in infant formulae.

Generally, differences have been noted in the exposure level and acquisition frequency of bacteria early in life between infants born in developing or industrialized countries. In industrialized countries, obstetric and hygienic procedures and practices in the maternity and neonatal facilities aim to minimize the spread of pathogens, may delay development patterns and may even prevent the successful colonization by several bacterial groups [23]. And not many of the microbes that newborns encounter are able
to colonize the various gastrointestinal habitats and therefore disappear shortly following birth [2].

Infant feeding choices has been implicated in gross differences in the composition of the intestinal microflora and the gastrointestinal disease incidence as exemplified by breast- and formula-feeding [18, 22, 24-27]. These differences could be due to human milk components that currently cannot be replicated in infant formulae. Human milk is species-specific and the standard for infant nutrition. It contains a myriad of components such as secretory immunoglobulin A (SIgA), peptide and non-peptide hormones, growth factors, proteins and peptides, lipids, milk membrane fractions and oligosaccharides, that have significant bioactive and immunomodulatory roles [28]. Infant formulae cannot replicate such bioactive and immunomodulatory properties because of complex quantitative and qualitative component differences [29]. This may be one reason why long-term epidemiological research has demonstrated that breast-fed infants are better protected against infections of the gut, respiratory and urinary tracts compared to those who are formula-fed [30, 31].

Other dietary factors that could affect gut colonization is the effect that certain milk proteins exert upon the intestinal microflora through regulation of gut motility, antibacterial action and bifidogenic properties [32, 33]. In addition, human milk oligosaccharides (HMO) are known to be potent inhibitors of microbial adhesion to epithelial cells by acting as receptor analogues for mucosal adhesion molecules [34, 35]. Among the HMO, lacto-N-tetraose and lacto-N-neotetraose act as cell surface receptors for Streptococcus pneumoniae; fucosylated oligosaccharides are receptors for Escherichia coli; sialated oligosaccharides are recognized receptor sites for influenza viruses A, B and C, Campylobacter pylori and Mycoplasma pneumoniae [34].

**Microbially associated factors**

Colonic bacteria must find a suitable intestinal attachment site (habitat) and attach to it. Four characteristic bacterial habitats have been described in the intestine: the intestinal lumen, the unstirred mucus layer or mucus gel that overlies the intestinal epithelium, the deep layer of mucus gel in the intestinal crypts and the mucosal epithelial cells surfaces [3, 23].

Obligate anaerobes, which are the numerically predominant indigenous gastrointestinal bacteria, associate intimately with the gut wall to form layers upon the mucosal epithelium [3]. Microbial surfaces that contact host’s cells must be sufficiently similar to host antigens to avoid triggering immunological reactions [2]. To persist in the gut, colonizing microbes should be able to survive the gut’s physicochemical conditions (e.g. digesta flow, pH and redox potential) and compete successfully with established species and others attempting to occupy the same intestinal sites. Microorganisms require adequate nutrient supplies to support their sufficient growth and generation. Newly divided bacteria replace dead bacterial cells, those washed away from colonization sites by peristaltic flows of digesta and bacteria that are removed with epithelial cells sloughed as epithelial cells turnover.

**Host-related factors**

Differences exist with respect to the composition and colonization levels in different anatomical regions of the gastrointestinal tract. In adult humans, the oral cavity contains an indigenous microflora of approximately 200 species with a dense bacterial population in the dental plaque (10^{11} bacteria g^{-1}) and a transient bacterial population in saliva. The human stomach and the upper-mid part of the small intestine (i.e. duodenum and jejunum) contain relatively low numbers of microbes (10^{2}-10^{4} bacteria ml^{-1} of gastric or intestinal contents). These are limited by a low pH and rapid flow of digesta. These microbes are mostly acid tolerant lactobacilli and streptococci and are transients from the oral cavity rather than being indigenous to the upper mid gut [2, 3]. The distal small intestine (ileum) maintains a more diverse microflora and higher bacterial populations (10^{8} bacteria ml^{-1} intestinal contents) and is the interface zone between the sparse microflora of the upper bowel and the enormous colonic microflora residing in the
colon ($10^{10}$-$10^{11}$ bacteria g$^{-1}$ intestinal contents). The large intestine is the primary site of microbial colonization because of the combination of nutrient supply, with a slow flow of digesta and a low redox potential [4].

Various other host related factors can influence and determine conditions for microbial colonization in particular regions of the host’s gastrointestinal tract. Such factors include age, sex, intestinal pH and redox potential, endogenous nutrients, various physiological functions such as adrenal function, peristalsis (gut motility), secretions of digestive enzymes, hydrochloric acid, bile and mucus, host immune mechanisms, bacterial mucosal receptors and emotional stress [2, 23, 36].

Regulation of the composition and localization of microbial communities in the gastrointestinal tract is a multifactorial process whereby any or all of these many forces may come into operation. For any given community, these factors must balance delicately to maintain the community’s structure [2].

**Composition and metabolic activities of the infants’ intestinal microflora**

An array of bacteria attempts to colonize the newborn. It gives rise to a diverse intestinal flora. The predominant bacterial genera and species isolated from infant faeces are given in table I. During the first week of life, facultative anaerobic bacteria such as enterobacteria (e.g. *E. coli*) and streptococci appear to be initial gut’s colonizers gut. These are followed subsequently by more strictly anaerobic bifidobacteria and bacteroides [24-26]. The gut’s initial colon-

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**Table I: Predominant bacterial genera and species colonizing the infant intestine.**

<table>
<thead>
<tr>
<th>Faculative anaerobes</th>
<th>Strict anaerobes</th>
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<tr>
<td><em>Escherichia</em></td>
<td><em>E. coli</em></td>
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<td><em>Bifidobacterium</em></td>
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<tr>
<td><em>B. breve</em></td>
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<td><em>B. longum</em></td>
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<tr>
<td><em>B. adolescentis</em></td>
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<td><em>B. bifidum</em></td>
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<tr>
<td><em>B. infantis</em></td>
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<tr>
<td><em>Staphylococcus</em></td>
<td><em>S. aureus</em></td>
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<td><em>S. epidermidis</em></td>
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<tr>
<td><em>Bacteroides</em></td>
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<tr>
<td><em>B. fragilis</em></td>
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<td><em>B. distasonis</em></td>
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<td><em>B. vulgatus</em></td>
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<td><em>B. ovatus</em></td>
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<tr>
<td><em>B. thetaiotaomicron</em></td>
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<td><em>B. uniformis</em></td>
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<tr>
<td><em>Streptococcus</em></td>
<td><em>S. faecalis</em></td>
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<td><em>S. faecium</em></td>
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<tr>
<td><em>Clostridium</em></td>
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<tr>
<td><em>C. perfringens</em></td>
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<tr>
<td><em>C. difficile</em></td>
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<td><em>C. butyricum</em></td>
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<tr>
<td><em>C. tertium</em></td>
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<tr>
<td><em>C. paraputrificum</em></td>
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<tr>
<td><em>Enterobacter</em></td>
<td><em>E. cloacae</em></td>
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<td><em>Lactobacillus</em></td>
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<td><em>L. acidophilus</em></td>
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<td><em>L. fermentum</em></td>
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<tr>
<td><em>L. brevis</em></td>
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<tr>
<td><em>L. salivarius</em></td>
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<td><em>L. plantarum</em></td>
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<tr>
<td><em>Klebsiella</em></td>
<td><em>K. pneumoniae</em></td>
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<td><em>Eubacterium</em></td>
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<td><em>E. aerofaciens</em></td>
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<tr>
<td><em>E. lentum</em></td>
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<td><em>E. rectale</em></td>
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<tr>
<td><em>Proteus</em></td>
<td><em>P. mirabilis</em></td>
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<td><em>Veillonella</em></td>
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<td><em>V. parvula</em></td>
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<tr>
<td><em>Citrobacter</em></td>
<td><em>C. freundii</em></td>
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<td><em>Peptococcus</em></td>
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<tr>
<td><em>P. saccharolyticus</em></td>
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<tr>
<td><em>Pseudomonas</em></td>
<td><em>Ps. aeruginosa</em></td>
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<td><em>Peptostreptococcus</em></td>
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<tr>
<td><em>P. productus</em></td>
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<tr>
<td><em>P. anaerobius</em></td>
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</table>
nization by facultative anaerobes mediates reduction of the redox potential in the intestinal lumen that in turn is thought to be a prerequisite for subsequent colonization by anaerobes [25].

**Intestinal microflora of breast-fed and bottle-fed infants**

Various studies demonstrate that the composition of the human infant’s intestinal microflora is very much dependent upon the type of feeding. The breast-fed infant’s intestinal microflora appears to be dominated by bifidobacteria. Formula-fed infants seem to develop a more complex microflora with facultative anaerobes, bacteroides and clostridia at higher levels and frequency than in breast-fed infants [18, 22, 25, 27]. A bifidobacterial flora predominance in formula-fed infants is also common, although in lower numbers and frequency than observed in breast-fed infants of the same age [22, 25, 26, 37-40]. However, depending upon constituents of experimental infant formulae, there are cases in bottle-fed infants in whom bifidobacteria are not predominant. Instead, bacteroides [18, 19, 39], coliforms [19, 24, 41] and enterococci [41] were seen to be prevalent (Table II).

The intestinal microflora of breast-fed infants who also receive formula appears to shift towards the pattern of formula-fed infants [24]. When solid foods are introduced, differences between breast-fed and formula-fed infants are lost and the microflora resemble that of adults by about the second year of life [23, 25, 40].

Human milk has a lower buffering capacity, which allows luminal contents of breast-fed infants to be acidified more easily following bacterial fermentation in the proximal colon [24]. This also may have an inhibitory effect on the growth of clostridia, bacteroides and other anaerobes, which as a result appear in lower numbers in the faeces of breast-fed infants. The bifidobacterial predominance usually observed in the faeces of both breast- and formula-fed infants also may be due to the fact that these bacteria can tolerate a less reduced environment for growth than other anaerobes [42].

The composition of the human infant’s intestinal microflora is very much dependent upon the type of feeding. The breast-fed infant’s intestinal microflora appears to be dominated by bifidobacteria. Formula-fed infants seem to develop a more complex microflora with facultative anaerobes, bacteroides and clostridia at higher levels and frequency than in breast-fed infants.

A lower buffering capacity resulting from reduced protein and phosphorous contents in formulae also may contribute to an increased prevalence of bifidobacteria [43]. However, bifidobacteria and/or lactobacilli predominance was not observed in a study conducted by Rose

**Table II: Counts of predominant populations of bacterial genera (log_{10} CFU/g faeces) calculated from various studies undertaken at weeks 1, 3-4 and 6-12 of age in exclusively breast-fed (BF) and formula-fed (FF) infants (calculated from the respective data of ref. [18, 19, 21, 24, 26, 39, 41]).**

<table>
<thead>
<tr>
<th>Predominant bacteria genera</th>
<th>W1 BF</th>
<th>W3-4 FF</th>
<th>W6-12 BF</th>
<th>Adult*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bifidobacteria</td>
<td>8.3 8.0</td>
<td>9.5 9.0</td>
<td>9.1 9.1</td>
<td>10.2</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>7.9 6.6</td>
<td>7.1 7.6</td>
<td>7.3 7.9</td>
<td>9.6</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>7.1 7.8</td>
<td>7.7 8.7</td>
<td>8.3 8.4</td>
<td>11.3</td>
</tr>
<tr>
<td>Clostridia</td>
<td>6.1 6.1</td>
<td>5.9 6.8</td>
<td>5.6 6.5</td>
<td>9.8</td>
</tr>
<tr>
<td>Coliforms</td>
<td>8.6 8.3</td>
<td>8.6 8.7</td>
<td>9.2 9.1</td>
<td>8.6</td>
</tr>
<tr>
<td>Streptococci</td>
<td>6.5 8.0</td>
<td>6.7 8.5</td>
<td>7.1 8.9</td>
<td>8.9</td>
</tr>
</tbody>
</table>

* Data for the adult human included for comparison originate from Salminen et al. [13]
[44], irrespective of the type of feeding and level of buffering capacity in infant diets. Enterobacteria were present in greater numbers in each group at all times.

From the bifidobacterial species isolated from faeces of 35 breast-fed and 35 bottle-fed infants (mean bifidobacterial count: 10.74±0.81 log CFU/g faeces and 10.62±0.49 log CFU/g faeces, respectively) aged 28 to 46 days, B. breve occurred most frequently in both breast-fed and bottle-fed infants (89% and 83%, respectively), followed by B. adolescentis (49% and 37%), B. longum (43% and 43%) and finally B. bifidum (14% and 26%). There were no statistically significant differences with respect to the bifidobacterial species counts and frequency between the two groups of infants. B. infantis was not isolated from any of the samples [38].

In the study of Yuhara et al. [37], bifidobacteria was the predominant genus isolated from the faeces of 30 breast-fed and 40 bottle-fed infants (mean count: 10.7±0.9 log CFU/g faeces and 10.0±2.2 log CFU/g faeces, respectively) aged 33-135 days and 3-134 days, respectively. The frequency of occurrence of B. breve, B. adolescentis, B. longum and B. bifidum was 90, 40, 67 and 70%, respectively, for breast-fed infants, and 93, 53, 45 and 23% for bottle-fed infants. Although the frequency of occurrence of B. bifidum in bottle-fed infants was much less than for breast-fed infants, overall there was no statistically significant difference in the numbers and frequencies of occurrence of each species between the two groups. B. infantis occurred at very low frequencies of 7% and 8% in breast-fed and bottle-fed infants [37].

Similarly, in the study of Mevissen-Verhage et al. [22], the bifidobacteria predominated. Species most frequently isolated from breast-fed and formula-fed infants were B. breve, B. adolescentis, B. longum and B. bifidum. Again, B. infantis was isolated infrequently. On the contrary, the most predominant bifidobacterial species in the studies of Kleessen et al. [40] was B. infantis, followed by B. bifidum, B. breve, B. longum and B. adolescentis.

Lactobacilli appear and disappear inconsistently from birth until weaning [18, 25]. This suggests that they are unable to form stable populations in the infant gut. For example, not all studies shown in table II reported detectable counts of lactobacilli. None of the lactobacilli present in maternal vaginal flora appeared to colonize the digestive tracts of normally delivered full-term infants [16].

For Bacteroides, the species most frequently isolated belong to the B. fragilis group and are mainly B. fragilis, B. distasonis and B. vulgatus. Generally, bottle-fed infants are more likely to have higher bacteroides counts (Table II) and colonisation frequency compared to those observed in breast-fed infants [18, 22, 27, 38, 40]. Dietary iron, which is incorporated into some infant formulae, is associated with increased numbers of bacteroides [22, 40]. In a study carried out by Benno et al. [38], bacteroides were significantly lower (p<0.05) in breast-fed (8.91±1.76 log CFU/g faeces) compared to iron supplemented formula-fed group of infants (9.5±0.61 log CFU/g faeces). The most prevalent Bacteroides species was B. fragilis.

Breast-fed infants have significantly less clostridia both in terms of counts and colonisation frequency when compared to formula-fed infants [37, 38, 40] (Table II). The most common Clostridium species isolated have been C. difficile, C. perfringens, C. paraputricum and C. tetrium [38]. C. perfringens was isolated most frequently (60-80%) from the faecal specimens of breast-fed and formula-fed infants who were 3-6 weeks old [22].

The development of the anaerobic microflora in infants delivered by Caesarean section appears to be delayed and bifidobacteria did not reach normal levels for 4-8 weeks [45]. Bifidobacteria and lactobacilli colonization rates in Caesarean delivered infants reached the rates of vaginally delivered infants at 1 month and 10 days, respectively [21]. C. perfringens colonized 26% and 90% of the infants delivered by Caesarean section within 48 hours after birth and first 14 days of life, respectively. Breastfeeding led to the repression of C. perfringens, whereas formula feeding allowed its maintenance [10].

New opportunities for the study of microbial ecology using molecular techniques

The microflora composition residing in the gastrointestinal tract of infants has been largely determined in studies of faeces by standard cul-
ture methods and techniques with phenotypic characterizations. These methods are based on colonial morphology and various biochemical markers such as growth requirements, enzyme activities and metabolic end products. However, only a proportion of the overall microbial diversity occurring in the infant colon can be elucidated through traditional cultural methods. These are applicable only to cultivable bacteria and quite often the media chosen are not sufficiently selective for the required bacterial genera or species [46, 47].

Developments in molecular biology have initiated the generation of new and more reliable information on the diversity of gut microflora in animals and humans. The application of nucleic acid probes, which are fragments of single stranded DNA that bind to complementary DNA or RNA (target nucleic acid), has created opportunities for the rapid identification of microorganisms [48].

In prokaryotes, the comparative analysis of ribosomal RNAs (rRNAs), in particular the 16S and 23S rRNA genes, has been a breakthrough for the identification of bacteria from genus down to species or strain level [49-53]. In particular, techniques such as the polymerase chain reaction (PCR) [54], gene sequencing [55] and in situ hybridisation [48] are used routinely. Such methods for the analysis of the intestinal microflora and have been reviewed recently in detail by McCartney [56].

PCR using 16S rRNA targeted primers has been applied successfully to the detection and quantification of predominant anaerobes in human adult and infant faeces [52], and in tracking of probiotic Bifidobacterium in the stools of infants fed an instant milk formula containing this strain [57]. Millar et al. [58] used 16S rRNA gene PCR combined with denaturing gel gradient electrophoresis (DGGE) in research targeting potential causative agents of necrotizing enterocolitis in infants.

In a study by Harmsen et al. [27], the development of intestinal flora in breast- and formula-fed infants during the first 20 days of life was investigated using oligonucleotide probes and fluorescent in situ hybridization (FISH), and a conventional cultural approach. Both groups of infants were colonized initially by a diverse (adult-type) flora during the first 6 days of life, but in the following days a more bifidobacterial dominant flora was established in breast-fed infants. In most formula-fed infants, similar numbers of Bacteroides and Bifidobacterium were found. Their study highlighted questions regarding the relative proportions of various genera in overall gut microbial populations when results from conventional methods are compared with those obtained by molecular methods. It is expected that the use of new, more powerful molecular techniques in gut microbiology will rapidly advance knowledge and understanding of gut microbial ecology and diversity.

Microflora fermentation metabolites and associated characteristics

Fermentation by colonic microflora results in the production of SCFA as major end products, and gases, including hydrogen, carbon dioxide and methane [4, 5]. SCFA are absorbed rapidly by the colonic mucosa facilitating water absorption from the colonic lumen and thus may confer some protection against diarrhoea [5].

Acetate, propionate and butyrate are the main SCFA produced during saccharolytic metabolism in the colon. Lactate, ethanol, succinate, formate, valerate and caproate also constitute significant products. The molar ratios of SCFA formed from carbohydrate fermentation depend on the type of substrate that is fermented [5, 59].
Faecal SCFA profiles in infants differ mainly according to the type of feeding. In breast-fed infants, acetic acid accounts for most of the total SCFA. Acetate is the predominant SCFA in faeces of formula-fed infants, but propionate, and to a lesser extent butyrate, have higher molar ratios when compared to those found in breast-fed infants (Fig. 1). Butyrate attracts attention for its possible biological properties against colon cancer [13]. However, the lower butyrate levels in infant faeces and in vitro faecal incubations with carbohydrates indicated to some that this metabolite might not be as important to colonic enterocytes in the developing intestine of preweaned human neonates than is suggested in adults [13, 60].

Generally, higher amounts of faecal SCFA have been determined in formula-fed compared to breast-fed infants. In the study of Midtvedt and Midtvedt [61], infants who received both breast milk and formula had values of SCFA intermediate to those in infants who received either breast milk or formula. It was hypothesized that these differences occurred because human milk is utilized better, thus less unabsorbed components reach the colon and subsequently less SCFA can be produced. Generally, faecal SCFA concentration in infants is lower than is found in adults [62].

Breast-fed infants tend to have a more acidic stool pH ranging from pH 5 to 6 compared to neutrality found in the faeces of formula-fed infants despite the fact that breast-fed infants have lower amounts of faecal SCFA. This possibly could be explained by human milk’s lower buffering capacity.

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![Figure 1: Molar ratios of faecal short chain fatty acids (SCFA) of breast-fed (BF) and formula-fed (FF) infants. Data originate from [65] and [60]. The respective values for the adult [62] have been included for comparison.](image-url)
(breast milk vs formula) fed in this age interval affected the faecal enzymatic activities. Faeces from formula-fed infants had significantly higher urease activity at 1-2 months of age and higher median activity of β-glucuronidase at 6 months of age [64].

**Carbohydrate fermentation capacity**

Lactose is the main carbohydrate in human milk and infant formulae. Irrespective of the type of feeding, a proportion of lactose escapes digestion and absorption in the small intestine. It becomes available for fermentation by the colonic microflora in both breast-fed and formula-fed infants. Since lactose is the main carbohydrate fermented in the colon, differences in faecal SCFA profiles could be due mainly to variability in the intestinal microflora between the two feeding groups, and possible differences in its ability to ferment it [60, 65] (Table II). Another reason for these differences also could be that human milk contains a significant amount of complex oligosaccharides not found in bovine milk or infant formulae [34]. HMOs have potential anti-infective function in human newborns [34, 35]. HMOs undergo fermentation in the large intestine since they mainly escape digestion and absorption in the small intestine [66, 67]. In particular, N-acetylgalcosamine oligosaccharides have been shown to favour the growth of *Bifidobacterium* species [34]. Bifidobacteria are recognized producers of lactate and acetate and their predominance in the intestinal microflora of breast-fed infants, possibly explains a higher proportion of acetate in the faeces of this group of infants.

The microflora of formula- and breast-fed infants have similar fermentation capacities for simple sugars and oligosaccharides, and equally poor for fermenting complex carbohydrates. In all cases, the fermentation capacity in both groups was lower than in adults [60, 62]. *In vitro* fermentation capacities for complex carbohydrates of breast-fed infants (i.e. soyabean polysaccharide and guar gum) increase progressively and are not developed significantly until late weaning [68]. The production of propionate and butyrate gives evidence for the development of a complex flora because SCFA are produced by bacteria belonging mainly to the bacteroides and clostridia genera [5]. The introduction of solids in the diet of formula-fed infants does not result in a major disturbance in the microbial ecology of the large bowel, as is the case for that of breast-fed infants [25]. In this sense, the more complex microflora of formula-fed infants may confer a significant adaptational advantage to the consumption of dietary complex carbohydrates when weaning occurs, but this will have to be determined in future studies.

**Conclusions**

Of all the issues dealing with microbial colonization of the GI tract of humans and animals and the development of an indigenous gut microflora, significant progress has been made towards the determination of their composition, metabolic activities and associated characteristics in the newborn and adult. Differences in these exist between breast-fed and formula-fed infants. These differences show that modulation of the composition and activities of the intestinal microflora through the diet is possible. However, although the theories of gut colonization seem sound, little is known about the very effi-
icient microbial selection system that newborn animals appear to possess, that enables the modification of various bacterial ecosystems and contributes to gut homeostasis. In the years to come, our knowledge on the gut microflora will increase substantially. This will be enabled by modern technologies and advances in molecular biology. The valuable knowledge remaining to be gained will lead to the design of nutritional management strategies that will improve and protect animal and human health substantially.

References


