The intestinal bacterial colonization in preterm infants: a review of the literature

E.A.M. Westerbeek,1 A. van den Berg,1 H.N. Lafeber,1 J. Knol,2 W.P.F. Fetter,2 R.M. van Elburg1

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1Department of Pediatrics/Division of Neonatology, VU University Medical Center, Amsterdam;
2Department of Biomedical Research/Section Gut Biology and Microbiology, Numico Research BV, Wageningen
Abstract

The aim of this study is to review the normal development of the intestinal microflora of preterm infants and the factors influencing its development. Preterm infants have an increased intestinal permeability, which may lead to bacterial translocation to systemic organs and tissues. In combination with immaturity of the immune system the risk of systemic infections might be increased. Especially potentially pathogenic bacteria are able to translocate. The intestinal microflora of breast-fed term infants, dominated by bifidobacteria and lactobacilli, is thought to suppress the growth of potentially pathogenic bacteria. Attempts have been made to stimulate the presence of bifidobacteria and lactobacilli by means of changes in the diet and ingredients such as prebiotics and probiotics.

After selection, six studies investigating the intestinal bacterial colonization of preterm infants were included. In general, these studies show that the intestinal bacterial colonization with beneficial bacteria is delayed in preterm infants. The number of potentially pathogenic bacteria is high. Antibiotic treatment influences the intestinal colonization. Many preterm infants receive prophylactic antibiotic treatment at birth. As antibiotic treatment delays the normal intestinal colonization, caution should be given to treatment with broad spectrum antibiotics in preterm infants at birth and every attempt has to be made to restrict the period of treatment.
Introduction

The gastrointestinal tract is one of the largest organs of the body serving as an important barrier between ingested elements from the external environment and the internal milieu of the body. In children and adults, the intestine is able to discriminate between pathogenic micro-organisms and the commensal intestinal microflora. Moreover, it is able to select the beneficial nutrients from the ingested food. In preterm infants, these functions are not completed yet.\textsuperscript{100,101} The development of the intestinal microflora starts at birth and is influenced by various factors such as gestational age, mode of delivery, local environment, type of feeding, and antibiotic treatment.\textsuperscript{35,102-105} In breast-fed term infants, bifidobacteria become the predominant bacteria in the intestinal microflora, whereas in formula-fed term infants the intestinal microflora becomes more diverse with apart from bifidobacteria, also clostridia, enterobacteria, \textit{Bacteroides} spp. and streptococci.\textsuperscript{106-108} Therapies have focused on the development of a bifidogenic intestinal microflora, e.g. with prebiotics and probiotics. Although recent studies with prebiotics and probiotics show promising results, more studies are needed to determine the role of prebiotics and probiotics in preterm infants.\textsuperscript{109-113}

Bacteria in the lumen of the intestine can be grouped according to their degree of pathogenicity.\textsuperscript{114} Three groups of bacteria can be recognized: (1) beneficial; (2) potentially pathogenic and (3) pathogenic bacteria. Beneficial effects are inhibition of growth of pathogenic bacteria, production of vitamins, degrading and fermentation of food ingredients, stimulation of feeding tolerance and stimulation of immune functions. Pathogenic effects of bacteria include feeding intolerance, inflammation, infections and especially in preterm infants necrotizing enterocolitis. Potentially pathogenic bacteria belong to the normal microflora of the intestine, but may become pathogenic if present in high numbers: enterobacteria, enterococci, \textit{Escherichia coli}, \textit{Bacteroides} spp. and streptococci. Pathogenic bacteria, such as staphylococci, clostridia, \textit{Proteus} spp. and \textit{Klebsiella} spp. may become pathogenic, even if present in low numbers.\textsuperscript{100,114}

Due to the immaturity of the gastrointestinal tract, bacteria may translocate to systemic organs and tissues and, in combination with immaturity of the immune system, increase the risk for systemic infections.\textsuperscript{115} Especially (potentially) pathogenic bacteria have the potential to translocate.\textsuperscript{116} An intestinal microflora of anaerobic bacteria such as bifidobacteria and lactobacilli favors protection, because it may suppress the growth of pathogenic bacteria.\textsuperscript{117,118}

Few studies have determined the developmental aspects of the intestinal bacterial colonization of preterm infants. As preterm infants often require intensive care treatment with an increased risk of serious infections, insight in the development of the intestinal bacterial colonization of preterm infants is important. In addition,
various factors that may influence the intestinal bacterial colonization of these infants need to be studied. It is not known whether prematurity itself influences the intestinal bacterial colonization, but preterm infants often need intensive care treatment in their first days of life. It is likely that this influences the intestinal bacterial colonization. Preterm infants often need respiratory support and due to the immaturity of the gut parenteral nutrition is often required. Furthermore, preterm infants are susceptible for infections and often need antibiotic treatment.

Insight in the development of the intestinal bacterial colonization in preterm infants and the factors involved might help to establish conditions that prevent a potentially pathogenic development of the microflora and may stimulate the presence of a microflora comparable to that seen in healthy term infants. The aim of this study is to review the literature on the normal development of the intestinal microflora of preterm infants and the factors influencing its development.

**Methods of literature review**

A literature search was performed by a PubMed search from January 1970 till December 2005 using the following keywords and limits:

(Intestines[MeSH] OR intestin* OR gut OR gastrointestin* OR enteric) and (flora OR microbiolog* OR microbiology[MeSH] OR microflora OR bacteria OR bacterial OR enterobacteria* OR colonisation OR colonisation OR microbes OR microbial) and (neonat* OR infant*) AND (premature OR preterm OR pre-term OR low birth weight OR low weight OR small for gestational age) and (english[la] OR german[la] OR dutch[la]) NOT (Probiotics[MeSH] OR Oligosaccharides[MeSH] OR prebiotic*[ti] OR necrotising enterocolitis OR necrotic enteric colitis OR “Enterocolitis, Necrotising”[MeSH]).

Forward citation tracking was performed with the retrieved publications via the Web of Science. Related articles in PubMed and in the Web of Science were reviewed as well as references described in these publications.

Inclusion criteria:
- preterm/very low birth weight (VLBW) infants;
- intestinal colonization from birth.

Exclusion criteria:
- necrotizing enterocolitis;
- prebiotics/probiotics.

The publications were analyzed for:
1. Methods used for collecting and analyzing the fecal samples;
2. Investigation of intestinal colonization over time and collection of fecal samples at least once a week with exact description of the day of collection;
3. Availability of data on the number (or percentage) of infants colonized with the tested bacteria;
4. Possible confounding factors, such as gestational age, mode of delivery, type of feeding, initiation of feeding, antibiotic treatment, length of stay at a neonatal intensive care unit.

Results

Results of the literature search
With the initial search, 11 relevant publications were found.\textsuperscript{24,25,103-105,119-124} The selected studies were published between 1979 and 2003. Five studies were excluded from this review because of:

1. Unclear description of sampling;\textsuperscript{105,119}
2. Heterogeneity of the study population;\textsuperscript{103,104}
3. Lack of data on the number of infants colonized with the tested bacteria.\textsuperscript{123}

Characteristics of included studies
Of the six selected studies, five studies used culturing techniques on selective media to determine the colonization pattern. One study used culturing techniques on selective media and gas–liquid chromatography. Mean gestational age of the infants varied from 26 weeks\textsuperscript{121} to 33 weeks.\textsuperscript{124} Mean birth weight of the infants varied from 782\textsuperscript{121} to 1920\textsuperscript{124} g (Table 1). In the included studies fecal samples were collected at different postnatal ages. For comparison, four groups were defined according to postnatal age at sampling (Table 2):

1. within 48 hours after birth;
2. day 5–9 (week 1);
3. day 10–16 (week 2);
4. from day 20 (≥ week 3).

Intestinal bacterial colonization of preterm infants

Beneficial bacteria
Bifidobacteria. In five studies bifidobacteria were determined in the feces.\textsuperscript{24,25,121,122,124} In two of three studies, bifidobacteria were not detected directly after birth.\textsuperscript{24,124} In these two studies, bifidobacteria increased over time. In two studies, bifidobacteria were rarely found during the whole study period.\textsuperscript{25,121} In only one study, high numbers of bifidobacteria were found even directly after birth.\textsuperscript{122} In conclusion, low numbers of bifidobacteria are found in the feces of preterm infants.

Lactobacilli. In five studies, lactobacilli were determined in the feces.\textsuperscript{24,25,120,122} In none of the studies, lactobacilli were found at birth. In the study of Sakata et al. growth of lactobacilli increased after 2 weeks.\textsuperscript{24} In the other studies, growth of lactobacilli remained low.\textsuperscript{25,120,122} In conclusion, low numbers of lactobacilli were found in the feces of preterm infants.
### Table 1. Included studies

<table>
<thead>
<tr>
<th>Author / year</th>
<th>n</th>
<th>Gestational age (wk)</th>
<th>Birth weight (g)</th>
<th>Methods</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blakey et al. / 1982</td>
<td>28</td>
<td>30 (25–36)</td>
<td>1125 (560–1500)</td>
<td>Feces culture first 3 weeks</td>
<td>Identify micro-organisms colonizing the gut of preterm infants admitted to a special care nursery</td>
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<tr>
<td>Stark and Lee / 1982</td>
<td>26</td>
<td>33 (30–35)*</td>
<td>1920 (1440–2300)*</td>
<td>Feces culture first 4 weeks</td>
<td>Define the early bacterial colonization of the large bowel of preterm infants fed expressed breast milk and compare it to the colonization of term infants who have been breast or formula fed</td>
</tr>
<tr>
<td>Rotimi et al. / 1984</td>
<td>23</td>
<td>29 (24–36)</td>
<td>1728 (750–2400)</td>
<td>Feces culture first 6 days</td>
<td>Investigate the development of bacterial colonization of preterm infants admitted to the special baby care unit and whose mothers received antenatal care</td>
</tr>
<tr>
<td>Sakata et al. / 1985</td>
<td>13</td>
<td>30 (26–35)</td>
<td>1077 (810–1350)</td>
<td>Feces culture first 7 weeks</td>
<td>Investigate the development of the intestinal flora in VLBW infants in comparison with term infants</td>
</tr>
<tr>
<td>Hall et al. / 1990</td>
<td>98</td>
<td>32 (25–33)</td>
<td>1140 (620–2510)</td>
<td>Feces culture &amp; gas liquid chromatography day 10 &amp; 30</td>
<td>Determine whether a characteristic pattern of colonization with lactobacilli and bifidobacteria can be identified in term and in preterm infants</td>
</tr>
<tr>
<td>Gewolb et al. / 1999</td>
<td>29</td>
<td>26</td>
<td>782</td>
<td>Feces culture day 10, 20 &amp; 30</td>
<td>Serially characterize the aerobic and anaerobic stool microflora in a cohort of preterm infants &lt;1000 g at birth</td>
</tr>
</tbody>
</table>

Gestational age and birth weight are means or *median (ranges).

In summary, low numbers of beneficial bacteria are found in the intestinal microflora in preterm infants.

**Potentially pathogenic bacteria**

**Enterobacteria and E. coli.** In five studies, enterobacteria were determined. In four studies analyzing E. coli, E. coli was found in high numbers. In two studies, enterobacteria were sparsely found. In one study, growth of enterobacteria was rarely found during the whole study period. The study of Sakata et al. analyzed enterobacteria, without further culturing of E. coli. Enterobacteria
were found in high numbers in all infants during the whole study period. In conclusion, high numbers of enterobacteria and *E. coli* are found in the feces of preterm infants.

**Bacteroides.** In five studies *Bacteroides* spp. were determined.\textsuperscript{24,120-122,124} In three studies, *Bacteroides* spp. were found in high numbers.\textsuperscript{120,122,124} In one study *Bacteroides* growth increased during the study period.\textsuperscript{24} In one study, *Bacteroides* spp. were rarely found during the whole study period.\textsuperscript{121} In conclusion, high numbers of *Bacteroides* spp. are found in the feces of preterm infants.

**Enterococci and streptococci.** In four studies, enterococci were determined.\textsuperscript{24,120-122} In two studies, streptococci were found in high numbers during the whole study period.\textsuperscript{24,122} In one study enterococci growth increased during the study period.\textsuperscript{121} In one study, enterococci were not found.\textsuperscript{120} In conclusion, high numbers of enterococci are found in the feces of preterm infants.

In summary, high numbers of potentially pathogenic bacteria are found in the intestinal microflora of preterm infants.

**Pathogenic bacteria**

**Clostridia.** In five studies, clostridia were determined.\textsuperscript{24,120-122,124} In two studies clostridia were sparsely found.\textsuperscript{120,121} In two studies low numbers of clostridia were found at the start of the study, but the prevalence increased over time.\textsuperscript{24,124} In the study of Rotimi et al. clostridia were only found within 48 hours after birth.\textsuperscript{122} In conclusion, colonization with clostridia varies in the feces of preterm infants.

**Staphylococci.** In four studies, staphylococci were determined.\textsuperscript{24,120-122} In two studies, staphylococci were sparsely found.\textsuperscript{120,121} In the two other studies, high numbers of staphylococci were found during the whole study period.\textsuperscript{24,122} In conclusion, high numbers of staphylococci are found in the feces of preterm infants.

**Pseudomonas.** In four studies, *Pseudomonas* spp. were determined.\textsuperscript{24,120-122} In all studies, *Pseudomonas* spp. were sparsely found. In one study, *Pseudomonas* growth slightly increased over time.\textsuperscript{24} In conclusion, low numbers of *Pseudomonas* spp. are found in the feces of preterm infants.

**Klebsiella.** In two studies, *Klebsiella* spp. were determined.\textsuperscript{120,122} In both studies, *Klebsiella* growth increased over time during the study. In conclusion, high numbers of *Klebsiella* spp. are found in the feces in preterm infants.

In summary, high numbers of pathogenic bacteria are found in the intestinal microflora of preterm infants.
Conditions that modify the intestinal bacterial colonization

Preterm versus term infants
Three studies determined intestinal bacterial microflora in preterm and term infants. In the study of Sakata et al. enterobacteria and streptococci were dominant at day 1 in both groups. At day 4, bifidobacteria became dominant in the term group, whereas bifidobacteria became dominant at day 20 in the preterm group. The dominance of bifidobacteria was weaker in the preterm group (ratio bifidobacteria to enterobacteria remained 10:1 at 7 weeks of life) than in the term group (ratio 1000:1 at day 7). In preterm infants, Stark and Lee found that colonization with bifidobacteria was significantly delayed during the first week of life.

Hall et al. found a significantly lower prevalence of coliforms (p < 0.01) and lactobacilli (p < 0.005) in the preterm group at 10 days. At 30 days, there was a significantly lower prevalence of lactobacilli in preterm infants (p < 0.01).

Breast-fed versus formula-fed infants
One study compared the intestinal microflora of breast-fed and formula-fed preterm infants. In the study of Gewolb et al. breast-fed infants showed a more diverse intestinal microflora than formula-fed infants with lower numbers of pathogenic micro-organisms.

Antibiotic versus no antibiotic treatment
Three studies determined the influence of antibiotic treatment on the intestinal bacterial colonization. In the study of Gewolb et al. all infants received at least 2 days of antibiotic treatment during the first month of life. They found an inverse correlation between the number of days of antibiotic treatment in the first month of life and the number of bacterial species (r = 0.491; p = 0.007) and the total number of organisms (r = 0.482; p = 0.008) in the fecal samples at day 30. Only in one infant lactobacilli and bifidobacteria were found. Blakey et al. found that in infants receiving antibiotics after birth lactobacilli were never isolated in the first 20 days of life. Before 12 days of age, the prevalence of clostridia was reduced in infants receiving antibiotic treatment. At 10 days of age, Hall et al. found that infants treated with parenteral antibiotics had a significantly reduced colonization with lactobacilli (p < 0.01).

In summary, colonization with bacteria, especially beneficial bacteria such as lactobacilli, is delayed in infants receiving antibiotic treatment after birth.

Vaginal delivery versus cesarean section
One study determined the influence of mode of delivery of birth. Rotimi et al. found that colonization with Bacteroides spp. and clostridia is delayed in infants delivered by cesarean section.
Table 2. Intestinal bacterial colonization in preterm infants at different postnatal ages

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<tr>
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<th>&lt;2d</th>
<th>1wk</th>
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<th>3wk</th>
<th>&lt;2d</th>
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<th>2wk</th>
<th>3wk</th>
<th>&lt;2d</th>
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</tbody>
</table>

Data are expressed as percentage of the number of preterm infants with a positive culture.

**Molecular techniques**

The studies discussed in this review used conventional cultivating techniques to determine the intestinal microflora. In recent years, molecular techniques were developed for directly detecting different groups of bacteria in fecal samples without further cultivation. However, only few studies used molecular techniques to study the intestinal microflora in preterm infants. Schwertz et al. used PCR-denaturing gradient gel electrophoresis to study the intestinal microflora of preterm infants. They found an increase in similarity of bacterial communities in hospitalized preterm infants as compared to breast-fed term infants.
Discussion

Our review shows that in preterm infants colonization of bifidobacteria and lactobacilli is delayed, whereas colonization with potentially pathogenic bacteria (especially *E. coli*) is increased. The type of feeding did not influence the intestinal bacterial colonization in preterm infants, although the longer time to full enteral feeding may have influenced this finding. Early introduction of enteral nutrition is thought to stimulate the intestine of preterm infants. This might maximize the immune function of the intestine, reduce the risk of infections and improve the outcome of preterm infants. Prebiotics and probiotics may to stimulate the presence of a bifidogenic microflora and thus may have a positive effect on health. The intestinal microflora is difficult to study. Only a limited number of bacteria may be detected using conventional cultivation techniques. Several studies have shown that fluorescent in situ hybridization (FISH) analyses provide quantitative data on the relative amount of the different bacterial groups, while cultivating techniques are insufficiently selective and unsuitable for quantitative analysis.

In this review, emphasis is on studies using conventional cultivation techniques and possibly intestinal colonization may differ from that in studies using molecular techniques. Cultivation techniques have a high sensitivity for *E. coli*, so the included studies may find relatively high numbers of *E. coli*. In this review, data are expressed as the number of infants colonized with the specific bacteria. This is not a sensitive method because every infant will be colonized with at least one bacterium of every type of bacteria. Quantitative data would be more reliable, but unfortunately most studies in this review did not provide these data.

The intestinal microflora has been implicated in the pathogenesis of necrotizing enterocolitis, which is an important cause of morbidity and mortality in preterm infants. In this review, studies of patients with necrotizing enterocolitis were excluded, because the pathogenesis is multifactorial and specific unrecognised bacteria that are not normally found in the intestine might be involved. It is concluded that the intestinal bacterial colonization with beneficial bacteria is delayed in preterm infants, while the number of potentially pathogenic bacteria is high. This review shows that antibiotic treatment may delay the normal intestinal bacterial colonization. Caution should be given to treatment with broad spectrum antibiotics in preterm infants and every attempt should be made to restrict the period of antibiotic treatment to a minimum.