ARTICLE

Early Life Exposures and the Development of the Infant Gut Microbiome: A Review

Expositions lors des premiers stades de vie et développement du microbiome intestinal du nourrisson : examen

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ABSTRACT

The influence of the intestinal microbiota on metabolic, nutritional, and immunological processes is widely reported. With increasing literature associating altered microbial compositions with adverse health outcomes, it is important to understand how early life exposures may impact the development of gut microbial colonization and subsequent risk of altered metabolic and immune regulation.

The purpose of this review is to describe factors in the maternal prenatal and perinatal period that may impact the intestinal microbiome over the first 2 years of life. A comprehensive search of MEDLINE, EMBASE, and the Cochrane Library using Medical Subject Headings (MeSH) and keywords for studies reporting on determinants of gut microbiota in infants (0 to 24 months) born at full term was conducted. Articles using culture techniques were included but not those that exclusively used molecular techniques that lacked sensitivity. Each citation title and abstract was independently assessed for inclusion for full text review. Findings related to the maternal prenatal period, mode of birthing, infant diet and antibiotics were included. Bifidobacteria and Bacteroides abundance were consistently greater in the first weeks of life in children born vaginally, and increased Bacteroides presence persisted throughout the first year. Bifidobacteria abundance was greater in breastfed children. Introduction of solid food was associated with greater presence of bacteria of the Firmicutes phylum. Although these studies advance our knowledge of how exposures in prenatal, intrapartum, and early life may impact colonization, larger studies with longitudinal follow-up are needed to improve our understanding of how perturbations may contribute to early origins of disease.

KEYWORDS

infant, gut microbiota, diet, breastfeeding, antibiotics, mode of delivery

This article has been peer reviewed.

RÉSUMÉ

On fait largement état de l'influence du microbiote intestinal sur les processus métaboliques, nutritionnels et immunitaires. Comme de plus en plus d'ouvrages associent l'altération des compositions microbiennes avec des effets indésirables sur la santé, il importe de comprendre comment les expositions lors des premiers stades de la vie sont susceptibles d'avoir une incidence sur le développement de la colonisation microbienne de l'intestin et le risque ultérieur d'altération de la régulation métabolique et immunitaire.

Cet examen vise à décrire les facteurs qui, durant la période prénatale et périnatale maternelle, peuvent influer sur le microbiome intestinal au cours des deux premières années de vie. À l'aide des Medical SubjectHeadings (MeSH) et de mots-clés, une recherche exhaustive de MEDLINE, EMBASE et la Bibliothèque Cochrane a été réalisé afin de trouver des études sur les déterminants du microbiote intestinal desnourrissons (de 0 à 24 mois) nés à terme. Les articles qui faisaient état du recours à des techniques de culture ont été inclus, mais non ceux qui employaient exclusivement des techniques moléculaires qui manquaient de sensibilité. Chaque titre abrégé et chaque résumé ont été évalués indépendamment en vue d'une inclusion dans l'examen du texte intégral. Nous avons inclus les constatations liées à la période prénatale maternelle, au mode d'accouchement, au régime alimentaire des nourrissons et aux antibiotiques. Il y avait invariablement beaucoup plus de bifidobactéries et de bactéroïdes durant les premières semaines de vie des enfants nés par voie vaginale, et la présence accrue de bactéroïdes a persisté tout au long de la première année. Il y avait une plus grande abondance de bifiobactéries chez les enfants nourris au sein. L'introduction des aliments solides a été associée avec une plus grande présence de bactéries du phylum Firmicutes. Bien que ces études fassent progresser notre connaissance de l'incidence des expositions durant les périodes prénatale et intrapartum et les premiers stades de vie sur la colonisation, de plus vastes études assorties d'un suivi longitudinal sont nécessaires pour améliorer notre compréhension de la

contribution possible des perturbations aux origines précoces des maladies.

MOTS-CLÉS
nourrisson, microbiote intestinal, régime alimentaire, allaitement, antibiotiques, mode d'accouchement

Cet article a été évalué par un comité de lecture.

INTRODUCTION

The intestinal microbiota is essential to metabolic, nutritional, physiological, and immunological processes, including digestion of complex starches and polysaccharides, production of nutrients such as short-chain fatty acids and vitamins (e.g., folic acid and vitamins K and B12), and immunological changes important for defense against pathogens. Research prior to 2008 primarily used bacterial culture and polymerase chain reaction–based methods to assess microbial community and diversity. Molecular methods such as temperature or denaturing gradient gel electrophoresis (TGGE or DGGE) or terminal restriction fragment length polymorphism (T-RFLP), used to identify organisms in the gut microbiome, have routinely underestimated diversity or resulted in low reproducibility, thus limiting our understanding of actual gut colonization patterns. Recently, advances in sequencing technology have made it possible to profile bacterial communities efficiently and thus measure the effect of external factors that influence development of the gut microbiota.

The adult gut microbiota varies significantly among individuals but appears stable to perturbations; although transiently altered by diet, infection, and antibiotic use, the microbiome in most individuals will return to its pre-perturbance compositions.[1–4] In adults, a broad range of adverse health outcomes have been accompanied by distinguishable gut microbiome differences.[5–16] Although less is known about the early colonization of the intestinal microbiome, it begins to appear at birth and is believed to be established by 3 years. As perturbations in the development of the infant gut microbiota are hypothesized to have long-term effects on immune development and metabolic function, factors that influence the development of the microbiome in early life may be associated with long-term health outcomes.[9,10,17] Indeed, compared to that of adults, the infant microbiota is dynamic and relatively unstable and could provide a critical window for the development of adult health outcomes. While other investigators have synthesized the literature to study microbial differences related to specific factors such as exclusive breastfeeding[18] and mode of delivery,[19] we undertook this review to provide a comprehensive understanding of what is known about the determinants of infant microbiota, including which bacteria initially populate the gut and how they transition over the early years.

METHODS

We undertook this review with a systematic search strategy in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[20] A librarian assisted our search, undertaken in January 2017, using Ovid MEDLINE, Ovid Embase, and the Cochrane Library. We used individual and comprehensive search strategies, Medical Subject Headings (MeSH), and keywords to search (with no language restrictions) for studies reporting on gut microbiota and determinants in infants (0–24 months) born at full term. Secondary literature, such as review papers and meta-analyses, were excluded, as were studies published only as abstracts. We included studies using culture techniques because of their contribution to the early understanding of the gut microbiome. Studies that used exclusively DGGE, TGGE, or T-RFLP were excluded, because these molecular techniques are considered to lack sensitivity and have been replaced by next-generation sequencing of the 16S rRNA gene.

Whereas pre-existing health conditions are known to affect microbial compositions in adults, maternal gut microbiota characteristics related to a chronic illness may be passed to the gut microbiota of the infant.

Two reviewers independently assessed each citation title and abstract. Consensus was reached regarding inclusion for full-text review, which was then undertaken in early life stages dependently using a descriptive summary form. Because of the large number of results to date, the search was not updated, and we limited the findings reported in this paper to factors associated with the maternal prenatal period, mode of giving birth, early environmental exposures, and infant feeding. [Table 1 shows the bacterial dynamics at different early life stages.]

FINDINGS

Maternal Factors

Uncertainty remains as to when the human gut is first inoculated with microbes. Preliminary evidence appears to indicate that development occurs in utero with maternal-fetal transmission of bacterial species,[21,22] although this has recently been questioned.[23] In many studies, mother and infant stool contain the same species of bacteria [although difficult to prove without detailed strain-level analysis], leading some researchers to conclude that mother-to-infant transmission occurs perinatally.[24–28] Because the fetus' first environment is within the mother's womb, select maternal factors related to diet, body composition, and health status were examined due to their vast impact on many aspects of fetal development, including the gut microbiome.[3,26,27,29–31] Whereas pre-existing health conditions are known to affect microbial compositions in adults,[6,8,12,15] maternal gut microbiota characteristics related to a chronic illness may be passed to the gut microbiota of the infant.[26,29¬,30,32] For example, in a study of pregnant women with diabetes and their infants, mothers and infants both had fecal microbiota enriched in *Bacteroides, Parabacteroides,* and *Lachnospiraceae*,[29] a signature profile of diabetes in adults. However, the transmission of condition-specific gut microbiota from mother to infant is not consistent across all maternal conditions and illnesses. For example, breast milk with detectable human immunodeficiency virus type 1 [HIV-1] RNA from African mothers showed greater bacterial diversity and Lactobacillus sp. abundance, despite no detectable differences in the composition of the gut microbiota of infants receiving breast milk with detectable HIV-1 RNA and those not receiving such breast milk.[33]

In the studies reviewed, while some authors have targeted prolonged maternal exposure, such as chronic health conditions,[29,30,33] others have focused on impacts of acute maternal exposures during pregnancy, such as prenatal stress and diet.[31,32,34,35] When compared to mothers with low prenatal stress, infants from mothers with prenatal high stress had a greater abundance of *Proteobacteria* and a lower abundance of *Lactobacillus, Lactococcus, Aerococcus,* and *Bifidobacterium* in their stool, findings the authors attributed to increased inflammation.[30] In investigating gestational exposures such as weight gain and body composition during pregnancy, one small study reported higher *Bifidobacterium* abundance at 6 months among infants born to women of normal weight [body mass index [BMI] < 25] women, compared to infants born to women with a higher BMI [≥ 25].[32] Further, the amount of

Table 1. Summary of Microbial Composition Change in Early Life

Factor	Microbial Outcome and Interpretation	Microbial Isolation and Analysis Techniques
Mode of birth	— Increased levels of *Bacteroides* in VB children at birth,[26,41,43,45] 1–3 months,[41–42,48] and through the first year of life[41,43] — Increased colonization of *Bifidobacterium* in VB children at birth[43–44] within the first month of life[39,43,47] — *Bacteroidetes* is persistently lower in CS-born infants than in VB infants[40,43,49] — CS-born infants show greater colonization by potentially pathogenic microbes, including members of the *Enterobacteriaceae* family[46] and *Clostridium* genus — Lower alpha diversity among CS-born infants within the first 2 years of life[41] with overall lower abundance[48,49]	— DNA extraction and 16S rRNA gene sequencing from stool[26,41,43,44,48,49] — DNA extraction and 16S rRNA gene sequencing from targeted culture[46] — DNA extraction and qPCR[39,47] — Metagenomic analyses[40,42] — Molecular analyses (HITChip microarray)[45]
Breastfeeding	— Exclusively EFF infants had greater colonization of *E. coli, C. difficile,* and *B. fragilis* compared to EBF infants;[34] however, reports on *lactobacilli* are inconsistent[34,69] — Some studies show that EBF infants have greater colonization of *Bifidobacteria*,[24,49] while other studies do not[34]	— DNA extraction and 16S rRNA gene sequencing from stool[49] — DNA extraction and qPCR[34] — DNA extraction and qPCR-DGGE[24] — Fluorescence in situ hybridization of bacterial cells[69]
Intrapartum antibiotic prophylaxis (IAP)	— Reduced abundance of *Bifidobacterium*,[34,51,52] further exacerbated by formula feeding[51] prior to the introduction of solid food — IAP-exposed infants consistently show lower microbial richness at 3 months[54]	— DNA extraction and 16S rRNA gene sequencing from stool[54] — DNA extraction and qPCR[34,51,52]
Introduction to solid food	— Increased overall microbial richness after introduction to solid food[50,72] — Increased colonization of *Clostridium,*[61] *Enterococcus,*[58] *Faecalibaterium,*[72,74] *Blautia,*[72] and *Prevotella*[74] were seen in varying populations after solid food introduction — Nutrient fortification[73] and macronutrient content[40] contribute to the changes in infant gut microbiome during and after the introduction of solid food	— DNA extraction and 16S rRNA gene sequencing from stool[50,58,61,72–74] — Metagenomic analyses[40]
Weaning from breast milk	— In the weaning and postweaning periods, there is a greater abundance of *Bacteroides* and *Clostridium*[40,61] while the dominant presence of bifidobacteria declines[40,61,24] — There may be an interaction effect between weaning and introduction to solid foods wherein EBF infants have greater counts of *Bacteroides, Lactobacillus,* and *Eggerthella,* and EFF infants have greater counts of bacteria from the Clostridiales family[72]	— DNA extraction and 16S rRNA gene sequencing from stool[61,72] — DNA extraction and qPCR-DGGE[24] — Metagenomic analyses[40]
Early postpartum environmental exposures	– Geographical environments[80–82] may affect the microbiome, as population density, hygiene, and environmental factors may vary widely across different cultural practices[60,81]	– DNA extraction and 16S rRNA gene sequencing from stool[60,80,82] – DNA extraction and qPCR-DGGE[81]

Notes: CS, cesarean section; DGGE, denaturing gradient gel electrophoresis; DNA, deoxyribonucleic acid; EBF, exclusively breastfed; EFF, exclusively formula-fed; HITChip, human intestinal tract chip; qPCR, quantitative polymerase chain reaction; VB, vaginally born

Bacteroides, Clostridium, and *Staphylococcus* was significantly greater among infants of mothers with a higher pre-pregnancy BMI and greater pregnancy-related weight gain.[32] The abundance of *Bacteroides,* independent of maternal BMI, has been reported to be lower in the meconium of infants born to women who consumed a high-fat diet in pregnancy, compared with infants born to women who consumed a low-fat diet—a pattern persisting for 4 to 6 weeks of the infant's life.[34] Other studies have also reported no differences or associations of the infant gut microbiome among infants whose mothers followed a diet that included salmon twice a week,[35] followed an organic or biodynamic diet,[31] or used probiotics or antibiotics during pregnancy.[31] Despite the limitations of relatively small sample sizes and the likelihood of confounding, these studies indicate the possibility that prolonged conditions or maternal health factors may affect the development of the infant's gut microbiota to a greater degree than do acute events such as short-term dietary interventions. Although maternal diet during pregnancy may be an important modifiable factor, much work needs to be done to elucidate its impact on infant gut development.

Intrapartum Factors

Mode of Birth

Maternal vaginal and rectal microorganisms are thought to seed the infant's microbiome, and infant exposure to these microorganisms is influenced by mode of birth.[21,36,37] While Del Chierico et al. reported a "core" microbiota that was independent of delivery mode and lactation stage, they suggested that highly specialized microbes act as seminal colonizers of the gut, where Proteobacteria and Firmicutes are the major phyla present at all time points,[38,39] followed by microorganisms from the Actinobacteria, Verrucomicrobia, and Bacteroidetes phyla in lesser amounts[38,40] (Figure 1). Overall, the infant gut microbiota in vaginally born (VB) and in cesarean section (CS)–born infants develop similarly, with a gradual decrease in Proteobacteria from 1 week to 2 years, peaking of Actinobacteria at 3 months, detectable Firmicutes from 3 months onward, and the emergence of Verrucomicrobia at approximately 6 months.[41] However, shifts in the abundance of Verrucomicrobia have also been reported within the first month in CS-born infants.[38] There is evidence that considerable mother-to-infant transmission from birth to 1 year among VB infants is as high as 72% of meta–operational taxonomic units (see Appendix 2) matched to the mother's stool, compared to 41% seen in CS-born infants,[42] and CS was found to significantly affect gut bacterial species at initial time points. While these differences gradually decreased between 4 and 12 months, the CS-born infant microbiome remained more heterogeneous, with increased beta diversity (see Appendix 2) as compared to VB infants at all time points.[42] Given physically different birth passages, mode of birth clearly plays an important role in determining whether infant exposure to the mother's vaginal, rectal, and fecal microbiota and how subsequent colonization occurs.

Several longitudinal studies have identified changes in early colonization patterns between VB and CS-born infants as early as the first week of life.[21,24,38,39,41,43,44] Even when antibiotic use during labour is controlled for, alpha diversity (see Appendix 2) is lower in the gut microbiome of CS-born infants in the first 2 years,[41] and microorganisms within the Bacteroidetes[41] and Firmicutes phyla are less prevalent, diverse, and abundant.[45] While some bacteria (such as *Enterococcus faecalis*) were found in all samples irrespective of mode of birth,[41] others (such as *Bacteroides*, a genus within the Bacteroidetes phylum) were less frequently found in CS-born infants.[40,46] This difference is consistent among longitudinal studies varying in follow-up duration to 3 years of age;[24,38,40,41,43–46] within the first year specifically, reduced *Bacteroides* in CS-born infants compared to VB infants was reported within the first week of life[24,38,41,43,45] and at 1 month,[43,45] 6 weeks,[46] 2–3 months,[40,41] 5–6 months,[41,43] and 10–12 months[41] (Figure 2). Indeed, Azad et al. noted that Bacteroidetes was detected in only 38% of samples at 4 months of life and were absent in all CS-born infants, regardless of feeding status.[40] In another study, rates of breastfeeding in the CS-born and VB groups were similar;[43] the authors suggested that the differences in the abundance of the bacterial populations were likely not influenced by infant feeding. When compared with the VB infants in these studies, the CS-born infants had persistently low levels of microorganisms from the Bacteroidetes

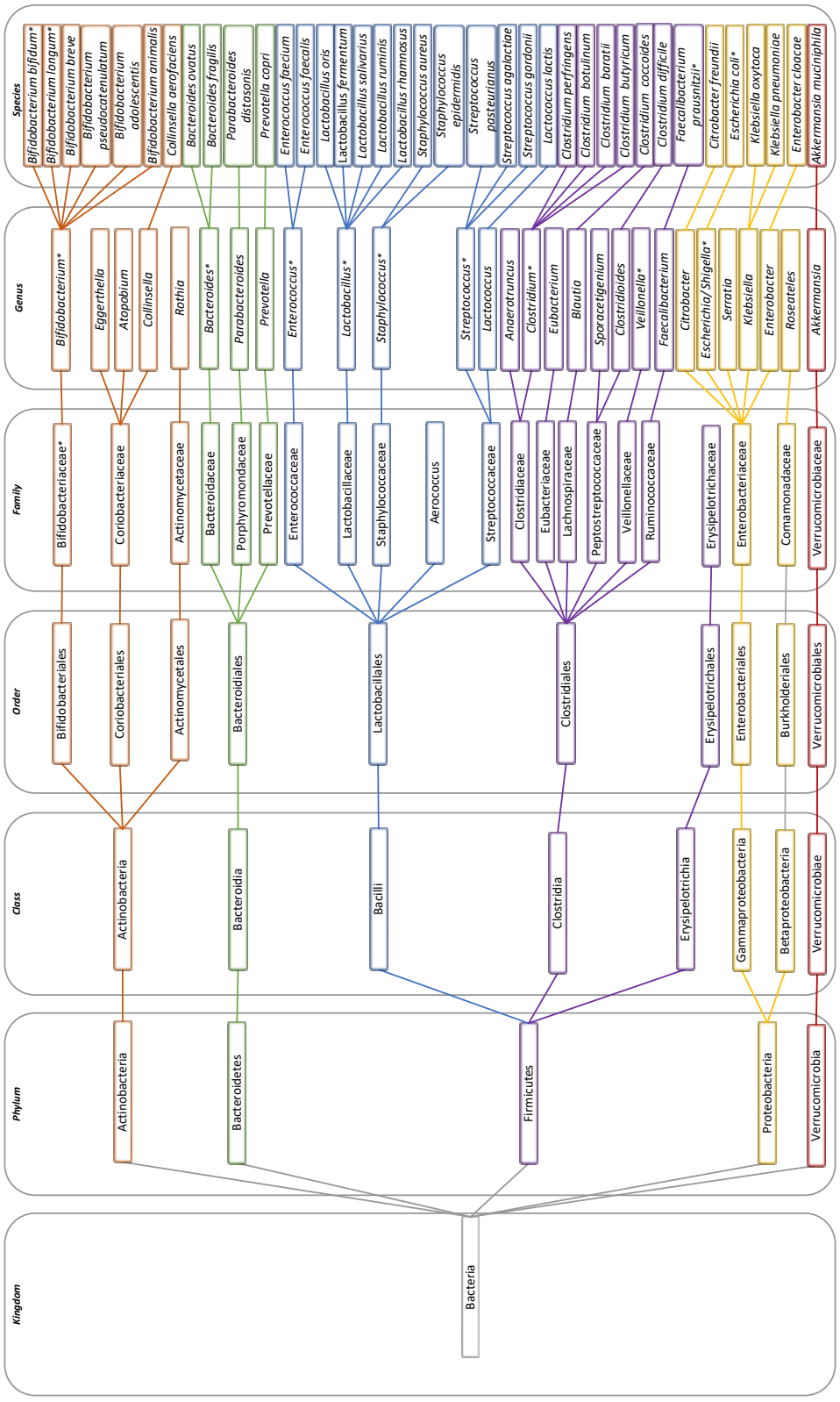


Figure 1. Taxonomic summary of microorganisms reported within the infant gut microbiome

* Colonizers of the infant gut microbiome

phylum (specifically, from the genus Bacteroides), experienced later colonization by Bacteroidetes, and were colonized with a significantly different microbiome.[24,38,40–46]

Despite these changes, mode of birth does not affect all downstream microbial species similarly. Although no change was observed in the abundance of *Lactobacillus* sp. (a microbe with low abundance throughout infancy),[45] dramatic shifts in phylum-level abundances have been reported. Brumbaugh et al. reported that the ratio of Firmicutes to Bacteroidetes at 6 weeks differed 381-fold by mode of birth among VB and CS-born infants (a median ratio of 381:1 for CS-born infants, a 1:1 ratio for VB infants).[45] Although dramatic, these differences are consistent with other reports that the CS-born infant gut is dominated by microorganisms from the Firmicutes and Proteobacteria phyla throughout the first year of life.[24,39] Indeed, in one small study comparing VB infants (n = 25) to elective CS-born infants (n = 16) within the first week of life, VB infants showed *E. coli* and *Bifidobacterium longum as dominant microbes, while in CS-born infants*, *Staphylococcus* sp., *Clostridium* sp., *Enterobacter* sp. and *Streptococcus* sp. were more common.[24] Lower abundance of *Bifidobacterium* has also been reported in CS-born infants at 6 days,[43] 3 weeks,[43] 1 month,[47] 6 weeks,[46] and 6 months[44] in studies varying in size. Higher abundance of microorganisms of the Enterobacteriaceae family and *Veillonella* genus, among other microorganisms, was also reported to be in CS-born children at various time points before 6 months.[41,43]

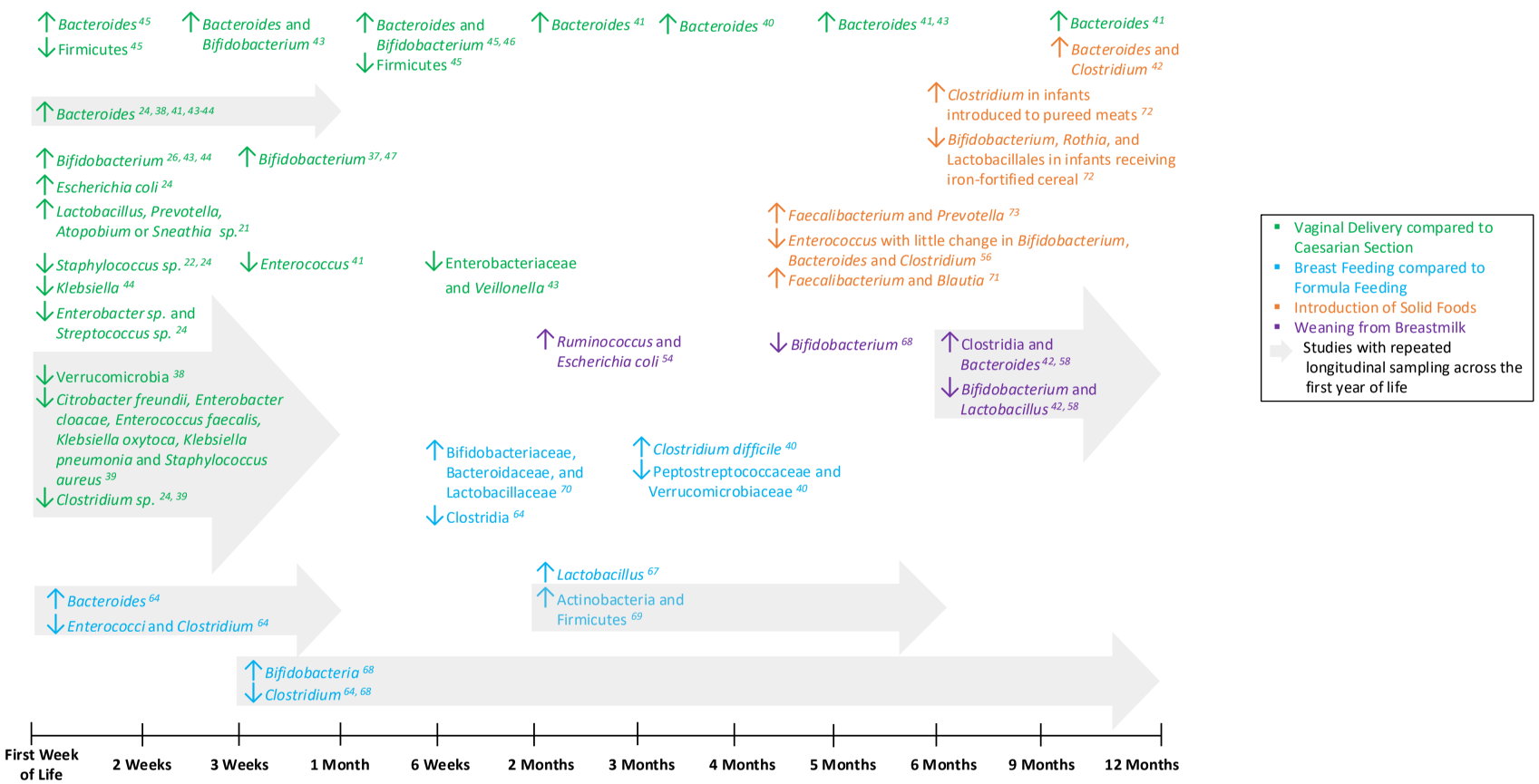
Overall, most studies investigating mode of birth have reported differences between the composition of the gut microbiome at early time points following CS and that at early time points following vaginal birth; even transient differences are noted between infants born through elective CS and those born through emergency CS.[39] Reporting of decreased or no Bacteroidetes in CS-born infants,[24,38,40–46] as well as reduced *Bifidobacterium,*[43,44,46,47] is quite consistent; delays in microbial establishment[41] and an overall lower abundance, richness, and diversity are also noted.[40,46] This pattern appears to be consistent across ethnic groups[24,39–41,47] and gestational ages[47] and regardless of type of feeding.[40,43] Some of these studies were limited by small or moderate sample sizes and little consideration of potential confounders. In all but one of these studies,[41] for example, those born by CS were exposed to intrapartum antibiotic prophylaxis.

Exposure to Antibiotics

Intrapartum antibiotic prophylaxis (IAP) has been associated with decreases in the abundance of **Bifidobacterium,**[31,48,49] *Escherichia*,[50] and microorganisms of the phylum Bacteroidetes[51,52] at various time points after antibiotic exposure. Aloisio et al., in a cohort of 52 VB infants (n = 26; IAP: 2 g ampicillin), found that infants exposed to IAP had a significantly lower abundance of Bifidobacterium, the dominant colonizer,[31,53,54] as early as 3 days after birth.[48] Similarly, Corvaglia et al. reported that IAP-associated decreases in bifidobacteria were further exacerbated by formula feeding as compared to exclusive breastfeeding in their study of 84 infants (n = 35, IAP) at 7 days of life.[49] Exposure to IAP was related to a greater overall diversity of the infant microbiome (as measured by the Shannon diversity index) in a group of 333 infants born at term (n = 133 IAP).[51] Using an approach that reports on coabundance groupings of bacteria, Sordillo et al. found that IAP was associated with greater levels of Lachnospiraceae and Clostridiales (both of the Firmicutes phylum) and lower levels of Bacteroidetes, *Bacteroides, Escherichia,* and *Bifidobacterium.* Of interest, these findings were similar to the reported profile for CS-born infants.[51]

In further exploring the different impacts of IAP for group B streptococcus compared to CS, Azad et al. found that although IAP resulted in decreased microbial richness at 3 months of age, IAP for emergency CS resulted in greater microbiota diversity and significant community differences out to 1 year.[52] Although Firmicutes and Proteobacteria levels were elevated at 1 year in infants exposed to IAP for CS, no differences in individual taxonomic composition between infants exposed to IAP for CS and infants exposed to IAP with vaginal birth were reported. Overall, while studies are often limited by their methods, small sample sizes, and the confounding effect of mode of delivery, findings indicate both quantitative and qualitative effects of IAP administration on the colonization of the infant's microbiome.

Figure 2. Summarization of bacterial dynamics within the first year of life.



Key: Green labels represent increases and decreases in bacteria from infants that were vaginally born in comparison to infants that were delivered by cesarean section; blue labels, increases and decreases in bacteria from infants that were breast fed in comparison to infants that were formula fed; orange labels, increases and decreases in bacteria from infants during the introduction of solid foods; purple labels, increases and decreases in bacteria from infants during weaning. Light grey arrows represent studies with multiple time points of variations in sampling ages. The dark grey arrow shows sampling time points within the first year of life.

Infant Dietary Factors

The human gut microbial profile is thought to begin with a bifidobacteria-dominated composition in infancy and to evolve within the first years of life to one resembling a complex adult composition.[51,55–58] During infancy, the developing microbiome is not only influenced by the type of milk consumed but also by two important dietary changes: (1) weaning from breast milk and (2) the introduction of solid foods.[59] In the following, we explore the impact of infant dietary factors on the development of the infant gut microbiome.

Breast Feeding versus Formula Feeding

Although early culture-based studies identified differences in gut microbial makeup among exclusively breastfed (EBF) infants and exclusively formula-fed (EFF) infants, findings regarding specific ecological patterns were inconsistent and sometimes contradictory.[60–65] Clearly, the early microbiome is diverse. In a study focusing exclusively on culturable bifidobacteria, 173 strains were identified in neonates at 1, 4, and 26 weeks of age.[25] Of the genus *Bifidobacterium*, irrespective of infant feeding, *B. longum* was most prevalent, followed by *B. breve* and *B. bifidum*. Of interest, greater strain diversity was identified in the first few months of life in these infants than what is generally seen in the fecal microbiota beyond 1 year of age.[25] It was hypothesized that this might be because the "young" bifidobacteria have higher mucosal adhesive properties than the adult strains. Furthermore, it is also thought that breast milk itself might be a bacterial inoculant.[66]

Culture-independent methodologies have also been used to examine the influence of breast milk compared to formula on the infant fecal microbiota. Many cross-sectional studies have used culture-independent methodologies to investigate feeding approach on the gut microbiota in infants between 1 and 4 months of age.[31,40,67] Using a targeted approach, Penders et al. determined that EFF infants were more often colonized with *Escherichia coli, Clostridium difficile, B. fragilis,* and lactobacilli, compared to EBF infants. However, only *C. difficile* counts were found to be significantly higher in EFF infants and infants fed a combination of formula and breast milk (after adjusted analysis of 700 EBF, 232 EFF, and 98 combination-fed infants at 4 months).[31] Gomez-Llorente et al. observed that only the Lactobacillus group was associated with EBF in their cohort of 31 EBF and 27 EFF infants.[67] However, they noted many differential clusters of bacterial composition between these groups, where EBF infants were characterized by *Bifidobacterium/Enterobacteriaceae, Lactobacillus/Bacteroides,* and *Clostridium coccoides/Atopobium,* and EFF were characterized by *Bifidobacterium/* Enterobacteriaceae, *Bacteroides,* and *C. coccoides.* Although Penders et al. did not report differences in bifidobacteria between the EBF and EFF infants,[31] Rogers et al. found greater colonization by bifidobacteria in breastfed infants than in formula-fed infants and greater colonization by *Clostridium* in formula-fed infants than in breastfed infants, findings similar to previously reported culture-based findings.[68] Azad et al. observed that non-breastfed infants had a significantly higher abundance of Firmicutes (Peptostreptococcaceae) and Verrucomicrobiaceae, higher prevalence of *C. difficile*, and higher microbial diversity and richness.[40] These findings contrast with those of other researchers, who report a greater abundance of bacteria from the Actinobacteria and Firmicutes phyla in EBF infants.[69] It is interesting that the gut microbiota from infants randomized to goat's milk was more similar to that of EBF infants than the gut microbiota of infants randomized to cow-based formula.[70] Greater abundances of Bifidobacteriaceae, Bacteroidaceae, and Lactobacillaceae were found in EBF infants than in goat- and cow-based formula-fed infants, as well as less Peptostreptococcaceae, Erysipelotrichaceae, Lachnospiraceae, and Enterococcaceae.[70]

Introduction of Solid Food

Initial exposure to solid food has been found to significantly increase overall microbial diversity and richness in infants,[51,71] and many studies have catalogued microbial-specific changes. Bernal et al. found that levels of *Enterococcus* were reduced within 2 months of cereal introduction to EBF infants, but there was little change in the *Bifidobacterium, Bacteroides* and *Clostridium* populations.[56] In other studies, a greater abundance of Bacteroides and *Clostridium*, both of which are prevalent microbes

in adults,[42] was associated with a more diverse carbohydrate, protein, and fat intake during the introduction of solid food. Similar results have been found in infant cohorts both in developed and developing countries with varying weaning food items.[59,71–73]

That the selection of food items for introduction is an important dietary factor for microbial colonization in infants is not surprising. Although little is known about food-specific effects in an infant diet, Krebs et al. noted that compared to fortified cereals, measures of *Clostridium* were more abundant in infants introduced to pureed meats.[72] Of interest, this study also found that micronutrient-specific fortification also has an impact on the microbiome: *Bifidobacterium, Rothia,* and *Lactobacillales* were reduced in infants receiving iron-fortified cereal but not in those receiving cereal fortified with both iron and zinc.[73] A variety of infant cohort studies also found increases in *Faecalibacterium*[70–72] and *Blautia,*[71] as well as *Prevotella,*[73] after the introduction of solid foods.

Weaning from Breast Milk

Magne et al. were among the first to investigate the effect of weaning from breast milk on the microbiome. In a study of 11 infants over 42 weeks, EBF infants were followed in a preweaning period, during which infants had a mix of breast milk and formula, and in a postweaning period, when breast feeding was stopped.[54] Although others, using untargeted approaches, have reported that bifidobacteria were rarely major colonizers,[55] Magne et al. reported that *Bifidobacterium* was the dominant genus during all periods and showed little change during the 42-week study follow-up. Weaning and postweaning periods were characterized by a greater abundance of *Ruminococcus* and an increase in *E. coli.*[54] Roger et al., in their cohort of 14 infants over an 18-month period, also found that bifidobacteria dominated in the breastfeeding, preweaning, and weaning phases when compared to other species, but found significant decreases during the later weaning stages.[68] In more recent, larger longitudinal cohort studies using 16S rRNA gene profiles[59] and metagenomics,[42] notably lower levels of bifidobacteria and *Lactobacillus* were confirmed when infants weaned from breast milk whereas *Clostridium* and *Bacteroides* species increased.

There appears to be some interaction between weaning and the introduction of solids on the gut microbiome. Roger et al. reported that changes in overall composition over time were less drastic in formula-fed infants than in breastfed infants.[68] During weaning, the gut microbiota composition of breastfed infants evolved to look more like the microbiota profile of formula-fed infants. Interindividual differences in the gut microbiota profile were less pronounced during weaning than during the preweaning period; this was especially evident in the formula-fed group as compared to the breastfed group.[68] Thompson et al. noted changes in response to the introduction of solid food when comparing non-EBF to EBF infant [71] and reported that EBF infants had greater *Bacteroides, Lactobacillus, Eggerthella, Ruminococcaceae* and less *Staphylococcus* and *Roseateles*, whereas non-EBF infants had higher levels of *Clostridiales, Bifidobacterium, Faecalibacterium, Eubacterium,* and *Anaerotruncus.*

Of interest, non-EBF infants also showed a more dramatic shift in microbiota composition in response to the introduction of solid food; there was a distinct clustering in their profiles before and after solid food introduction.[71] Although these studies are limited by small size, design, and microbial techniques, they show that the introduction of anything other than breast milk had a significant influence on the ecological development of the gut microbiome in humans.[51,56,58,71–75]

Early Postpartum Environmental Exposures

Despite their limitations, studies using culture methods indicate an environmental effect on the establishment of the infant gut microbiome, pointing to, for example, infant-to-infant and staff-to-infant transmission of gram-negative bacteria in hospital nurseries. This is theorized to correlate with poor handwashing among staff[76] and nosocomial [hospital-acquired] sources of colonization.[77] Studies using targeted 16S rRNA gene methods such as quantitative polymerase chain reaction [see Appendix 1] reported contrasting findings with infants from various countries and cultural postpartum environments, where highly hygienic practices may be linked to delayed colonization

> Factors that influence the development of the microbiome in early life may be associated with long-term health outcomes.

and higher levels of *Staphylococcus*[78] (similar to patterns seen in premature infant microbiota[53]) in comparison to cultures without such practices.[50]

Fallani et al. took a broader interest in geographic origin on the establishment of the microbiome across Europe, using a cross-sectional design to sample and analyze the microbiota of 606 6-week-old infants.[79] Observations showed a "geographic gradient" in the composition of European neonatal microbiomes, which changed significantly from north to south across the continent. Higher proportions of *Bifidobacterium, Atopobium, C. perfringens,* and *C. difficile,* as well as higher numbers of total bacteria, were seen in northern European countries; southern European countries had higher proportions of Bacteroides, *Enterobacteria* and lactobacilli.[79] That other researchers have also observed distinct microbial differences across geographical areas that vary in population density and cultural practices is interesting.[58,80,81] Kemppainen et al. noted that 18-month-old infants from Finland and the US state of Colorado had a lesser abundance of *Bifidobacterium* compared with infants from Sweden and the US state of Washington[80] and that 6-month-old infants in rural Malawi had a greater abundance of *Lactobacillus* compared to infants in southern Finland.[78] Although these findings indicate that geography and culture are possibly important determinants in gut microbiota development, it is important that such findings be interpreted in a greater context. For example, a study conducted in a restricted geographical area in Norway found alpha diversity to be highest at 2 years of age and lowest at 4 months of age, and beta diversity to be highest in the newborn period,[82] indicating that gut development could be distinct not only across geography but also in regard to age.[58,80,81] Overall, although their findings are not explained, studies show that immediate postpartum exposures (including hygienic practices, geographic location, and culture) potentially influence early gut microbiome development.

DISCUSSION

The ability to describe the human gut microbiome and its dynamic development is ever increasing with the rapid pace of technological advances. The current state of knowledge is in the early formative stages. We have reported on factors that appear to influence the intestinal microbiota of infants, including mode of delivery, intrapartum antibiotic exposure, infant diet, and early environmental exposure. A list of all organisms reported on is provided in Figure 1, and a summary of the influence of these key factors on the microbiome is provided in Figure 2 and Table 1. The findings must, however, be treated as preliminary because many studies reviewed are limited by their sample size, the approaches taken to microbial analyses. and the exclusion of potential confounding factors. To date, for example, many studies reporting on the effect of mode of birth fail to account for breastfeeding or the use of antibiotics, and studies that investigate methods of infant feeding fail to account for mode of birth.

Nonetheless, several patterns emerged from our review. The infants of women with a high BMI or who consumed a high-fat diet during pregnancy were more likely to have offspring with a gut microbiome in which *Bacteroides* was underrepresented. Across studies, infants born vaginally have higher levels of Bacteroides and bifidobacteria and fewer organisms

from the phyla Firmicutes, Proteobacteria, and Verrucomicrobia as compared to infants born through CS. This is true in the early stages of life and continues over the first year. The organisms seen more frequently in CS-born infants are more representative of those derived from skin as opposed to vaginal and rectal origins and likely reflect the infant's earliest exposures. It has been proposed that the changes found among CS-born infants may reflect the lack of competing organisms usually inoculated through vaginal exposure.[37] Similarly, differences based on infant feeding approaches are found. Compared to formula-fed infants, breastfed infants appear to have more *Bacteroides, Lactobacillus,* and Actinobacteria and relatively fewer Clostridia and *C. difficile,* Proteobacteria, and Verrucomicrobia.

Although the importance of the differences in the microbiome profiles is not yet clear, it is likely that some differences have shorter-term and perhaps longer-term affects. For example, *Bacteroides* and *Bifidobacterium*, found at relatively high levels among VB infants and among breastfed infants, are likely important in infancy, as they play a role in catabolizing human milk oligosaccharides. This is confirmed by the observation that when an infant is weaned from breast milk, the proportion of bifidobacteria decreases. We are in the very early stages of understanding if there are developmental windows where the presence or absence of particular microbes may influence subsequent development. These early studies provide an important step, but further research is needed to fully understand the impacts of these various prenatal, intrapartum, and infant exposures on microbiota.

The technological pace of the field is increasing rapidly. Although next-generation sequencing [high-throughput 16S rRNA gene sequencing [see Appendix 1]] provides nonselective profiling of bacterial communities, there are limitations of this technology, including wide variance in the accuracy of taxonomic classification and profiling among sources of 16S-rRNA gene sequencing [culture vs. stool], reference database, and downstream analyses.[83] Thus, much work using more-consistent methodologies is required for further understanding [1] the normal ecological development of the human gut microbiome and [2] factors causing perturbations that may contribute to early disease that later becomes manifest in adulthood.

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APPENDIX 1: Methodological Techniques

quantitative polymerase chain reaction (qPCR): sometimes called real-time PCR, a culture-free technique to quantify the amount of a targeted DNA sequence in a sample through monitoring the accumulation of amplification product. There are several primers for regions of the bacterial 16S rRNA gene that can be used in qPCR to target either all bacteria or a selected group of bacteria.

high-throughput 16S rRNA gene sequencing: a culture-free technique (also known as next-generation sequencing) to infer the entire microbial community within a sample. This involves the PCR amplification of a region of the bacterial 16S rRNA marker gene and subsequent sequencing with a technology such as Illumina technology. This method aims to nonselectively profile the bacterial community and involves complex bioinformatics analysis to infer bacterial abundance and taxonomy.

culturing of microorganisms: a means by which microorganisms are multiplied in a controlled laboratory environment. Microbial culture can occur in broth, on agar plates, or in stab cultures. This is performed with media types that are optimal for the bacteria of interest and results in a pure (axenic) culture.

APPENDIX 2: Microbiome Terms

bacterial taxonomy: the rank-based classification system used to categorize a specific organism. Within the bacterial kingdom, individual organisms are classified under the following main taxonomic ranks: phylum, class, order, family, genus, and species.

operational taxonomic unit clustering: a method used to study a group of bacterial 16S rRNA gene sequences with high similarity (a threshold of 97% similarity is commonly used). Sequences with high similarity are often clustered together; a single sequence is selected as the representative and used for the study of bacterial taxonomy.

alpha diversity: the diversity within a specific site or sample. Common measures of alpha diversity include estimated species richness; Shannon diversity (H) and Simpson index (D), which are estimates of species richness (i.e., number of species); and the relative distribution of species (or evenness) within a given sample.

beta diversity: the diversity between two sites (or samples). Beta diversity is interpreted with distance or dissimilarity measures that indicate how different communities are from one another. A common measure of beta diversity is the Bray-Curtis dissimilarity index.

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