CHILD HEALTH AND THE GUT MICROBIOME

N. Moes¹,², A. Smet²,³

¹Department of Pediatrics, Antwerp University Hospital, Antwerp, Belgium
²Laboratory of Experimental Medicine and Pediatrics. Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium
³Infla-Med Research Consortium of Excellence, University of Antwerp, Antwerp, Belgium

Corresponding Author: Annemieke Smet, MD; e-mail: annemieke.smet@uantwerpen.be

Abstract: In this review, we summarized the most important, accessible, and relevant literature published between April 2019 and March 2020 on the gut microbiota in child health. The first part of the review focuses on literature describing changes in microbiota composition in a wide spectrum of pathologies, while the subsequent section focuses on the impact of antimicrobial treatment and nutritional intervention on the pediatric microbiota during early infancy and development.

Keywords: Pediatric population, Nutrition, Dysbiosis, Development, Antibiotics.

GUT DISBIOSIS IN THE PEDIATRIC POPULATION

Disturbances in microbial homeostasis (or dysbiosis) have been implicated in the pathogenesis of several pathologies. Sixteen research papers relating to gastrointestinal diseases, autoimmune diseases, acute lymphocytic leukemia, infectious diseases, neurologic and cardiac disorders in children were identified.

Gastrointestinal and autoimmune diseases

Inflammatory bowel diseases (IBD) are the exemplar medical conditions of the microbiome era in which disturbances in microbial populations were first described and associated to disease pathogenesis. Sila et al¹ analysed the intestinal microbiota in newly diagnosed pediatric IBD patients and compared it to the patients’ healthy siblings with same genetic and environmental background and to healthy unrelated controls. Microbial diversity differed significantly between IBD patients, healthy siblings and unrelated controls. In the pediatric IBD cohort, the genera Eubacterium, Lactobacillus, Enterobacter and Clostridium were significantly reduced whereas Streptococcus, Prevotella and Escherichia were abundantly present¹. Anti-Saccharomyces cerevisiae antibody (ASCA) is associated with the diagnosis of Crohn’s disease (CD) and may reflect a loss of tolerance to yeast, possibly exacerbated by the presence of an inflamed more permeable gut wall. ASCA is highly predictive but poorly sensitive for CD diagnosis². Kansal et al² explored the association between ASCA and the gut microbiota in children with CD and unveiled that ASCA-positive and ASCA-negative CD patients have significant different gut microbial composition, which could possibly influence the phenotype of the disease. Obesity has been associated with a wide spectrum of liver abnormalities. Zhao et al³ investigated the intestinal microbiota in obese children with nonalcoholic fatty liver disease (NAFLD). Deep sequencing of gut microbiota in stool samples revealed large differences...
in gut microbiota between obese children with and without NAFLD compared to healthy controls which reflected in several metabolic pathways differentially enriched among these groups. Interestingly, Faecalibacterium prausnitzii was the only species that discriminated between obese children with and without NAFLD. Another study further confirmed the lower proportion of Bacteroidetes in obese children. At the genus level, Faecalibacterium, Phascolarctobacterium, Lachnospira, Megamonas and Haemophilus were more abundant in the obese group compared to the normal weight group whereas Oscillospira and Dialister were significantly reduced. The authors also highlighted that gut microbial diversity decreased with increasing body weight, further underlining the role of gut dysbiosis in the development of obesity in children. In recent years, the impact of Helicobacter pylori infection on the gut microbiota has also gained more attention. Miao et al. explored this relationship in the pediatric population by examining gastric mucosal biopsies from children with gastrointestinal symptoms. As similar for the adult population, H. pylori-positive children were mainly dominated by the genus Helicobacter. They also concluded that the characteristics of the gastric microbiota were mainly affected by H. pylori infection rather than the disease state (i.e., gastric ulcers) itself. The function and composition of the gut microbial community have also been associated with the risk of autoimmune diseases, including type 1 diabetes. Paun et al. investigated whether immune responses to gut microbiota are associated with autoreactivity to host tissue outside the gut. Their analysis revealed associations between antibody responses to intestinal bacteria and diagnosis of type 1 diabetes. Furthermore, they presented a platform to investigate antibacterial antibodies in biological fluids useful to study therapeutic interventions in autoimmune diseases.

**Acute lymphocytic leukemia**

Haematological malignancies are the most common type of pediatric cancers, with acute lymphocytic leukemia (ALL) the most frequently occurring during childhood. Haematopoietic stem cell transplantation (HSCT) is employed as curative immunotherapy. This procedure can be accompanied by severe side effects, such as bloodstream infections, acute graft-versus-host disease (aGvHD) and death. The gastrointestinal tract is the predicted reservoir for most bloodstream infections after HSCT. Three research papers investigated the microbial signature associated with side effects related to HSCT. Kelly et al. studied which bacteria were associated with bloodstream infections in ALL patients undergoing HSCT and whether these species originated from the gut. They showed that bloodstream infections were mainly caused by Escherichia coli and Enterococcus faecium which also dominated the gut prior to occurrence of the infection. Another research performed a longitudinal study of immunological markers and microbial composition in relation to clinical outcomes in children undergoing HSCT. They revealed that high concentrations of human beta-defensin 2 prior to transplantation in patients with high abundances of Lactobacilli associated with moderate to severe aGvHD and exhibited high mortality. On the contrary, high abundance of obligate anaerobes, including Ruminococcaceae, associated with no or mild aGvHD and low mortality. Biagi et al. described a gut microbial signature (i.e., depletion of Blautia and abundance of Fusobacterium) predictive for developing aGvHD in children undergoing HSCT. A final study investigated the effect of chemotherapy on the gut microbiota in ALL patients. ALL patients exposed to chemotherapy resulted in a decrease of the gut microbiota diversity. Interestingly, mucolytic Gram-positive bacteria, including Ruminococcus gnavus and Ruminococcus torques, were abundantly present during the chemotherapy regimen, which may contribute to the development of gastrointestinal complications in ALL children following chemotherapy.

**Neurological disorders**

Two papers explored the faecal microbiome in children with autism spectrum disorder (ASD). Arnold et al. investigated the benefit of probiotics in children with ASD and gastrointestinal symptoms. Outcome improvement significantly correlated with abundance of Lactobacillus without observable changes to microbiota composition and diversity. Variation of maternal
gut microbiota may increase the risk of ASD in the offspring. Li et al\textsuperscript{13} examined gut microbial signatures in children and their mothers with the aim to identify bacterial biomarkers with discriminative power. Mothers of ASD children had more \textit{Proteobacteria}, \textit{Moraxellaceae} and \textit{Acinetobacter} than mothers of healthy children. Interestingly, ASD children carried unique bacterial biomarkers, including \textit{Alcaligenaceae}, \textit{Enterobacteriaceae} and \textit{Clostridium}. The identified microbial mother-child patterns may be important for risk assessment and prevention of ASD via microbiota modulation\textsuperscript{13}. A final study explored the gut microbiota in children with attention-deficit/hyperactivity disorder (ADHD) and showed that significant microbial differences (i.e., reduction of \textit{Veillonellaceae} and \textit{Faecalibacterium}) in ADHD children may play a role in gut-brain axis alterations and in this way could contribute to ADHD symptoms\textsuperscript{14}.

Other diseases

Chronic kidney disease (CDK) is associated with high risk of cardiovascular disease. Hsu et al\textsuperscript{15} investigated the correlation between gut microbiota-dependent metabolites and the cardiovascular risk in children with CKD. They highlighted that microbial-derived methylamines associated with increased risk for cardiovascular diseases in pediatric CKD but warranted that further studies are necessary for usage of these microbial markers in CKD progression\textsuperscript{16}. The relationship between pulmonary tuberculosis and gut microbial homeostasis is still poorly understood in the pediatric population. Li et al\textsuperscript{16} shed more light on gut microbial signatures present in children with pulmonary tuberculosis. Based on their findings, they hypothesized that gut dysbiosis, characterized by \textit{Prevotella} and \textit{Enterococcus} abundance and a decrease of \textit{Bifidobacteriaceae} and \textit{Ruminococcaceae}, in children with tuberculosis affects the pathogenesis of the disease by dysregulation of the hosts’ immune status via the gut-lung axis\textsuperscript{16}. Kortekangas et al\textsuperscript{17} studied whether common childhood infections, i.e., gastroenteritis and respiratory tract infections (as morbidity) predict gut microbiota composition. The authors hypothesized that gastrointestinal infections would be predictors for lower microbiota maturity and diversity. However, their findings showed only lower maturity at 12 months after recent diarrhoea. Respiratory Infections at the age of 12 months caused on the contrary increased microbiota diversity. Morbidity symptoms seem to be associated with changes in microbial community composition and influence abundance of certain species. However, there was no clear consistent pattern in these associations and therefore no conclusion could be drawn that reduction in disease burden would lead to a healthier microbiota environment or the other way around that that influencing the microbiota environment would impact on disease burden\textsuperscript{17}.

IMPACT OF ANTIBIOTIC TREATMENT ON THE PEDIATRIC GUT MICROBIOTA

The pediatric gut microbiota is established during the first years of life, representing a critical time window where the gut microbiota is especially vulnerable to external influences, including antibiotics. Coker et al\textsuperscript{18} evaluated the potential impact of intrapartum antibiotic usage on the infant gut microbiota in the first year of life. Exposure to intrapartum antibiotics associated with lower microbial diversity, reduction of \textit{Bacteroidetes} and \textit{Bifidobacterium} taxa and a significant increase of \textit{Veillonella}\textsuperscript{18}. Another study investigated the effect of intrapartum antibiotics on the oral microbiome composition. Phyla \textit{Actinobacteria} and \textit{Bacteroidetes} were more abundant after intrapartum antibiotic exposure whereas \textit{Lactobacillus} genera were more dominant in neonates not exposed to antimicrobial agents. Further function analysis demonstrated that LPS biosynthesis and amino acid metabolic function were enriched in neonates exposed to antibiotics while carbohydrate metabolic pathways were more abundant in the group without antimicrobial treatment. They concluded that intrapartum antibiotic treatment is a key regulator of the initial neonatal oral microbiota\textsuperscript{18}. Early antibiotic exposure may also have a negative impact on bacterial composition, causing dysbiosis and thus increasing the risk for disease development. Zhou et al\textsuperscript{19} showed that perinatal antibiotic treatment induced dysbiosis in the mother’s vagina and the neonatal gut, characterized by a significant decrease of \textit{Lactobacillus} abundance. Furthermore, they also suggested an association between perinatal antibiotic exposure and the risk for early-onset sepsis in infants\textsuperscript{19}. Four other research papers\textsuperscript{21-24} associated exposure of antibiotics to the risk of overweight,
development of asthma, allergic rhinitis, hypertension or celiac disease in children, further providing support for a conscious approach regarding antimicrobial usage in early childhood. On the contrary, antimicrobial usage can also have beneficial effects in the treatment of disease. One study investigated the impact of metronidazole, with or without azithromycin, on the microbiota composition in pediatric CD and whether certain microbial features can act as predictors for CD remission. Both antimicrobial regimens lead to distinct microbiota signatures in pediatric CD at remission, but the authors warranted that larger cohorts are necessary to confirm these findings25.

**IMPACT OF NUTRITIONAL INTERVENTION ON THE PEDIATRIC GUT MICROBIOTA**

Twelve papers were identified investigating the impact of nutritional intervention on the infant gut microbiota. Kamng’ona et al26 described the effect of lipid-based nutrient supplements (LNSs), such as multiple micronutrients or iron and folic acid, on mature infant microbiota composition. Supplements were given to mothers during pregnancy and 6 months postpartum and to their infants from 6 to 18 months. Prenatal and postnatal LNS intake influenced gut microbiota diversity at 18 months of age, but did not alter microbiota maturation26. A second study publishing results from the same study cohort looked at additional environmental exposures, like stool sample collection (dry or wet), maternal education, water source, domestic animals, maternal HIV status, which could influence microbiota composition. The results showed that adverse environmental exposures affect the abundance of several bacterial OTU’s and genera. Low maternal education is furthermore associated with higher maturity and diversity. The authors further point out that these adverse environmental factors are indirectly reflecting the socio economic status and suggested that improvement of these environmental factors could have an important impact on health outcome27.

Three studies described nutritional interventions during pregnancy or early life. Drall et al28 looked at gut microbiome profile differences between exclusively breastfed-children, partially-breastfed-children and formula-fed children at the age of 2-4 months who were colonized with *Clostridioides difficile*. They showed that *C. difficile* colonisation was associated with a different gut microbiome in exclusively breast fed vs. exclusively formula fed children. In exclusively breast-fed children, an abundance of *Firmicutes* and *Proteobacteria* and less *Bifidobacteria* was observed. In formula fed infants, the microbial flora did not adapt to *Clostridium* infection suggesting that these infants are more prone to symptoms when colonized with *C. difficile*. Mancino et al29 studied the transfer of *Bifidobacteria* from mother to child in an animal model. *Bifidobacteria* are the most abundant group in the gut microbiota of healthy infants. This study showed mother to pup transmission via the vertical route, but very interestingly, the same *Bifidobacterium* strain was also found in mother and pup after caesarean section. This could possibly suggest existence of colonization at preterm level29. Moossavi et al30 investigated the influence of maternal and early life factors on microbiota composition and variation on mother-infant dyads from the CHILD cohort. Milk microbiota at 3-4 months of age were inversely dominated by *Proteobacteria* and *Firmicutes*. Composition was associated with maternal factors as for example BMI, parity, mode of delivery and breast-feeding practices. An interesting finding, which needs more investigation, is that pumped breast milk is associated with enrichment of potential pathogens and depletion of *Bifidobacteria* in a sex-dependent manner. This finding supports the retrograde inoculation hypothesis whereby infant oral cavity impacts on milk microbiota30. A paper written by Azad31 summarizes the results of the same CHILD cohort study on the role of bioactives and microbiota in infant feeding. Breastfeeding was associated with lower richness and diversity of gut microbiota at 3-4 month of age with relative abundance of *Bifidobacteria*. This probably reduces the risk of obesity later on. In infants with risk of overweight there was abundance of *Lachnospiraceae*31. Robinson et al32 investigated the differences in gut microbiota composition in pregnant women with and without ketonuria at 16 weeks gestation. They concluded that the genus *Roseburia* is highly abundant in the gut microbiota of pregnant women with ketonuria, which is a butyrate producing bacterium increasing serum ketone levels. Hu et al33 showed in a mouse model that time restricted feeding during childhood induced altered microbiota differences in diversity and specific groups of bacteria33. Two studies compared the effect of enteral vs. parenteral nutrition. Kaplan et al34 found unexpected weak anti-B antibodies in 2 patients.
with group O on parental nutrition and specific enteral feeds. Parental nutrition affects microbiota composition by reducing Firmicutes and increasing Bacteroides and Proteobacteria. This could be the cause of microbial dysbiosis which has an effect on the immune system but the authors warrant further study. D’Amico et al published a paper on promotion of gut microbiome homeostasis recovery after hematopoietic stem cell transplantation (HSCT) with enteral feeding in comparison to parenteral nutrition. Prompt recovery of structural and functional gut microbiota was only observed in the enteral nutrition group, possibly reducing the risk of systemic infections and graft versus host disease onset. El Manouni et al analyzed the effect of a daily intake of the Lactobacillus casei strain Shirota (LcS) on microbial diversity and dynamics in healthy children (aged 12-18 years) from the Netherlands. They concluded that LcS ingestion did not result in a more diverse and stable gut microbiota composition. Two final studies described study protocols for nutritional intervention in children. Mostafa et al published a proof-of-concept study where they will look at the use of microbiota directed complementary food in the treatment of moderate malnutrition in Bangladesh. Worldwide 33 million children are affected by malnutrition, of whom 2 million children in Bangladesh. Weight gain, microbiota composition and biomarkers will be analyzed at baseline (start of intervention), after 1 month, 3 months (end of intervention), 1 month after the end of the intervention and every 6 months for 4 years. Another study protocol described by Lind et al will investigate the impact of protein reduced Nordic diet on faecal microbial composition. The diet comprises increased intake of fruits, berries, vegetables and whole grain and a decrease in the intake of sweets, dairy products, meat and poultry. The children will be randomly allocated in the Nordic Food group or the regular diet group. At age of 6-18 months, fecal microbial composition will be analyzed.

ROLE OF GUT MICROBIOTA IN EARLY INFANCY, GROWTH AND DEVELOPMENT

Thirteen studies fulfilled our selection criteria for the role of microbiota in early infancy and growth. Four of them dealt with microbiota features in pregnancy and the effect on mother and new-born child. The paper by Avershina et al focussed on spore-forming bacteria in the gut of mother-child pairs selected from the IMPACT study cohort in Norway. These bacteria (mainly Clostridium) comprise a large part of the human gut microbiota, but they are difficult to culture. The investigators developed a new culture-independent DNA technique [based on 16S rRNA gene sequencing after a two-step purification technique with ethanol and ethidium monoazide (EMA)]. The results indicated a low abundance of endospore forming species shared between mothers and their children suggesting that these bacteria are mainly acquired from the environment. Tanabe et al investigated the association between the maternal microbiome and the prevalence of dermatitis in early infancy. They concluded that the diversity of Proteobacteria and relative abundance of Acinetobacteria were reduced in maternal faeces during pregnancy in cases of dermatitis in infancy, suggesting that this early trigger could be a good predictor of subsequent allergy and allergic march through infancy. Another study from the same research group investigated the association between gut microbiota composition and metabolism via glycoalbumin level during pregnancy in healthy Japanese women. They found no evident differences in microbiota composition, but they indicated that their study was limited in power so further larger studies are required. Sato et al showed that maternal gut microbiota are associated to new-born anthropometrics in a sex-specific manner. Genus Parabacteroides and Eggerthella showed negative associations with new-born head circumference and weight, respectively in males. A higher proportion of Streptococcus was associated with smaller anthropometrics in females. These findings suggest that maternal microbiome is an important factor for intrauterine growth.

One paper and one protocol looked at the association between microbiome and child neurological development. Leong et al presented a protocol to study the epigenetic influences on neurodevelopment at 11 years of age [longitudinal peri/postnatal epigenetic twin study (PETS@11)]. This analysis will include questionnaires, blood, faecal samples, and brain MRI-scans. Atukunda et al performed a follow-up education trial in Uganda looking at child development, growth, and microbiota. The trial included mother-child pairs recruited when the children were 6-8 months and divided in an intervention group where mothers received...
nutrition, stimulation and hygiene education and a control group with routine health care. Interestingly, gut microbiota composition did not differ significantly between both groups. Korpela et al. published a cohort profile from the Finnish Early life Microbiota (HELMi) longitudinal birth cohort consisting of healthy term infants born in 2016-2018, mainly in the capital region of Finland. The intestinal microbiota development is characterized based on faecal samples and extensive online questionnaire that collected data at weekly to monthly interval on diet, other exposures, family lifestyle, health, and growth of the child. Motor and cognitive screening was performed at 18 months of age. Infant DNA, mothers breast milk and parental samples were collected. Findings to date do not yet include microbial data. Li et al. looked at the impact of delivery mode (vaginal vs. caesarean section) in very low birth weight infants’ microbiome of the neonatology intensive care from the Shenzhen University Hospital in China. The results demonstrated that oral bacterial communities were dominated by Proteobacteria and Pantoea were more prevalent, whereas Corynebacterium, Methylobacterium and Variovorax were more prevalent in caesarean born infants. There were also metabolic differences (mainly vitamin and amino-acid pathways) between the different modes of delivery. The group of Huang (Shengzen University Hospital, China) also described the characteristics of intestinal microbiota in very low birth weight infants with extrauterine growth restriction (EUGR). The intestinal bacterial communities of EUGR infants are dominated by Proteobacteria. The relative abundance of Aeromicrobium and Serratia is decreased, whereas genera Parabacteroides, Ruminococcus, Blautia and Aeromonas were more prevalent. Moran-Ramos et al. looked at environmental and intrinsic factors shaping gut microbiota composition and diversity and its relation to metabolic health in children and early adolescence. Microbiota of 926 children (ORSMEC cohort) aged 6-12 years in Latin America were characterised. For theen clinical and environmental co-variates were identified explaining 15.7% of inter-individual gut microbial variation. Extrinsic factors, such as socioeconomic status, showed major influence on the most abundant taxa. Age was positively correlated to higher diversity in normal weight children. The authors concluded that these results will contribute to a better understanding of gut microbiome and ultimately develop therapeutic approaches to improve metabolic status. Rouhani et al. concluded that diarrhoea is a potential cause and consequence of reduced gut microbial diversity among undernourished children in Peru. Children who were still severely stunted at time of sampling had the greatest diarrhoea-associated reductions in diversity and the slowest recovery. Increased diversity was, on the other hand, predictive of reduced, subsequent diarrheal episodes. Another study showed that bacteriophages isolated from Bangladeshi stunted children (< 38 months) can regulate gut bacterial communities in an age-specific manner. Stunted children harbor distinct bacteriophages relative to their non-stunted counterparts. Proteobacteria from non-stunted children increased in the presence of phages from younger stunted children, suggesting that phages could contribute to the bacterial community changes observed. Campylobacter infection is associated with impaired growth of children, also in the absence of symptoms. Rouhani et al. studied the associations between Campylobacter infection, linear growth, and fecal microbial community features in a prospective birth cohort with a high burden of diarrhea and stunting in the Amazonian lowlands of Peru. As Campylobacter infection was common in this cohort, clear disruptions in microbial communities were noted which could help explain the observed effects of asymptomatic infections on growth in early life.

CONCLUSIONS

In this review, evidence was given for the role of gut dysbiosis in a wide spectrum of diseases in children. Substantial progress has also been made in the understanding of the impact on antimicrobial treatment and nutritional intervention on microbial composition in childhood health and development. As the microbiota establishes during childhood, this may be the prime time for microbiota interventions for health promotion and/or disease prevention. It is therefore crucial to further unveil pediatric microbial gut signatures in health and disease and to identify the underlining mechanisms and confounding factors involved in disease onset and development in children.
Conflict of Interest
The authors declare no conflict of interest.

REFERENCES


