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THE PAEDIATRIC GUT MICROBIOME: A YEAR IN REVIEW

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Abstract – This review explores one year of the most important, accessible and relevant original scientific publications published between April 2020 and March 2021 exploring the microbiome of infants, children and adolescents. This review encompasses 40 studies describing changes in microbiota composition observed in paediatrics in a wide spectrum of pathologies in addition to the development of the microbiome during infancy and early childhood and the impact of nutritional intervention and antibiotics on the gut microbiome.

Keywords: Paediatric, Microbiome, Dysbiosis, Development, Nutrition, Antibiotics.

BACKGROUND

This review offers an overview of papers published between 01/04/2020 and 31/03/2021 surrounding the paediatric gut microbiome. A single PubMed search was undertaken with queries of "paediatric" OR "pediatric" AND "microbiome". Additional filters were added for infant, child or adolescent ages and to human studies in English. A total of 240 abstracts were reviewed in duplicate by the authors of this review, with studies included if they contained original research, recruited children, explored the gut microbiome in some way and were deemed interesting enough to discuss further. Papers put forward by two authors were accepted automatically, with those put forward by a single author being subject to a casting vote by a third author. Following groupings into distinct clinical areas, 40 original papers were taken forward for manuscript writing.

INFLAMMATORY BOWEL DISEASES

Microbial population disturbances (or dysbiosis) were first described and implicated in the pathogenesis of inflammatory bowel diseases (IBD); Crohn's disease (CD) and ulcerative colitis (UC). Dysbiosis is defined as a gut microbiota pattern different from the established patterns of normality. Six research papers relating to IBD were identified, with a particular focus on treatment and therapy of CD. Four papers explored the effect of therapies on the gut microbiota of paediatric patients including exclusive enteral nutrition (EEN) therapy, infliximab (IFX) therapy and intravenous corticosteroids (CS).

Hart et al[1] recruited 30 children at the time of initiation of EEN therapy or CS for the induction of remission of CD or UC. Diederen et al[2] characterised changes in faecal microbiota by collecting stools from 43 children prior to initiation, during and after the conclusion of EEN

therapy, and comparing to those of healthy controls. Both these studies commented on the microbial diversity of subsequent responders and non-responders prior to and after initiation of therapy.

Hart et al[1] showed that in participants who progressed from an active disease to full remission, had greater abundances of *Blautia, Sellimonas* and bacteria from the family *Ruminococcaceae* and reduced abundances of *Granulicatella, Haemophilus*, and *Streptococcus* regardless of therapy type. Similar remission rates were achieved for both treatment options, however by week 2 of treatment, a significant difference in Shannon diversity was observed between participants achieving remission and those that would not. Following 8 weeks of treatment, regardless of disease phenotype or therapy received, microbiota communities were significantly more clustered than prior to treatment in participants who achieved remission compared to participants with ongoing active disease[1]. Similarly, Diederen et al[2], showed that the overall composition of the microbiota differed between responders and non-responders prior to and after therapy. These combined findings suggest that the successful induction of remission, independent of induction therapy, results in microbial communities shifting toward a similar healthy remission microbiota profile and may also allow for future prediction of therapy response.

In addition to commenting on the differences between subsequent responders and non-responders, Diederen et al[2], showed that the overall microbial diversity of controls was very different from that of CD patients at all time points. *Escherichia coli, Ruminococcus gnavus, Dorea longicatena* and *Blautia* were present in greater relative abundance in CD, whereas a reduction in the relative abundance of beneficial organisms, including *Eubacterium rectale, Bifidobacterium longum* and *Ruminococcus bromii* was observed.

Interestingly, Diederen et al[2] showed that the proportional abundance of the *Blautia* genus was generally higher in patients with CD at the time of diagnosis versus healthy controls, was reduced in patients with CD during EEN therapy and was associated with non-responders. In contrast, Hart et al[1] showed a greater abundance of *Blautia* overall in patients who progressed from an active disease state to full remission.

Salamon et al[3] investigated the quantitative changes in four selected species of bacteria, *Bacteroides fragilis, Lactobacillus fermentum, Lactobacillus rhamnosus* and *Serratia marcescens,* during the course of EEN or IFX in the treatment of CD, alongside a healthy control comparator group. Both EEN and IFX treatment showed a significant decrease in the Paediatric Crohn's Disease Activity Index (PCDAI) indicating response to treatment. Significantly less *L. fermentum* was observed pre-treatment in those subsequently receiving EEN. Following EEN treatment, the number of *L. fermentum* increased and was significantly higher than that of controls. In contrast, *L. fermentum* counts following IFX was significantly lower than that of controls as well as before therapeutic intervention. Additionally, the number of *S. marcescens* was found to be significantly lower both before and after EEN therapy in comparison to the control group. Both treatment options were found to modify the microbiome in CD patients, albeit that they remained different to that of healthy controls.

Wang et al[4], investigated the effect of infliximab therapy on the gut microbiome structure. The study found paediatric CD patients exhibited lower relative abundances of short-chain fatty acid (SCFA) producing bacteria including *Faecalibacterium, Clostridium clusters IV and XIVb*, *Roseburia* and *Ruminococcus.* IFX treatment was shown to enrich the bile salt hydrolase producing bacteria in CD participants. Additionally, a sustained response to IFX therapy was associated with higher abundances of *Methylobacterium, phingomonas, Staphlyococcus* and *Streptococcus*. This suggests that the effects of IFX might partially be mediated by enriching bacteria that produce SCFAs and bile salt hydrolases thereby inhibiting inflammation.

A multi-centre clinical cohort study investigated changes in the microbiota associated with mucosal healing in established paediatric CD patients 5. In total 25 paediatric patients with CD, with disease over 6 months and receiving maintenance biological therapy were recruited and divided into two groups based on the presence of mucosal healing as indicated by low faecal calprotectin levels. No significant differences in α- or ß-diversity between groups was observed. However, there was a significant sixfold increase in the relative abundance of *Dialister* genus in patients with low calprotectin levels compared to high calprotectin. The relative abundance of *Dialister* negatively correlated with faecal calprotectin. H-NMR-based

metabolic profiling identified an increase in pentanoate in the low calprotectin group which was correlated with *Dialister* relative abundance. The relationship between *Dialister* and pentanoate could be a novel therapeutic target to promote mucosal healing in CD.

A Canadian group investigated whether polymorphisms in NOD2 affected the microbiome of healthy first-degree relatives (FDRs) of individuals with CD 6. NOD2 is an intracellular pattern recognition receptor that senses bacterial peptidoglycan and stimulates the host immune response. Interestingly the minor allele frequency of all three of the NOD2 risk SNPs was higher in the FDRs of the CD cohort than in the 1000 genomes healthy control cohort. No association between the individual risk SNPs and α-diversity was detected however the rs2066845 SNP was significantly associated with an increased relative abundance of the family *Erysipelotrichaceae.* Overall, no significant influence on the composition of the microbiome was observed with the NOD2 genotype, indicating it is not a major determinant of the overall composition of the microbiome in a healthy FDR cohort.

OBESITY

Childhood obesity is a growing global pandemic urgently requiring greater understanding to reduce prevalence and long-lasting adverse sequelae. The role of the gut microbiome on obesity is well established and a continuing source of imperative research. Six papers on gut microbiome and paediatric obesity were identified.

Korpela et al[7] investigated the foetal microbiome by analysis of the first-pass meconium with subsequent follow-up. Following meconium collection, newborns were followed with stools collected and growth data recorded at 1, 2 and 3 years of age. The meconium samples of overweight versus normal weight children at 3 years of age differed, with overweight children having a higher proportion of *Bacteroidetes*, specifically *B. fragilis*, indicating an association between obesity and the microbiome may begin *in utero*7.

In a further study[8] the gut microbiota of children with and without obesity in Harbin, China were compared. All diversity indexes of the normal weight children were higher than children with obesity, the *Firmicutes* to *Bacteroidetes* (F:B) ratio in obese children decreased, while the relative abundance of *Lactobacillus* and *Bifidobacterium* decreased and *Akkermansia* increased in the obesity group. Additionally, using murine models the authors identified nine bacterial strains (two significantly) that inhibited weight gain.

Mexico has one of the highest rates of both childhood obesity and caesarean births (C-section) in the world. A descriptive study was conducted to determine the association between delivery mode and gut microbiota profiles in healthy Mexican infants9. Distinct microbiota profiles were observed in infants born by C-section with low levels of *Bacteroidetes*, high levels of *Firmicutes* especially Clostridium and Enterococcus and high F:B ratio. While vaginally delivered infants had low F:B ratios and high levels of *Bacteroidetes*. Surprisingly, all samples, regardless of delivery mode, had high abundance of *Proteobacteria*[9].

Prader-Willi syndrome, a rare genetic disorder is associated, amongst other manifestations, with excessive weight gain and obesity. Garcia-Ribera et al[10], identified gut microbiota associated with the weight status of these patients. Obese patients had lower phylogenetic diversity of gut microbiota compared to normal weight patients, with the genera *Alistipes*, *Klebsiella* and *Murimonas* differentially more abundant in the normal weight patients than obese patients. An inverse relationship was noted between *Alistipes* abundance and adiposity, glucose and lipid metabolic indicators and meat intake. An additional study[11] into Prader-Willi syndrome aimed to characterise the gut bacterial and fungal communities associated with PWS and to determine the associations with hyperphagia. Overall bacterial α-diversity was not found to be different between PWS and matched controls, however, higher Chao1 richness of *Actinobacteria* was observed in PWS compared to controls. Additionally, LEfSe identified a higher abundance of *Prevotella* in PWS, while *Oscillospira* and unclassified *Enterobacteriaceae* were more abundant in healthy controls. Patients with PWS showed a distinct fungal community structure from that of the matched controls. The genera *Candida, Mrakia* and *Agaricomycetes* was in higher abundance in PWS while *Saccharomyces* was in higher abundance in controls. Hyperphagia scores were found to be associated with fungal α-diversity and relative abundance of *Staphylococcus, Clostridium, SMB53* and *Candida.*

The gut microbiota together with genetic contributions, are important in the pathogenesis of Non-alcoholic fatty liver disease (NAFLD) in obese children. Monga Kravetz et al[12] explored the relationship between gut microbiota and NAFLD, while simultaneously investigating the role of PNPLA3 rs738409, a genetic variant and strong contributor to the disease. Patients with NAFLD had decreased bacterial diversity compared to obese children without NAFLD, and NAFLD subjects having higher F:B ratio and lower abundance of *Bacteroidetes*, *Gemmiger* and *Oscillospira* even when controlling for the PNPLA3 rs738409 genetic variant.

Faecal microbiota transplant (FMT) as a treatment modality for adolescent obesity was investigated by Leong et al[13] in 87 obese adolescents in a randomised, double-masked, placebo-controlled trial in New Zealand. FMT had no effect on body mass index (BMI) standard deviation at 6 weeks, yet there was a change in total gut microbiome composition in the FMT group. Those with metabolic syndrome in the FMT group benefited from resolution of this condition as well as a reduction in visceral adiposity.

ALLERGIES, ATOPY, ASTHMA

While asthma, atopic and allergic diseases are multifactorial, research into the pathogenesis and protective effects played by the gut microbiome is increasing. Four studies on these diseases were identified.

The total antibiotic resistant gene (ARG) profile or resistome was described in healthy infants at risk of developing eczema over time by Loo et al[14], in a South-east Asia cohort (a region with high endemic bacterial resistance). Seventy-five infants at risk of eczema were followed over their first year of life, with metagenomic analyses conducted on stool samples. All infants were found to harbour antibiotic resistant genes at some point with mean ARG numbers increasing with age but few persisting. Resistance to aminoglycosides, beta-lactam, macrolide and tetracycline antibiotics were the most common ARGs identified. Beta-lactam resistant *Klebsiella pneumoniae* and *Escherichia coli* were present in 42.7% and 5.3% of infants respectively[14].

Chan et al[15] published a clinical trial protocol for a 2 year longitudinal cohort study following 1250 Hong Kong Chinese infants, assessing the gut microbiome and environmental influencers on the aetiopathogenesis of eczema. The same research group then published a 4-month pilot study examining the microbial influence on the development of eczema in 152 newborns in Hong Kong. Microbial DNA extraction from stool samples and 16S rRNA sequencing identified a higher abundance of *Bifidobacterium* in the eczema group than the control group16. This preliminary evidence supports the full study that is still underway, aiming to better define the associations between the gut microbiota and development of eczema.

The effect of the gut microbiome on airway disease, and more specifically asthma, was investigated in the Protection against Allergy: Study in Rural Environments (PASTURE) birth cohort[17]. The gut microbiome of Infants aged 2-12 months was modelled for maturation using 16S rRNA sequence data. Farm environments rich in microbial stimuli specifically with regular contact with hay and animal sheds, strongly influenced maturation of the gut microbiome and prediction of butyrate production. The asthma-protective farm effect points towards a gut-lung axis in humans, through the identification of two potential asthma-protective amplicon sequence variants (ASVs) in the genera *Roseburia* and *Coprococcus*. Networks of bacteria within these two genera are directly associated with butyrate production (with immunomodulatory and anti-inflammatory properties) and other SCFAs which in turn was shown to be protective against asthma in the first year of life[17].

AUTOIMMUNE DISEASES

The microbiome of children with Type 1 Diabetes (T1D), a common autoimmune disease, was explored in three papers. Biassoni et al[18], employed machine-learning analyses to identify significant taxa associated with T1D. The relative abundance of *Bacteroides stercoris, Bacteroides fragilis, Bacteroides intestinalis, Bifidobacteriumn bifidum, Holdemania, Synergistetes*

and *Gammaproteobacteria* were significantly higher in patients with T1D in comparison to healthy controls. In addition, patients had significantly lower levels of *Bacteroides vulgatus, Deltaproteobacteria, Parasutterella, Lactobacillus* and *Turicibacter.* Two studies by the same research group[19,20] investigated the role the microbiota plays in the risk of T1D development in an Italian paediatric population. Collectively these studies showed that higher levels of Firmicutes is a risk factor for T1D whilst an abundance of *Bifidobacteirum* in the gut was a protective factor against T1D. Additionally an abundance of *Akkermansia muciniphila* was shown to be a protective factor against diabetic ketoacidosis. Further work in the field of T1D is needed to determine whether strategies to modify the gut microbiota could control T1D development.

A single study explored the gut microbiota in children with juvenile idiopathic arthritis (JIA), the most common autoimmune rheumatic disease in children. Qian et al[21], found that the Chao1 and Shannon-Wiener indexes in the JIA group were significantly lower than the healthy control group. The relative abundance of *Anaerostipes, Dialister, Lachnospira,* and *Roseburia,* was found to be significantly decreased in the JIA group, all of which are microbes that produces SCFAs.

OTHER PATHOLOGIES OF INTEREST

In addition to the diseases discussed above, there were several diseases or areas of interest with a single paper, demonstrating the increasing interest in the role of the microbiome in a wide range of childhood disease.

Chen et al[22], investigated the correlation between systematic inflammation and altered gut microbiota in children with Kawasaki disease (KD). Acute KD children were found to exhibit a significantly reduced faecal microbial diversity compared to matched controls. LEfSe identified higher abundances of *Enterococcus, Acinetobacter, Helicobacter, Lactococcus, Staphylococcus* and *Butyricimonas* in KD patients. *Enterococcus* and *Helicobacter* were additionally found to be positively correlated with IL-6 measures of inflammation indicating that gut microbiota alteration is closely associated with systemic inflammation in children with acute KD.

The link between gut bacteria and autism spectrum disorder (ASD) has been explored in the past after ASD presentation. Laue et al[23], explored this association further by prospectively characterizing the infant and toddler gut microbiome from 6 weeks to 3 years of age and assessing ASD-related social behaviour at 3 years of age using the Social Responsiveness Scale (SRS-2). Several taxa, including many in the *Lachnospiraceae* family, were found to be associated with SRS-2 performance at 1, 2 and 3 years of age. At 1-year, greater relative abundance of *Adlercreutzia equolfaciens*, *Ruminococcus torques*, *Eubacterium dolichum*, and *Lachnospiraceae* were associated with poorer social behaviour. This study supports the notion that there is a potential association between early-childhood gut microbiome and social behaviours.

A case study[24] following a 14-year-old male with severe graft-versus-host disease (GvHD) explored the longitudinal dynamics of the gut microbiome following four doses of FMT. FMT has emerged as a treatment for severe colitis associated with GvHD. Initially the patients gut bacterial microbiota was characterized by low diversity, however following each FMT dose, the bacterial community gradually recovered with increasing diversity after each treatment. The mycobiota change however was opposite to that of the bacterial microbiota change. The gut fungi community initially showed expansion of several species however a decrease in diversity was seen following multiple FMTs. Lastly the gut virome community varied substantially over time. This study has shown that bacterial, fungal and viral communities respond differently to FMT.

The association of the microbiome and the growth velocity of children with environmental enteric dysfunction (EED) was explored in a cohort of Malawian children25. EED is a gut inflammatory process that is endemic to children living in low- and middle-income countries which leads to increased childhood morbidity and mortality. Thirty bacterial taxa were found to be significantly differentially abundant and associated with linear growth. Interestingly 10 of these bacterial taxa were only present in samples from children who had subsequent

adequate growth and were completely absent in children who went on to have poor growth. In addition, 3 differentially abundant bacteriophages, all from the *Caudovirales* species, were found between the growth velocities. A positive correlation between bacteria and bacteriophage richness was observed in children with subsequent adequate/moderate growth. This finding was not observed in children with subsequent poor growth, suggesting that a disruption in the equilibrium between bacteria and bacteriophage communities might be associated with subsequent poor growth velocity.

DEVELOPMENT OF THE GUT MICROBIOME IN EARLY INFANCY

Microbial colonisation of the infant gut has been extensively studied for neonatal, paediatric and adult disease causality. The establishment of the infant gut microbiota (up to 3 years of age) is crucial for the long-term health of the adult gut microbiome, as these communities are intricately involved in immunomodulatory capacities, conferring protective immunity against pathogenic organisms while creating immune tolerance towards commensal organisms. Five publications examining development of the infant gut microbiota were identified.

Rackaityte et al[26], investigated the presence of bacterial colonisation of the foetal intestine *in utero*. Foetal intestines from terminated pregnancies, meconium samples and control samples underwent scanning electron microscopy and 16S rRNA sequencing to indicate the presence of highly specific and limited bacterial species mid-gestation. The taxa *Micrococcaceae* and *Lactobacillus* were most prevalent in foetal meconium, while *Micrococcus luteus* in the intestine was isolated only in the presence of monocytes, in nutrient-poor environments and in the presence of pregnancy hormones. These very early gut colonisers have the potential to influence immune development later in life.

The nature biodiversity hypothesis with specific reference to the infant gut microbiome was assessed by Nielsen et al[27], through exploration of the associations between close living proximity to natural ecosystems in an urban environment and gut microbial composition and diversity in four month old infants in Canada. Double stratified results indicated that close proximity to natural environments together with pet ownership in formula-fed infants was associated with reduced gut microbial diversity in these infants. Future studies are required to further understand the implications of these findings.

Duan et al[28] investigated the gut microbiota and metabolites in breast-fed infants with and without breast milk jaundice (BMJ) using gut microbiome-metabolomics. Infants with BMJ had significantly increased faecal *Streptococcus* while *Enterococcus* was significantly decreased. SCFA detection through gas chromatography-mass spectrometry revealed reduced levels of acetic and propionic acid. These two findings may be interconnected and important in the pathogenesis of BMJ.

While bacterial species in the gut microbiome have the greatest role in maintaining health, viruses and fungi also have important functions. As yeasts may play an important role in this microenvironment, Kondori et al[29] prospectively followed Swedish infants from birth to 3 years investigating the colonisation rates, composition and species distributions of yeasts in the gut microbiome through quantitative culture methods. Yeast colonisation increased rapidly up to six months of age, then decreased by 3 years. *Candida albicans* was the predominant species cultured. Yeast colonisation rates between vaginally and caesarean section delivered infants were not significantly different, however lower colonisation rates were present in breast-fed infants. The study demonstrated the importance of yeast commensals, specifically *C. albicans* and *C. parapsilosis* within the infant gut microbiome.

Hayden et al[30] postulated that the gut microbiome may affect endocrine functions in infants with cystic fibrosis (CF) which in turn may result in linear growth abnormalities. The researchers identified differences in early faecal dysbiosis in CF infants with poor linear growth compared to CF infants with normal growth. The faecal dysbiosis showed decreased abundance of *Bacteroidetes* and increased abundance of *Proteobacteria*, taxa important in gut health, nutrient distribution and endocrine signalling pathways. The authors concluded faecal dysbiosis with concomitant gut metabolite changes as a result of malabsorption, disturbs early growth in infants with CF.

IMPACT OF NUTRITIONAL INTERVENTION ON THE GUT MICROBIOME

The development and composition of gut microbiota in newborn infants during their first year of life is predominantly dependent on breast milk intake. Regulation of the microbiome during this early period has crucial implications on long-term health outcomes. Seven papers on the effect of nutrition on the infant gut microbiota were identified.

Ma et al[31] prospectively studied 91 term infants, divided into three groups of exclusively fed either breast milk (n=30), formula A (n=30) or formula B (n=31) for the first 4 months of life, with stool samples collected at 40 days, 3 months and 6 months of age. The breast-fed group had lower α diversity at 40 days of age than the formula-fed groups, however this dramatically increased at 6 months of age after the introduction of solid foods. *Bifidobacterium* followed by *Enterobacteriaceae* represented the first and second most predominant genera in all groups at all time points. A healthy infant gut is initially characterised by low diversity, in contrast to that in adults. The different feeding types resulted in differences in bacterial composition; with *Streptococcus* and *Enterococcus* significantly lower in breast-fed group compared to formula-fed A and *Lachnospiraceae* lower in breast-fed compared to formula-fed B. *Veillonella* and *Clostridioides* were also lower in breast-fed than formula-fed infants. The bacterial composition between the two formula-fed groups were distinct, raising questions about long-term health effects between the different feeding options.

Brink et al[32] investigated infant faecal microbiota and metabolites at different time points (3, 6, 9 and 12 months), comparing three diets; breastfed (BF), dairy-based milk formula (MF) and soy-based formula (SF). BF infants had the lowest α-diversity at 3, 6 and 9 months of age, followed by MF and then SF groups. The presence of *Bifidobacterium* in the BF cohort was 2.6 to 5 times higher than the SF group through the first year of life. Higher levels of the metabolites; butyric acid, D-sphingosine, kynurenic acid, indole-3-lactic acid, indole-3-acetoc acid and betaine were present in BF infants than formula fed infants. There were different associations of metabolites to microbiota profiles with the three diets at different ages. The SF group had the highest bacterial diversity and altered metabolic pathways than the BF and MF cohorts, indicating the impact of neonatal diets on the microbial growth, development and resultant metabolite profiles.

Another study[33] looked at differences in neonate feeding and resultant gut microbiome as well as assessing intestinal biomarkers as indicators of gut maturation. This randomised, double-blinded controlled trail enrolled 203 healthy term infants who were allocated to two different supplemented formula-fed groups and a reference breast-fed group. Measurements of stool microbiome profiles and faecal biomarkers at 1, 2, 4 and 8 weeks showed lower calprotectin levels in the formula-fed group supplemented with prebiotics (Bovine Milk-derived Oligosaccharides). Gut maturation biomarkers included calprotectin, elastase, α-1 antitrypsin and neopterin, all of which were reduced in the prebiotic supplemented formula group compared to the control formula. Both prebiotic supplemented formula and breastfed groups showed significantly higher *Bifidobacterium* levels than the control formula group.

Differding et al[34] published a paper on the effect of infant complementary feeding timing and breastfeeding duration on microbial composition and BMI at 5 years of age. Early introduction of complementary food was defined as ≤ 4 months, and those breastfed more than 4 months with early introduction of solid food had a differential relative abundance of 6 bacterial taxa, lower *Roseburia* and 0.3 higher BMI-z at 5 years of age. While early solids in infants breastfed less than 4 months was associated with a differential bacterial composition of 9 taxa but not with BMI. The timing of complementary feeding is associated with changes in bacterial composition, with breastfeeding duration affecting these changes. A higher abundance of *Roseburia* was found in infants still breastfeeding at 4 months of age and introduced to solids later, which may be of significance to childhood BMI. While *Ruminococcus bromii* was higher and *Bifidobacterium animalis* lower in children breastfed less than 4 months and introduced to complementary foods earlier. However, there was no evidence that the timing of complementary food intake caused changes in α or ß microbial diversity. Yet childhood BMI-z was higher in those infants breastfed when solid foods were introduced early.

Kurath-Koller et al[35] investigated the effect of different hospital regimens on gut microbiome development in very low birth weight pre-term infants in the first two weeks of life. This prospective controlled multicentre cohort study compared three different neonatal intensive

care protocols for prophylaxis of necrotising enterocolitis and their resultant effect on the microbiome profile. Initially meconium samples contained bacterial signatures and were not sterile, thereafter differing hospital regimens resulted in differences in microbiome composition, diversity and load, the latter increased earlier when probiotics were used. *Lactobacillus* and *Bifidobacterium*, the probiotic genera used, were present in very low quantities within the faecal microbiome in the absence of probiotics. Administration of oral antibiotics to neonates did not negatively influence diversity or abundance of microbiome signatures. After two weeks of age, all samples achieved similar diversity indexes, although there was centre-specific clustering.

Hughes et al[36], hypothesised that gut microbiota of undernourished infants may have an effect on growth and inflammation in the context of lipid-based nutrient supplementation (LNS). This randomised controlled trial in Malawi found the genera *Clostridium, Ruminococcus* and *Firmicutes* appeared to modify growth in infants obtaining LNS, while inflammation was modified by *Faecalibacterium* and *Streptococcus.* However, correction for multiple hypothesis testing provided no evidence of effect modification in this analysis.

Liang et al[37] reported that neonatal viral community establishment in the gut takes place in specific steps, and partially or fully breastfed infants have lower viral abundance than formula fed babies. Initial gut seeding bacteria induce bacteriophages with resulting virus-like particles, thereafter viral replication within host cells signifies the second phase of neonatal virome assembly. Breastfeeding is associated with differences in the both the phage and viral communities, suggesting that breast milk may have infectious viral protective effects.

IMPACT OF ANTIBIOTIC TREATMENT ON THE GUT MICROBIOME

The first few years of life is a critical time for the development of the gut microbiota where it can be impacted by external influences including antibiotics. Three studies were selected which investigated the impact of antibiotic treatment on the early gut microbiome. Uzan-Yulzari et al[38], investigated the long-term impact of antibiotic treatment on 12,422 full term children. Neonatal antibiotic exposure was associated with significant differences in the gut microbiome, with decreased abundances and diversity of Bifidobacterium until 2 years of age. Significant attenuation of weight and height gain was observed in the first 6 years of life following neonatal antibiotic exposure in boys but interestingly not in girls.

The effect of long-term low dose antibiotic prophylaxis on the gut microbiota was investigated in children receiving treatment during the acute phase of febrile urinary tract infection. Stool samples were collected at various time points from children who received continuous antibiotic prophylaxis for treatment of vesicoureteral reflux and children with no reflux who only received initial antibiotic treatment. Within two weeks after initiation of treatment almost all enteric bacteria belonged to Lactobacillales and gut microbiota diversity had decreased compared to pre-treatment levels. Within 1-2 months diversity recovered in both groups. In the antibiotic prophylaxis group, a smaller proportion of Enterobacteriales was observed. These findings suggest that the effect of continuous long term antibiotic prophylaxis was insignificant; however, it may selectively suppress bacteria belonging to the order of Enterobacteriales, which are the main causative bacteria in febrile urinary tract infections39.

The carriage rates and proportions of resistant Enterobacteriaceae in the gut were assessed in a study looking at antibiotic use in children with mild respiratory infections in Vietnam[40]. Antibiotic use was found to be inappropriately used in over 90% of children included in this study. The consequence of this was the selection of antibiotic resistant Enterobacteriaceae in stool samples from patients. The proportion of individual patients with resistant colonies was already high at the time of presentation however showed further significant increases by day 8 of treatment.

CONCLUDING REMARKS

This review has encompassed the breadth of microbiome research into the paediatric gut from the last year. This research makes substantial progress into the understanding of the

microbiome on the development of disease as well as the impact of nutritional intervention and antibiotic treatment on microbial composition during childhood development. Future research will likely offer new insights into paediatric microbial gut signatures in health and disease and be able to identify novel microbial targets that influence disease development.

Conflict of Interest

The authors declare no conflict of interest.

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