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The gut microbiota in paediatric population

J. Motion[1], H.S. Bishop[2], R. Hansen[3]

[1]Medical Paediatrics, Royal Aberdeen Children's Hospital, Aberdeen, UK
[2]Paediatrics Oncology, Royal Aberdeen Children's Hospital, Aberdeen, UK
[3]Paediatric Gastroenterology, Royal Hospital for Children, Glasgow, UK

Corresponding Author: Richard Hansen, MBChB, Ph.D, FRCPCH; e-mail: richard.hansen@nhs.net

Abstract: This review explores one year of original scientific publications exploring the microbiome of infants, children and adolescents between 01/04/2018 and 31/03/2019. 48 studies are reported herein, encompassing children with inflammatory bowel disease, obesity and diabetes, autism spectrum disorder, atopic diseases, cystic fibrosis and problems with the ears, nose and throat. In addition, multiple studies are presented on the topic of early life colonisation and neonatal disease, alongside the development of the microbiome throughout childhood. This manuscript therefore offers a state-of-the-art overview of paediatric microbiome research and offers a succinct synopsis of published studies on the topic.

Keywords: Microbiome, Paediatrics, Neonatology, Obesity, IBD, Otolaryngology, Ulcerative Colitis, Crohn's Disease, Diabetes, Autism Spectrum Disorder, Allergy, Asthma, Cystic Fibrosis, Ketogenic Diet, Microbiota.

METHOD

This review offers an overview of paediatric papers published between 01/04/2018 and 31/03/2019 covering the microbiome. A single PubMed search was undertaken to query "paediatric AND microbiome", limiting results to the date range specified and to human studies in English. Additional filters were added for infant, child or adolescent ages. 193 abstracts were reviewed in duplicate by the authors of this review, with studies included if they contained original research, recruited children, explored the 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 the third author. 60 papers were taken forward to manuscript writing and grouped into distinct clinical areas. Clinical topics with a single paper were discarded at this stage (largely for reasons of word count). 45 original papers were ultimately included in this review.

INFLAMMATORY BOWEL DISEASE

Inflammatory bowel disease (IBD) is probably the exemplar medical condition of the microbiome era, with the first descriptions of a microbial population disturbance (or dysbiosis) being implicated in IBD pathogenesis. Eight research papers relating to IBD were identified, with an unexpected focus on ulcerative colitis (UC) and predicting response to therapy being a feature.

Three papers were published from the Predicting Response to Standardised Paediatric Colitis Therapy (PROTECT) study. This multicentre North American inception cohort

encompassed 428 UC patients recruited at diagnosis from 29 centres. Mucosal biopsies were collected at baseline and 52 weeks, with faecal samples at baseline, and 4, 12 and 52 weeks. Schirmer et al[1] reported the use of baseline data to predict treatment outcomes and identified 12 operational taxonomic units (OTUs) positively associated with steroid-free remission at 12 weeks and 4 at 52 weeks, with 2 negatively associated at this later timepoint. Clostridia and *Erysipelotrichaceae* were prominent in the 12-week model and *Ruminococcaceae* at both timepoints[1]. In contrast, 4 baseline OTUs were positively associated with colectomy and 17 negatively associated, including *Ruminococcaceae* again. Interestingly, oral-associated bacteria, particularly *Haemophilus parainfluenzae*, in the gut seemed to be a bad prognosticator in this study. In Haberman et al[2] the same cohort was used to explore pre-treatment rectal gene expression in conjunction with faecal microbiota profiling. In doing so, they implicated colonic mitochondriopathy in disease pathogenesis in particular all 13 mitochondria-encoded genes involved in ATP production. Furthermore, the authors linked genes and pathways to specific OTUs associated with both positive and negative outcomes. Whilst this was not the core analysis of the paper, similar organisms to those highlighted as predictive of treatment response, including Clostridia, *Erysipelotrichaceae* and *Ruminococcaceae*, were amongst those linked. In the final paper from PROTECT, Hyams et al[3] modelled baseline predictors of corticosteroid-free remission at 52 weeks, identifying low clinical severity, high haemoglobin and week 4 clinical remission as important predictors, achieving an area under the curve of 0·70 (95% confidence interval [CI] 0·65-0·75) in a logistic model. Interestingly, abundance of *Ruminococcaceae* (odds ratio 1·43, confidence interval 1·02-2·00) and *Sutterella* (odds ratio 0·81, 95% CI 0·65-1·00) were both independently associated with achieving week 52 corticosteroid-free remission. Clearly, *Ruminococcaceae* are a bacterial family of significant importance in the UC story, worthy of further study.

One further paediatric paper explored UC, with Nusbaum et al[4] looking at microbiome predictors of treatment success after faecal microbial transplant (FMT) in seven children, four of whom responded to the therapy. Whilst a small study, the authors reported an increase in alpha diversity after FMT and an increase in the relative abundance of Clostridia in responders.

Two Crohn's disease (CD) papers met our criteria. The first by Mouzan et al[5] looked at fungal diversity in stool and mucosal samples from 15 children with CD and 20 controls and demonstrated the accuracy of fungal dysbiosis as a classifier of CD (AUC 0.85 +/- 0.057 in stool and 0.71 +/- 0.067 from biopsies). The authors went on to explore the fungal population at species level and identified both a reduction in prevalence of most species and a lower diversity in stools than in biopsies, suggesting the importance of biopsy sampling in exploring the fungal microbiome. Svolos et al[6] presented a three-part study supporting a new dietary approach to the management of CD termed Crohn's Disease Treatment with Eating (CD-TREAT). They showed proof of principle comparability of microbiome changes between healthy volunteers and rats with gut inflammation given both exclusive enteral nutrition and their solid food CD-TREAT diet. They then went on to show open-label efficacy, achieving clinical remission in 3/5 children with active CD, coupled with a significant reduction in faecal calprotectin.

Armstrong et al[7] reported a novel methodology to study both IgG-bound and unbound bacteria from terminal ileal aspirates from 15 children with CD, 7 with UC, and 10 non-IBD controls. They suggested that IgG-bound organisms may be good targets for study as potential pathobionts in IBD and demonstrated this by describing their increased capacity for cell invasion compared to non-bound organisms. Finally, Zhang et al[8] explored the microbiome and host proteome from mucosal aspirates taken from 25 children with CD, 22 with UC, and 24 non-IBD controls during colonoscopy. Importantly, in this study the proteome of aspirates was compared to extracellular vesicles (EV) harvested from the same site. Host defence proteins in EV samples, reactive oxidant-producing enzymes in particular, were implicated in intestinal oxidative stress. Exploring the microbiome, the authors found a reduction in *Bacteroides* and an increase in *Faecalibacterium* in IBD, with strain-level dysbiosis and an expansion of strain L2-6 over A2-165 evident in the latter, with A2-165 being more adept at butyrate production. *Faecalibacterium prausnitzii* has long been thought of as a beneficial organism in the CD story, though the biology now seems more complex and strain-level studies will be needed to validate and replicate these findings.

OBESITY AND DIABETES

The emergence of an obesity pandemic has prompted renewed interest in environmental drivers of weight gain, with the microbiome being a prominent target for research. Seven papers were identified, four regarding obesity and three relating to diabetes.

Stanislawski et al[9] presented a prospective cohort of 165 children, followed from birth to 12 years, exploring their microbiome in the first 2 years and subsequent body mass index at 12 years. The authors found that the strength of predicting obesity *via* the microbiome at age 12 increased with age, peaking at 53% predictability by 2 years of age. Importantly, obesity was not yet established in these children by 2 years of age, suggesting microbial changes were evident before obesity, and not consequential to an overweight state. Finally, this study demonstrated an overlap between the microbiome of overweight/obese mothers and that of their children who would go on to develop obesity by age 12, with 6/10 species most associated with maternal overweight/obesity also predictive of obesity in the children. Related to this concept, Soderborg et al[10] colonised germ-free mice with stool microbes taken from children, born to either obese or normal weight mothers, and demonstrated increased intestinal permeability, reduced macrophage function and reduced cytokine production in those colonised from children of obese mothers. Exposure of the mice colonised with the "obese" microbiome to a Western diet led to excessive weight gain and signs in keeping with non-alcoholic fatty liver disease. López-Contreras et al[11] reported microbiome differences between 71 Mexican children with obesity and 67 normal weight counterparts, demonstrating a significant increase in *Bacteroides eggerthii* in obesity which correlated with body fat. Conversely, *Bacteroides plebeius* and an unclassified *Christensenellaceae* were significantly increased in normal weight children. Nirmalkar et al[12] also studied 172 Mexican children and adolescents grouped into obesity or normal weight cohorts. They identified significant positive associations between particular bacteria and markers of endothelial dysfunction, notably *Veillonellaceae* and vascular cell adhesion molecule 1, and *Ruminococcus* and intracellular adhesion molecule 1.

The Environmental Determinants of Diabetes in the Young (TEDDY) study, reported by Vatanen et al[13] described >10,000 longitudinal faecal metagenomes from 783 children over the first 5 years of life. 101 developed type I diabetes mellitus (DM) and 267 demonstrated persistent autoantibodies to islet cells but not full diabetes. This paper offered important insight into early life colonisation patterns, but the authors conclude, "the taxonomic and functional signals we detected in case-control comparisons were modest in effect size and statistical significance", demonstrating the inherent challenges in exploring microbiome changes during the development of disease even in the largest and most comprehensive of studies. Cinek et al[14] described the faecal microbiome of 73 children with DM from four distinct, geographically distant countries. They reported a significant positive association between diabetes and *Escherichia coli*, and negative associations with *Eubacterium*, *Roseburia* and Clostridial clusters IV and XIVa. Finally, Leiva-Gea et al[15] explored the microbiome of 43 children: 15 with maturity-onset diabetes of the young 2 (MODY2), as a monogenic cause of diabetes, 15 with DM and 13 who were healthy. The microbiomes of the three groups appeared distinct with MODY2 having significantly increased *Prevotella* and significant decreases in *Ruminococcus* and *Bacteroides* against both DM and healthy controls. These findings suggest there may be distinct microbial influences from both the clinical phenotype of diabetes and the autoimmune processes involved in DM.

AUTISM SPECTRUM DISORDER

Three papers described the faecal microbiome of autism spectrum disorder (ASD). Shaaban et al[16] used rtPCR in 30 children with autism whilst reviewing their symptoms after a course of a triple probiotic containing *Lactobacillus acidophilus*, *Lactobacillus rhamnosus* and *Bifidobacterium longum*. Unsurprisingly, the levels of both *Lactobacilli* and *Bifidobacteria* rose after supplementation. The authors also noted significant reductions in gastrointestinal and autism behaviour symptom scores, attributing this to probiotic supplementation. Plaza-Díaz et al[17] looked at 48 children with ASD with (n=18) and without (n=30) mental regression against 57 normally developed children. Encouragingly, the authors acknowledged and sought to explore the impact of different diets between the three groups, an important potential confounder in ASD. The ASD microbiome was different to that

of healthy controls, with increases in Actinobacteria and Proteobacteria at phylum level, and Actinobacteria, Bacilli, Erysipelotrichi, and Gammaproteobacteria at class level. Looking at the two ASD phenotypes, Proteobacteria, *Thermoactinomycetaceae* and *Enterococcus* were increased in those with mental regression whilst *Corynebacteriaceae* and *Clostridiales* Family XVII were increased in those without. Rose et al[18] recruited 103 children to a paired stool and blood sampling study aimed at exploring immunological function alongside host microbiome in children with ASD, with and without gastrointestinal symptoms, and compared these to normally developing children with and without gastrointestinal symptoms. The authors suggest that children with ASD and gastrointestinal symptoms produced more mucosa-associated cytokines (IL-5, IL-15, IL-17) under lipopolysaccharide (LPS) stimulation experiments and generally produced less TGF 1 against comparator groups. Looking at the microbiome in children with gastrointestinal symptoms, *Bacteroidaceae*, *Lachnospiraceae*, *Prevotellaceae* and *Ruminococcaceae* were increased in ASD but not in normally developing children. This pattern was not repeated when contrasting the two groups without gastrointestinal symptoms.

MICROBIOME IN EARLY INFANCY

Characterisation of the gut microbiome in the early life is increasingly being utilised to look at disease mechanisms and direct novel treatment strategies in neonatal medicine. Sixteen studies were identified pertaining to the microbiome in early infancy with a breadth of topics being covered. This is summarised in Figure 1.

Three studies characterised the natural history of microbiome development in preterm infants. Grier et al[19] mapped and explored the gut and respiratory microbiome of 38 preterm and 44 term infants and identified a predictable chronological progression of "community state types" in the first year of life in the gut, nose and throat. In preterm infants, initial community state types in all sites were distinct from those born at term, with progression becoming similar at 50 weeks corrected gestation. The predominant organism in preterm infants was *Staphylococcus* in all sites. Ho et al[20] also followed the developing microbiome in 45 preterm infants. They described an increasing abundance of Proteobacteria over time, comprising 46% of all sequencing reads at less than 2 weeks, rising to 77% by week four. Gammaproteobacteria predominated, with *Klebsiella* the dominant genus. Across all infants, vaginal delivery and antenatal steroid exposure were significant determinants of increasing gammaproteobacteria abundance, supporting vertical transmission and iatrogenic factors as important influences on early colonisation. Brown et al[21] followed the development of the microbiome of 35 premature infants during the first 3 months of life. Most were colonised by *Enterococcus faecalis*, *Klebsiella pneumoniae* and *Staphylococcus epidermidis*. Nine clus-

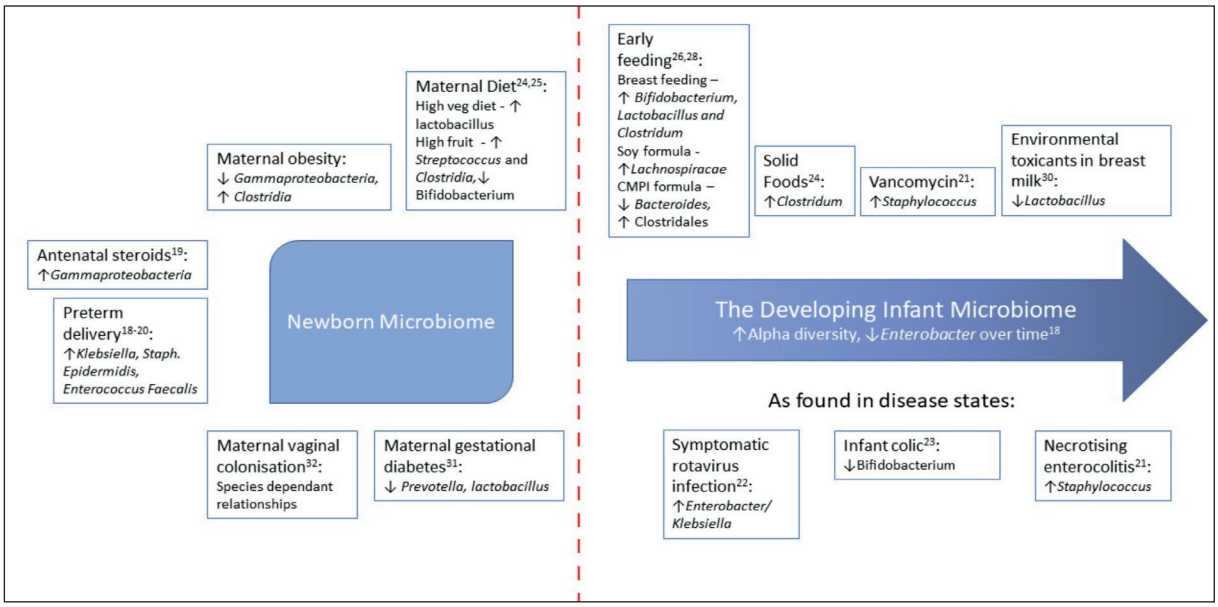

Figure 1. Summary of findings in the microbiome of the newborn and of early infancy.

ters of microbial communities, based on species membership and abundance, were identified with seven occurring in multiple infants. Infants often switched clusters during colonisation, sometimes on more than one occasion. Low microbiome diversity correlated with antibiotic administration. No specific species or communities were associated with necrotising enterocolitis (NEC).

Three studies investigated how the microbiome was affected by different disease states. Romano-Keeler et al[22] investigated 10 infants with NEC requiring surgery against controls requiring surgery for other reasons. Mucosal samples were obtained intraoperatively in addition to the first stool sample postoperatively. In tissue samples from patients with NEC there was a significant reduction in microbial richness compared to controls. In tissue samples from infants with NEC there was an overrepresentation of Firmicutes, specifically *Staphylococcus* and *Clostridium*. Increased abundance of *Staphylococcus* was found to be independently associated with NEC.

Ramani et al[23] investigated how neonatal rotavirus infections are modulated by human milk oligosaccharides and the milk and infant gut microbiome. They described significant differences in microbiome composition between rotavirus symptomatic neonates and asymptomatic neonates both positive and negative for rotavirus. *Enterobacter/Klebsiella* were significantly increased in symptomatic neonates. An increased relative abundance of *Staphylococcus* and *Streptococcus* was seen in both groups of asymptomatic infants, indicating a potential protective effect.

Rhoads et al[24] investigated gut inflammation and microbiome profiles of 37 infants with colic against 28 healthy controls. This study combined samples from 2 randomised control trials (RCTs) collected before receipt of study interventions. The stool of infants with colic had a significant decrease in *Actinobacteria*, independent of feeding mode. *Bifidobacterium* was significantly lower in infants with colic. These findings, combined with raised faecal calprotectin, led the authors to suggest that colonisation by opportunistic proinflammatory microbes might be a mechanism for the development of colic.

Ten studies investigated factors that influence the microbiome in early infancy. Four investigated the impact of maternal diet during pregnancy and the method of infant. Savage et al[25] explored maternal diet during pregnancy against the microbiome in 323 infants. High intake of vegetables and low intake of processed meats and deep-fried foods was positively associated with Shannon diversity, but not significantly in adjusted analysis. There was an independent positive association with high vegetable intake and low processed meat and deep fried foods and *Lactobacillus* spp. Lundgren et al[26] investigated 145 mother/infant dyads from the New Hampshire Birth Cohort Study. Among the key associations found, maternal fruit consumption in pregnancy was associated with microbiome composition of vaginally delivered infants. They found increased *Streptococcus* and *Clostridium* and reduced *Bifidobacterium* in this group. Maternal dairy intake in infants delivered by Caesarean section was associated with a high abundance of *Clostridium*. Baumann-Dudenhoeffer et al[27] studied pre- and postnatal factors affecting microbiome development in 60 healthy twins from birth to 8 months. Alpha diversity increased with time and maternal fruit/vegetable intake and reduced with maternal antibiotics. *Bifidobacteriaceae* enrichment was found in infants predominantly breastfed or fed formulas containing galacto-oligosaccharide (GOS). Soya formula was positively associated with *Lachnospiraceae* and pathways suggesting a short chain fatty acid rich environment in all 6 soya-fed infants in the study, potentially linked to inflammation, allergies, and hepatic steatosis. Flaherman et al[28] performed an RCT in 164 exclusively breastfed newborns with significant weight loss. Infants were randomised to receive 10 ml of infant formula to supplement feeds until the onset of copious maternal milk production or to exclusive breastfeeding. 15 infants in the study had their microbiome investigated. The use of early limited formula did not decrease *Lactobacillus* or increase *Clostridia* as described in exclusively formula-fed infants. Díaz et al[29] investigated the natural history of the microbiota following exclusion diet for non-IgE mediated cow's milk protein allergy (CMPA) compared to healthy controls. Infants on any exclusion diet had significantly lower *Bacteroides* and increased Clostridiales. Three infants on rice-based formula did not develop tolerance to cow's milk protein by the end of a six months exclusion. These patients had a distinct colonisation pattern with low abundance of *Bifidobacteria*. This offers an interesting insight into microbiome changes in infants receiving specialised formulas, whose use has increased in recent times.

Vatanen et al[30] used the DIABIMMUNE study to investigate the development of the microbiome in children at risk of autoimmune diseases in Finland, Estonia and Russian Karelia. They found that early growth, household location and antibiotic courses during pregnancy were associated with early gut microbial composition.

Iszatt et al[31] investigated the common environmental toxicants that pass to breastmilk and the effects of these on infant gut microbiome in 267 mother/infant pairs in Norway. They found that relatively high (>80th percentile) exposure to toxicants led to lower abundance of *Lactobacillus*. Specifically, they found that polybrominated flame retardant 28 and the surfactant perfluorooctanesulfonic acid were associated with reduced microbial diversity. They concluded that environmental toxicant exposure could significantly influence the gut microbiome during the first month of life.

Su et al[32] recruited 20 infants whose mothers had gestational diabetes and 14 controls. They found significantly lower alpha diversity in infants of mothers with gestational diabetes alongside increased Proteobacteria and Actinobacteria and a significant decrease in Bacteroidetes.

Finally, Gabriel et al[33] explored the significance of maternal vaginal bacteria on infant microbiome development in 79 maternal infant pairs in Poland. They found correlation between maternal vaginal and infant stool colonisation when analysed by culture-dependent methods. Maternal *Streptococcal* colonisation influenced the intestinal colonisation of *Staphylococci*, *Clostridium difficile* and *Candida*. Vaginal *Lactobacilli* influenced gut colonisation by reducing *C. difficile* counts.

ATOPIC DISEASES

Numerous studies implicate microbial factors in the pathogenesis of allergy and asthma, particularly in westernised societies. We found four studies in this broad area.

To investigate the role of the microbiome in the development of food allergies Feehley et al[34] colonised germ-free mice with stool from infants with and without CMPA. Mice colonised from healthy infants were protected against an anaphylactic response to βlactoglobulin (BLG), the cow's milk allergen. In this mouse model anaphylaxis was evidenced by a drop in core body temperature and production of BLG-specific IgE and IgG. By correlating ileal bacteria with gene regulation in the ileal epithelium of mice, *Anaerostipes caccae* was identified as protective against an allergic response to food. This suggests one possible mechanism for microbial colonisation influencing the development of allergic reactions to food. These results are complimented in the study by Kourosh et al[35] who characterised the microbiome of 22 food allergic American infants against non-allergic siblings and controls. They found food allergic subjects had enrichment of *Oscillobacter valarcigenes*, *Lachnoclostridium boltae* and *Faecalibacterium*. *Clostridia* colonisation separated allergic and non-allergic subjects, with differing species in this class seen in the two groups. Fozlollahi et al[36] found contrasting results when investigating the gut microbiome of 141 infants with egg allergy against healthy controls. They found an increase in alpha diversity in egg allergy. Three bacterial families were significantly different in allergic patients: *Lachnospiraceae* and *Streptococcaceae* were enriched and *Leuconostocaceae* were diminished.

Finally, Liwen et al[37] looked to characterise the microbiome of children in China with wheezing diseases. They analysed the microbiome of 90 infants diagnosed with bronchiolitis or asthma against healthy controls. They showed that there was a statistically significant decrease in *Bifidobacterium* in children with bronchiolitis and asthma compared to healthy controls. There was a significant negative correlation between *Bifidobacterium* and levels of IgE, Th12 cytokine IL-17A and Th2 cytokine IL-4, and a positive correlation with Th1 cytokines IFN-gamma. This might propose a mechanism for the protective effects of breastfeeding against asthma as breastfed infants are shown to have increased *Bifidobacterium*.

OTHER AREAS OF INTEREST

In addition to the areas of paediatric microbiome research summarised to date, our search strategy identified several areas with single or small numbers of papers, demonstrating the increasing interest in, and understanding of, the role of the microbiome in childhood health and disease.

Two papers focus on the development of the microbiome over time during childhood. Kim et al[38] described the diversity of the gastrointestinal microbiota in Korea in healthy adolescents and adults and against the reference population in the Human Microbiome Project. They showed differences in the composition, function and diversity in the adolescent compared to adult cohorts, leading them to conclude that the microbiome had not yet reached

adult maturity. Stewart et al[39], using the cohort from the TEDDY study, elucidated the evolution of the microbiome in 903 children from 3 to 46 months of age using amplicon and metagenomic sequencing. They classified the microbiome into three phases: developmental from 3 to 14 months; transitional from 15 to 30 months; and stable after 31 months. Breastfeeding in this study positively correlated with *Bifidobacterium* and cessation was associated with faster maturation of the microbiome. *Bacteroides* were more prominent during the developmental phase in children delivered vaginally. These two papers support a dynamic microbiome throughout childhood, modified by factors, such as mode of delivery and diet.

In cystic fibrosis (CF), Vernocchi et al[40] reported an innovative study using combined metagenomics and metabolomics from 31 CF patients to describe the microbiome against healthy controls. They described an enterophenotype specific to the underlying disease, unaffected by age, and with microbial diversity most similar to younger healthy controls, suggesting persistence of an immature microbiome into later childhood in CF. They eloquently delineated that the observed dysbiosis was independent of the degree of pancreatic insufficiency and only influenced in limited fashion by antibiotic exposure. This led to the novel hypothesis that deranged microbial colonisation in children with CF may be a direct effect of impairment of cystic fibrosis transmembrane conductance regulator function within the gut mucosa. De Frietas et al[41] used fluorescent *in situ* hybridisation to describe the microbiome of 19 children with CF and 17 healthy controls and correlated it to inflammation using faecal calprotectin. CF patients had higher levels of calprotectin. *Bacteroides*, Firmicutes, *Eubacterium rectale* and *F. prausnitzii* were all significantly decreased in CF patients. *C. difficile*, *E. coli* and *Pseudomanas aeruginosa* were all increased. In common with the work of Vernocchi, they did not demonstrate that dysbiosis was influenced by antibiotic use.

Another emerging area of study is the development of microbiome-altering diets, and we identified studies on the ketogenic diet for refractory epilepsy and the phenylalanine-restricted diet for children with phenylketonuria (PKU). In the first, Zhang et al[42] described the microbiome in a population of 20 children treated by ketogenic diet and showed that alpha diversity was reduced six months after initiation. In the subset of patients whose epilepsy did not respond, Clostridiales, *Ruminococcaceae*, *Rikenellaceae*, *Lachnospiraceae*, and *Alistipes* were enriched, suggesting that the microbiome may interact with the ketogenic diet and influence its efficacy. In the second study in this area, Verduci et al[43] studied the microbiome of PKU patients on a phenylalanine-restricted diet compared with a population of children with mild hyperphenylalanaemia on an unrestricted diet. Phenylalanine restriction was associated with lower microbial diversity and faecal butyrate levels, with depletion of butryrate-producing *Faecalibacterium* and *Roseburia*. The authors suggested that the high carbohydrate content of the diet may contribute to these findings.

Moving to otolaryngology and infection, Johnston et al[44] investigated the longstanding hypothesis that the adenoid microbiota may act as a reservoir of bacteria leading to otitis media by comparing the microbiota of the adenoids, tonsils, and middle ears of children undergoing adenotonsillectomy and grommet insertion for otitis media with effusion. Only one of the ten patients evaluated had significant correlation between the middle ear and adenoids, suggesting that the pathogen reservoir hypothesis may be erroneous. Separately, in a population of 170 children in Botswana, 16s rRNA PCR of nasopharyngeal samples was used by Kelly et al[45] to identify that 56% of children were colonised by *Streptococcus pneumoniae*. In addition to clinical parameters, such as older age, lack of electricity in the home, and the use of wood as a cooking fuel, colonisation was also more prevalent in children with a relative abundance of *Corynebacterium* and *Staphylococcus*.

CONCLUSIONS

This review has demonstrated the quality and breadth of microbiome research within paediatrics, with high impact papers describing microbial colonisation and identifying potential mechanisms underpinning various chronic diseases with potentially lifelong reach. Children offer a window into the initiation of disease, relatively free of comorbidities or therapy/lifestyle confounders, which is increasingly being used by clinical researchers to offer new insights into pathogenesis with potentially paradigm-shifting impact. Future studies will likely transform this insight into novel microbial therapeutics to prevent and influence disease development to the benefit of all of human health.

Conflict of Interest

RH has received consultancy fees and conference travel support from Nutricia and 4D Pharma. The other authors declare that they have no conflict of interest.

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