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REVIEW ARTICLE

Paediatricians play a key role in preventing early harmful events that could permanently influence the development of the gut microbiota in childhood

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ABSTRACT

Aim: The development of the gut microbiota occurs primarily during infancy, and growing evidence has emphasised its positive role and implications for human health. The aim of this review was to provide essential knowledge about the gut microbiota and to describe and highlight the importance of the factors that influence the gut microbiota in early life and their potential harmful effects later in life.

Methods: The European Paediatric Association, the Union of the National European Paediatric Societies and Associations, convened a panel of independent European experts to summarise the research on microbiota for general paediatricians. They used PubMed and the Cochrane Library to identify studies published in English up to June 2018.

Results: A number of clinical conditions can disrupt the development of a stable gut microbiota. Changes in the microbiome have been documented in many chronic diseases, mainly immune-mediated gastrointestinal and liver diseases, and distinct patterns have been associated with each specific disease. The gut microbiota can be positively modulated with probiotics, prebiotics, synbiotics, paraprobiotics and postbiotics.

Conclusion: Paediatricians can play a key role in preventing harmful events that could permanently influence the composition and/or function of the gut microbiota. Various treatment strategies can be used.

INTRODUCTION

The microbial communities hosted by the human gut have been forged over millions of years of co-evolution with humans, to achieve a symbiotic relationship leading to physiological homoeostasis. The gut microbiota has become a new, fascinating and promising area of research, which enables us to understand the development of gut functions and some health disorders and diseases, as well as their treatment or prevention.

The development of the gut microbiota occurs primarily during infancy. Evidence regarding the implications of the gut microbiota in children is increasing, and new insights have been reported about the development of the microbiome during early life. For example, advances in genome sequencing technology and metagenomic analysis are increasing our broader understanding of the gut microbiota and highlighting differences between healthy and diseased states. Healthcare professionals involved in paediatric care may find it difficult to interpret the complex data published in specialised literature. However, this information is of considerable importance in paediatric practice. Different definitions have also caused confusion. These include the interchangeable use of the basic terms microbiome and

Key notes

- The aim of this review was to describe what is already known about the gut microbiota, by focusing on the factors that influence its early development and potential harmful effects later in life.
- Our review showed that changes in the microbiome have been documented in many chronic diseases, mainly immune-mediated gastrointestinal and liver diseases.
- Paediatricians can positively modulate the gut microbiota by using probiotics, prebiotics, synbiotics, paraprobiotics and postbiotics.

microbiota by the medical community and the general public when they are talking about the local mini-ecosystem of a collection of microorganisms in the gut.

METHODS

The European Paediatric Association, the Union of the National European Paediatric Societies and Associations, convened a panel of eight independent European experts from five countries to outline the essential elements of the current knowledge on the gut microbiota that may be useful for general paediatricians in their practice. The panel was chosen based on the experts' scientific profiles and publication history, and all members were active participants in the work and activities of the association. The panel held their first meeting with regard to this review at the 8th Europaediatrics Congress in Bucharest in June 2017, where they discussed relevant issues about the definition and function of the gut microbiota. They decided that a particular focus of this review would be to highlight the factors that influence the gut microbiota in early life, as well as their potential harmful effects in later life, for the benefit of general paediatricians. Each panel member was responsible for reviewing the literature on a given topic, according to their specific expertise. They searched for papers published in English up to June 2018 by using PubMed and the Cochrane Library. The members then summarised the relevant findings on their given topic, and the panel discussed the findings discussed in a series of meetings until they reached a final consensus.

RESULTS

A microbiological approach to understanding the gut microbiota

Previously called the gut microflora, the microbial communities are composed of approximately 10^{14} bacteria, which is approximately 10 times the number of cells in the human body (1). The term gut microbiota refers to the organisms that comprise the microbial community, while the term microbiome refers to the collective genomes of the microbes, including bacteria, bacteriophages, fungi, protozoa and viruses that live inside and on the human body. The gut microbiota may be considered a human organ that can be transplanted, and it has its own functions, such as modulating the expression of genes involved in mucosal barrier fortification, angiogenesis and postnatal intestinal maturation of several gut-associated systems (2).

The gut microbiota comprises more than 2000 microbial species. Its diversity has been revealed by the application of metagenomics: 16S ribosomal ribonucleic acid gene or deoxyribonucleic acid (2). *Firmicutes* and *Bacteroidetes* are the two dominant bacterial phyla in most individuals. Other phyla include *Proteobacteria, Actinobacteria*, *Fusobacteria* and *Verrucomicrobia* (2). Groups of bacterial families have been classified into enterotypes on the basis of their functions. The term enterotype and its definition remain debated. For example, the classification may be based on the metabolism of dietary components and the ability to metabolise drugs. The aim of this classification is to help us to understand the role of the gut microbiota in health and disease. Ageing is associated with changes in the diversity of noncultured species that current laboratory culturing techniques are unable to grow in the laboratory. These are a greater proportion of *Bacteroides*, a distinct abundance of *Clostridium* clusters, an increased enterobacteria population and a lower number of bifidobacteria. The taxonomic alterations may be due to changes in diets, such as less fibre, and/or, the increased use of antibiotics with advancing age (3).

There is no definition of a normal microbiota, since the bacterial species vary in different groups of individuals. The vast majority of microbial species give rise to symbiotic host–bacterial interactions that are fundamental for human health. Disrupting the development of a stable gut microbiota, which is known as dysbiosis, may be associated with several clinical conditions. These include nosocomial infections, necrotising enterocolitis in premature infants, inflammatory bowel disease, obesity, autoimmune diseases, allergies or even functional bowel disorders or behavioural problems.

Factors influencing neonatal intestinal colonisation

Foetal colonisation and prematurity

The sterility of the gut of the foetus *in utero* has been challenged by studies that have identified bacteria, bacterial deoxyribonucleic acid or bacterial products in the meconium, amniotic fluid and placenta. These indicate the initiation of microbial colonisation from the mother to offspring (4,5). Therefore, during developmental phases, the foetus could encounter bacteria *in utero* that might contribute to establishing the microbiota before delivery. This prenatal bacterial colonisation of the foetal gut might be a source of microbial stimulation, providing a primary signal for the maturation of a balanced postnatal innate and adaptive immune system. However, studies stating the existence of this *in utero* microbiota remain controversial (3,4). Importantly it has been shown that meconium with low bacterial diversity has been associated with a more frequent onset of sepsis in very low birth weight babies (6).

The first and most important phase of normal colonisation occurs when the newborn foetus passes through the birth canal and ingests maternal vaginal and faecal microorganisms. These bacteria proliferate further when oral feeding is initiated. After 48 hours, the number of bacteria is already as high as around10^4–10^6 colony-forming units per millilitre of intestinal content. However, many factors can influence this process and they may potentially impair the establishment of what is known as symbiosis (7) (Fig. 1).

The pattern of bacterial colonisation in preterm infants differs from the pattern observed in the healthy gut of full-term infants during the neonatal period (7). This abnormal colonisation, which is mostly due to the routine use of sterile formula and antibiotics in neonatal intensive care units, could play a central role in feeding intolerance. It

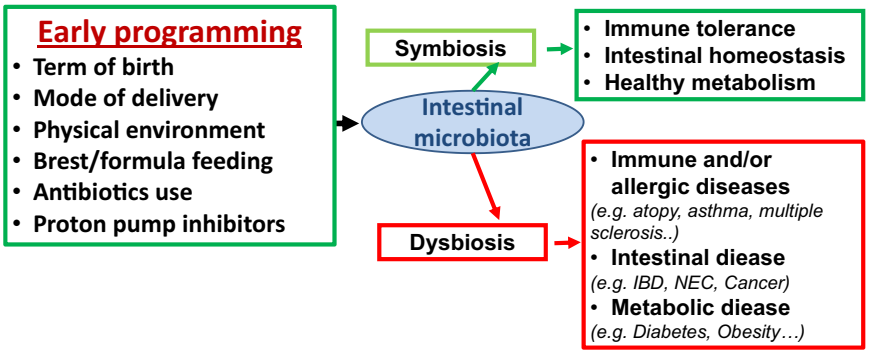

Figure 1 Role of neonatal bacterial colonization in establishing symbiosis.

could also be indicated in the development of necrotising enterocolitis, which is a severe disease primarily that affects premature infants and often leads to death or short bowel syndrome, which requires an extensive bowel resection (6).

Mode of delivery

The microbiota of vaginally delivered infants mirrors the vaginal and gut microbiota of the mother. Infants delivered by Caesarean section have reduced bacterial biodiversity, and colonisation by *Bifidobacteria* can be delayed by up to six months, in contrast to vaginally delivered infants (7,8). Infants delivered by Caesarean section exhibit bacterial communities composed of prominent genera, such as *Lactobacillus*, *Prevotella*, *Escherichia*, *Bacteroides* and *Bifidobacterium*. After a Caesarean section, the gut microbiota is characterised by a reduced number of *Bifidobacteria* species. Although vaginally delivered neonates exhibit individual microbial profiles, these are characterised by predominant groups, such as *Bifidobacterium longum* and *Bifidobacterium catenulatum*. Dominguez-Bello et al. used multiplex 16S ribosomal ribonucleic acid gene pyrosequencing to characterise the bacterial communities of mothers and their neonates. Interestingly, they reported that vaginally delivered infants acquired bacterial communities that resembled their own mothers' vaginal microbiota and that these were dominated by *Lactobacillus*, *Prevotella* or *Sneathia* spp. In contrast, infants delivered by Caesarean section harboured bacterial communities similar to those found on the skin surface and these were dominated by *Staphylococcus*, *Corynebacterium* and *Propionibacterium* spp. (8).

Influence of feeding

The mode of oral feeding may influence the composition of the gut microbiota in infants. Breastfeeding has been associated with higher diversity, as assessed using the Shannon index (9). Human milk contains beneficial factors for the gut microbiota, such as oligosaccharides (10). Oligosaccharides function as prebiotics, by stimulating the growth of *Bifidobacterium* and *Lactobacillus* species, thereby selectively altering the microbial composition of the intestine (10). It is likely that evolutionary selective pressure has equipped *Bifidobacterium longum* subsp. *infantis* with multiple enzymes to deconstruct human milk glycans. As a result, this subspecies is able to outcompete other *Bifidobacteria* as well as other commensals and pathogens in the gut lumen of healthy breastfed infants (10). In formula-fed infants, *Enterococci*, *Bacteroides* and *Clostridia* predominate. When breastfed infants are one month of age, there is a direct association between the levels of secretory immunoglobulin A in intestinal secretions and the number of *Bifidobacteria* in the gut. Furthermore, the level of the proinflammatory cytokine interleukin-6 in intestinal secretions is inversely related to the number of *Bifidobacterium fragilis* organisms in the gut at one month of age. It has been suggested that human milk oligosaccharides do not just stimulate *Bifidobacterium longum* subsp. *infantis* proliferation, they also activate important genes involved in the proinflammatory and anti-inflammatory balance in the intestinal mucosa (11). These observations provide additional evidence of the beneficial effects of breastfeeding for the newborn infant (Fig. 2). In addition to human milk oligosaccharides, human milk contains other glycans with antimicrobial and prebiotic activity that are thought to have beneficial effects on the infant (12). On the other hand, there is accumulating evidence that human milk is not sterile, but contains maternally derived bacterial molecular motifs that are thought to influence the development of the newborn infant's immune system (13). This mechanism, which has been called bacterial imprinting, requires further research (13). However, comparative studies with formula-fed infants have not carefully documented the effects of formula feeding on the gut microbiota or health-promoting bacteria. There is growing evidence that the microbiota does not reach its adult composition until two to three years of age (14). Finally, host defences can be improved by breastfeeding, which helps the immature intestinal mucosal immune system to develop and respond appropriately to highly variable bacterial colonisation and food antigen loads. Later in life, the type of food consumed influences the profile of the gut microbiota (15) and short-chain fatty acids play a central role (16). Short-chain fatty acids are organic fatty acids that are produced in the distal gut by the bacterial fermentation of macro-fibrous material that

escapes digestion in the upper gastrointestinal tract and enters the colon. They are central to the physiology and metabolism of the colon. Resident bacteria can also metabolise dietary carcinogens, synthesise vitamins and assist in the absorption of various molecules. Research has shown that 90-95% the short-chain fatty acids present in the colon are made up of acetate (60%), propionate (25%) and butyrate (15%). Butyrate is a major energy source for the colonic epithelium. Short-chain fatty acids have been associated with improved metabolic functions in individuals with type 2 diabetes mellitus, as they help to control blood glucose levels, insulin resistance and glucagon-like peptide-1 secretion (16).

Gut microbiota predators

The use of broad-spectrum antibiotics significantly reduces the relative abundance of *Bacteroidetes* and increases the abundance of *Firmicutes* at the same time. A reduction in microbial diversity is often observed in infants under one year of age who have received oral antibiotics. Complete recovery of the initial bacterial composition is not always achieved. The response depends on the type of antibiotics, the duration of administration and the baseline microbiome. Studies have reported that antibiotics that target specific pathogenic infections and diseases may alter the gut microbiota ecology, and interactions with the host metabolism, to a much greater degree than previously assumed (17).

The prolonged use of antibiotics, which is common in preterm infants, profoundly decreases microbial diversity and promotes the growth of predominant pathogens, such as *Clostridium*, *Klebsiella* and *Veillonella*, which have been associated with neonatal sepsis. It has been suggested that there may be healthy microbiota present in extremely premature neonates that may ameliorate the risk of sepsis (6). More research is needed to determine whether different antibiotics, probiotics or other novel therapies could re-establish a healthy microbiome in neonates. It has also been reported that when low-dose antibiotic exposure disrupted the microbiota during maturation, this altered the host metabolism and adiposity in mice (18). A study that gave mice low-dose penicillin immediately after birth demonstrated that metabolic alterations and changes in the ileal expression of genes were involved in immunity (18). Administering low-dose penicillin sufficiently perturbs the microbiota to modify body composition, even when these drugs are limited to early life. This indicates that microbiota interactions in infancy may be critical determinants of long-term host metabolic effects.

Other xenobiotics, such as proton pump inhibitors, may alter the gut microbiota. Meta-analyses have shown that the use of proton pump inhibitors potentially increased the risk of enteric infections caused by *Clostridium difficile*. They have also led to small intestinal bacterial overgrowth, spontaneous bacterial peritonitis, community-acquired pneumonia, hepatic encephalopathy and adverse outcomes of inflammatory bowel disease (19).

The role the gut microbiota plays in gut maturation

A study by Hooper et al, published in 2001, reported that a single bacterial species, *Bacteroides thetaiotaomicron*, which is a prominent component of normal mouse and human intestinal microbiomes, modulated the expression of genes involved in several important intestinal functions. These included nutrient absorption, mucosal barrier fortification, xenobiotic metabolism, angiogenesis and postnatal intestinal maturation (20). Another study that covered gastrointestinal motility, found that bacterial metabolites, such as short-chain fatty acids and deconjugated bile salts, generated potent motor responses (21). Colonised mice have been shown to have a faster intestinal transit time than germ-free mice (20). Collectively, the gut microbiota influences tissue regeneration, the permeability of the epithelium, the vascularisation of the gut and tissue homoeostasis.

Role of the gut microbiota in the development of the gut immune system

The intestine is an important immune organ that harbours approximately 60% of the total immunoglobulins and more than 10^6 lymphocytes per gram of tissue. The largest pool of

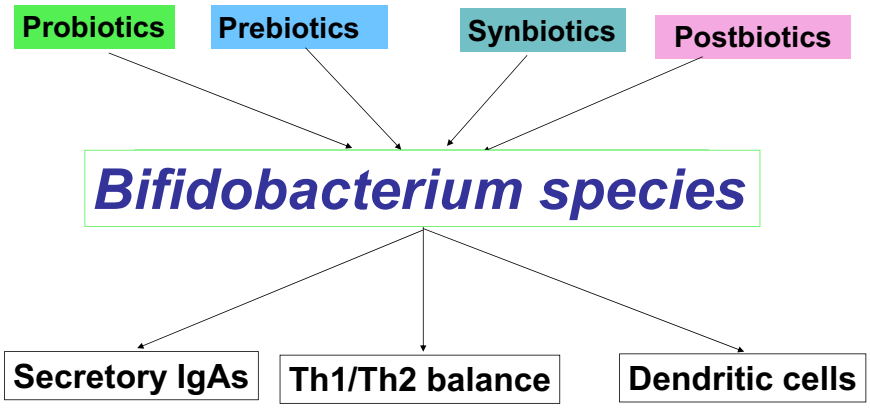

Figure 2 The central role played by Bifidobacterium species in the development of the gut associated immune system.

immune-competent cells in the body is housed in the intestinal mucosa. The number of T lymphocytes and plasmocytes within the intestinal lamina propria increases markedly in response to intestinal colonisation. Although immunoglobulin A producing cells are virtually absent in germ-free mice, high levels are detectable in the mucosa when bacterial colonisation occurs (22). The gut microbiota exerts positive stimulatory effects on the intestinal innate and adaptive immune systems, by modulating the development of the intestinal mucous layer and lymphoid structures, immune-cell differentiation and the production of immune mediators (23,24). The innate immune system must discriminate between pathogens and the harmless commensal bacteria of the gut microbiota. Pathogen recognition receptors, such as Toll-like receptors and nucleotide-binding oligomerisation domain receptors, enable us to recognise a restricted number of bacterial motifs. These can be either microbe-associated molecular patterns or, in the case of pathogens, pathogen-associated molecular patterns (24). Both types of pathogen recognition receptors are naturally expressed by the intestinal epithelial and antigen-presenting cells, such as dendritic cells or macrophages, and this enables them to sense any bacterial motifs easily. The intestinal epithelial barrier is protected by a highly viscous microfilm to avoid permanent and unwanted stimulation of the innate immune system. This prevents close contact between the commensal bacteria and intestinal epithelial cells.

The intestinal mucosal barrier function can be defined as the capacity of the intestine to host commensal bacteria and molecules, while preserving the ability to absorb nutrients and prevent the invasion of host tissues by resident bacteria. The dense communities of bacteria in the intestine are separated from body tissues by a monolayer of intestinal epithelial cells. The assembly of the multiple components of the intestinal barrier is initiated during foetal development and continues during early postnatal life. This means that the intestinal barrier is not completely developed soon after birth, particularly in preterm infants. The secretion of mucus-forming mucins, secretory immunoglobulin A and antimicrobial peptides reinforces the mucosal barrier on the extra-epithelial side, while a variety of immune cells contribute to mucosal defence on the inner side. Thus, the mucosal barrier is physical, biochemical and immune in nature. In addition, the microbiota may be viewed as part of this system because of the mutual influence of the host and the luminal microorganisms.

Altered mucosal barrier function, accompanied by increased permeability and/or bacterial translocation, has been linked to a variety of conditions. These have included metabolic disorders, such as type 2 diabetes mellitus, insulin resistance, obesity and inflammatory bowel diseases (25). Genetic and environmental factors may converge to evoke defective functioning of the barrier, which, in turn, may lead to overt inflammation of the intestine as a result of an exacerbated immune reaction towards the microbiota. Inflammatory bowel diseases may be both precipitated and treated by either stimulation or downregulation of the different elements of the mucosal barrier, and the outcome depends on the timing, the types of cells affected and other factors. Fermentation products of commensal bacteria have been shown to enhance the intestinal barrier's function, by facilitating the assembly of tight junctions through the activation of adenosine monophosphate-activated protein kinases (26). On the other hand, removing the entire detectable commensal gut microbiota by using a four-week course of four orally administered antibiotics – vancomycin, neomycin, metronidazole and ampicillin – led to more severe intestinal mucosal injury in a mouse colitis model induced by dextran sulphate sodium (27). Early treatments with broad-spectrum antibiotics have been shown to alter the gastrointestinal tract's gene expression profile and intestinal barrier development (28). This finding underlines the importance of normal bacterial colonisation in the development and maintenance of the intestinal barrier. Antibiotic therapy between birth and five years of age might increase the risk of Crohn disease by disrupting the pattern of gut colonisation (29). A meta-analysis confirmed that antibiotic use was associated with an increased risk of new-onset Crohn disease, but not of ulcerative colitis (30).

In summary, the gut microbiota protects against pathogens, influences the development of the intestinal barrier and its functions and plays many roles in the development of the gut immune system. It acts by competing for nutrients and receptors, by producing antimicrobial compounds and by stimulating a multiple-cell signalling process that can limit the release of virulence factors.

Role of the gut microbiota in health and disease

As emphasised above, microorganisms colonise the human gut from birth, and even before that, and stimulate the development of the local and systemic immune systems. In addition, the newly developed immune system shapes the gut flora, which means that it is unique for every individual. An imbalance or alteration in the composition and/or function of the microbiota, which is usually called dysbiosis, has been found to be associated with many chronic diseases (31). However, in this relationship, it is almost impossible to delineate the causes from the consequences, as few studies have shown that changes in the microbiota precede inflammation (31).

Inflammatory bowel disease

The current hypothesis of the aetiology of inflammatory bowel disease suggests that the inflammation is a consequence of an unrestrained or aberrant immune response to the gut flora, which is shaped by different environmental factors in a genetically predisposed individual (32).

The most consistent changes that have been described have been a reduction in the diversity of the gut microbiota, increased abundance of *Bacteroidetes* and *Proteobacteria* and the loss of *Firmicutes* (32). Furthermore, the loss of certain specific beneficial microbes, such as *Faecalibacterium prausnitzii* and members of *Clostridium* clusters XIVa and IV, has previously been described (33). The importance of these specific microorganisms has been

further demonstrated by their ability to inhibit inflammation and affect the differentiation of regulatory T cells. More precisely, *Faecalibacterium prausnitzii* has the ability to stimulate the production of interleukin-10 and inhibit proinflammatory cytokines such as interleukin-12 and interferon-gamma. Other mechanisms could also be involved, such as decreased production of short-chain fatty acids, which then affects the differentiation and expansion of regulatory T cells and the growth of epithelial cells.

Another well-described feature of patients with inflammatory bowel diseases is altered intestinal barrier function, and this has mainly been increased permeability and decreased mucus production. Both of these factors can be influenced by the microbiota, but they can also give bacteria easier access to the mucosa, allowing them get closer to immunocompetent cells. Patients with inflammatory bowel diseases exhibit increased colonisation by bacteria that are able to adhere to the intestinal epithelium, causing altered permeability of the intestine (34). This adherence can be further promoted by the increased number of mucolytic bacteria, such *Ruminococcus gnavus* and *Ruminococcus torques* (35). In addition, the number of sulphate-reducing bacteria, such as *Desulfovibrio*, is increased in patients with inflammatory bowel disease. This has been shown to result in the production of hydrogen sulphate, which damages intestinal epithelial cells and induces mucosal inflammation (36).

Functional gastrointestinal disorders

The pathogenesis of functional gastrointestinal disorders has not yet been fully explained, but the proposed mechanisms include mild gastrointestinal inflammation, visceral hypersensitivity, an altered brain–gut axis and altered gut microflora (37).

The most notable changes in microbial intestinal colonisation during the first weeks and months of life have been described in infants with infant colic (38). These infants were reported to have decreased faecal-bacterial diversity, increased gram-negative bacterial colonisation and a lack of *Actinobacteria* and *Firmicutes*, which appears to have a protective effect. More specifically, infants with colic have been shown to have more *Proteobacteria* and less *Bifidobacteria* and *Lactobacillus* (39). Although a cause versus effect phenomenon has not been fully described, there is evidence that changes in the gut microbiota precede the development of infantile colic (40).

Similar changes in the microbiome have been reported in older children with functional gastrointestinal disorders. One meta-analysis, published in 2017, identified downregulated colonisation of *Lactobacillus*, *Bifidobacterium* and *Faecalibacterium prausnitzii* in patients with irritable bowel syndrome, particularly in irritable bowel syndrome where diarrhoea predominated (41). Furthermore, a greater proportion of the *Proteobacteria* phylum and of genera, such as *Dorea, Haemophilus*, *Ruminococcus* and *Clostridium* species, were found in the same group of patients, (42). These changes might have altered or influenced visceral perception, gut motility, gut permeability and intestinal gas production, which can lead to functional gastrointestinal disorders where pain is the predominant complaint.

Allergies

The immune system of the gastrointestinal tract is in close proximity to many antigens that originate mainly from food and the gut microbiota, both of which can affect immune tolerance. The normal commensal microflora play an essential role in inflammatory homoeostasis and appropriate immune regulation and may therefore influence the development of allergic diseases. It has been suggested that alterations in the microbiota can disrupt mucosal immune tolerance, leading to allergic diseases, such as food allergies, atopic dermatitis and even asthma (43). The early microbiota of children who later developed allergies has been characterised by lower bacterial diversity, with predominant *Firmicutes*, higher counts of the *Bacteroidaceae*, increased numbers of the anaerobic *Bacteroides fragilis*, *Escherichia coli*, *Clostridium difficile*, *Bifidobacterium catenulatum*, *Bifidobacterium bifidum* and *Bifidobacterium longum*. In contrast and decreased numbers of *Bifidobacterium adolescentis*, *Bifidobacterium bifidum* and *Lactobacillus* have been reported (44). When the microbiota of children with allergies was assessed at the onset of allergic symptoms in one study, it showed a different pattern, with higher counts of *Bacteroides*, lower counts of *Akkermansia muciniphila, Faecalibacterium prausnitzii* and *Clostridium* and overall lower bacterial diversity (45).

The potential mechanisms underlying an increased risk of sensitisation and allergy development, detected as a consequence of dysbiosis in animal models, have been related to various alterations in mucosal regulatory T cells. Other reported effects were defects in the epithelial barrier function, as evidenced by increased mucosal permeability, diminished secretory immunoglobulin A production and excretion and altered dendritic and B-cell function (44).

Obesity and liver disease

Studies have shown that gut microbiota could also play an important role in the etiopathogenesis of obesity and other prevalent chronic liver diseases, such as nonalcoholic fatty liver disease and nonalcoholic steatohepatitis.

Nonalcoholic fatty liver disease has become one of the most frequent causes of liver disease and represents a spectrum of pathologies, varying from steatosis to nonalcoholic steatohepatitis, with or without cirrhosis, and possible evolution to hepatocellular carcinoma. Nonalcoholic fatty liver disease is a multifactorial disease that is affected by genetic, metabolic, dietary and environmental factors. The most commonly proposed theory is the multiple hit hypothesis, which also involves changes to the gut microbiota (46).

The gut microbiota plays an important role in obesity, and this is primarily based on its influence on energy balance. Dysbiosis affects short-chain fatty acid production and metabolism and adipocyte lipid deposition, with a decrease in mitochondrial fatty acid oxidation. Human studies have reported that the balance between

Bacteroidetes and *Firmicutes* has been related to obesity. Lean subjects have more *Bacteroidetes* in their gut microbiota, and diet that restricted fats and carbohydrates was shown to increase the ratio in favour of *Bacteroidetes* (47).

With regard to chronic liver disease, the proposed mechanisms for the negative effects of dysbiosis include small intestine bacterial overgrowth, altered release of inflammatory cytokines, alteration of the intestinal barrier, choline metabolism, endogenous ethanol production, regulation of hepatic toll-like receptors expression in patients with nonalcoholic fatty liver disease or nonalcoholic steatohepatitis and an alteration in bile acid metabolism (48).

Furthermore, there is evidence that gut dysbiosis promotes the progression of nonalcoholic steatohepatitis to cirrhosis and hepatocellular carcinoma via an increase in tumour necrosis factor alpha and interleukin-8, the activation of toll-like receptor-4 and toll-like receptor-9 and the production of interleukin-1beta in Kupffer cells, favouring lipid accumulation, hepatocyte death, steatosis, inflammation and fibrosis (49).

There have been many animal studies that have evaluated the gut microbiota differences associated with nonalcoholic fatty liver disease or nonalcoholic steatohepatitis, but few studies have been performed in humans and they have produced inconsistent results. Patients with nonalcoholic steatohepatitis, including children, have been reported to have lower levels of *Bacteroidetes* than patients with liver steatosis or healthy individuals (50). *Firmicutes* have been found in higher levels in individuals with nonalcoholic fatty liver disease than in healthy subjects (51), but the results have not been consistent (52).

Modulation of the gut microbiota

The gut microbiota can be modulated to achieve health-promoting effects (53). The beneficial manipulation of the composition and metabolic footprint of the gut microbiota can be achieved by using probiotics. These can be defined as a preparation of, or a product containing, viable microorganisms in an adequate number to enable such dietary preparations to favourably modulate the gut microbiota (53,54). The ability to exert a beneficial modulation on the gut microbiota may be enhanced by combining probiotics with other ingredients (64), namely prebiotics, which are capable of favouring the growth and/or activity of microorganisms. Prebiotics appear to be poorly understood by the general public in this regard (55). It is important to correctly define, and understand, prebiotics and their potential when they are combined with probiotics. This information needs to be disseminated beyond the scientific community, so that regulatory agencies, the food industry and healthcare professionals can correctly describe them and suggest how they should be used. The combined use of prebiotics and probiotics may be described as synbiotic if the net health benefit is synergistic and scientifically validated (56). Finally, the terms paraprobiotic and postbiotic describe nonviable bacterial cells and soluble factors that are secreted as metabolic by-products by live bacteria. Such products, which could also be released after bacterial lysis, can provide additional physiological benefits to the host organism. That is why they have received increasing attention from scientific researchers and industry, due to their potential food and pharmaceutical applications (Table 1).

Probiotics

Probiotics have been defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (53,57). The term probiotics is used widely, but not always properly, in the scientific literature and by the industry. Their fundamental characteristics have been described extensively in the literature (53), including their microbial origin, their viability and their benefit to the health of the host (Table 2). The microbial origin of a probiotic product must be guaranteed by identifying a taxonomically defined microbe or combination of microbes. A probiotic must therefore be properly identified by strain-genotypically and phenotypically characterised. An essential characteristic of a probiotic is its viability (57), as it must be a live microorganism that is able to survive the acidity of the stomach in order to reach and colonise the intestinal tract. Moreover, a probiotic must be guaranteed to remain viable and stable throughout the technical procedures, during its production, use and storage.

A consensus statement was issued by the International Scientific Association for Probiotics and Prebiotics in 2013 with regard to the possible benefits of probiotics to human health. The statement sought to further clarify the appropriate use and scope of the term probiotic and stated that probiotics should exert specific general benefits, which it defined as core benefits (58). These benefits include contributing to establishing and sustaining a healthy gut microbiota. They are expected to be obtained by creating a favourable intestinal environment through nonstrain-specific beneficial actions that are shared by most probiotics, which sustain a healthy digestive tract and immune

Table 1 Probiotics, prebiotics, synbiotics, paraprobiotics and postbiotics in clinical practice. Definitions

- Probiotics: Food or food supplements containing viable microrganisms, able to modify the microflora of their hosts, with potential beneficial outcomes on their health
- Prebiotics: Food or food supplements containing nondigestible components, able to selectively stimulate the activity and, or, growth of autochthonous bacteria
- Synbiotics: Products containing a combination of probiotics and prebiotics
- Paraprobiotics: Nonviable, inactivated microbial cells containing products that have shown dose-related beneficial effects in selected groups of patients
- Postbiotics: Products containing inactivated (nonviable) bacterial products or metabolic by-products from probiotic microorganisms, able to exert potentially beneficial biological activity on their hosts

Table 2 Attributes of probiotics

- Human origin
- Not a pathogen
- Resistant to technical procedures
- Resistant to gastric acidity
- Capable of adhering to intestinal epithelium
- Capable of colonising the intestinal tract
- Capable of producing antimicrobial substances
- Acts as immunomodulator
- Influences human metabolic activities

system. In fact, some effects of probiotics can be observed across taxonomic groups and are achieved through general mechanisms, such as the inhibition of pathogens and the production of beneficial metabolites. These effects should be distinguished from other benefits, such as neurological or endocrinological effects, which are strain specific.

An important aspect of probiotic activity is identifying the adequate amount that is able to confer health benefits on the host and a specific accepted definition of this is not currently available. Nevertheless, some regulatory approaches in Canada and Italy (59,60) have suggested that a probiotic product should contain at least $1^{\wedge}10^{9}$ colony-forming units per serving to be able to exert the claimed beneficial effects.

The 2013 Statement also describes the different categories of live microorganisms for human use, in order to distinguish what can and cannot be considered a probiotic, according to health claims (58). Products claiming to contain live and active cultures should not be considered probiotics, because the simple use of the terms live and active does not imply any probiotic activity. Foods or supplements that state they contain probiotics have no specific health claims, and their expected effects are those related to the core benefits, as demonstrated by well-conducted human studies. Products containing probiotics that make specific health claims are those that claim to have any beneficial health effects, according to documented evidence from well-designed observational studies. Products containing probiotics that claim they can prevent or treat a specific disease need to be backed up by appropriate trials to meet the regulatory standards for drugs.

Probiotics are commonly used in paediatric practice, and a summary of the indications and limitations is reported in Table 3. Their use includes preventing common and nosocomial infections, allergies and antibiotic-associated diarrhoea, treating acute gastroenteritis and functional abdominal pain disorders and preventing and treating infantile colic. Guidelines by Hojsak et al. on using probiotics in clinical practice for children were published in 2018 (53), and the study reported that they seemed to be safe in general, even when provided in high doses. The authors provided a detailed description of the correct conditions for their use, together with specific positive instructions (53) for the use of strictly defined strains for various clinical conditions. These conditions include preventing upper respiratory tract infections in children attending day care centres, nosocomial diarrhoea and antibiotic-associated diarrhoea and treating acute gastroenteritis and infantile colic in breastfed infants.

Prebiotics

The definition of prebiotics has undergone an important evolution over time. They were initially referred to as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria already residing in the colon (S61). Several studies have focused on the nondigestible oligosaccharides fructans, namely fructooligosaccharides and inulin, and galactans, namely galactooligosaccharides, and how they exert their effects through the enrichment of *Lactobacillus* and/or *Bifidobacterium* spp. (S62).

Prebiotics have been described as nondigestible compounds that confer a beneficial physiological effect on the host (S63). They do this by metabolising microorganisms in the gut, which then modulate the composition and/or activity of the gut microbiota. In 2017, the International Scientific Association for Probiotics and Prebiotics Consensus Statement proposed a new definition for prebiotics (S64). The document discussed the concept of selectivity with respect to fermentation by bacteria and suggested that prebiotics were defined as substrates that are selectively utilised by host microorganisms and confer a health benefit on the host (51) (Table 4). Incorporating the concept of selectivity in the definition is important, as it distinguishes between prebiotics and other substances. The term selective does not mean that only lactobacilli and bifidobacteria are affected by prebiotics. It means that a broader range of microorganisms, but not all, can be affected. Substances that can affect the composition of the microbiota, but are not selectively used by microorganisms, are not prebiotics.

The use of prebiotics in paediatric clinical practice is currently limited. Human milk oligosaccharides are a group of prebiotics that can influence a newborn infant's gastrointestinal health by favouring the development of a healthy gut microbiota through some metabolic and immunological activities. It has been demonstrated that an infant's consumption of human milk oligosaccharides increases the proportion of human milk oligosaccharide-consuming *Bifidobacteriaceae*, particularly *Bifidobacterium longum* subsp. *infantis* and *Bacteroidaceae* (S65). The mechanisms of action in the newborn infant's intestine include immune regulation and preventing the adhesion of pathogens to the intestinal epithelium, which protects the infant from infections (S66). Some compounds that are equivalent to human milk oligosaccharides or bovine milk oligosaccharides are obtained by enzymatic synthesis. It is still a matter of debate whether these are able to exert beneficial effects on human health by selectively stimulating the microbiota and thus acting as prebiotics. The existing literature does not provide definitive conclusions, but some human milk oligosaccharides may be considered candidate prebiotics. Studies have reported that prebiotics containing

Table 3 Use of probiotics in children

Preventing common infections	Preventing nosocomial infections	Preventing allergies	Preventing antibiotic associated diarrhoea (AAD)
• Children attending day care centres during winter months: if probiotics are considered for preventing upper respiratory tract infections, only lactobacillus rhamnosus (LGG) could be considered. However, evidence is limited and meta-analyses confirming its efficacy are lacking. • Preventing gastrointestinal infections in day care centres: the use of probiotics is not supported by convincing evidence.	• Preventing nosocomial diarrhoea: if the use of probiotic is considered, only LGG can be recommended (at least 109 CFU/day for the duration of their hospital stay). • Preventing nosocomial respiratory tract infections: insufficient evidence to recommend probiotics in these conditions.	• Preventing atopic diseases: based on the currently available evidence, probiotics cannot be recommended	• Preventing AAD: LGG or *S. boulardii* should be considered. • Preventing C difficile-associated diarrhoea: evidence indicates that *S. boulardii* can be considered *Notes:* • Other strains of probiotics, on their own or in combination, are currently not recommended. • No safety data are available on the use of probiotics for preventing AAD in severely ill children. Their use in these patients should undergo special evaluation.
Treating acute gastroenteritis (age)	**Treating functional abdominal pain disorders**	**Preventing and treating infantile colic**	**Safe probiotic use**
• LGG and *S. boulardii* may be considered as an adjunct to the oral rehydration therapy. (LGG should be administered for 5–7 days, at a dose of ≥1010 CFU/day, while *S. boulardii* should be administered for 5–7 days, at a dose of 250–750 mg/day) • No recommendation are currently available for the use of other strains or products containing single or multiple strains of probiotics *Note:* • Probiotic administration initiated early in the course of diarrhoea is recommended to maximise results. Spell out terms in yellow in full please.	• Recommendations for the use of probiotics in these conditions are limited by the scarcity of the available evidence and the lack of current guidelines.	• A probiotic treatment can be considered. However, L. reuteri DSM 17938 is the only strain that has proved to be effective treating infantile colic in breastfed infants. (It should be used at a dose of at least 108 CFU/day for 21–30 days). *Notes:* • Limited evidence on the use of L. reuteri DSM 17938 in preventing infantile colic precludes specific recommendations. • Insufficient evidence is currently available for the use of other strains of probiotics or products containing probiotic mixtures	• The use of probiotics in children seems to be safe in general, even when provided in high doses. • Probiotics should be used with caution in special situations, such as prematurity, immunocompromised patients, critically ill patients, central venous catheters, cardiac valvular disease and short-gut syndrome. • Some probiotic strains are not recommended for children (namely Enterococcus faecium SF68), due to the possible transfer of vanomycin-resistance genes. • *S. boulardii* has been effective in children with C. difficile infections. However due to the potential for infections spread, special caution is required in critically ill patients.

Table 4 Prebiotics selectively used by host microorganisms

- CLA, conjugated linoleic acid
- PUFA, polyunsaturated fatty acid
- FOS, fructooligosaccharides
- GOS, galactooligosaccharides
- MOS, mannanoligosaccharide
- XOS, xylooligosaccharide
- HMOs, human milk oligosaccharides
- Phenolics and phytochemicals
- Readily fermentable dietary fibres

immunoactive oligosaccharides could effectively prevent atopic dermatitis in low-atopy risk infants and that they could potentially be used to prevent adolescents becoming overweight. However, the clinical significance and efficacy of prebiotics and their possible widespread use in paediatric practice still needs to be clarified (S67-69).

Synbiotics

Synbiotics are commonly described as a combination of probiotics and prebiotics in functional food compounds. Functional food is a food that has been modified and claims to improve a person's health or well-being by providing benefits that extend beyond the traditional nutrients it contains. Examples of functional foods include bread, cereals and drinks that are fortified with vitamins or selected herbs. They can also contain nutraceuticals, which have physiological benefits or provide protection against chronic disease.

Studies have reported that their combined use has facilitated the survival of live microbial dietary supplements and their implantation in the gastrointestinal tract (S66–68). This mechanism has been reported to generate a

beneficial effect in the host organism, by the metabolic activation of a restricted type of bacteria, which is considered to be health promoting, and the selective stimulation of its growth (S69). It has been suggested that these combined conditions have improved the host's welfare (S70).

Single products containing an appropriate combination of probiotics and prebiotics have been reported to guarantee a greater effect than when they have been used separately. In fact, the synbiotic activity of foods containing a combination of prebiotics and probiotics is based on their elective action in two different areas of the gut. Probiotics are mainly active in the small and large intestine, while prebiotics are mainly active in the large intestine (S69). Synbiotics act in combination in two main ways: by improving the viability of probiotic microorganisms and by providing specific benefits for the host's health.

The rationale of using a synbiotic formulation of prebiotics is because they function as a selective medium, favouring the growth of certain probiotic strains, their fermentation and their intestinal passage. Furthermore, several studies have reported that prebiotics have positively influenced the ability of probiotic microorganisms to develop higher tolerance to particular situations caused by the presence of possibly challenging conditions. These include oxygenation and the pH and temperature of the intestines (S71). In brief, the main reason for using synbiotics is that the survival of probiotics in the digestive system is challenging in normal conditions and when an appropriate prebiotic is not present.

Therefore, using prebiotics to stimulate the effectiveness of probiotics appears to be a good way of inducing the beneficial modulation of the metabolic activity of probiotics in the intestine. At the same time, this preserves the intestinal biostructure, favours the development and maintenance of a beneficial microbiota and inhibits the growth of potential pathogens in the gut.

In general, the beneficial outcomes of synbiotics for the host's health have been related to significant increases in short-chain fatty acid levels, ketones, carbon disulphides and methyl acetates. In particular, the potential beneficial activity of synbiotics in clinical practice has been described in different clinical conditions (Table 5). The reported potential therapeutic properties of synbiotics include anticarcinogenic, anti-allergic and antibacterial effects (S67). A few studies, which need to be confirmed or validated, have also suggested that synbiotics could be used to prevent constipation, diarrhoea and osteoporosis and in treating brain diseases associated with altered hepatic function (S72).

Studies suggest that the synbiotic activity exerted by a combination of prebiotics and probiotics in functional food products is mainly due to their ability to modulate the host's immune system. This means that they can be used in clinical practice for selected conditions. It has been reported that healthcare professionals have used synbiotics in clinical practice before using antibiotics and surgery interventions and that their use may be related to cost-effectiveness and safety considerations. Finally, the availability of synbiotic-based commercial products is rapidly increasing, due to the large number of possible existing combinations of prebiotics and probiotics. This may offer increased therapeutic options in the near future (S72).

Table 5 Activity of synbiotics in humans

- Improving the viability of probiotics
- Expanding Lactobacillus and *Bifidobacterium* genus counts
- Sustaining immune system modulation abilities in hosting organisms
- Increasing hepatic functions in patients affected by cirrhotic dysfunctions
- Preventing bacterial translocation in individuals in restricted communities
- Preventing hospital-acquired infections in patients receiving surgery and/or postoperational procedures
- Reducing risk factors for colon cancer
- Providing preventive effects in selected clinical conditions (namely osteoporosis, allergic disorders, constipation and diarrhoea)

Paraprobiotics and postbiotics

In addition to the factors provided by the host organisms, further regulatory elements are able to support the maintenance and growth of the gut microbiota by favouring bacterial development, reproduction, protection from external insults and intercellular communication (S73). Data from the literature have emphasised that bacterial viability, which characterises probiotic activity, is not the exclusive factor involved in exerting health-promoting effects (S74). In this regard, the term paraprobiotics is used to identify nonviable, inactivated microbial cells that have shown dose-related beneficial effects for consumers. It has been suggested that paraprobiotics are safer than viable bacterial products for selected groups of patients, such as individuals with impaired immune systems, because they pose a reduced risk of infection, microbial translocation or potential inflammatory responses. Inactivated bacterial cells are typically obtained artificially, through chemical or physical methods such as heating, acid deactivation, freeze-drying, sonication and ultraviolet treatment. This means that they are able to modify the cell structure and/or the physiological functions of the bacteria while preserving the beneficial properties of their viable forms (S74).

The term postbiotics describes soluble factors that may be secreted by viable bacteria or by-products resulting from bacterial lysis (S73, S74) (Table 6). Several bacterial strains have shown the ability to express a wide range of soluble factors of different natures, including cell surface proteins, vitamins, enzymes, peptides, teichoic acids, plasmalogens and organic and short-chain fatty acids. These cell-free supernatant metabolites have been reported to possess antimicrobial, antioxidant and immunomodulatory properties. These can positively influence microbiotal homoeostasis as well as the metabolic and/or signalling pathways of the host organism. The active structure and mechanism of action that enable postbiotics to produce a beneficial effect in the context of physiological, immunological, neuro-

Table 6 Composition and function-based distinction of postbiotics

Composition element based	
• Lipids (butyrate, dimethylacetyl-derived plasmalogen, propionate)	• Organic acids (propionic acid, 3-phenyllactic acid)
• Carbohydrates (galactose-rich polysaccharides, teichoic acids)	• Various complex molecules (cell wall associated peptidoglycans, lipoteichoic acids)
• Proteins (lactocepin, p40 molecules)	
Physiological function based	
• Immunomodulation (*Lactobacillus* sp., *Bifidobacterium* sp., Fecali bacterium sp.)	• Hypocholestrolemic (*Bifidobacterium* sp.)
• Anti-inflammatory (*Lactobacillus* sp.)	• Antihypertensive (*Lactobacillus* sp.)
• Antiproliferative (*Lactobacillus* sp.)	• Anti-obesogenic (*Lactobacillus* sp.)
• Antioxidant (*Lactobacillus* sp., *Bifidobacterium* sp., *Strep salivarius* ssp.)	• Hepatoprotective (*Lactobacillus* sp., Enterococcus lactis)
	• Antimicrobial (*Lactobacillus* sp.)

hormone biological and metabolic reactions in the host have not yet been clarified. Investigations are currently in progress to explain the beneficial health effects of postbiotic products reported in the literature. These health effects have been previously been related to their possible anti-inflammatory, antiproliferative, antioxidant, hypocholesterolaemic, antihypertensive, anti-obesogenic, hepatoprotective and antimicrobial activities (S74).

The definitions of probiotics and prebiotics have a long history, particularly with regard to the standing of probiotics in international regulations, but there is still no consensus on their definitions. It is unclear whether these terms will be maintained or changed in future.

Specific commercial products use fermentation technology, such as fermented milk-based infant formulas, and these can be used in clinical practice to beneficially modulate the gut microbiota and gut immunity. Selected lactic acid bacterial strains are used in industrial processes to ferment cows' milk, and these are combined with heat treatment. The end products are formulas that contain no viable bacteria or prebiotic components, but do contain specific active factors resulting from the fermentation process (S75). Metabolites produced through fermentation processes are used as raw materials for pharmaceutical products, healthcare supplements and functional foods. Experimental *in vitro* and *in vivo* studies have indicated that specific fermentation products are involved in establishing immune balance and oral tolerance, although the mechanism of action underlying these functions has not yet been fully explained (S75).

Changes in the microbiome have been documented in many chronic, mainly immune-mediated, gastrointestinal and liver diseases and distinct patterns have been associated with each specific disease. However, causality and the mechanisms by which the gut microflora influences the aetiopathogenesis of a disease have not been fully explained, so they may be considered limiting factors of this review. Paediatricians are on the frontline when it comes to caring for children's health and well-being. The strength of this review was that we have emphasised the key roles that paediatricians' play in minimising preventable early harmful events that could permanently influence the composition and/or function of the gut microbiota.

CONCLUSION

The basic science relating to the gut microbiota is changing rapidly, as clinical data provide evidence of the importance of diversity in the microbial community and point to the general accepted role of so-called protective bacteria. Gut microbes are moving rapidly from being considered potentially dangerous to being considered as a positive influence on health when they are properly implemented. In clinical practice, modulation of the gut microbiota may be achieved by using several approaches, including probiotics, prebiotics, synbiotics, paraprobiotics and postbiotics.

A better understanding of the potential impacts of the gut microbiota on human health, and of the use of related commercially available products, would lead to more appropriate use of these products in clinical practice by healthcare professionals.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article:

Appendix S1 References.

