The contrasting human gut microbiota in early and late life and implications for host health and disease

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Abstract. The gut microbiota plays a significant role in health and development from birth and continues to affect several processes throughout life and into old age. During both infancy and old age, the trajectory of the gut microbiota changes with contrasting consequences at both stages for the host. The infant gut is unstable, and colonization is influenced by a variety of perinatal and postnatal factors. Many of these factors can contribute to an altered microbiota profile in infancy which can be associated with negative consequences later in life such as allergies, obesity, and neuropsychiatric disorders. The late-life gut microbiota is influenced by physiological changes within the host, illness, diet and lifestyle that impact its composition and functionality. Indeed, reduced microbial diversity, loss of beneficial microorganisms and increased pathobionts are key signatures of the elderly microbiome. Such changes have been associated with degenerative diseases including inflammaging, Alzheimer's disease, Parkinson's disease, and increased risk of infection with *Clostridioides difficile*. Here, we examine early- and late-life factors that contribute to contrasting gut microbiota disturbances and the consequences associated with these disruptions. Finally, we provide compelling evidence of nutritional and probiotic/prebiotic interventions that may help alleviate the effects of gut microbiota changes into old age.

Keywords: Gut microbiota, early life, late life, gut-brain axis, diseases, prebiotics, probiotics

1. Introduction

With an estimated 10^{13} microorganisms, the human gut is recognized as one of the most densely populated ecosystems on the planet. It is home to microorganisms from all domains of life including bacteria, archaea, and eukarya along with prokaryotic and eukaryotic viruses. The microbiota refers to the 'living' component – bacteria, archaea, protists and fungi, while the microbiota and its combined nucleic acids, including eukaryotic and prokaryotic viruses, microbial metabolites and microbial structural elements are referred to as the gut microbiome [1]. It bestows a myriad of health benefits on its host [2]. Most information to date relates to its bacterial component, indeed, the gut microbiota contains

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100 trillion bacteria. The functions provided by these microorganisms include production of nutrients and bioactive metabolites that are essential for host health such as vitamins and short chain fatty acids (SCFAs). It is responsible for programming host immunity and plays an important role in maintaining intestinal barrier integrity. Through colonization resistance, it protects the host from pathogen colonization and infection. It regulates energy homeostasis and communicates with the central nervous system. Its dysfunction has been associated with many diseases including inflammatory diseases [3], cardiovascular disease [4], gastrointestinal diseases [5], neurological diseases [6], and metabolic syndrome and obesity [7]. However, not all studies report the same observations which could be a consequence of laboratory techniques used, geographical location/dietary patterns etc. of the cohort under investigation, and evidence for causality remains to be elucidated.

Despite our understanding of the role the gut microbiome plays in host health, describing the healthy gut microbiome at any stage of life has proven extremely challenging for scientists. This is due to the vast inter-individual variation of the gut microbiota. Indeed, the gut microbiome could be described as a malleable, emergent system within the body influenced by many factors including host genetics [8], diet [9], stress and emotions [10], illness [11], antibiotics [12, 13], hormonal changes [14], to name but a few. Its establishment at birth is also influenced by a number of factors from birth mode to gestational age and type of feeding. Yet, correct microbial establishment in early life has been demonstrated to be central to the immunological, cognitive and metabolic development of the child, whereby altered seeding of this ecosystem during the first days of life can have long-term health consequences, such as predisposition to many disease states later in life.

The established healthy gut microbiota throughout adulthood is marked by a gut microbial diversity and richness that is significantly reduced in several disease states. Furthermore, certain bacterial members, beyond the noted probiotic members bifidobacteria and lactobacilli, are now linked with beneficial effects on the host including members of Ruminococcaceae, Lachnospiraceae, Christenellaceae, and Eubacteriaceae [15], which can be reduced in illness and certain conditions.

However, such signatures of a healthy gut microbiome have been shown to wane in later life – with loss of beneficial gut members, increased pathogens, and reduced microbial diversity representing common features of the elderly microbiota. Indeed, altered microbiota composition as a result of health perturbations at an advanced age induces a shift in the bacterial-host relationship from a symbiotic to a non-desirable/pathogenic one. Whether the extent of these changes in old age is linked to the establishment of the infant gut microbiota is as yet unknown as this science is still too young [16]. However, understanding the trajectory of the gut microbiome in infancy and old age, the two extremes of life, is vital to ensuring the optimal health of the host throughout its life cycle, given that physiological and external factors at both stages render significant consequences for the gut microbiome and subsequently host health.

With this in mind, this review aims to summarize recent findings on the factors that influence microbial development in early- and late-life, and disease states that may arise as a cause of perturbations to this delicate ecosystem. The review highlights the centrality of the gut microbiota in human health at the most vulnerable stages and discusses nutritional and probiotic/prebiotic interventions which may alleviate the consequences of gut microbiome dysbiosis in later life.

2. The microbiota in early life

2.1. The development of the infant gut microbiota

One of the most critical developmental events in early life is the bacterial colonization of the infant gut. The direct microbiota-host interactions of this biological 'superorganism' serve to seed immunological, cognitive and physiological development – where this delicate ecosystem is perturbed, disease often ensues [17]. According to the long accepted sterile womb paradigm, the human fetus develops in a sterile environment and microbial colonization of the gastrointestinal (GI) tract occurs by both vertical and horizontal transmission during and after birth [18]. Recent studies have begun to contest this belief however, with findings (often controversial) reporting the presence of bacterial communities in the placenta, amniotic fluid and meconium [19], suggesting this colonization process in fact begins *in utero* [20].

Whilst it has been reported to have six times less OTUs (operational taxonomic units) than adults, the early infant gut microbiota is a highly dynamic entity which develops rapidly over the first two years of life
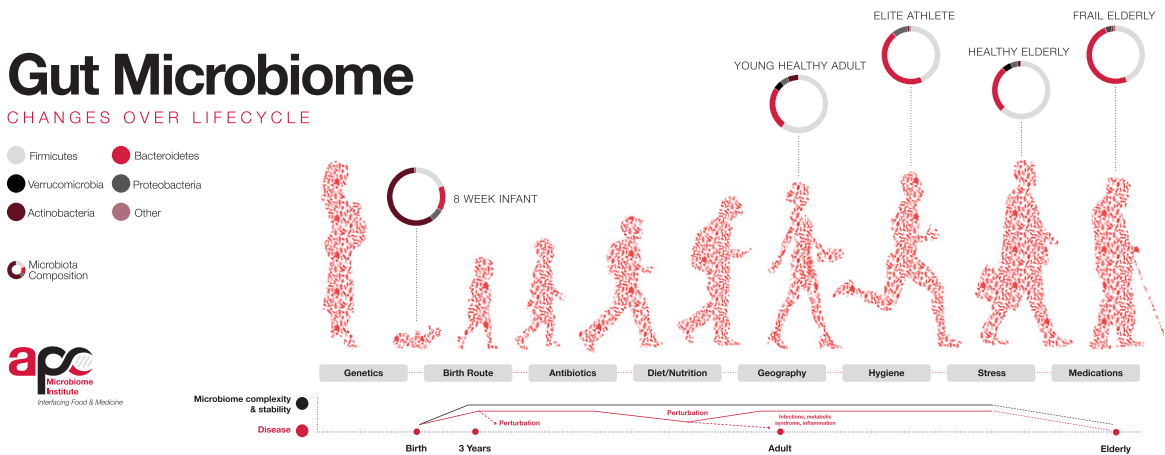
Fig. 1. Factors influencing the colonisation of the gut microbiota throughout life. Bacterial diversity increases from infancy until it matures at ~3 years of age and resembles an adult-life composition. Following adulthood, diversity again begins to decline with age. Source: APC Microbiome Ireland, 2018.

[21, 22]. Following normal vaginally delivered birth, the infant GI tract is swiftly colonized by an arsenal of bacteria, which is influenced by factors such as mode of delivery, gestational age, antibiotic exposure, diet, close family members and exposure to animals (Fig. 1). The aerobic environment of the infant gut following birth influences the initial colonizers which are facultative anaerobes such Proteobacteria (*Enterobacteria*) and Firmicutes (S*treptococcus, Staphylococcus*). These microbes produce a reduced, oxygen-deficient environment favourable for later colonization by Actinobacteria (*Bifidobacterium*), *Clostridium, Lactobacillus, Ruminococcus*, Bacteroidetes (*Bacteroides*) and *Escherichia* [23–25]. At ~1 month after birth, the diversity of the infant gut is increased and dominated by Actinobacteria (*Bifidobacterium*), Bacteroidetes (*Bacteroides*) and Gammaproteobacteria (*Enterobacteria*) which are required for the metabolism of specific carbohydrates found in breastmilk [22]. Following the introduction of solid foods at ~6 months, the infant gut shifts again with relative abundances of *Bifidobacterium* and Clostridiales decreasing, and Firmicutes levels increasing [26]. At the end of the first year of life, microbiota profiles display inter-individual variation but begin to converge towards that found in an adult-like GI tract [27].

Alpha-diversity (α-diversity) and beta-diversity (β-diversity) are measurements allowing for a higher-level description of the microbial communities in a sample. They measure microbial diversity or evenness, and variation, respectively between different environments, to display a broad change in microbial communities, rather than abundance or specific taxa. The α-diversity of the gut microbiota increases and β-diversity decreases as the infant ages, and microbial profiles become more complex and more dissimilar between individuals [28], until the infant gut microbiota stabilizes and reaches an adult-like composition and diversity at about two to five years of age [29, 30]. The exact time point of maturation remains unclear however, and findings are not universal. Studies have reported the gut incapable of reaching compositional and functional maturity before the age of four [31], the age of five [32], and even before pre-adolescence, with 7–12 year olds being found to possess significantly lower levels of *Bacteroides* spp. and more complex composition compared to adults, suggesting ongoing microbial development [30]. On the contrary, a 2019 study reported a cohort of 281 school-aged children between six and nine years old to have an adult-like alpha diversity and a similar functional repertoire to that of adults [33], whilst a 2020 study reported four-year olds to have an adult-like gut microbiota, with significantly increased levels of adult gut-dominating phyla Bacteroidetes and Firmicutes and decreased infant-gut phyla *Actinobacteria* and *Proteobacteria* [34].

2.2. Factors that influence the early gut microbiota

2.2.1. Gestational age at birth

Gestational age is a major determinant in the establishment of the neonatal gut microbiota [34, 35]

and may continue to influence this process up until four years of age, with the alpha diversity of full-term infants being substantially higher than that of pre-term infants [36]. Disruption to critical developmental stages of the final trimester of pregnancy due to premature birth result in infants possessing an immature GI tract with delayed bacterial colonization, aberrant intestinal barrier function, and poor nutrient-absorbing capabilities [37]. Aberrant gut physiology predisposes pre-term infants to illnesses such as systemic inflammation, sepsis and necrotizing enterocolitis (NEC) due to a 'leaky gut' and increased translocation of microorganisms into the bloodstream [38].

NEC is a life-threatening GI emergency, and one of the leading causes of premature infant death, with an incidence rate of between 2–13% and a mortality rate of up to 45% [39, 40]. Whilst antibiotics are commonly used to treat NEC, recent studies have shown promise with probiotics containing both *Lactobacillus* and *Bifidobacterium* being reported to reduce the incidence of the disease in pre-term infants by up to 50% [41]. Such results have meant that many neonatologists now consider it almost "unethical not to treat" premature babies with probiotics following birth.

The pre-term infant gut microbiota remains largely understudied; however, it is postulated that it is uncharacteristic relative to the full-term counterpart, being largely unbalanced and lacking in microbial diversity [42, 43]. This may be due to the frequent delivery of premature infants by caesarean-section (CS) and an almost universal exposure to the microbiologically restrained environment of the neonatal intensive care unit and exposure to antibiotics in the first days of life [44]. Intestinal microbial colonization of preterm neonates has been reported to occur in a four-phased pattern, in the first 50 weeks post-birth as indicated by the dominance of *Staphylococcus, Enterococcus, Enterobacteriaceae* and *Bifidobacterium* [45]. The pre-term GI tract is reported to be primarily dominated by *Proteobacteria* and *Firmicutes* and members of the *Enterobacteriaceae* family [46, 47]. High levels of inter-individual variation have been reported between microbiota profiles of these infants; however, abundances of the beneficial commensals *Bifidobacterium, Bacteroides* and *Lactobacillus* are consistently reduced compared to full-term infants [22, 36, 46]. Furthermore, the abundances of potentially pathogenic bacterial groups such as *Enterococcus*, *Klebsiella*, *Staphylococcus, Streptococcus* and *Clostridium* spp. are increased in the pre-term infant gut [47, 48], although, breastfeeding has been shown to be capable of restoring the gut-microbiota to that of a full-term infant, by significantly increasing levels of *Bifidobacteriaceae* and reducing *Staphylococcaceae* and *Clostridiaceae* [44, 49].

2.2.2. Birth mode

Another pivotal factor in shaping the infant gut microbiota is birth mode. Vaginally-delivered (VD) infants are exposed to the vaginal microbiota of the mother, and consequently, these infants initially harbour microbial communities that resemble the maternal vaginal microbiota such as *Escherichia, Bifidobacterium, Lactobacillus, Bacteroides, Shigella* and *Prevotella* [50–52]. VD infants are widely reported to display higher taxonomical diversity than that of infants born by CS [52, 53], and CS delivery has been associated with the development of non-communicable diseases such as asthma [54], eczema [55], allergies [56], adiposity [57] and diabetes [58] later in life.

Infants born by CS do not come into direct contact with the maternal birth canal and as a result acquire microbial communities from alternative sites such as the maternal skin microbiota and the hospital environment [29]. As a result, CS-born infants harbour gut bacteria such as *Enterococcus, Staphylococcus, Streptococcus, Propionibacterium* and *Klebsiella* spp. [51, 52, 59], and lower abundances of Actinobacteria (*Bifidobacterium*) and Bacteroidetes (*Bacteroides*) than VD-born infants [53]. CS infants have been associated with higher levels of opportunistic pathogens such as *Clostridioides difficile* and *Lactobacillus* genera, which coincide with lower luminal SCFA levels and higher pH due to decreased abundances of Actinobacteria and Bacteroidetes [60]. Alternatively, microbial populations of the VD infant GI tract such as *Bacteroides fragilis* and *Bifidobacterium* spp. produce SCFAs which maintain a lower intestinal pH that inhibits the growth of pathogens such as *Clostridium perfringens* [60, 61]. CS-born infants have a delayed bacterial colonization and lower microbial diversity; however they eventually 'catch up' [61], and birth mode-related compositional differences have been shown to largely diminish in the first two years of life [62]. More recently, we have shown that C-section in mouse models results in enduring effects on physiology and behaviour. [63]. Whether such effects also occur in humans requires further investigation.

2.2.3. Antibiotic intervention

Premature birth and CS delivery are often confounded with antibiotic administration for the treatment of infections [64]. Early life antibiotic interventions have been shown to influence the gut microbiota, delaying microbial maturation for between six to 12 months after birth and causing significant alterations in the bacterial taxa over the first two years of life [62]. Additionally, antibiotic treatments have been linked to adverse health outcomes in later life such as adiposity [65] and the development of allergies and asthma [66]. Decreased microbial diversity has been associated with antibiotic treatment [67], and infants receiving antibiotics host lower abundances of *Bifidobacterium, Bacteroides* and *Lactobacillus* and increased levels of *Proteobacteria* and Firmicutes such as *Clostridium* and *Staphylococcus* [68, 69]. Intrapartum antibiotic prophylaxis (IAP) is a common antibiotic treatment to which neonates are exposed and is administered to group B *Streptococcus* positive mothers, to reduce the risk of early onset neonatal sepsis [70]. IAP treatment in the mother has been reported to subsequently affect the infant developing gut microbiota, by causing a diminution in the relative abundances of *Bifidobacterium* spp. and *Bacteroidetes* and increased *Proteobacteria* and *Enterobacteriaceae* family members [71, 72]. Reduced phylogenetic diversity and richness associated with antibiotic exposure have been reported to be dependent on the duration of treatment [64], however influences of antibiotics on the developing infant gut microbiota have been shown to occur irrespective of infant health [73]. Furthermore, the increased relative abundances of *Proteobacteria* namely *Enterobacteriaceae* by early exposure to antibiotics promote a pro-inflammatory state, inducing a microbial imbalance in the developing gut [74], which requires prolonged recovery time compared with that of antibiotic-treated adults [75].

In addition to alteration of the infant developing intestinal microbiota, antibiotics also pose the threat of enriching the reservoir of antibiotic resistance genes (ARG, also termed 'the resistome') which is easily influenced in early life [76]. Multidrug-resistant members of the genera *Escherichia, Klebsiella* and *Enterobacter* which are common amongst nosocomial infections, have been reported to dominate the pre-term gut microbiota [77]. Further, infants exposed to IAP antibiotics have been reported to have significantly increased abundances of ARG [78], with one study reporting that out of 241 AGR genes identified, 29% and 25% were encoded by

Escherichia and *Staphylococcus,* respectively [79]. Studies of antibiotics on the developing infant gut microbiota and resistome warrant further investigation.

2.2.4. Infant diet

Milk is the first food to which the infant is exposed postpartum and it is widely understood to directly influence establishment of the gut microbiota [80, 81] and may continue to do so throughout early life [25]. Interestingly, mode of feeding in early life and gut microbiota composition at six to nine years have been significantly, positively correlated [33]. Breastmilk contains > 700 bacterial species at a concentration of ~1000 colony forming units (CFU)/ml, therefore breast-fed infants are estimated to ingest ~800,000 bacteria a day [82].

Often regarded as the 'gold standard' for infant nutrition, breast-milk has been widely reported to provide the infant with both short- and long-term health benefits such as reduced susceptibility to morbidity, childhood obesity, GI disease and allergies [83, 84]. Breastmilk aids in "jump-starting" early life by providing the new-born infant with a plethora of bioactive molecules such as human milk oligosaccharides (HMO), IgA and essential fatty acids which, in addition to optimally shaping the infant gut microbiota, influence cognitive development and maturation of the immune system [85–87]. The maternal milk microbiota is primarily composed of *Staphylococcus* and *Streptococcus,* followed by *Bifidobacterium* and *Lactobacillus, Enterococcus,* and members of the *Enterobacteriaceae* family [88–90], which coincides with these being amongst the earliest colonizers of the infant gut microbiota [91]. The transfer of microbes from mammary gland to infant gut has been suggested to occur via vertical transmission [75, 92], and infants who are breast-fed during their first month of life have been observed to share 28% of their fecal microbiota with their maternal milk microbes [81]. Infant formula is often used in lieu of breastmilk in instances where breastfeeding is not possible due to an unavailability of milk or maternal reasons. Whilst it is a sufficient source of nourishment for the newborn, infant formula is nutritionally inferior, lacking the diversity of components of breastmilk, such as HMOs which serve to promote the colonization of probiotic *Bifidobacterium* and *Bacteroides* commensals [93]. Many studies exist that report differences in the gut microbiota of breast-fed and formula-fed infants, with those who are formula-fed possessing a more complex microbiota with

higher relative amounts of facultative anaerobes such as *Proteobacteria* and *Firmicutes* [69, 94]. Contrastingly, breast-fed infants possess a less stable, simpler microbiota, with significantly higher abundances of *Bifidobacterium* and *Bacteroides* than formula-fed infants [50, 95, 96]. Breast-fed infants are reported to have lower *Streptococcus, Viellonella, Clostridiodes* and *Enterococcus* levels [97], whilst formula-fed infants have been shown to possess higher abundance of *C. difficile* [91]. Previous findings suggest that around two years of age, the observed differences between breastfed and formula-fed infants diminish, and the bacterial communities more or less coincide to resemble an adult-like microbiota [95].

2.2.5. Prenatal stress

There is increasing recognition of prenatal stress as a disruptive factor in the development of the infant gut microbiota [98]. Prenatal exposure to stress has been linked to health issues such as pre-term birth [99], lower birth weight [100], as well as obesity and metabolic dysfunctions later in life [101]. Additionally, infants pre-exposed to stress *in utero* have been linked to increased antibiotic treatments in childhood [102] and higher GI disturbances such as diarrhoea and gastroenteritis during the first three months of life [103]. Prenatally stressed infants have been reported to host microbial communities that are characteristic of increased inflammatory levels including higher abundances of pathogenic Proteobacteria (*Escherichia, Enterobacter* and *Serratia* related) and also lower abundances of lactic acid bacteria (*Lactobacillus, Lactococcus*), bifidobacteria and *Akkermansia* [103, 104]. Interestingly, a recent study reported increased maternal hair cortisol concentrations to be negatively associated with Actinobacteria, Proteobacteria and Firmicutes levels, although findings were not significant [104].

Infants exposed to prenatal stress have also been shown to have increased levels of Firmicutes (*Weissella*) and Enterobacteriaceae (unclassified genera) in the meconium [105], the latter of which has been found to be characteristic of depression in later life [106]. Animal studies have also found similar results, whereby prenatally stressed monkey infants were seen to have an altered microbiota composition compared to control infants, with significantly lower levels of lactobacilli and bifidobacteria [107]. Further, consequent to these shifts in gut microbial communities as observed in prenatally stressed infants is an aberrant gut physiology, with increased permeability and reduced gut barrier integrity, leading to the predisposition of various GI and immune diseases later in life [108].

Based on a series of preclinical studies, the gut microbiota has also been postulated to be a paramount contributor to the neurodevelopment of the infant via the gut-brain axis [109]. Prenatal stress in mice has been shown to alter the gut and vaginal microbiota of the mother and consequent transmission of these dysbiotic microbiota to the infant has been implicated in increased risk of neurodevelopmental disorders through a disruption in their own microbiota development (discussed further below) [110, 111]. Maternal prenatal stress and subsequent disruption of the offspring developing gut has also been speculated to delay the development of the hypothalamus-pituitary-adrenal axis (HPA), a series of mechanisms which have been implicated in aberrant behavioural and emotional development of the child [112].

2.3. Diseases associated with the gut microbiota in early life

2.3.1. Allergies

In recent years, the incidence of IgE-mediated atopic diseases such as asthma, atopic dermatitis (eczema) and food allergies has risen sharply [113]. Reduced bacterial diversity in the first year of life is associated with increased risk of allergies in first six years of life [114], and increased bacterial richness at three months may provide the infant with up to 55% reduced risk of developing a food allergy in the first year of life [115]. In general, children with food allergies have been reported to have an increased Firmicutes/Bacteroidetes ratio, with increased levels of genera such as *Bifidobacterium, Clostridium, Enterococcus, Lactobacillus* and *Akkermansia* and under-representations of *Streptococcus* and *Bacteroides* [116]; however, exact microbiota profiles depend on the type of food allergy (e.g. wheat, peanut soy, etc.) [117].

Gut microbial communities of children with eczema have been characterised by a lower alpha diversity and lower relative abundance of Actinobacteria, whilst Bacteroidetes were highly enriched, compared to healthy controls [118]. Probiotics have shown promise in this area, with *Lactobacillus fermentum* and *Lactobacillus salivarius* both capable of significantly reducing the severity of eczema in children [119]. Additionally, *Lactobacillus plantarum* was found to improve eczema severity, by lowering IL-

4, IL-17 and IFN-γ levels [120]. CS delivery and subsequent colonization of *C. difficile* in the infant gut has been linked to development of asthma in six to seven year olds, whilst vaginally-born children were only pre-disposed if a parent suffered with an atopic disease [121]. A putative therapeutic role for probiotics in the treatment of asthma has been suggested [122].

2.3.2. Obesity

In parallel with the increased consumption of the 'Western diet' rich in processed foods, is an increasing prevalence of the worldwide "globesity" epidemic. According to the World Health Organisation, approximately 38 million children under the age of five were reported to be overweight in 2019, representing a three-fold increase since the 1970s [123]. Obesity and adiposity can have major health implications such as diabetes and cardiovascular disease [113]. Whilst often due to an increased energy intake and reduced energy output, studies over recent years have begun to uncover a role of the gut microbiota in the process [124], with mounting evidence suggesting the establishment of the gut microbiota in early life plays a pivotal role in adiposity and the development of obesity in childhood [125].

Obesity has been widely associated with alterations in the gut microbiota in adults, albeit findings are inconsistent. Some studies report an increased Firmicutes/Bacteroidetes ratio, as a result of obesity [126–128], and other studies report the converse, or, no relationship [129, 130]. Consequently, relative changes in these phyla cannot be currently classified as a biomarker for obesity. Whilst findings of microbiota alterations in children due to obestiy are limited, it has been suggested that relative abundances of *Escherichia coli* and *Staphylococcus* are increased, whilst *Bifidobacterium* counts are reduced [131, 132]. Additionally, levels of *Dorea* have been reported to be increased, whilst the genera *Ruminococcus, Akkermansia* and *Parabacteroidetes* were found to be decreased in obese children compared to non-obese children [133]. Predisposition to adiposity may occur *in utero*, with infants' fecal microbiota profiles being reported to be related to maternal BMI, weight and weight gain during pregnancy [134]. Infants of overweight mothers have been shown to have an altered gut microbiota, with higher levels of *Bacteroides, Enterococcus* and *Staphylococcus* over the first six months of life [135, 136]. Moreover, feeding has also been reported to influence the prevalence of obesity in childhood, with infants who are breastfed being less likely to develop obesity than their formula-fed counterparts [137]. This may be in part, explained by lower abundance of *Bifidobacterium* observed in formula-fed infants versus breastfed infants which has been linked to increased adiposity at 18 months [138]. CS delivery has also been associated with a higher prevalence of adiposity during childhood, which may extend into adulthood [139]. Antibiotic exposure in infancy has been associated with adiposity and obesity later in childhood, which may occur through a disrupted microbial colonization [140, 141], however, it has been purported that the GI microbiota become less susceptible to alterations by antibiotics as it matures [142]. Interestingly, antibiotics and adiposity have been reported to have stronger links in boys which may be due to sex-specific GI responses such as antibiotic drug metabolism or host-microbe interactions [143, 144]. Moreover, only high levels of antibiotic treatments (≥4 courses) were linked to a higher body mass index trajectory in childhood in Irish children between the ages of two and five years [142].

2.3.3. Neuropsychiatric disorders

The gut-brain axis is a bi-directional communication network involving several aspects of the nervous system and the gut microbiota [145]. The GI tract is capable of influencing the brain in terms of mood states, which in turn can have consequent effects in GI motility, secretion and immune function [146]. Evidence suggests a co-maturation between the two in infancy [147]. Behavioural and cognitive processes may affect brain-gut axis function and have been implicated in functional disorders such as irritable bowel syndrome and increased intestinal permeability [108]. Conversely, dysbiosis of the gut has been associated with CNS disorders such as autism, anxiety and depression [148]. The HPA axis is one arm of the human stress response system, activation of which involves stimulation of the hypothalamus and a downstream systemic release of cortisol [146]. The other arm is the sympathetic nervous system (SNS), which is able to alter gut function, including gut permeability ([149], reviewed in [150]). Increased translocation of bacteria across the intestinal lumen as seen in prenatally stressed mice results in the upregulation of inflammatory markers and proinflammatory cytokines which are potent activators of the HPA axis [151]. Disruptions to the HPA axis caused by variations in gut microbiota have been shown to exacerbate anxiolytic behaviours in mice [152]. In addition, exaggerated responses of the SNS have also

been demonstrated in chronic anxiety and implicated in the aetiology of cardiovascular disease [153].

As aforementioned, intestinal microbial communities are known to be central to the maturation of an infant's GI tract and immunity, with this extending to aspects of cognitive development. Mounting evidence suggests that microbial colonization of the GI tract in early life contributes to the parallel sequential maturation of the brain up to three years of age [147], and the gut microbiome composition of infants has been implicated in childhood temperament, with greater sociability being associated with a higher gut phylogenetic diversity [154]. Moreover, a 2018 study reported high GI alpha-diversity to be negatively associated with cognitive performance and reported the gut microbiota composition and diversity at one year of age to be predictive of cognitive performance at two years of age [155]. The same study also found infants with higher abundances of *Bacteroides* and lower levels of *Faecalibacterium* to have better cognitive function.

More recently, research has begun to unveil the role of the gut microbiota in neuropsychological disorders, further highlighting the possible pathophysiological role of this biological 'supersystem' in human health and disease. Increased abundances of Bacteroidetes (*Bacteroides uniformis, Bacteroides ovatus)* and *Sutterella stercoricanis* have been reported in children with attention deficit disorder [156]. Alterations in the gut microbiota have also been implicated in schizophrenia [157], with a recent study reporting the schizophrenia gut to be specifically enriched in *Streptococcus vestibularis* [158]. Gut dysbiosis has also been implicated in autism spectrum disorder, with severely autistic children harbouring higher levels of Bacteroidetes, *Clostridium, Lactobacillus* spp. and *Desulfovibrio* spp. and lower levels of Firmicutes and *Bifidobacterium* in comparison to control, non-autistic subjects [159–161].

Animal studies have also highlighted the importance of gut-brain interactions in early life development, and germ-free (GF) mice have been shown to have aberrant anxiety responses [162]. Remarkably, when adult GF mice are inoculated with specific-pathogen-free mice microbiota in adulthood, altered anxiolytic phenotypes are seen to persist, suggesting that a critical window exists for the development of stress systems in early life [163]. Studies reporting the gut-brain axis in the context of neuropsychiatric disorders in childhood are limited and underlying mechanisms of the development of these disorders have yet to be elucidated. It is worth noting however,

that the 'medical accessibility' of the human gut microbiota in comparison to the human genome may represent a promising therapeutic strategy for the treatment and prevention of neuropsychiatric disorders in the future [113].

3. The microbiota in later life

3.1. The gut microbiota in old age

While the healthy adult gut microbiota is relatively stable over long periods [164], ageing (>65 years generally) has a significant impact on its composition and functionality [165]. Key signatures of the elderly gut microbiota include increased abundance of opportunistic pathogens, a decrease in microbiota diversity, reduction in SCFA-producing species, and an increase in facultative anaerobes [166–168]. A change in the ratio of Firmicutes to Bacteroidetes has been observed in older individuals consuming a Western-type diet, which decreased from 10.9 in adults (25–45 years) to 0.6 in the elderly cohort (70–90 years), resembling the ratio of 0.4 in infants (three weeks to 10 months old) [169]. Inter-individual variation is also extensive in the elderly group and much greater than that observed in healthy adults, with Firmicutes proportions in an elderly Irish cohort ranging from 7% to 94%, and the dominant phylum, Bacteroidetes, ranging from 3% to 92% [170]. Other observed trends include decreases in *Bifidobacterium*, *Faecalibacterium* groups, and *Blautia coccoides* (*Clostridium* XIVa group) [171–175]. Country-specific trends have also been observed and are discussed in detail by Salazar et al. [165]. As an example, the *Bacteroides-Prevotella* group has been found to be over-represented in Irish [170], Japanese [173], German, Swedish [176], Austrian [177] and Finnish [178] elderly cohorts but under-represented in English [179], Italian [176] and Spanish [167] cohorts. However, as Salazar et al. state, it is unclear if such differences are related to different geographical populations or the consequences of methodological differences [165].

3.2. Factors that influence the elderly gut microbiota

Both physiological and lifestyle changes that ultimately influence diet and nutrition undoubtedly contribute to the changes in the ageing gut microbiota. In terms of physiological alterations, these can

include changes in appetite, mood, medications, changes in salivary function and dentition, which can lead to imbalanced nutrition due to poor dietary choices, and changes in gastrointestinal motility that can cause a reduction in digestion and absorption of nutrients [180]. Medication and drugs are more frequently prescribed for the elderly and these too may impact the gut microbiota. For example, it has been reported that polypharmacy in older hospitalized patients significantly associated with gut microbiota dysbiosis (reduced species richness and significant variations in the relative abundances of a large number of taxa), while, in contrast, such dysbiosis was not found in active healthy older people without polypharmacy [181]. In old age, the normal functioning of the immune system also wanes, which can contribute to increased infection with gut pathogens [173]. The elderly GI tract is also marked by increased immune activation and a thinning of the intestinal lining [182]. In terms of lifestyle, changes in living conditions have been shown to significantly influence the elderly gut microbiota as a consequence of dietary changes [172]. Indeed, distinct gut microbiota profiles were observed across elderly individuals either living in the community, attending day-hospital, in rehabilitation or long-stay residential care [172], and these profiles overlapped with the diets associated with these locations (low fat/high fibre, moderate fat/high fibre, moderate fat/low fibre, high fat/low fibre, respectively). The low-fat/high fibre diet of community dwellers correlated with a gut microbiota that resembled that of healthy young adults. In contrast, the high fat/low fibre diet of long-stay care residents correlated with a gut microbiota that was less diverse than that of healthy community dwellers. Loss of community-associated microbiota in long-stay care residents such as *Prevotella* and *Ruminococcus* associated with frailty. In another study, the important butyrate producer, *Faecalibacterium prausnitzii* was less abundant in frailer individuals while the pathogens *Eggerthella lenta* and *Eubacterium dolichum* were more abundant with frailty [183]. Likewise, residents of an elderly nursing home in America consuming a typical low-fibre-nursing-home diet had lower levels of butyrate producers and greater abundances of dysbiotic species [184]. A more recent study by the same group revealed that the dysbiotic gut microbiota of elderly nursing home residents – characterised by pro-inflammatory bacteria and reduced anti-inflammatory microbes – associated with *C. difficile* colonization [185].

3.3. Degenerative conditions and diseases associated with the elderly gut microbiota

3.3.1. Clostridioides difficile infection

The most common bacterial infection of the elderly, particularly those that are institutionalised, is *C. difficile*, causing mild to fatal diarrhoea. Mortality risk from *C. difficile* infection (CDI) increases with age [186]. Carriage rate of the pathogen has been shown to increase to 21% in institutionalised elderly individuals compared with a carriage rate of 1.6% in community-living elderly individuals [187]. Gut microbiota disruption as a consequence of antibiotic treatment is a leading factor in CDI. The resulting decrease in microbiota diversity and the elimination of health-associated taxa enable *C. difficile* to become established in the gut [188]. Yet, as previously mentioned, the dysbiotic microbiota of elderly nursing home residents also allows *C. difficile* to flourish [185]. CDI in hospitalised elderly individuals was also associated with under-representation of gut microbes with potential protective properties including *Bacteroides*, *Alistipes*, *Lachnospira*, and *Barnesiella*, and over-representation of opportunistic pathogens including *Klebsiella, Escherichia/Shigella, Sutterella, Enterococcus, Citrobacter, Veillonella, Proteus, Helicobacter, Morganella, Hafnia, Corynebacterium* and *Staphylococcus* [189].

3.3.2. Inflammaging

Inflammaging is described as the "long-term result of the chronic physiological stimulation of the innate immune system, which can become damaging during ageing . . . " [190]. It is a risk factor for numerous diseases including cardiovascular disease, diabetes mellitus, chronic kidney disease, cancer, dementia, sarcopenia, and depression [191]. Given the impact of the gut microbiota on local and systemic immune responses [192], it is unsurprising that the gut microbiota plays a central role in inflammaging. Indeed, the gut microbiota changes that occur with ageing promote a pro-inflammatory environment. Evidence of the inflammaging potential of the elderly gut microbiota became apparent when transfer of an aged microbiota to young GF mice resulted in inflammaging in the young mice [193]. Low levels of *Akkermansia* and increased levels of TM7 and Proteobacteria were associated with the inflammaging effect of the aged microbiota following transfer. It is interesting to note that centenarians and semisu-

percentenarians have been shown to have increased levels of health-associated bacteria such as *Akkermansia*, which could be a signature of longevity.

Furthermore, SCFA production in the elderly gut has been shown to decrease with age. This could be due to a drop in the metabolic activity of certain microbiota as observed in elderly individuals > 80 years of age [192] or the result of a reduction in SCFA-producing bacteria due to a low-fibre diet [170]. Thus, unsurprisingly, aging has been associated with a progressive and statistically significant reduction in fecal SCFAs [194]. Yet, SCFAs play an essential role in immune homeostasis. For example, in mice, microbiota-derived SCFAs have been shown to promote Th1 cell IL-10 production via G-protein coupled receptor 43 (GPR43), which results in intestinal homeostasis [195]. The SCFA butyrate has been shown to down-regulate the expression of genes involved in inflammatory pathways in non-inflamed intestinal tissue [196]. The results suggest that the low levels of SCFA-producing bacteria in the elderly gut, and consequently reduced SCFAs, contribute to inflammaging.

Data from a non-human primate model suggest that aging increases colonic permeability as a result of remodeling of intestinal tight junction proteins [197]. Moreover, gut microbiota lipopolysaccharide (LPS) was reported to accelerate inflammaging in mice [198], suggesting that the gut microbiota can contribute to inflammaging via translocation of LPS across the poor intestinal barrier in the aged population.

3.3.3. Alzheimer's disease

The gut-brain axis refers to the network of connections between the gut microbiota and the brain [199, 200]. As a consequence, the gut microbiota influences the central nervous system (CNS) through a variety of mechanisms. The gut microbiota can produce neurotransmitters such as gamma aminobutyric acid (GABA) [201]: Microbial metabolites such as SCFAs, indole, secondary bile acids, tyramine, p-aminobenzoate, and α-tocopherol promote serotonin biosynthesis in colonic enterochromaffin cells [202]. In preclinical models, SCFAs have been shown to influence the CNS by regulating neuroplasticity [203, 204]. Numerous studies have correlated the gut microbiota with neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease [199, 205]. Alzheimer's disease is the most prevalent form of dementia affecting older people [206] and is characterized by plaques in several parts of

the brain. These plaques are composed of β-amyloid (Aβ) fibrils and hyperphosphorylated tau proteins. Interestingly, Aβ's activities have been recently proposed to possess a protective/damaging duality. Aβ has been nominated as an antimicrobial peptide, which elicts a protective role in innate immunity. Recent studies of infection in mouse and worm models of Alzheimer's disease revealed that Aβ protects against microbial infection, via reduced microbial adhesion to host cells. Further, amyloid precursor protein (APP) – knockout (APP-KO) mice were found to display lower survival rates following bacterial infection [207]. It is also believed that gut microbial LPS and amyloids may contribute to the pathogenesis of Alzheimer's disease in older individuals by passing through the leaky gut barrier [206]. Indeed, bacteria produce a range of amyloids that have been implicated in the pathogenesis of Alzheimer's disease. It is also hypothesized that increased inflammation in the gut, and the dysbiosis that ensues promotes the production of antimicrobial calprotectin, which supports amyloid fibril formation in the gut and the brain [206]. The gut microbiota of cognitively impaired, amyloid-positive patients was found to harbor an increased abundance of pro-inflammatory bacteria (*Escherichia/Shigella*) and a reduction in anti-inflammatory bacteria [208]. Furthermore, in a mouse model of Alzheimer's disease, transfer of a healthy microbiota via fecal microbiota transfer reduced amyloid and tau pathology in the recipient animal suggesting that restoration of intestinal homeostasis may prove beneficial for Alzheimer's disease treatments [209].

3.3.4. Parkinson's disease

Parkinson's disease is a very common age-related neurodegenerative disorder. It involves the accumulation and aggregation of alpha-synuclein (α-syn) within the CNS and other neural structures [210]. Gastrointestinal dysfunction is a feature of Parkinson's disease with patients reporting nausea, constipation, defecatory dysfunction, as well as irritable bowel syndrome [211, 212]. It has been proposed that inflammation stemming from gut microbiota changes may be a contributing factor to α-syn misfolding [213, 214] and several studies have reported gut microbiota changes preceding or taking place during the disease [205, 215]. A 2015 study by Scheperhans et al. reported that the abundance of *Prevotellaceae* was reduced by 77.6% in Parkinson's disease patients compared with healthy controls. Furthermore, severity of postural instabil-

ity and gait difficulty was positively associated with the relative abundance of *Enterobacteriaceae* [216]. Another study observed similar microbiota profiles in Parkinson's disease patients along with a reduction in Bacteriodetes, but the study also measured fecal SCFA levels and found that they were significantly reduced in the patients [217]. More recently, a 2020 study by Zhang et al. reported that eight inflammation-associated bacterial genera (*Parabacteroides*, *Akkermansia*, *Coprococcus*, *Bilophila*, *Collinsella*, *Methanobrevibacter*, *Eggerthella*, *Adlercreutzia*) may play a role in the progression of the disease [218]. Given that faecal microbiota transplantation in Parkinson's disease mice has been reported to exert neuroprotective effects [219], the gut microbiota may be a target in the treatment of the disease.

3.4. Nutritional/probiotic interventions that modulate gut microbiota and health

Dietary and therapeutic interventions with probiotics and prebiotics continue to generate promising results in terms of beneficially modulating the gut microbiota based on randomized, controlled trials in humans in many cases, resulting in improved clinical manifestations in the host. Fibre is essentially the food of the gut microbiota and is found in fruits, vegetables and cereals, thus increasing the servings of these consumed in the daily diet, increases fibre in the gut [220]. Growing attention is now being focused on enhancing fibre for modulating the microbiota-gut brain axis [221, 222]. Humans lack the necessary enzymes to degrade fibre but the gut microbiota encodes the enzymatic repertoire to breakdown fibre into fermentable monosaccharides [2], and the resulting end-products of microbial fermentation are SCFAs. Prebiotics are defined as "a substrate that is selectively utilised by host microorganisms conferring a health benefit" [223] and these can be added to the diet as supplements, while probiotics are defined as "live microorganisms which when administered in adequate amounts, confer a health benefit on the host" [224]. Probiotics can be taken as supplements or via probiotic-enriched foods, such as fermented milks and yoghurts [225].

Approximately, 30% of individuals over 50 years of age are said to fall short of several nutrients in their diet including fibre, B vitamins, folic acid, vitamin D and iron [226, 227]. Dietary fibre intake in the elderly should directly affect SCFA levels. This was confirmed in a study involving 32 institu-

tionalised elderly subjects ranging in age from 76 to 95 years, which investigated the impact of different dietary fibres on fecal SCFA concentrations [228]. Indeed, potato intake was directly associated with SCFA concentrations, apple intake associated with propionate, cellulose associated with acetate and butyrate, and insoluble pectin partly associated with propionate. Therefore, ensuring the aging population consumes adequate fibre in the diet should go some way to preventing/delaying the potentially detrimental gut microbiota modifications observed in the elderly. However, changes in dentition etc. may preclude older individuals from consuming fibre-rich foods. In this case, prebiotics are a viable alternative.

Several studies have investigated the impact of prebiotic supplementation on the elderly gut microbiota as well as other parameters of host health, and the results have revealed the ability of prebiotics to increase beneficial gut bacteria, and improve immune parameters as well as certain clinical indices of old age [229]. For example, daily consumption of 5.5 g of the prebiotic galactooligosaccharides (GOS) by elderly volunteers for four weeks in a randomized, double-blind, placebo-controlled, crossover study significantly increased *Bifidobacterium* and *Bacteroides* and generated immune alterations [230]. Consumption of 4 g of GOS by men and women aged 50 years and older twice daily for three weeks in a randomized, cross-over study significantly increased fecal *Bifidobacterium* levels [231]. Continuous culture systems of fecal inocula from three volunteers representing different large bowel regions revealed increased butyrate levels in the vessel similar to the proximal colon. Daily consumption of the prebiotic fructooligosaccharides (FOS) also generated a bifidogenic effect with fecal bifidobacteria counts increased [232]. Following a randomized, double-blind, clinical trial to investigate the impact of prebiotic supplementation on frailty syndrome in the elderly, it has been reported that the prebiotic in question significantly improved exhaustion and handgrip strength following 13 weeks of daily consumption [233]. A more recent placebo-controlled, randomized, double-blind study reported that prebiotic supplementation in the elderly significantly reduced frailty levels, especially in individuals with higher frailty levels to begin with [234].

In the elderly, probiotic supplementation has been shown to increase beneficial bacteria in the gut and improve certain immunity parameters [229]. For example, daily consumption of *Bifidobacterium lactis* HN019 by elderly subjects (68 to 84 years

of age) improved immunity [235]. Consumption of a probiotic mixture consisting of strains of *Lactobacillus gasseri*, *Bifidobacterium longum*, and *Bifidobacterium bifidum* by elderly volunteers in a randomized, double-blind, placebo-controlled cross-over study, not only significantly increased IL-10 concentrations, but significantly increased beneficial bacteria including bifidobacteria, lactic acid bacteria and the butyrate producer *F. prausnitzii* [236]. Consumption of a probiotic-containing cheese in a randomized, controlled, cross-over clinical trial was associated with lower counts of *C. difficile* in the elderly volunteers [237]. However, a high dose of probiotics proved ineffective to prevent antibiotic-associated diarrhoea in older people admitted to hospital in a multicentre, randomized, double-blind, placebo-controlled, parallel-arm trial [238]; despite this, a trend for reduced *C. difficile* diarrhoea was noted in the probiotic arm. A 2019 retrospective, case-control study by Nagamine et al. also reported that administration of a combination of probiotics has potential to reduce the risk of CDI in patients undergoing proximal femoral fracture surgery [239]. A probiotic-containing biscuit consumed daily for one month by elderly individuals in a randomized, double-blind, plaebo-controlled trial reverted age-related increases in a number of opportunistic pathogens including *C. difficile* [240].

Probiotics have also generated promising results in individuals suffering from cognitive decline. A double-blind, placebo-controlled clinical trial reported that *B. breve* A1 supplementation resulted in a significant improvement in cognitive function in older individuals with suspected mild cognitive impairment [241]. Synbiotics consist of probiotics and prebiotics which act synergistically on the host. Such a mixture was shown to improve constipation in individuals with Parkinson's disease in a randomized, double-blind, placebo-controlled trial [242]. Consumption of a multi-strain probiotic (Hexbio®) by Parkinson's disease individuals in a randomized, controlled trial was shown to improve bowel opening frequency and whole gut transit time [243].

4. Discussion

The trajectories of the gut microbiota at infancy and old age, particularly in terms of α-diversity, move in opposite directions. As the infant grows, the α-diversity of the gut microbiota increases until it resembles that of an adult. In old age, α-diversity is seen to decline. At a certain point, the ratios of Firmicutes to Bacteroidetes in infants and the elderly almost converge (0.4 and 0.6, respectively) [169]. But while the healthy infant gut microbiota goes on to acquire rich species diversity with an abundance of commensals and beneficial bacteria, the elderly gut microbiota is marked by reduced microbiota diversity, loss of beneficial members, and higher levels of pathogens/"undesirables". Many of these alterations have been linked with dietary changes in old age [170, 192], thus it has proven difficult to find associations between aging itself and specific microbiota changes [192]. But the impact of other factors that may inflict elderly individuals including loneliness and depression should also be investigated in terms of gut microbiota changes.

The acquisition of the healthy infant gut microbiota involves a delicate assembly of timely species where full-term, vaginally born, breast-fed infants are recognised as having the most favourable opportunities to acquire a microbiota that optimally benefits its host. This delicate assembly is thus compromised by several factors including gestational age at birth, birth mode, antibiotics, diet and prenatal stress. Indeed, preterm birth, CS delivery, antibiotic intervention, formula-feeding, and maternal stress during pregnancy, can each shape a microbiota that is less abundant in beneficial microbes, lacking in microbial diversity with an increased abundance of potentially pathogenic bacteria, alterations that resemble the ageing microbiota.

In the elderly, inevitable physiological changes associated with old age, lifestyle changes, illness and medication contribute to the altered microbiota composition and functionality. And in both age-groups, the aberrant microbiota alterations have been associated with diseases and conditions, even though the majority of these studies do not prove a causal effect. Indeed, association studies, while essential in cataloguing particular microbiota profiles and physiological signals, lack the specificity required to pin down the exact microbes responsible for these effects. But the reported associations are necessary to guide the way for causal studies in the highly complex biological system of a holobiont (host and microbiota together). In children, atopic diseases, neuropsychiatric disorders, and obesity have been linked with sub-optimal microbiota structures. The microbiota alterations in old age have been linked with degenerative conditions such as Alzheimer's and Parkinson's diseases and increased risk of infection with pathogens, especially *C. difficile*.

In infants, time and dietary changes have seen the altered microbiota 'catch up' with its healthy counterpart although the full consequences of sub-optimal microbiota succession have yet to be delineated. In the elderly, dietary interventions, probiotics and prebiotics have generated promising results in terms of reversing age-related microbiota changes, which have manifested in improved clinical parameters such as reduced frailty and improved cognitive function, though it must be emphasized that evidence for causality is few and far between. As such, defining the links between association and causality in gut microbiota research at these delicate stages of life should enable scientists to develop strategic interventions that revert or prevent aberrant microbiota alterations, and thus provide a layer of protection against age-associated diseases.

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Conflicts of interest

The authors declare no conflict of interest.

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