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COMPOSITIONAL DYNAMICS OF THE HUMAN INTESTINAL MICROBIOTA WITH AGING: IMPLICATIONS FOR HEALTH

B. LAKSHMINARAYANAN[1,2], C. STANTON[1,3], P.W. O'TOOLE[2,3], R.P. ROSS[1,3]

1. Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland; 2. Department of Microbiology, University College, Cork, Ireland; 3. Alimentary Pharmabiotic Centre, University College, Cork, Ireland. Corresponding author: R. Paul Ross, Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland. Email: paul.ross@teagasc.ie, Tel: 00353 (0)25 42229, Fax: 00353 (0)25 42340.

Abstract: The human gut contains trillions of microbes which form an essential part of the complex ecosystem of the host. This microbiota is relatively stable throughout adult life, but may fluctuate over time with aging and disease. The gut microbiota serves a number of functions including roles in energy provision, nutrition and also in the maintenance of host health such as protection against pathogens. This review summarizes the age-related changes in the microbiota of the gastrointestinal tract (GIT) and the link between the gut microbiota in health and disease. Understanding the composition and function of the gut microbiota along with the changes it undergoes overtime should aid the design of novel therapeutic strategies to counteract such alterations. These strategies include probiotic and prebiotic preparations as well as targeted nutrients, designed to enrich the gut microbiota of the aging population.

Key words: Gastrointestinal tract, microbiota, aging, host health.

Introduction

The human gut is home to a complex ecological system harboring trillions of bacteria which vary according to their location in the gastrointestinal tract (GIT). This ecological niche provides an excellent site for complex interactions between the host and its microbial inhabitants. Molecular methods indicate that there are approximately 1,100 prevalent bacterial species in the intestine with as many as 10^{12}-10^{14} microorganisms present, of which 70-80 % remain uncultured (1-6). In its entirety, the human intestinal microbiota is estimated to contain 150-fold more genes than its own host's genome (7). The mutualistic relationship between the gut microbiota and the human host contributes to the maintenance of health and well-being in a number of ways. These include protecting the host against colonization of pathogenic bacteria, metabolizing complex carbohydrates, producing bioactive peptides and lipids, synthesizing vitamins and hormones and stimulating the immune system (8-11). Reduced proportions of protective bacteria such as lactobacilli and bifidobacteria in the gut have been linked to the severity of disorders such as inflammatory bowel diseases or colonic inflammation (12, 13). This review summarizes the age-related changes that occur in the human GIT and makes the link between the bacterial communities and human health in an effort to understand the relationship between the two.

Changes and succession of human gut microbiota from infancy to elderly

The establishment of a stable microbial population involves complex processes such as bacterial succession and host-microbe interactions (14, 15). The colonization of the microbial population in the human gut begins immediately after birth, and results in an unstable composition in infants, which then undergoes marked changes until it develops into a relatively stable community in the adult (15, 16). The initial bacterial population of an infant depends on a number of factors including mode of delivery, feeding type, antibiotic usage and the surrounding environment (17, 18). The microbiota of infants delivered by Caesarean (C-) section have been reported to harbor relatively lower numbers of bifidobacteria and higher numbers of Clostridium difficile and Escherichia coli than infants delivered vaginally (17). Colonization is also delayed in infants delivered by C-section when compared to vaginally delivered infants. Not surprisingly, bacteria involved in the initial colonization of the gut of vaginally delivered infants were predominated by lactobacilli and Prevotella species which have been shown to reflect their mother's faecal and vaginal bacteria (19). Antibiotic administration has also been shown to influence the intestinal colonization in infants, whereby antibiotic treated infants have lower numbers of enterococci and lactic acid bacteria (17).

The diet of a newborn is one of the major factors affecting the intestinal colonization and studies using molecular techniques showed that the gut microbiota of the breast-fed infant is predominantly comprised of E. coli, streptococci and bifidobacteria and the gut microbiota of formula-fed babies is predominantly comprised of Bacteroides, clostridia and enterobacteria (17, 20). Changes to the intestinal microbiota then occur after the introduction of solid food leading to a remarkably complex and stable gut microbial population (20). The impact of diet on the gut microbiota of European children (1 to 6 years of age) consuming a Western diet was compared to the gut microbiota of children from a rural African village consuming a diet rich in fiber (21). The Western diet was associated with reduced microbiota diversity, higher

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proportions of Firmicutes and Proteobacteria and lower proportions of Bacteroidetes and Actinobacteria compared with the African diet.

After several years of development into the typical adult microbiota, the species composition of the bacterial community remains relatively stable (14) but can be transiently altered by some extrinsic factors (4, 22, 23). While both anaerobic and aerobic bacteria inhabit the GIT (3), the majority of genera are composed of anaerobic bacteria such as bifidobacteria, lactobacilli, clostridia and Bacteroides (2). Recent 16S rRNA gene compositional studies had shown that the diversity of the healthy adult intestinal microbiota was mostly distributed among the phyla Firmicutes and Bacteroidetes followed by Actinobacteria, Proteobacteria and others (24-26).

The composition of the intestinal microbiota in elderly subjects changes from that of healthy adults due to factors related to aging, including nutritional behaviour, lifestyle modification, mobility, dentition, stress and reduced intestinal functionality (27, 28). Alterations in the gut morphology and physiology also occur during the aging process. These include difficulty in swallowing, decreased gastrointestinal motility, prolongation of gastric emptying and decline in splanchnic blood flow (28, 29). The microbial composition in elderly subjects was typically characterized by a decreased diversity of bacterial species, including the levels of beneficial bacteria (30, 31). Assessment of the gut microbiota of the elderly revealed lower levels of bifidobacteria and lactobacilli and higher levels of Enterobacteriaceae and clostridia when compared to younger adults (32, 33). This age related breakdown in the balance between the beneficial and detrimental bacteria has been associated with an increase in many intestinal inflammatory disorders. Indeed, a healthy intestinal microbial population supports human longevity. A recent Italian study supports the hypothesis that complex remodeling of metabolism and gut microbiota functionality are the key regulatory processes that marks longevity in humans (34).

Changes in the intestinal microbiota of the elderly may derive from dietary changes, changes in immune response, hospitalization, prolonged intestinal transit times, lack of physical activity, recurrent infections and frequent use of antibiotics and other medications (31, 35-38). The most dramatic changes in the composition of the intestinal microbiota in the elderly include reduction in numbers and species diversity of Bacteroides and bifidobacteria and increased abundance of Ruminococcus, enterobacteria and lactobacilli (30, 32, 39, 40). Interestingly, a high level of total presumptive aerobes were recorded in the gut microbiota of elderly when compared to the younger adult (41). Isolation of potential pathogens such as Clostridium perfringens and C. difficile (causative agent of C. difficile associated diarrhoea (CDAD)) from elderly subjects has also been reported (33, 42).

The gut microbiota of the elderly has been reported to show different microbial composition and greater inter-individual variations compared to younger adults (31, 43). A Finnish study showed that members of Firmicutes, Bacteroidetes and Actinobacteria were reported to be more abundant in the intestinal microbiota of younger adults compared to the elderly (44). Similarly, Claesson *et al* have also reported that the Firmicutes/Bacteroidetes ratio was lower in elderly people when compared to young adults (31). However, Biagi *et al* did not find significant differences among the Firmicutes/Bacteroidetes ratios of Italian centenarians, elderly and young adults (36).

Age related changes in the intestinal microbial composition have also been shown to be location/geography-dependent (45). The intestinal abundance of Clostridium cluster XIVa has been reported to decrease in elderly Japanese, Italian and Finnish people (44-46), whereas an inverse trend was noted in elderly German people (45). An increase in the relative abundance of Bacteroides group was also observed in the Austrian elderly (47), whereas decreased proportions of Bacteroides group were reported in elderly Italian people (45). Benno *et al* reported that elderly Asian subjects had lower levels of bifidobacteria and higher levels of lactobacilli and Enterobacteriaceae (48).

One of the well documented aging effects is the decrease in the prevalence of Faecalibacterium prausnitzii, a member of Clostridium cluster IV, as found in the elderly Austrian and Italian subjects (36, 45, 47). Analysis of the faecal microbiota of IBD patients previously showed reduced numbers of Faecalibacterium prausnitzii (49, 50). A decrease in the Faecalibacterium population is also associated with hospitalization and antibiotic therapy (35). The gut ecosystem is dynamic. In order to fully understand the human biology in respect to the human microbiota, it is crucial to understand the impact of the gut microbiota on the host health. Summarization of the earlier studies on elderly has reported a large inter individual variations among older subjects (2, 31, 36). Any deviation in the diversity of the intestinal microbiota profile seems to be one of the indicators of aging process and have been reported to be associated with inflammatory disorders. Overall, the maintenance of microbial homeostasis in the GI tract is essential for healthy ageing.

Pathogenesis and alterations in the gut microbiome during intestinal dysbiosis

The gut microbiota plays an important role in both human health and disease (51) and a vital role in the maturation of host immunity and defence against enteropathogens (52-55). The stability that coexists between the host and the gut microbiota has a profound impact on human health, relating to ageing and longevity; as alterations to the intestinal microbiota composition (sometimes referred to as “dysbiosis”) (56) are associated with pathogenesis of many diseases, including liver disease (57), Inflammatory Bowel Disease (IBD) (58), cancer (59, 60), malnutrition (61, 62), diabetes (25), imbalance in the regulation of body weight (63, 64) and several chronic conditions (including obesity, frailty, sarcopenia and cognitive

impairment). In addition, changes in the diet and lifestyles are also involved in disease manifestation. The changes of gut microbial communities during these diseased states are discussed in detail.

Bowel Diseases

The absence or low levels of bifidobacteria in the intestine of the elderly may have health consequences for the host, such as affecting the immune system or colonization resistance in the bowel (39). Irritable Bowel Syndrome (IBS) is an intestinal disorder that is characterized by recurrent intestinal pain, diarrhoea and / or constipation with other non-colonic symptoms, which include features such as constant backache, urinary symptoms, non-cardiac chest pain and fibromyalgia (65, 66). Abdominal bloating, passage of mucus and faecal urgency are the most common features of IBS and are often rated as the worst symptoms by IBS patients (66). Several studies have implicated the role of the gut microbiota in immune changes in IBS (67-71).

Crohn's disease (CD) and Ulcerative Colitis (UC), are collectively known as chronic inflammatory bowel disease (IBD). Diagnostic methods for the elderly are the same as for other age groups. It would be of benefit to elderly IBD patients to receive proper diagnosis as misdiagnosis in the initial stages of IBD are common, which can be explained by the number of clinical conditions that mimic IBD, particularly ischemic colitis or colitis associated with diverticular disease (66, 72). The IBD pathogenesis includes impairment of mucosal barrier function, superficial or deep ulcers, rectal bleeding, higher rates of anemia, electrolyte disturbance and malnutrition; which are more common in older patients compared to younger patients (73) .

Crohn's disease can involve any part of the gastrointestinal tract from the mouth to the anus; however, the colon seems to be most affected in the elderly (74). Recurrence after surgery is usually common in CD. Depending on the localization and extent of disease, the clinical picture can be variable and the patients may present with abdominal pain, tiredness, fever, anemia, weight loss and symptoms of bowel obstruction (74). Although the initial mortality rate in elderly CD patients is similar to that in younger patients, the elderly do show a higher mortality rate a few years after the onset of CD (72). Ulcerative colitis generally affects the rectum and colon to a varying extent, and can be cured by surgery. The pathogenesis involves impairment of mucosal barrier function and superficial or deep ulcers. Common features include bloody diarrhea, passage of mucous, abdominal pain and sometimes fever (74). Pouchitis is a common complication occurring in mostly in the first 10 years of disease (74).

In general, a large reduction in the microbial diversity was reported in patients with CD, including a decline in proportions of Bacteroidetes and Firmicutes (49, 58, 75). Similarly, studies observed the disruption of the commensal microbiota in UC, including a decreased proportion of bifidobacteria (76), reduction in the proportions of members belonging to the phyla Bacteroidetes and Firmicutes (58), decreased levels of F. prausnitzii (50), along with increased Peptostreptococcus species numbers compared to healthy individuals (76) and the occurrence of C. difficile during relapse of UC (77).

Antibiotic-associated diarrhea (AAD) occurs in 5-30 % of patients receiving antibiotic therapy (78). The common risk factors include hospitalization, health of the host and exposure to pathogens, such as toxigenic C. difficile, a spore-forming gram-positive toxigenic anaerobic bacterium that is commonly associated with patients in hospitals and long-term care facilities (42). The overgrowth of this bacterial species in the intestinal tract disrupts the normal microbiota leading to gastrointestinal illness, decreased digestion, infections including sepsis, perforation of the colon, pseudo membranous colitis and sometimes death (79-82).

Colon Cancer

The most common bowel cancer is colon or colorectal cancer (CRC),which is the second largest cause of cancer deaths in Western countries (83). Colorectal cancer is a major source of morbidity and mortality in the elderly population with advancing age (84). More likely both colon and rectal cancer in the older adults are diagnosed at an advanced stage, which may contribute to a decreased survival among this population (85). The standard treatment for advanced colorectal cancer includes chemotherapy or combination of therapies, during which the patients experience diarrhoea, vomiting, especially neurotoxicity, which can cause functional decline and poorer quality of life (86). It has been suggested that both dietary supplements and intestinal bacteria may play a role in the initiation of colon cancer through production of carcinogens (87). Results from a human study indicated that Bacteroides species (B. vulgatus and B. stercoris) are associated with a higher risk of colon cancer (87) and especially with a high-fat diet; mainly because fat stimulates bile flow, which in turn is thought to stimulate the growth of Bacteroides species. Other studies have supported a similar finding with members of Bacteroides species being shown to convert bile to metabolites and fecapentaenes, which are considered co-carcinogenic or mutagenic (88). Studies have observed changes in the bacterial species of individuals that are associated with CRC. Scanlan et al. observed that colon cancer patients exhibit a significantly increased diversity and reduced stability of clostridia compared to healthy individuals (89). Interestingly, Sobhani *et al* demonstrated (using qPCR) that all the bacterial species belonging to the Bacteroides/Prevotella group were more abundant in colon rectal cancer patients than in normal individuals (90).

Obesity

The prevalence of obesity is increasing in all age groups. In general, obese individuals with a BMI ≥30.0 are considered to have a higher mortality risk than those who are considered overweight (BMI 25.0–29.9) (91). In older adults, obesity can cause serious medical complications, which can lead to considerable cardiovascular morbidity, osteoarthritis, hypertension and impaired quality of life (91, 92). Increased BMI is associated with an increased risk of knee osteoarthritis and higher rates of certain types of cancers including breast, colon, renal and cervical in older population (91). Obesity increases the risk of frailty and sarcopenia in older adults (92). Obesity is also a recognized contributing factor to urinary incontinence in older population, especially in older women (93). Increased abdominal obesity in older people can cause metabolic changes which lead to insulin resistance, a key risk factor in the development of type II diabetes (92). Aging causes a progressive decrease in renal function and obesity is considered a significant risk factor for end-stage renal disease (92). Increased chest wall stiffness in obese adults may contribute to difficulty in breathing (93).

On the whole, obesity is a complex and increasingly alarming health issue in humans so it is not surprising that obesity-related gut microbiota studies have received a lot of attention in recent years. Ley and co-workers characterized the faecal microbiota of 12 obese individuals consuming low calorie diets and observed significantly higher proportions of Firmicutes than Bacteroidetes when compared to the faecal microbiota of lean individuals (63). This was in agreement with the findings by Turnbaugh *et al*, where they observed individuals with high BMI have decreased proportion of Bacteroidetes compared to lean individuals (64). In contrast, a study by Schwiertz *et al* showed that the obese individuals showed higher proportions of Bacteroidetes compared to lean counterparts. They also observed that the concentrations of SCFAs were higher in the obese individuals than the lean individuals. The study concluded that rather than the Firmicutes/Bacteroidetes ratio, the metabolism of SCFAs might play a considerable role in the development of obesity (94). However, these specific changes remain controversial as other studies found no evidence that the proportions of Bacteroidetes and Firmicutes are associated with obesity (95, 96).

Diabetes

Type-2 diabetes is a metabolic disease associated with insulin resistance and compositional changes in the intestinal microbiota. Aging is associated with a reduced ability to metabolize glucose from food, which makes type-2 diabetes one of the most prevalent conditions in elderly (97). It is considered as the sixth-leading cause of death among the aged population, as it associated with increased risk of multiple medical conditions in older adults including cardiovascular events, fatal hypoglycemia, dementia and Alzheimer's disease (98, 99). Hypoglycemia is linked to cognitive impairment and incidence of dementia among older population (100). Age related insulin resistance appears to be associated with oral and dental issues, sarcopenia, under nutrition due to altered taste and smell, swallowing difficulties, hearing impairment (100).

Larsen and coworkers observed that the proportion of Firmicutes and Clostridium species were significantly reduced in diabetic patients compared to non-diabetic subjects (25). In addition, the Bacteroidetes/Firmicutes ratio and the ratio of the Bacteroides-Prevotella group to C. coccoides-Escherichia rectale group demonstrated a positive correlation with plasma glucose concentration (25). Recently, a study highlighted that diabetic individuals were characterized by a decrease in the abundance of butyrate-producing bacteria and an increase in several opportunistic pathogens, as well as an increase in functions relating to oxidative stress response and sulphate reduction (101).

Other diseases

Intestinal permeability and increased bacterial translocation (migration of bacteria from the intestinal lumen to mesenteric lymph nodes or other extra-intestinal sites) especially by E. coli, Klebsiella, enterococci and other streptococci species are a common complication in patients with cirrhosis (102).Other frequent complications observed in these patients include spontaneous bacterial peritonitis (an infection of ascitic fluid), urinary tract infections, respiratory tract sepsis (pneumonia and spontaneous bacterial empyema) and bacteremia (102, 103).

Celiac disease is a chronic inflammatory disorder of the small intestine, where patients show permanent intolerance to cereal gluten proteins. The only therapy is adherence to a strict lifelong gluten-free diet. Recent studies on healthy adults consuming a gluten-free diet (GFD) have shown reductions in beneficial gut bacteria populations (including bifidobacteria and lactobacilli) and corresponding alterations in host immunity (104). Similar results were seen in a preliminary study, where subjects on a GFD showed decreased populations of beneficial bacteria, while populations of E. coli and total Enterobacteriaceae increased. In addition, production of pro-inflammatory cytokines and chemokines (TNFα, IFNγ and IL-8) and anti-inflammatory cytokines (IL-10) were remarkably reduced as a consequence of the GFD (105).

Using a combination of flow cytometry, 16S rRNA hybridization and DNA-staining, Vaahtovuo and colleagues compared the composition of the intestinal microbiota of patients with early rheumatoid arthritis (RA) and fibromyalgia (FM). The RA patients had significantly lower levels of bifidobacteria, members of the Bacteroides-Porphyromonas-Prevotella group, B. fragilis subgroup and Eubacterium rectale–C. coccoides group when compared to patients with FM (106).

Although it is clear from the studies described above that the

intestinal microbiota are likely to be involved in various diseases but is difficult to draw definite conclusions on the role of any particular bacterial groups. Further well-designed studies are required to examine the disease progression and outline the link between the gut microbiota and various health measures.

Geriatric syndromes

It has been suggested that decreased levels of SCFA production by the gut microbiota of older people may contribute to the onset of some distinctive conditions such as frailty, malnutrition, diabetes and sarcopenia (107). Frailty can be generally defined as state of decreasing reserves to functions in the elderly such as mobility, physical fitness, comorbidity, hearing, weight loss and vision and stress factors (108). Frailty in the elderly is associated with poor health conditions, less cognitive function and greater mortality (109). It is typically a multisystem impairment and its prevalence advances with age. Serum levels of IL-6 and CRP have shown to be elevated in the community-dwelling frail older adults (110).

Cognitive decline occurs at varying degrees in older adults (111). The basic cognitive functions most affected by age are attention and memory. The other factors associated with cognitive aging include hypertension, diabetes mellitus, and dietary factors such as vitamin D deficiency (112, 113). The study conducted by Barnes *et al* included women aged 65 and above being assessed for cognitive function of a 15 year period. About 9 % maintained optimal cognitive function, 58 % showed minor decline and 33 % experienced major decline. The group with optimal cognitive function was shown to have access to a positive social network, lack of diabetes, lack of hypertension and have moderate alcohol consumption (113). Maintaining a balanced diet, regular exercising have been considered as protective factors for cognitive decline related to aging (112).

Sarcopenia is an important syndrome characterized by progressive loss of skeletal muscle mass and strength, which occurs as a consequence of aging and is usually associated with decreased motility and poor physical inactivity (114). IL-6 is shown to be strongly associated with adverse physiologic effects such as sarcopenia, loss of weight and increased susceptibility to infections (110). Nutritional supplementation, in particular dietary protein intake, is relevant for the maintenance of this condition (114).

Immune disorders

The survival of the host against the effects of pathogenic microbes depends on the protective immune system. Aging is a complex process that negatively impacts the functionality of the immune system resulting in a low-grade inflammatory status often referred to as inflamm-aging (115). This causes a persistent inflammation of the intestinal mucosa, increased susceptibility to diseases such as type-2 diabetes, sarcopenia, arthritis, osteoporosis and Alzheimer's diseases (116). The high prevalence of cardiovascular risk factors and morbidity increases with age and significantly contributes to the higher circulating levels of proinflammatory cytokines in older people (117). Increased levels of proinflammatory cytokines including interleukin-6 (IL-6) and C-reactive protein (CRP) can influence Alzheimer's disease and mortality in the elderly (118). It has been shown that higher production of IL-10 has a significant influence in the attainment of longevity (119). Immunosenescence (deterioration of the immune system) may lead to significant biological changes in the elderly population (115, 120, 121), which are detailed in Table 1.

Table 1
Age-related immune changes

Affected cell type	Change	Reference
Phagocytes	Reduced in number of cells	(122, 210)
B-lymphocytes	Reduced antibody production	(120, 123)
Lymphokines	Declined production of IL-2	(120)
Antigens*	Declined proliferation of T-cells	(121, 123)
Memory cells	Decreased functionality	(120)
Cytokines profile	Increased production of proinflammatory cytokines	(210)

*response to stimulation of antigens

The aging process also affects innate immunity, with a reduction in the levels of natural killer (NK) cells and phagocytes making the elderly more prone to infections (122, 123). In this respect, the composition of the commensal microbiota may be disrupted which may favour the growth of opportunistic pathogens (pathobionts) (124). Aging has been shown to be associated with reduced antigen-specific IgA antibody responses (125), involuntary weight loss (126), diminished ability to generate high affinity antibodies after immunization (120), reduced secretion of IL-7, an essential cytokine for development of lymphocyte responses and altered composition/type of lymphocytes in the spleen and lymph nodes (121).

In the healthy human intestine, a fine homeostatic balance exists between the immune cells and the gut microbes (127). The interaction of an altered microbiota could contribute to maintaining a low-grade, systemic inflammation (128). The aging gut microbiota enriched in facultative anaerobes including streptococci, staphylococci, enterococci, and enterobacteria (often classified as pathobionts) and depleted in immune modulatory species such as Clostridium clusters IV and XIVa are hypothesized to contribute to the development of an overall pro-inflammatory profile (107).

In this context, in a recent study a comparison of aging microbiota between community-dwelling and long-stay individuals showed that the microbiota of people in a long-stay care environment had a high proportion of Bacteroidetes,

whereas individuals living in the community had a high level of Firmicutes (37). Levels of clinical markers such as IL-6, IL-8 and C-reactive protein (CRP) were significantly high in long-stay subjects than in community dwellers confirming the status of systemic inflammation. In addition, metabolome data suggested that changes in gut microbial populations are responsible for an altered production of short-chain fatty acids, which was more pronounced in community group than in long-stay subjects. Taken together, the major trends in the microbiota that separated healthy community subjects from less healthy long-term care correlated with increased frailty, inflammation and other clinical markers (37).

Fermentation of non-digestible prebiotic substances by certain anaerobic bacteria accounts for the production of short chain fatty acids (SCFA), in particular butyrate, acetate and propionate. In aging people, reduction of butyrate levels was correlated with decreased amounts of F. prausnitzii and bacteria belonging to the E. rectale/Roseburia group, which are butyrate producers. Therefore, the decrease of anti-inflammatory SCFA-producing bacteria may lead to an easier entry of pathogens into the intestinal mucosa, especially Enterobacteriaceae, which has been positively correlated with the serum levels of two proinflammatory cytokines in old Italian people (36). It has also been demonstrated that the level of Bifidobacterium species is negatively correlated with the serum levels of the pro-inflammatory cytokine TNF-α and the regulatory cytokine IL-10, indicating that the modulation of the faecal Bifidobacterium microbial population may represent a mean of influencing the inflammatory responses (129). Although, it seems plausible that the intestinal microbiota play an important role in regulating the inflammatory and immune responses, more studies are required for a better understanding of the intricate relationship between the human gut microbiota and the gut immune cells in the elderly.

Influence of diet on the gut microbiota

Dietary habits are considered as one of the major factors that influence the intestinal microbiota composition in human studies (21, 37) and animal models (130) and this review provides a link to the changes in the gut microbiome of elderly and other subjects based on diet. The study by De Filippo et al indicated that both the composition and the fermentation pattern of the gut microbes were influenced by diets rich in dietary fiber. The African children had significantly higher levels of Actinobacteria and Bacteroidetes, which possess enzymes that encode for hydrolysis of complex polysaccharides. Furthermore, higher abundance of Enterobacteriaceae, Prevotella and Xylanibacter species that possess enzymes required for cellulose hydrolysis were found in the African children which were absent from the European population (21). The authors postulated that the microbiota of the rural African population allow them to maximize energy extraction from the consumed fiber rich diet.

A more recent molecular study by our ELDERMET consortium compared the dietary pattern and faecal microbiota of elderly community-dwelling subjects (consuming diet rich in fibre but low in fat) and elderly subjects in long-term residential care (consuming diet low in fibre but rich in fat). Those in long-term care had a less diverse microbiota with a

Table 2
Mode of action of antibiotics commonly prescribed to the elderly

Antibiotic	Interferes with	Target site	References
Pencillins, cephalosporins, carbapenems and glycopeptides	Cell wall synthesis	Peptidoglycan layer	(211)
Fluoroquinolone	Nucleic acid synthesis	DNA gyrase enzyme	(212)
Rifampicin		RNA polymerase	(213)
Macrolides	Protein synthesis	Ribosomal subunits	(212)
Sulfonamides	Folic acid synthesis	Folic acid	(214)
Aminoglycosides	Elongation process	Peptidoglycan layer	(215)

Table 3
Side effects of antibiotic therapy in the elderly

Antibiotic	Side effects
Beta-lactams: penicillins, cephalosporins and carbapenems	Bronchitis, diarrhoea and rashes
Fluoroquinolones: ciprofloxacin, ofloxacin andgatifloxacin	Nausea, vomiting and seizures
Rifampicin	Drug interactions
Macrolides: erythromycin, clarithromycin and azithromycin	Gastrointestinal intolerance
Trimethoprim-Sulfamethoxazole	Rashes and drug induced fever
Aminoglycosides: amikacin, streptomycin and kanamycin	Nephrotoxicity and ototoxicity

higher proportion of the phylum Bacteroidetes, while the microbiota of community subjects exhibited a far greater level of diversity with a higher proportion of phylum Firmicutes. The microbiota of the community-dwelling subjects had an abundance of bacteria from the genus Prevotella, similar to the rural Burkina Faso children (21), confirming the association between carbohydrate rich diet and the genus Prevotella (37). Notably, the microbiota of elderly people in long-term care was significantly less diverse and a loss of the community-associated microbiota correlated with increased frailty and progression of disease in older people and hence indicates the relationship between diet, gut microbiota and the health status among the elderly (37).

In the same context, van Tongeren and colleagues assessed the faecal microbiota composition of 23 elderly volunteers (median age 86 yrs) living in the same environment and receiving similar diet. Based on the Groningen Frailty Indicator, the subjects were stratified into two groups, 13 subjects with low frailty score and 10 subjects with high frailty score. More differences in fecal microbiota composition were observed between elderly subjects with low and high frailty scores. Statistically significant reduction was seen in the number of lactobacilli (26-fold), F. prausnitzii (4-fold) and Bacteroides/Prevotella group (3-fold) were seen in the high frailty volunteers. In contrast, members of Enterobacteriaceae showed a 10-fold increase in high frailty group (131).

Diet and dietary patterns fluctuate over time, especially in older life, and can be modulated by mobility, appetite, taste and smell. Malnutrition is considered as one of the major factors leading to reduced immune responses in all ages of people (132). Both amino acid and protein deficiencies has been associated with impaired cellular immunity and decreased antibody response respectively (132). Gastric atrophy or Helicobacter pylori infection in the elderly has been associated with the malabsorption of vitamin B12 (133). A nutritionally-imbalanced diet may result in reduced mastication and taste sensation (134), coupled with dysphagia (135). Constipation, a common problem with advancing age, may be associated with inappropriate diet, depression, decreased physical activity resulting from chronic diseases and multiple medications (136, 137). Constipation maybe readily improved by laxative intake or by non-pharmological measures including increased fluid and fiber intake, consumption of legumes, fruits and vegetables (137).

Diet appears to be the main environmental modifier for microbiota composition. It has been shown by Wu *et al* that dietary protein and animal fat favour the growth of Bacteroides while carbohydrate is associated with increase in Prevotella (138). Diets rich in fat result in a phylogenetic shift in the intestinal microbiome associated with obesity (139). Diets low in vegetables but rich in fat and processed meat have been shown to be associate with increased faecal excretion of N-nitroso compounds, a promoter of colon cancer (140). A low fiber diet and low levels of calcium and selenium may also relate to the incidence of colonic diseases (141).

Diet influences metabolism of the bacterial species and specific items in the diet may have selective effects on the microbiota, which may be important for host health, irrespective of their age. It is been suggested that reducing the intake of food items rich in sulphur containing amino acids (such as cheese and eggs) from the diet of UC patients resulted in substantial therapeutic benefits (142). The ingestion of sulphur-rich food encourages the production of sulphide, which can damage the colonic mucosa by inhibiting butyrate oxidation- this is a characteristic defect in UC patients (142). Dietary protein may also reach the colon undigested and is then fermented by the gut microbiota to produce end products including ammonia, indoles, cresol and phenols, which favour the growth of malignant cells and may play a role in the etiology of bladder and bowel cancer (143). In contrast, a high-fiber diet was shown to lower urinary phenol and cresol concentrations in humans (144).

Kruis et al observed that a high-sugar diet (165 grams/day) prolonged the gut transit time and significantly increased the fermentative colonic bacterial activity and concentration of intestinal bile acids in the human colon (145). De Palma and co-workers observed that GFD could contribute to a reduction in pro-inflammatory signals (TNF-α, interferon-γ, IL-10 and IL-8) which was associated with modifications in the microbiota composition. These include decreased levels of bifidobacteria and lactobacilli and increased levels of E. coli (104). Based on the available data, the compositional differences in the intestinal microbiota are demonstrable among people living on different diets. These diet associated changes in the composition and function of the intestinal microbiota may in turn provoke immunosenescence. Albeit attempts to change the intestinal microbiota population by varying the diet have been successful in animal models, there is a relative paucity of human dietary intervention studies, which awaits further investigation.

Effect of antibiotic therapy on the intestinal microbiota of elderly subjects

While the development of antibiotics has lengthened the lifespan of humans, their use has led to a pervasive impact on the gut microbiota for a long period of time, sometimes up to one or two years, depending on the antibiotics used (146). Antibiotic therapy for the main part drastically alters the composition of the microbiota and can provide a pathway for the proliferation of pathogens. The intensity of the impact on the commensal bacteria depends on the antibiotic type, dosage, route and duration of therapy (147).

Assessing the temporal changes in the gut microbiota due to administration of broad spectrum antibiotics is one of the emerging fields of research and is a target for highly sophisticated molecular techniques. Several studies have demonstrated the long-term ecological impact on the gut

microbiota of antibiotic therapy (148, 149). Dethlefsen *et al* demonstrated that a short course of the antibiotic ciprofloxacin (member of the Fluoroquinolones) reduced the diversity and abundance of the intestinal microbiota with significant effect on about one-third of the bacterial taxa (26). Although the majority of the bacterial community returned to the pretreatment state within a period of four weeks, failure of several other species to recover demonstrated the permanent damage and changes caused by antibiotic therapy (26). In another study, the impact of the same antibiotic on 3 individuals was assessed over a 10 month period. The study highlighted that the microbiota response varied across the subjects with reduced diversity among the microbial population in all subjects. This diversity was restored following the completion of antibiotic treatment to the point that they had stabilized by end of the study (150). Using molecular approaches, Donskey *et al* have also demonstrated the disturbances in the gut population due to administration of different antibiotics (151). The study indicated that the anaerobic microbiota was minimally affected by ciprofloxacin but markedly reduced by clindamycin therapy.

It is important to understand the need for antibiotics in the elderly, in particular those living in long-term care facilities, as these vulnerable people are more prone to bacterial infections due to immunosenescence and compromised health. In the process of eliminating the pathogenic microbes, the antibiotic therapies extend their effect to the gut microbiota as a whole, leading to unintentional dysbiosis. Several studies have looked at the impact of antibiotics on the intestinal microbiota in the elderly. Antibiotic therapy decreased the proportion of Desulfovibrio and Faecalibacterium species (35) along with increasing levels of Lactobacillus species (152). Recently, Claesson et al investigated the overall impact of antibiotic administration on the gut microbial composition of the elderly and revealed that antibiotic therapy reduced proportions of Firmicutes and Proteobacteria and increased the proportion of Bacteroidetes (31). Bartosch *et al* also reported a 2.5-fold decrease in the Bifidobacterium species in elderly hospitalized subjects receiving antibiotics compared to those not on antibiotics (35).

As a consequence of the potential damaging effect of the antibiotics, a number of investigations have focused on the introduction of antimicrobials that can cause less collateral damage, over classical antibiotics. One such potent narrow spectrum antimicrobial peptide is Thuricin CD, a recently identified bacteriocin produced by Bacillus thuringiensis, which is specifically active against C. difficile (153). The efficacy of broad spectrum antimicrobials, such as vancomycin, metronidazole, lacticin 3147 and the Thuricin CD bacteriocin, on the gut microbiota composition, in particular C. difficile was demonstrated in a human distal colon model (154). The introduction of broad spectrum antibiotics resulted in a major disturbance in the overall population with a dramatic proportional increase in Proteobacteria and Enterobacteriaceae and a decrease in the numbers of Bacteroidetes and Firmicutes. In contrast, the introduction of the Thuricin CD bacteriocin caused no significant alterations in the relative proportions of the dominant microbial population but had potent anti-C.difficile activity.

Another major concern with the use of antibiotics is the emergence of antibiotic resistant bacteria, and the potential transfer of antibiotic resistance genes to pathogenic bacteria (149, 155). Bacterial resistance to antimicrobial agents can be either intrinsic (inherent or natural) or acquired. The microbes develop intrinsic resistance by altering the target sites to avoid binding of the antibiotic or by decreasing the permeability of their cell walls and/or developing efflux pumps to pump out the antibiotic. In acquired resistance, the microbes acquire genes to produce different enzymes, such as beta-lactamases which deactivate the beta-lactam ring of penicillin. Treating the rapidly emerging antibiotic resistant bacteria, especially the hospital acquired microbes, is becoming more challenging (156). Sjölund *et al* demonstrated that all Enterococcus species isolated immediately after treatment with clarithromycin had high level resistance to the antibiotic due to the presence of the ermB gene. It was also observed that resistant enterococci persisted for one to three years even after treatment in 3 patients (157).

The frequency and severity of infectious diseases are higher in older people compared to younger individuals (156). In this respect, antibiotic administration is the cause of profound disturbances in the indigenous bacterial population which undoubtedly lead to compromised colonization resistance, causing adverse effects including AAD (158), unintentional state of dysbiosis (56), risk of CDAD especially in elderly hospitalized patients (42, 159, 160), bowel dysfunction (161), intestinal malabsorption (162), nephrotoxicity, ototoxicity, seizures, skin rashes, nauseas, abdominal cramps, renal dysfunction and acute liver injury (163, 164). The mechanism of action and the adverse effects of different antibiotics are presented in Tables 2 and 3, respectively. The benefits of antibiotic usage is straight-forward, nevertheless negative impacts like the promotion of antibiotic resistance and disruption of the ecology within the gut are quiet common. Antibiotic alternatives are an active area of research in terms of developing new approaches. The use of prebiotics and/or probiotics could be a promising effect in restoring impaired functions or enhancing desirable functions of the microbiota.

Modulation of the elderly human gut microbiota using probiotics, prebiotics and synbiotics

The increased prevalence of diseases and disorders associated with the gut microbial imbalance due to factors such as dietary habits, lifestyle and drug usage can be modulated through supplementation with probiotics, prebiotics or synbiotics.

Probiotics

Probiotics, a term derived from the Greek meaning 'for life', are defined as "live microorganisms, which, when consumed in adequate amounts, confer a health benefit on the host" (165). The most promising probiotics include organisms of the genera Lactobacillus, Bifidobacterium, Lactococcus, Streptococcus and non-pathogenic yeasts. Probiotic strains should be natural inhabitants of the host species and have some of the following properties, technologically suitable for industrial processes, acid-fast and bile-fast, viable, adhere to the gut epithelial tissue, modulate immune responses (166, 167), regulate cytokine secretion and produce antimicrobial substances such as bacteriocins (168, 169). A number of mechanisms have contributed to the health benefits of probiotic bacteria, including production of SCFAs, vitamins, bioactive peptides, bacterial-host signaling molecules, antimicrobial substances and triggering the immune response (167, 170).

Prebiotics

The term prebiotic was first introduced by Gibson and Roberfroid in 1995 and are defined as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or limited number of bacteria in the colon, and thus improves the health of the host" (171). Prebiotic usage avoids the drawbacks of using probiotic bacteria, such as maintaining viability during storage or en route to the intestine (171). Prebiotics of proven efficacy and commercially available are lactulose, galacto-oligosaccharides (GOS), fructo-oligosaccharides (FOS) and inulin, of which the last two are the most studied and well recognized (171). These carbohydrates, when fermented by gut bacteria can serve as energy sources for the intestinal epithelial cells. Through stimulation of bacterial growth and fermentation, prebiotics can influence many aspects of bowel functionality (172).

Health benefits of probiotics and prebiotics in elderly

Probiotic intervention is a promising dietary approach for exerting health benefits such as prevention of ADD (173, 174), restoring the intestinal population in IBS patients (175) and enhancement of intestinal immunity (176, 177, 178). Probiotics have been considered as a promising approach to modify the gut microbiota and its overall functions for decades (179). Sood and colleagues reported that the probiotic preparation VSL#3 reduced UC symptom severity in some patients and induced remission in patients with mild-to-moderately active UC (180).

Lactobacilli and bifidobacteria are well known for their beneficial effects as probiotics in human health and can cause significant improvements in providing longevity and alleviation of age related disorders. As a key member of the intestinal microbiota, bifidobacteria were shown to inhibit the growth of enteric pathogens and may prevent gastroenteritis in humans (181). Supplementation with Bifidobacterium strains have been shown to increase the levels of health-promoting bacteria in the elderly (182, 183). Some strains of the genus Bifidobacterium exhibit powerful anti-inflammatory properties by restoring appropriate cytokine production (184). Certain members of the genus Bifidobacterium have been involved in functional foods, conferring health-promoting effects, in particular reduction of chronic inflammatory diseases including UC and Pouchitis, reducing symptoms of allergy and lactose intolerance (185). A Finnish study has shown an increase in the frequency of bowel movements in elderly nursing home residents with natural food supplies containing the two probiotic strains, B. longum and B. lactis (186). Intervention studies with B. lactis HN019 have also shown positive effects on the immune system of the elderly, such as increased phagocytic activity and number of NK cells (176, 187). A more recent study demonstrated that one month consumption of a probiotic biscuit, containing mixture of two probiotic strains, B. longum Bar33 and L. helveticus Bar13 was effective in not only restoring some of the age-related dysbiosis of the intestinal microbiota but also reverting the age-related increase in the opportunistic pathogensincluding Clostridium cluster XI, C. difficile, C. perfringens, E. faecium and Campylobacter (188). Probiotic supplements containing lactic acid bacteria such as L. rhamnosus and L. acidophilus has been shown to alleviate constipation in aging people (189, 190). A randomized control study also demonstrated that dietary supplementation with L. casei DN-114001 in elderly subjects for three weeks showed potential for a 20 % reduction in the duration of ''winter infections'' (gastrointestinal or respiratory) compared to the controls (191).

Elderly people may be regarded as immune compromised due to their decreased ability to fight infections- this is one of the main targets for researchers to develop strategies that can boost their immunity level (192). Probiotic intervention is a potential nutritional approach to modulate immune functions in the elderly population. The probiotic strains Lactobacillus rhamnosus HN001 and B. lactis HN019 have improved innate immune functions in elderly subjects (193, 194). Recent studies have demonstrated that the consumption of probiotic cheese containing L. rhamnosus HN001 and L. acidophilus NCFM by elderly volunteers resulted in significantly enhanced innate immune function (195) and modified subpopulations of faecal lactobacilli and C. difficile (196). Some probiotics are able to increase the activity of NK cells (197) especially in those habitual smokers affected by decreased NK cell activity (198). Other studies addressing the use of specific probiotic strains and their involvement in improving the host health are shown in Table 4.

Prebiotics have been shown to exert beneficial effects on host health by minimizing the disruption to the baseline gut bacterial community. Guigoz *et al* reported an increase in levels of faecal bifidobacteria accompanied by a significant rise in

Table 4
Effect of probiotic therapy on human health

Probiotic strain or probiotic mix	Therapeutic effect	Reference
L. casei DG	Prevents recurrence of diverticular disease of the colon	(216)
L. rhamnosus GG, Saccharomyces boulardii and probiotic mixtures	Reduces the development of AAD	(217)
L. johnsonii La1 (NCC533)	Improves immune system and reduces duration of infection	(218)
L. casei DN 114001, L. bulgaricus and Streptococcus thermophilus	Reduces the incidence of AAD and CDAD	(219)
L. acidophilus SDC 2012 and L. acidophilus SDC 2013	Reduces abdominal discomfort in patients with IBS	(220)
L. casei DN 114001	Improves antibody responses to influenza vaccination	(221)
L. bulgaricus	Increases NK cell activity and reduces risk of common cold	(222)
L. acidophilus 145 and Bifidobacterium sp.420	Prevents recurrence of diverticular disease of colon	(223)
L. casei DN 114001	Reduces duration of respiratory infection	(224)
Bifidobacterium longum BB536	Increases NK cell activity and neutrophilic function	(225)

total lymphocyte counts in a group of frail elderly subjects treated with the prebiotic FOS for three weeks (199). Kleessen *et al* found that the intake of unabsorbed carbohydrates such as inulin improved constipation in elderly and significantly increased the bifidobacterial population (200). Consumption of the prebiotic mixture trans-galacto-oligosaccharide (B-GOS) showed a beneficial effect, by significantly increasing the level of bifidobacteria and the immune response in healthy elderly volunteers (201). Similarly, a four week ingestion of short chain fructo-oligosaccharides (scFOS) significantly increased the levels of faecal bifidobacteria in healthy elderly volunteers (202). A progressive increase in inulin ingestion from 20 grams (g)/day (d) to 40 g/d for 19 days in an *in vivo* study significantly increased the population of bifidobacteria without altering the total bacterial counts in elderly female subjects suffering from constipation (200). Inulin has also been used as an aid to treat UC and to inhibit C. difficile infections (203).

Synbiotic therapy is proving promising in the management of some health conditions. The use of synbiotic supplementation (combination of B. longum and fructo-oligosaccharides/inulin) in a placebo-controlled study was shown to reduce inflammation in patients with active UC (204). Similarly, their use in the treatment of CD has been documented, where the patients receiving synbiotics exhibited reduced CD activity (205).The consumption of the synbiotic combination with L. acidophilus NCFM and lactitol had an effect on the microbiota composition (206) further reinforcing the effects on microbiota reported previously for this combination(207). Bartosch *et al* have demonstrated that consumption of synbiotics modified the composition of intestinal bifidobacterial populations in healthy elderly volunteers (208). A similar synbiotic preparation has been used to increase the levels of bifidobacteria and lactobacilli (209). Supplementation with pre/pro/synbiotics has been proven to promote species diversity and increase resilience of microbial communities to challenges including various pathological conditions and antibiotic therapy.

Conclusion

The human gut is made up of a complex consortium of microorganisms that perform multiple important functions, including governing the health of the host by acting as a barrier against pathogens and improving the host immune system. With rapidly developing metagenomic methods, an in depth understanding of the physiological and metabolic activities of the intestinal microbes is being uncovered. Several factors promote shifts in the microbial diversity during aging, such as the use of antibiotics, dietary supplements and life style, resulting in susceptibility to infections and diseases. Studies hold promise that administration of functional foods containing probiotics and/or prebiotics on its own or in combination (synbiotics) as possible approaches to restore homeostasis between the gut microbiota and immune system of the elderly. However, there is very little evidence for long term colonization with probiotics suggesting that their effects are transient and occur at times following consumption. Future research and applications should help serve not only to investigate the complex diversity of the microbial community, but also to focus on the functional properties ensuring health benefits for the host.

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