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REVIEW ARTICLE

Intestinal bacteria and ageing

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Summary

Advancements in science and medicine, as well as improved living standards, have led to a steady increase in life expectancy, and subsequently a rise in the elderly population. The intestinal microbiota is important for maintenance of host health, providing energy, nutrients and protection against invading organisms. Although the colonic microbiota is relatively stable throughout adult life, age-related changes in the gastrointestinal (GI) tract, as well as changes in diet and host immune system reactivity, inevitably affect population composition. Recent studies indicate shifts in the composition of the intestinal microbiota, which may lead to detrimental effects for the elderly host. Increased numbers of facultative anaerobes, in conjunction with a decrease in beneficial organisms such as the anaerobic lactobacilli and bifidobacteria, amongst other anaerobes, have been reported. These changes, along with a general reduction in species diversity in most bacterial groups, and changes to diet and digestive physiology such as intestinal transit time, may result in increased putrefaction in the colon and a greater susceptibility to disease. Therapeutic strategies to counteract these changes have been suggested in ageing people. These include dietary supplements containing prebiotics, probiotics and a combination of both of these, synbiotics. Limited feeding trials show promising results with these supplements, although further longer-term investigations are required to substantiate their use in elderly healthcare fields.

Introduction

The elderly population

Ageing has been defined as 'the regression of physiological function accompanied by advancement of age' (Imahori 1992). Advancements in science and medicine, as well as improved living standards, have led to a steady increase in life expectancy and subsequently a rise in the elderly population.

Recent reports suggest that over one-third of the UK population will be 65 years old or over by the year 2050, putting immense demand on scarce healthcare resources.

Reduced income, social isolation and poor nutrition in this group can lead to many of the problems commonly associated with old age. For example, malnutrition is one of the main factors responsible for low immune responses in aged people (Lesourd *et al.* 1994). Development of preventative nutritional strategies to promote healthy ageing, maintain healthy living, as well as the independence and dignity of elderly people are therefore essential. Greater understanding of the anatomical and physiological processes involved in ageing and subsequent changes in the intestinal microbiota is crucial to this end.

Changes in the gastrointestinal tract in older people

Gastrointestinal (GI) function is indispensable for maintaining good nutrition. Thus, knowledge of age-related changes in the GI tract is important in the treatment and prophylaxis of diseases, and in maintenance of health among the elderly. Increased thresholds for taste and smell (Weiffenbach *et al.* 1982; Doty *et al.* 1984), resulting in foods tasting bland and uninteresting, coupled with masticatory dysfunction caused by loss of teeth and muscle bulk (Karlsson *et al.* 1991; Newton *et al.* 1993), and swallowing difficulties (Castell 1988) can lead to the consumption of a narrow, nutritionally imbalanced diet. In

the stomach, hypochlorhydria because of atrophic gastritis, is associated with a decreased absorption of calcium, ferric iron and vitamin B_{12} (Russell 1992), reducing micronutrient intake. Furthermore, decreased intestinal motility resulting in faecal impaction and constipation is a major problem in elderly people (Yagamata 1965; Brocklehurst 1972; Kleessen *et al.* 1997). One direct indication of physiological changes occurring in the GI tract of elderly people is the reported reduction in faecal weight with increased age (Woodmansey *et al.* 2004). Low faecal weights have been correlated to slow intestinal transit times and reduced excretion of bacterial matter (Stephen *et al.* 1987). Furthermore, increased retention time is associated with an increase in bacterial protein fermentation and consequently, the levels of ammonia and phenols generated in putrefactive processes in the gut (Macfarlane *et al.* 1989).

Changes in the GI tract, as well as modification of diet and host immune system, inevitably affect the colonic microbiota, thereby allowing bacterial population changes to occur. The normal intestinal microbiota is important for maintenance of host health, providing energy in the form of short-chain fatty acids (Cummings and Macfarlane 1991, 1997), nutrients such as vitamins K and B_{12} (Deguchi *et al.* 1985) and protection against invading organisms by exerting colonization resistance (Van der Waaij *et al.* 1971; Rolfe 1997). However, limited research into the bacteriological changes that occur with increased age prevents a clear understanding of the impact these shifts in population have on the beneficial functions normally provided by a healthy microflora, and subsequently, of possible therapeutic strategies that might be available to minimize or reverse such alterations.

Impact of ageing on the intestinal microflora

Changes with age in specific bacterial genera and species have been identified, with extensive interindividual variations occurring (Finegold *et al.* 1983b). Mean total anaerobe counts from faecal material are reported to remain relatively stable in elderly populations (Bornside 1978; Woodmansey *et al.* 2004). Nevertheless, shifts in composition of different genera are frequently observed. A summary of the changes in the microbiota of elderly people is shown in Fig. 1.

Numerous studies have shown a decline in viable counts of bacteroides with increased age, an observation that is unsurprisingly magnified following antibiotic therapy (Hopkins and Macfarlane 2002; Bartosch *et al.* 2004; Woodmansey *et al.* 2004). In addition, the species diversity within the genus *Bacteroides* is reportedly reduced in elderly compared with the healthy young volunteers (Woodmansey *et al.* 2004). *Bacteroides* species are nutritionally versatile and are able to utilize a wide variety of different carbon sources, consequently, they are believed to be responsible for the majority of polysaccharide digestion occurring in the colon (Salyers 1984; Macfarlane and Gibson 1991). Changes at species level of such a nutritionally important sub-population could have considerable consequences for the elderly host, because of alterations in metabolic activities, and for other bacteria in the ecosystem that rely on a complex cross-feeding network within the gut (Gibson *et al.* 1988). Reduction in amylolytic activity observed in healthy elderly population, and to a lesser extent, antibiotic-treated elderly patients (Woodmansey *et al.* 2004) further correlates with the nutritional importance of *Bacteroides* spp.

Previously regarded as being part of the genus *Bacteroides*, until fermentation acids were included in taxonomy (Holdeman and Moore 1974), fusobacteria are known to ferment amino acids, resulting in the production of several detrimental end-products such as ammonia and indoles (Holdeman *et al.* 1977). *Propionibacterium acnes* show similar fermentative activities. A rise in proteolytic bacteria such as fusobacteria, propionibacteria and clostridia has been reported in elderly populations (Hopkins

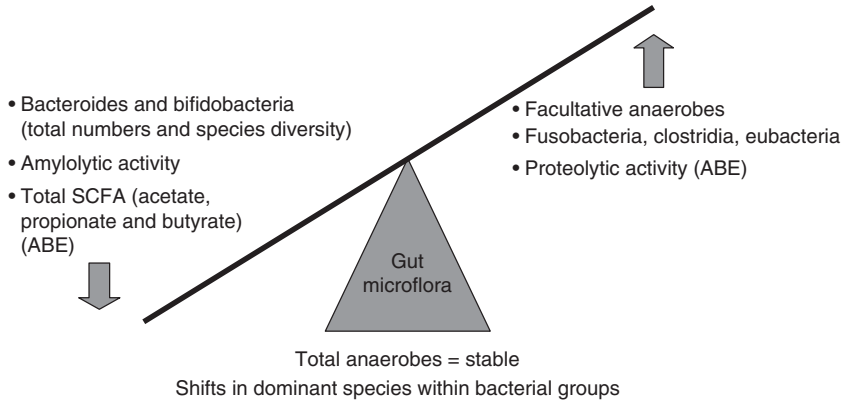

Figure 1 Summary of the key changes to the intestinal microflora observed in elderly populations. ABE, antibiotic-treated elderly.

et al. 2001; Hopkins and Macfarlane 2002; Woodmansey *et al.* 2004) and may indicate a trend towards putrefaction the large bowel, principally in patients undergoing antibiotic therapy, and concurs with the marked increase in proteolytic activity observed in these individuals.

The genus *Clostridium* comprises a heterogeneous group of micro-organisms, with highly diverse nutritional requirements and habitats. Ljungberg *et al.* (1990) previously observed a marked decrease in clostridia following administration of ciprofloxacin to young and elderly volunteers. In contrast, the majority of studies in the literature report an increase in clostridia in elderly subjects, particularly following antibiotic therapy, accompanied by a distinct increase in species diversity (Mitsuoka and Hayakawa 1972; Mitsuoka 1992; Hopkins and Macfarlane 2002; Woodmansey *et al.* 2004). In the latter study, an average of 2·1 different species of clostridia per person were detected in antibiotic-treated subjects, almost double that observed for the healthy young and healthy elderly groups, with the predominant species including *Clostridium bifermentans, Clostridium clostridioforme, Clostridium sordellii* and *Clostridium malenominatum*. Other species isolated include the pathogen *Clostridium difficile*, which was found in one volunteer, demonstrating some of the undesirable side-effects of antibiotic therapy, and *Clostridium sporosphaeroides,* which was not detected in either of the healthy groups. Unlike many other species of clostridia, *C. malenominatum* and *C. sporosphaeroides* are able to utilize pyruvate and lactate (Holdeman *et al.* 1977; Hippe *et al.* 1992), and their increased numbers and prominence in antibiotic-treated subjects may be related to increased numbers of lactobacilli and lactate production reported in this same study, as suggested by Hopkins and Macfarlane (2002).

Eubacteria have complex nutritional requirements, and some members of this genus are phylogenetically related to the clostridia. Their observed increase in elderly volunteers (Woodmansey *et al.* 2004) compared with their younger counterparts may have health consequences for the host, with a possible increase in the transformation of bile acids, creating potentially harmful metabolites in the gut. Additionally, cell material from *Eubacterium aerofaciens* has been reported to produce moderate to severe arthritis in rats, inoculated intraperitoneally with bacterial cell wall components (Severijnen *et al.* 1989). If this occurs *in vivo*, these bacteria could contribute to the rise in arthritic conditions in elderly people. Eubacteria have been reported to be second only to bacteroides in numbers isolated from the large intestine (Finegold *et al.* 1983b), however the fastidious nature of some members of this genus may explain reduced detection in many studies highlighting some of the problems associated with traditional culture techniques.

The beneficial properties of lactobacilli in fermented milk have long been known, and a rise in their numbers and species diversity with increased age and antibiotic therapy, has been reported in various studies (Mitsuoka and Hayakawa 1972; Mitsuoka 1992; Hopkins and Macfarlane 2002; Woodmansey *et al.* 2004). The ability of lactobacilli to persist through antibiotic treatment accentuates their value as probiotics, particularly against antibiotic-associated diarrhoea. Conversely, the presence of numerous plasmids, transposons and insertion sequences in various lactobacilli (Wells and Allison 1995) could be less useful, and may potentially provide a mechanism for the spread of antibiotic resistance genes within the gut ecosystem.

Bifidobacteria are numerically important colonic species that can occur in adults in excess of 10^{10} per gram dry weight in faeces (Finegold *et al.* 1983a). In conjunction with the shifts observed in the genus *Bacteroides*, the decline in beneficial bifidobacteria numbers is one of the most marked changes in the elderly gut, with a number of studies confirming these reductions (Mitsuoka *et al.* 1974; Benno *et al.* 1992; Mitsuoka 1992; Gavini *et al.* 2001; Hopkins *et al.* 2001; Woodmansey *et al.* 2004). Taken in conjunction with reduced species diversity, such changes indicate a decline in the stability of this population in the ageing colonic ecosystem. A wide range of bifidobacterial species are found in infants and young adults, however, in the elderly population, species diversity is reduced to one or two dominant organisms, in particular *Bifidobacterium adolescentis* or the phenotypically similar *Bifidobacterium angulatum* and *Bifidobacterium longum* (Mitsuoka *et al.* 1974; Gavini *et al.* 2001; He *et al.* 2001; Hopkins and Macfarlane 2002). One suggestion used to explain the decline in bifidobacterial species diversity in elderly people is reduced adhesion to the intestinal mucosa, although it is not clear if this is because of changes in the bacteria, or in the chemical composition and structure of colonic mucus (Ouwehand *et al.* 1999; He *et al.* 2001). Such a decline could result in a reduced functionality and immune responsiveness in the gut, and an increased susceptibility to gastrointestinal infections.

Further evidence to support changes in bacterial communities in the ageing gut is the marked rise in facultative anaerobes, particularly following antibiotic treatment (Gorbach *et al.* 1967; Hopkins *et al.* 2001; Hopkins and Macfarlane 2002; Bartosch *et al.* 2004; Woodmansey *et al.* 2004). In the latter study, numbers of enterobacteria, streptococci, staphylococci and yeasts were found to rise, particularly in the healthy elderly group, although enterococci, which were not isolated from a single healthy elderly individual, were found in highest numbers in antibiotic-treated donors. These findings can be linked to increased serum antibodies to commensal gut micro-

organisms, such as *Escherichia coli* and *Enterococcus faecalis*, as demonstrated by Percival *et al.* (1996).

Overall, the metabolic, and particularly, the bacteriological changes in the colonic microflora described in the literature highlight the ongoing microbial succession throughout the life of the host. The reduction in numbers and species diversity of many beneficial or protective anaerobes, such as bacteroides and bifidobacteria, as well as shifts in the dominant bacterial species can help to understand the decreased functionality of the microflora in some elderly people.

Modification of the intestinal microflora

A greater understanding of the colonic microbiota, as well as the increasing proportions of elderly people in western communities highlights the possibilities for therapeutic intervention using beneficial bacteria or probiotics in order to halt or reverse the decline in members of the community that can be advantageous to host health. The first significant introduction of the probiotic concept was by Metchnikoff in the early 1900s, who believed that the complex microbial population in colon was having an adverse reaction on the host through the so-called 'auto-intoxication effect', and reported that Bulgarian peasants who consumed large quantities of fermented milk experienced longevity (Metchinikoff 1907), attributed to the health-promoting effects of the live micro-organisms.

Currently, probiotics can be described as live microbial food supplements that change either the composition or metabolic activities of the microbiota, or modulate immune system reactivity in a way that benefits health (Macfarlane and Cummings 2002). A plethora of studies has investigated the use of probiotics against various microbial infections in humans. A detailed description of these investigations would be beyond the scope of this review; however, an overview of some therapeutic applications is summarized in Table 1. The mechanisms by which probiotics exert their effects are still uncertain, but are they thought to be multifactoral including chemical inhibition of pathogenic bacteria or stimulation of the immune response, competition for nutrients and adhesion receptors and immune clearance. The production of short chain fatty acids (SCFA) by lactobacilli and bifidobacteria, and other fermentative bacteria, decreases luminal pH providing antagonistic properties against invading organisms (Fooks and Gibson 2002), more specifically, certain probiotic bacteria have been reported to produce inhibitory compounds

Table 1 Overview of the effects of probiotics on microbial diseases in humans

Disorder	Probiotic	Effect	Reference
Infantile diarrhoea	*Lactobacillus* GG	Reduced duration of diarrhoea	Isolauri *et al.* (1991)
		Enhancement of humoral immune response	Isolauri *et al.* (1994)
			Kaila *et al.* (1992)
			Majaama *et al.* (1995)
	Lactobacillus reuteri	Reduced duration of diarrhoea	Shornikova *et al.* (1997)
	Bifidobacterium bifidum and *Stretococcus thermophilus*	Prevented rotavirus diarrhoea	Saavedra *et al.* (1994)
	Bifidobacterium breve	Prevented diarrhoea	Hotta *et al.* (1987)
Antibiotic-associated diarrhoea	*Bifidobacterium longum*	Decreased course of erythromycin-induced diarrhoea	Columbel *et al.* (1987)
	Lactobacillus GG	Decreased course of erythromycin-induced diarrhoea and other side effects of erythromycin	Siitonen *et al.* (1990)
	Streptococcus faecium	Decreased diarrhoea associated with antituberculosis drugs	Borgia *et al.* (1982)
	Saccharomyces boulardii	Reduced incidence of diarrhoea	Surawicz *et al.* (1989)
			McFarland *et al.* (1995)
			Elmer *et al.* (1996)
Relapsing *Clostridium difficile* colitis	*Lactobacillus* GG	Improved/terminated colitis	Gorbach *et al.* (1987)
			Bartlett *et al.* (1987)
	Lactobacillus GG	Erradicated associated diarrhoea	Biller *et al.* (1995)
Traveller's diarrhoea	*Lactobacillus acidophilus* and *B. bifidum*	Decreased frequency not duration of diarrhoea	Black *et al.* (1989)
	Lactobacillus GG	Decreased incidence of diarrhoea	Oksanen *et al.* (1990)
			Hilton *et al.* (1996)
Food-borne pathogen exclusion	*Lactobacillus* GG	Decreased shigellosis-associated diarrhoea	Sepp *et al.* (1995)
Helicobacter pylori-associated gastritis	*Lactobacillus johnsonii* fermented milk	Reduction in gastritis	Felley *et al.* (2001)

called bacteriocins shown to be antagonistic to various degrees against closely related species in some cases or a range of intestinal pathogens in others (Gibson and Wang 1994a,b,c; Itoh *et al.* 1995; O'Riordan *et al.* 1995; Jacobsen *et al.* 1999; Pessi *et al.* 1999; Todoriki *et al.* 2001). The ability to compete for limiting nutrients is an essential factor for all bacteria in the colon and determines the composition of the microbiota, with species unable to compete being rapidly eliminated from the ecosystem. Substrate availability is known to decrease with progression through the large intestine away from the proximal colon, furthermore, increasing lactobacilli or bifidobacterial numbers may enhance this phenomenon, consequently probiotics may reduce substrate availability to other bacterial populations. Competition for attachment sites is also an important mechanism whereby probiotic bacteria are thought to protect against colonization of allochthonous bacteria. Many studies have investigated adherence characteristics and mechanisms of probiotic preparations using Caco-2 cells, as they display typical features of intestinal cells. Various bifidobacteria strains have been shown to adhere to intestinal cell lines *in vitro, Bifidobacterium bifidum* strains, in particular, have demonstrated highly adherent properties (Crociani *et al.* 1995; Bibiloni *et al.* 1999; Aissi *et al.* 2001), although as Bibiloni *et al.* (1999) demonstrated, adherence does not always guarantee protection against enteropathogens. Adhesion of bifidobacteria to intestinal mucus is also thought to be important, and has been reported to occur in varying degrees by many authors. The decrease in adherence of bifidobacteria in elderly populations, as discussed in the previous section, may partially explain the decline of this genus and highlights the requirement for supplementation in this age group.

Studies on the immunostimulatory effects of probiotic preparations have heightened the understanding of the mechanisms by which these beneficial micro-organisms exert their effects, many of which are through immune regulation, particularly through the regulation of pro- and anti-inflammatory cytokines (Isolauri *et al.* 2001). Probiotics have been shown to enhance humoral immune responses, to promote the intestinal immunological barrier (Kaila *et al.* 1992; Isolauri *et al.* 1993) and to stimulate nonspecific host resistance to microbial pathogens (Perdigon *et al.* 1986, 1998), thereby facilitating immune elimination. Moreover, probiotic bacteria have been shown to modulate the host's immune responses to potentially harmful antigens with a potential to downregulate hypersensitivity reactions (Sutas *et al.* 1996), such as food intolerance (Pelto *et al.* 1996), and atopic eczema (Isolauri *et al.* 2000). Schiffrin *et al.* (1995) reported an increase in phagocytosis of *E. coli*, following probiotic ingestion by healthy volunteers, which persisted for 6 weeks after consumption had ceased. This may be particularly important in elderly populations, where the immune defence is adversely affected by the ageing process (Franceschi *et al.* 1995; Goodwin 1995). Furthermore, in a recent study, the proportions of T-lymphocytes and natural killer (NK) cells were increased in elderly subjects following supplementation with *Bifidobacterium lactis* HN019 (Gill *et al.* 2001), in addition, the *ex vivo* phagocytic capacity of mononuclear and polymorphonuclear phagocytes and the tumoricidal activity of NK cells were elevated.

To exert any effect in the large intestine, high numbers of probiotic bacteria must be delivered to the ecosystem. Delivery is usually in the form of viable bacteria, however, inactivated bacteria have also been shown to induce a beneficial response (Majaama *et al.* 1995). Studies show that not all commercial probiotic products satisfy this criterion (Hamilton-Miller *et al.* 1996), therefore caution must be applied to supplier and delivery medium of the therapy to ensure maximum survival through the GI tract. The combination of probiotics with synergistically acting components such as prebiotics can promote their growth and enhance their survival through the GI tract, this is termed a synbiotic (Gibson and Roberfroid 1995). Consequently, a stimulating effect is exerted on the probiotic bacteria as well as the lactic acid bacteria already present in the resident gut microflora.

Promising effects were observed in a recent feeding trial where *B. longum* BB 536 and *Lactobacillus acidophilus* NCFB 1748 were used in conjunction with oligofructose during use of the antibiotic cefpodoxime proxetil (Orrhage *et al.* 2000). Two further test groups, both on antibiotic therapy, received either the prebiotic oligofructose only, or a placebo. In the group given the synbiotic, *C. difficile* was isolated at a lower frequency than in the other two groups. Furthermore, the number of lactobacilli was significantly higher in the synbiotic group than in subjects receiving the placebo. Strong inhibition of enteropathogen growth has been observed *in vitro* using probiotic *Lactobacillus plantarum* and *B. bifidum*, combined with fructooligosaccharides (FOS), inulin and xylooligosaccharides (XOS) (Fooks and Gibson 2002). The authors suggested that antagonism against the pathogens was influenced by the carbohydrate provided, with a possible mechanism attributed to the production of the SCFA acetate and lactate.

Trials demonstrating the application of probiotics or synbiotics as a supplement and therapy in elderly people are limited. However, a recent double-blind feeding trial, in elderly individuals, with a synbiotic preparation containing high numbers of *B. lactis* BL-01 and *B. bifidum* BB-02, used in combination with an inulin-based prebiotic reported promising results (Bartosch *et al.* 2005). Significant increases in *B. bifidum*, total bifidobacteria and total lactobacilli numbers were observed in the synbiotic group during feeding, compared with the placebo. Moreover,

detailed examination of bifidobacterial species composition in the volunteers suggested that the rise in total bifidobacterial numbers was not only because of the addition of the exogenous probiotic organisms, but also as a result of stimulation of the indigenous bifidobacteria, in particular *B. adolescentis, B. angulatum* and *Bifidobacterium dentium* prevalence and numbers, during synbiotic feeding. In accordance with a recent synbiotic study (Malinen *et al.* 2002), synbiotic feeding had an adverse effect on *B. longum* as demonstrated by a reduction in numbers and prevalence of this species in the feeding period. Furthermore, the probiotic organisms were isolated from some volunteers up to 3 weeks after cessation of feeding, indicating that these organisms not only survived transit through the GI tract but also persisted in the colonic ecosystem. Although this study was performed using healthy elderly volunteers, the successful application highlights the potential of such supplementation in particular 'at-risk' groups with unbalanced gut ecosystems such as hospitalized patients or those using broad-spectrum antimicrobial therapy.

Present and future prospects for a healthy gut microflora in older people

In conclusion, studies to date demonstrate that distinct changes occur in the composition of the intestinal microflora of elderly people. The decline in numbers and species diversity of many beneficial or protective anaerobes with increased age, such as bacteroides and bifidobacteria, as well as shifts in the dominant bacterial species can help to understand the decreased functionality of the microflora in some elderly people. These changes in intestinal bacteria, and changes to diet and digestive physiology such as intestinal transit time, may result in increased putrefaction in the colon, and a greater susceptibility to disease such as gastroenteritis in the elderly, or infections caused by *C. difficile*, particularly following antibiotic treatment. However, larger, more detailed investigations are required to substantiate these claims.

The plethora of studies performed with probiotics (*in vitro* and in clinical trials) along with more recent studies using synbiotics in healthy elderly individuals has highlighted this as a possible method of therapeutic intervention to minimize or reverse the decline in beneficial bacteria in the elderly population. It is likely that hospitalized patients or those in long-term care facilities, with unbalanced gut ecosystems as a result of broad-spectrum antibiotic therapy may reap particular benefit from such intervention. However, further large-scale investigations into the colonic ecosystem of these at-risk groups are necessary to identify the optimum combination of probiotic organisms to suit the particular niche.

The future of the probiotic and synbiotic therapy depends on promotion of the scientific evidence that is accumulating in this area. This will not only help to satisfy the understanding of the medical community who could possibly apply such therapies, but should also highlight probiotics/synbiotics as an area for increased attention within regulatory bodies such as the Food and Drug Administration (FDA) in the United States or the Medicines and Healthcare Products Regulatory Agency (MHRA) in the United Kingdom. Such increased awareness should result in reassessment and upgrading of their classification from a functional food to either a medical device or pharmaceutical therapy depending on intention for use. As a consequence, more strict regulation of this area would positively influence the quality of scientific data to support new products. Furthermore, future studies to promote a stronger health economics rationale are recommended to link the benefits of probiotic therapy to, e.g. a reduction in antibiotic usage or development of hospital-acquired infections with subsequent impact on length of stay and ultimately cost reduction. Ultimately, future applications should help to promote intestinal health and quality of life for elderly people.

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