MICROBIAL ECOLOGY

Changes in predominant bacterial populations in human faeces with age and with *Clostridium difficile* infection

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The bacterial composition of human faeces can vary greatly with factors such as age and disease, although relatively few studies have monitored these events, particularly at species level. In this investigation, bacteria were isolated from faecal samples from healthy young adults and elderly subjects, and elderly patients with *Clostridium difficile*-associated diarrhoea (CDAD). The organisms were identified to species level on the basis of their cellular fatty acid profiles with the MIDI system. In some groups of bacteria, species diversity was found to change with age despite the overall numbers of organisms being similar at genus level. *Bacteroides thetaiotaomicron*, *B. ovatus* and *Prevotella tanneriae* were common gram-negative anaerobes isolated from young adults. *Bacteroides* species diversity increased in the faeces of healthy elderly people. Bifidobacterial species diversity decreased with age, with *Bifidobacterium adolescentis* and *Bif. angulatum* being the most common isolates. CDAD patients were characterised by greater diversity of facultative species, lactobacilli and clostridia, but greatly reduced numbers of bacteroides, prevotella and bifidobacteria. Such bacterial population changes in the normal microbiota could result in metabolic conditions favourable for the establishment of pathogenic micro-organisms, such as clostridia, and would have considerable effects on the biochemical capacity of the large intestine as a whole. Alterations in the community structure of bifidobacteria and lactobacilli have relevance for dietary and therapeutic interventions such as the use of pre- or probiotics that aim to modify the composition or metabolic activities of the intestinal microflora in a beneficial way, particularly in elderly people or individuals at risk of CDAD.

Introduction

A wide variety of host, microbiological, dietary and environmental factors affects bacterial colonisation of the human large bowel. While metabolic variations in the gut microflora associated with ageing have not been investigated in detail, a limited number of studies has indicated that structural changes occur in the ecosystem in elderly people. Species such as bifidobacteria, which are regarded as being protective, are thought to decline in numbers, whereas clostridia and enterobacterial populations, which are viewed as being detrimental to health, increase [1, 2]. Previous studies in this laboratory by culture and non-culture-based techniques supported some of these findings [3]. 16S rRNA analysis showed that faecal microbiotas in young adults and the elderly differed significantly from those of

children up to 8 years of age. Whereas bacterial populations of the elderly and young adults were not considered to differ significantly on the basis of 16S rRNA abundances, notable variations were detected in viable counts of bacteria making up these populations at genus level.

In broad terms, the colonic microbiota is generally viewed as being a stable entity within an individual [4], but it is probable that at the level of individual species, considerable variations in bacterial cell populations occur. This was shown in 10 human volunteers who were studied over a 12-month period; it was found that up to 1000-fold differences in counts of *Bacteroides* spp. (*B. fragilis* group) occurred during the course of the investigation [5]. Thus, differences at the species level are unlikely to be detected with current genus-specific nucleic acid probes.

Changes in the composition of the intestinal microbiota have been implicated in the initiation or maintenance

Received 31 Oct. 2001; accepted 16 Dec. 2001.

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of various disease states [6, 7]. A well-known example of this is *Clostridium difficile* infection causing *C. difficile*-associated diarrhoea (CDAD), which occurs predominantly in patients whose colonic microbiotas have been disturbed by antibiotic therapy [8]. CDAD can be a major problem in elderly hospitalised patients, with the PHLS Communicable Disease Surveillance Centre receiving over 14 500 *C. difficile* toxin-positive reports in England and Wales during 2000 (<http://www.phls.co.uk/facts/Gastro/Clostridium/closdifToxAnn.htm>). This places a considerable financial and operational burden on the National Health Service. Modification of the large intestinal microbiota by functional foods has been viewed as an attractive method of treatment or prophylaxis for such conditions [9, 10]. Several non-digestible oligosaccharides are known to stimulate the growth of bifidobacteria, which can in turn affect the growth and metabolism of other microorganisms in the bowel [11–13]. Carbohydrates such as lactulose and fructo-oligosaccharides (FOS) have been shown to affect the growth of *C. difficile* both *in vitro* and *in vivo* [14, 15]. Detailed information concerning the species composition of the gut flora is essential for understanding the mechanisms involved in such therapies and their suitability for treating different age groups within a population. This paper reports studies in which the predominant culturable bacterial species in the faeces of young adults were compared with those from healthy elderly subjects and patients with CDAD.

Materials and methods

Subjects

Fresh faeces samples were collected from three subject groups: healthy young adults, aged 21–34 years ($n = 7$); healthy elderly people, aged 67–88 years ($n = 4$); and elderly patients with CDAD, aged 67–73 years ($n = 4$). Healthy subjects had no history of gastrointestinal complaint or antibiotic therapy within the previous 2 months. CDAD patients had commenced metronidazole treatment and faecal samples were obtained <24 h after diagnosis. Faecal samples were collected in sterile plastic universal containers and processed within 1 h of voiding. Ethical approval and subject consent were obtained for this study.

Bacterial isolation and enumeration

Faeces were homogenised and serially diluted 10-fold in anaerobic half-strength peptone water. Samples were spread in duplicate on to pre-reduced agar medium and incubated in an anaerobic cabinet (H_2 10%, CO_2 10%, N_2 80%) for 48 h as described previously [16]. The following selective and non-selective agar media were used: Wilkins-Chalgren agar (total anaerobes), Wilkins-Chalgren agar plus GN selective supplements (*Bacteroides*, *Porphyromonas*, *Prevotella*), cycloserine-cefoxitin-fructose agar (*C. difficile*), Perfringens OPSP

agar (*C. perfringens*), Beerens agar [17] (bifidobacteria), Rogosa agar (lactobacilli) and blood azide agar (enterococci); MacConkey agar no. 2 (enterobacteria) and nutrient agar (facultative species) were incubated for 48 h at 37°C in air. Multiple samples of the predominant colony types were taken from the various media during the counting process for bacterial identification.

Bacterial identification

All isolates were identified on the basis of their cellular fatty acid (CFA) profiles obtained with the Anaer1 method [18] of the Microbial Identification System (MIDI, Microbial ID, Newark, DE, USA). Fatty acid methyl esters of strict anaerobes were extracted from bacterial cell mass obtained from c. 30 ml of overnight culture in peptone yeast extract broths, whereas facultative species were cultivated on trypticase soy agar [19]. Methylated fatty acids were identified by gas chromatography as reported previously [3], and bacterial identification was determined by CFA profile comparison with standard libraries, Moore and TSBA for strict and facultative anaerobes respectively (<http://www.midi-inc.com/pages/databases.html>).

Chemicals

Bacteriological culture media and selective antibiotic supplements were obtained from Oxoid (Basingstoke, Hants). Unless otherwise stated, all other chemicals were purchased from Sigma (Poole, Dorset).

Results

Overall, bacterial species diversity varied markedly between different subject groups in this investigation, with the lowest number of species being detected in CDAD patients (Tables 1–4). However, this reduced species diversity was not reflected at genus level because members of the genera *Lactococcus*, *Fusobacterium* and *Actinomyces* were not detected in the young adults or healthy elderly subject groups, but were isolated from patients with CDAD, in whom overall species diversity was low.

Bifidobacteria and lactobacilli

Bifidobacteria were frequently isolated from the faeces of young adults, particularly *Bifidobacterium angulatum*, which was found in six out of seven of these subjects and achieved populations as high as $\log_{10}$ 9.8 cfu/g wet weight faeces (Table 1). Other prevalent species included *Bif. longum*, *Bif. catenulatum* and *Bif. adolescentis*, although the latter two species generally occurred in lower numbers. *Bif. angulatum* was also the most common isolate in elderly patients, in whom it had the highest bifidobacterial cell count. *Bif. adolescentis* was the only isolate of this genus that

Table 1. Populations of bifidobacteria, lactobacilli and lactococci in the faeces of healthy young adults and elderly subjects and CDAD patients

Organism	Young adults (n = 7)			Elderly (n = 4)			CDAD (n = 4)		
	Number of isolates	Mean*	Range*	Number of isolates	Mean*	Range*	Number of isolates	Mean*	Range*
<i>Bifidobacterium adolescentis</i>	5	8.0 (0.9)	6.7–8.9	1	9.6 (0.1)	NA	1	7.7 (0.0)	NA
<i>Bif. angulatum</i>	6	8.8 (0.5)	8.4–9.8	2	8.7 (1.6)	7.5–9.8	–	–	–
<i>Bif. bifidum</i>	1	7.9 (0.1)	NA	–	–	–	–	–	–
<i>Bif. catenulatum</i>	4	7.8 (0.9)	7.0–8.8	–	–	–	–	–	–
<i>Bif. infantis</i>	1	8.3 (0.1)	NA	–	–	–	–	–	–
<i>Bif. longum</i>	3	8.9 (0.1)	8.7–9.0	1	9.2 (0.0)	NA	–	–	–
<i>Bif. pseudocatenulatum</i>	1	9.1 (0.2)	NA	–	–	–	–	–	–
Total	21			4			1		
<i>Lactobacillus acidophilus</i>	1	7.4 (0.0)	NA	–	–	–	–	–	–
<i>L. bif fermentans</i>	–	–	–	–	–	–	1	9.4 (0.2)	NA
<i>L. brevis</i>	–	–	–	1	6.1 (0.0)	NA	–	–	–
<i>L. buchneri</i>	1	5.4 (0.1)	NA	–	–	–	–	–	–
<i>L. cateniforme</i>	1	7.7 (0.0)	NA	–	–	–	–	–	–
<i>L. coryneformis</i> subsp. <i>coryneformis</i>	1	6.4 (0.0)	NA	–	–	–	–	–	–
<i>L. coryneformis</i> subsp. <i>torquens</i>	1	6.2 (0.1)	NA	–	–	–	–	–	–
<i>L. delbrueckii</i> subsp. <i>bulgaricus</i>	–	–	–	1	3.2 (0.0)	NA	–	–	–
<i>L. delbrueckii</i> subsp. <i>lactis</i>	3	4.8 (1.5)	3.3–6.3	1	7.7 (0.1)	NA	3	7.1 (1.0)	6.1–8.1
<i>L. paracasei</i> subsp. <i>paracasei</i>	4	5.5 (1.7)	3.9–7.5	3	5.0 (1.5)	3.3–6.0	1	6.3 (0.2)	NA
<i>L. paracasei</i> subsp. <i>tolerans</i>	–	–	–	–	–	–	1	8.9 (0.1)	NA
<i>L. pentosus</i>	–	–	–	–	–	–	1	5.6 (0.0)	NA
<i>L. plantarum</i>	1	5.9 (0.0)	NA	–	–	–	1	6.4 (0.0)	NA
<i>L. reuteri</i>	–	–	–	–	–	–	1	9.3 (0.0)	NA
<i>L. rhamnosus</i>	1	5.1 (0.1)	NA	–	–	–	–	–	–
<i>L. rogosae</i>	–	–	–	1	4.3 (0.0)	NA	–	–	–
<i>L. salivarius</i>	–	–	–	–	–	–	1	8.0 (0.1)	NA
<i>L. sharpeae</i>	1	3.8 (0.0)	NA	–	–	–	1	8.9 (0.0)	NA
<i>L. vaginalis</i>	–	–	–	1	5.9 (0.0)	NA	–	–	–
<i>Lactobacillus</i> species D10	1	9.2 (0.1)	NA	–	–	–	1	10.1 (0.1)	NA
Total	16			8			12		
<i>Lactococcus lactis</i>	–	–	–	–	–	–	1	5.9 (0.0)	NA

NA, not applicable; –, not detected.

*Results are expressed as mean log₁₀ cfu/g wet weight of faeces (SD).

was detected in any of the CDAD patients. Inter-individual variation in *Lactobacillus* populations was high; individual subject groups yielded 6–11 different species, but only *L. delbrueckii* subsp. *lactis* or *L. paracasei* subsp. *paracasei* were found in several subjects within the same group.

Clostridia and eubacteria

C. innocuum was the most frequently isolated member of this genus in all three subject groups, although numbers of this species were lower in the healthy young adults (Table 2). *C. ramosum* was also a common isolate in these individuals but was not detected in the faeces of any healthy elderly subjects. *C. difficile* was found only in the faeces of CDAD patients. The majority of clostridia were saccharolytic, although asaccharolytic species such as *C. malenominatum* in healthy young and elderly adults and *C. sporosphaeroides* in CDAD patients were also detected. Of the 13 *Eubacterium* spp. isolated from all patients, only *E. rectale*, *E. bifforme* and *E. limosum* were found in more than one subject, with *E. bifforme* achieving the highest faecal populations (log₁₀ 9.2 cfu/g wet weight).

Bacteroides and prevotella

Species from these two genera accounted for the majority of bacteria detected in both healthy young and elderly subjects. *Prevotella tanneriae* was prevalent in the faeces of healthy young adults, as were *B. thetaiotaomicron* and *B. ovatus*. *B. distasonis*, *B. fragilis* and *B. vulgatus* also occurred in numbers in excess of log₁₀ 9.5 cfu/g wet weight of faeces but were isolated from less than half of the faecal samples (Table 3). Elderly subjects had a similar profile with respect to the distribution of the major *Bacteroides* spp., although species diversity was higher with an average of five different *Bacteroides* spp. in the faeces of each subject. Only one unspiciated *Bacteroides* isolate was detected in one CDAD subject.

Facultative species

Table 4 shows that total counts of facultative bacteria were notably higher in CDAD patients (log₁₀ 9.1 cfu/g wet weight) than in the other two subject groups (log₁₀ 7.7 cfu/g wet weight). Enterobacteria and enterococci were isolated in similar numbers from the CDAD patients, whereas in healthy young adults enterobacterial populations were c. 10-fold lower than enterococci.

Table 2. Populations of clostridia and eubacteria in the faeces of healthy young adults and elderly subjects and CDAD patients

Organism	Young adults (n = 7)			Elderly (n = 4)			CDAD (n = 4)		
	Number of isolates	Mean*	Range*	Number of isolates	Mean*	Range*	Number of isolates	Mean*	Range*
<i>Clostridium clostridioforme</i>	1	7.2 (0.0)	NA	1	6.9 (0.1)	NA	1	6.3 (0.0)	NA
<i>C. difficile</i>	—	—	—	—	—	—	4	7.6 (1.2)	6.1–8.7
<i>C. innocuum</i>	3	6.1 (1.2)	5.3–7.4	3	7.1 (0.7)	6.4–7.7	3	9.0 (0.6)	8.6–9.7
<i>C. malenominatum</i>	2	6.5 (1.3)	5.6–7.4	1	5.6 (0.0)	NA	—	—	—
<i>C. novyi</i>	—	—	—	—	—	—	1	6.4 (0.1)	NA
<i>C. perfringens</i>	1	7.4 (0.1)	NA	1	8.3 (0.0)	NA	1	7.5 (0.2)	NA
<i>C. ramosum</i>	2	6.1 (1.3)	5.1–7.0	—	—	—	2	7.9 (1.8)	6.6–9.1
<i>C. sphenoides</i>	1	6.2 (0.2)	NA	—	—	—	—	—	—
<i>C. sporosphaeroides</i>	—	—	—	—	—	—	1	9.1 (0.3)	NA
<i>Clostridium</i> species P	1	6.3 (0.0)	NA	—	—	—	—	—	—
<i>Clostridium</i> species SPR	1	7.6 (0.0)	NA	1	7.6 (0.0)	NA	—	—	—
Total	12			7			13		
<i>Eubacterium aerofaciens</i>	1	8.5 (0.0)	NA	—	—	—	—	—	—
<i>E. alacalyticum</i>	—	—	—	—	—	—	1	9.0 (0.1)	NA
<i>E. bifforme</i>	1	9.2 (0.1)	NA	2	7.7 (1.3)	6.7–8.6	—	—	—
<i>E. contortum</i>	—	—	—	—	—	—	1	7.0 (0.0)	NA
<i>E. cylindroides</i>	—	—	—	1	6.6 (0.0)	NA	—	—	—
<i>E. limosum</i>	—	—	—	1	8.5 (0.0)	NA	1	8.0 (0.0)	NA
<i>E. plauti</i>	1	7.7 (0.2)	NA	—	—	—	1	8.7 (0.1)	NA
<i>E. rectale</i>	2	7.5 (1.3)	6.5–8.4	1	4.3 (0.0)	NA	—	—	—
<i>E. timidum</i>	1	7.3 (0.0)	NA	—	—	—	—	—	—
Total	6			5			4		
<i>Actinomyces</i> species D01	—	—	—	—	—	—	1	5.8 (0.1)	NA

NA, not applicable; —, not detected.

*Results are expressed as mean log₁₀ cfu/g wet weight of faeces (SD).**Table 3.** Populations of prevotella, bacteroides and fusobacteria in the faeces of healthy young adults and elderly subjects and CDAD patients

Organism	Young adults (n = 7)			Elderly (n = 4)			CDAD (n = 4)		
	Number of isolates	Mean*	Range*	Number of isolates	Mean*	Range*	Number of isolates	Mean*	Range*
<i>Prevotella loescheii</i>	1	9.1 (0.2)	NA	1	8.3 (0.0)	NA	—	—	—
<i>Pr. ruminicola</i>	1	9.5 (0.0)	NA	—	—	—	—	—	—
<i>Pr. splachnicus</i>	—	—	—	1	9.0 (0.0)	NA	—	—	—
<i>Pr. tanneriae</i>	4	9.1 (0.5)	8.7–9.8	1	8.2 (0.0)	NA	—	—	—
<i>Prevotella</i> species D64	1	8.0 (0.2)	NA	—	—	—	—	—	—
<i>Prevotella</i> species D88	1	8.7 (0.0)	NA	—	—	—	—	—	—
Total	8			3					
<i>Bacteroides caccae</i>	2	9.1 (0.2)	8.9–9.2	3	8.1 (0.4)	7.8–8.6	—	—	—
<i>B. distasonis</i>	3	9.3 (0.5)	8.9–9.9	3	9.3 (0.3)	9.0–9.5	—	—	—
<i>B. fragilis</i>	3	9.3 (1.0)	8.3–10.3	3	8.7 (0.2)	8.5–8.9	—	—	—
<i>B. ovatus</i>	4	9.1 (0.5)	8.5–9.6	1	8.6 (0.1)	NA	—	—	—
<i>B. thetaiotaomicron</i>	5	8.8 (1.1)	7.4–10.1	3	8.6 (0.8)	7.7–9.2	—	—	—
<i>B. uniformis</i>	1	9.9 (0.0)	NA	1	9.2 (0.0)	NA	—	—	—
<i>B. uniformis</i> cfa 2	2	9.1 (0.4)	8.8–9.4	1	9.3 (0.0)	NA	—	—	—
<i>B. vulgatus</i>	3	9.3 (0.5)	8.9–9.9	3	9.0 (0.2)	8.9–9.3	—	—	—
<i>Bacteroides</i> species AR	—	—	—	1	9.6 (0.0)	NA	—	—	—
<i>Bacteroides</i> species FL	—	—	—	1	8.6 (0.1)	NA	—	—	—
<i>Bacteroides</i> species FO	1	8.3 (0.1)	NA	—	—	—	—	—	—
<i>Bacteroides</i> species L	—	—	—	—	—	—	1	9.1 (0.1)	NA
Total	24			20			1		
<i>Fusobacterium prausnitzii</i>	—	—	—	—	—	—	1	9.0 (0.0)	NA
<i>F. russii</i>	—	—	—	—	—	—	1	8.1 (0.2)	NA
Total	—	—	—	—	—	—	2		

NA, not applicable; —, not detected.

*Results are expressed as mean log₁₀ cfu/g wet weight of faeces (SD).

Species diversity was reduced in healthy elderly subjects with only three different organisms being isolated, although enterobacterial populations accounted for a greater proportion of the total microbiota in these subjects.

Discussion

Although several studies have investigated bacterial populations in the adult large bowel, in various levels of detail [1, 20–22], relatively little information is

Table 4. Populations of facultative species in the faeces of healthy young adults and elderly subjects and CDAD patients

Organism	Young adults (n = 7)			Elderly (n = 4)			CDAD (n = 4)		
	Number of isolates	Mean*	Range*	Number of isolates	Mean*	Range*	Number of isolates	Mean*	Range*
<i>Escherichia coli</i>	3	5.7 (2.3)	3.3–7.9	3	6.2 (1.3)	4.8–7.4	2	8.5 (0.1)	8.4–8.5
<i>Klebsiella ascorbata</i>	1	5.5 (0.0)	NA	2	7.9 (0.9)	7.2–8.5	—	—	—
<i>K. pneumoniae</i>	2	5.3 (0.6)	4.9–5.7	—	—	—	—	—	—
<i>Morganella morganii</i>	2	6.1 (1.2)	5.2–6.9	—	—	—	1	7.3 (0.0)	NA
<i>Proteus mirabilis</i>	—	—	—	—	—	—	2	8.1 (0.6)	7.7–8.5
<i>P. vulgaris</i>	—	—	—	—	—	—	1	7.9 (0.1)	NA
<i>Serratia fonticola</i>	2	5.7 (0.2)	5.5–5.8	—	—	—	—	—	—
Total	10			5			6		
<i>Enterococcus faecalis</i>	4	6.4 (1.6)	5.0–8.5	3	6.8 (0.5)	6.3–7.3	3	8.4 (0.3)	8.2–8.7
<i>Micrococcus luteus</i>	—	—	—	—	—	—	1	7.6 (0.1)	NA
<i>Streptococcus mutans</i>	2	7.4 (1.2)	6.5–8.2	—	—	—	—	—	—
Total	6			3			4		

NA, not applicable; —, not detected.

*Results are expressed as mean log₁₀ cfu/g wet weight of faeces (SD).

available concerning the effects of age on these microbiotas. This study investigated differences in faecal bacteria with both advancing age and *C. difficile* infection and provided information on the species composition of the faecal microbiota. Measurements at species level could be of considerable importance, particularly in bacterial groups that contain pathogenic organisms or those with special nutritional and metabolic capabilities, which would be overlooked if the microbiota is studied at genus level. An example of this was found with the bacteroides; total counts for the genus were slightly higher in young adults but greater species diversity was observed in the elderly (5.0 and 3.4 different *Bacteroides* isolates per subject in healthy elderly and young adults, respectively).

Bacteroides are nutritionally versatile and can utilise a wide variety of carbon sources. These bacteria are thought to be responsible for the majority of polysaccharide digestion that occurs in the large intestine [23, 24]. Changes at species level of such a nutritionally prominent population could have considerable consequences for the host, as a result of alterations in microbiota metabolic profiles, and for other communities within the ecosystem, such as hydrogen-utilising syntrophs, that rely on a complex cross-feeding network within the intestine [25].

The microbiotas of elderly patients with CDAD were markedly different from those in healthy elderly subjects, and there was only one *Bacteroides* isolate from this entire group. Bifidobacteria also occurred in low numbers in these patients and this was reflected in the reduced number of anaerobic species isolated per subject relative to facultative species. The ratio of anaerobic:facultative species isolated per subject in CDAD patients was 3.5:1.0 compared with 5.9:1.0 in healthy elderly subjects. It was probably this reduction in competing anaerobes, which would usually overwhelm smaller populations on agar plates, that allowed

detection of fusobacteria and actinomyces, rather than these organisms not being present in other subject groups. This highlights one of the limitations of the viable count method and the enumeration of these bacteria would require the use of procedures that circumvent the necessity to culture and identify organisms, such as fluorescent in-situ hybridisation analyses.

When compared with the healthy elderly group, the CDAD patients not only had higher enterobacterial and enterococcal counts but also had a greater diversity of clostridia and lactobacilli. These changes in the gut microflora may have resulted from metronidazole treatment. Ideally, faecal samples from CDAD patients would have been obtained before antibiotic therapy, but even though faeces were always collected on the day of diagnosis, treatment could not be delayed for the purpose of this study. However, differences in bacterial composition could not all be attributed to the effects of metronidazole because CDAD patients were also found to have high prevalence and viable counts of anaerobic organisms such as clostridia and lactobacilli. This high incidence of lactobacilli with an average of three isolates per patient was unexpected, as these organisms have been used as probiotics to treat *C. difficile* infections [26–28]. This study points towards bacteroides and bifidobacteria as having a more prominent role in colonisation resistance against *C. difficile*, because these organisms were not detected in significant numbers in CDAD patients. This could also extend to prebiotic studies because bifidobacteria are known to have specific substrate preferences [29], and results from this investigation would indicate that substrates that *Bif. adolescentis* could utilise might be of benefit, because this was the only species detected in CDAD patients. However, the low overall occurrence of bifidobacteria in CDAD patients suggests that a synergic approach, incorporating both pre- and probiotic, would be more likely to be successful.

At genus level, the number of bifidobacterial isolates declined in healthy elderly subjects compared with young adults. *Bif. angulatum* was the most common bifidobacterial isolate in healthy young adults, but species diversity was found to decrease with age, with *Bif. bifidum*, *Bif. catenulatum*, *Bif. pseudocatenulatum* and *Bif. infantis* not being detected in the faeces of elderly subjects. Previous work with culture techniques has identified *Bif. adolescentis* as the most common bifidobacterial isolate in adults and centenarians [30], although the phenotypically very similar *Bif. angulatum* was not listed, and may have been overlooked because of the method of identification. The application of PCR found the *Bif. catenulatum* group and *Bif. longum* to be more common than *Bif. adolescentis* in adults, although again, *Bif. angulatum* was present in few subjects [31]. This latter study showed that the adult microflora generally harboured a combination of three to four different bifidobacterial species, and the results of the present study are in agreement with this observation (average of three bifidobacterial isolates per young adult subject). Prebiotics have been shown to stimulate the growth of species belonging to this genus *in vivo* [13, 32] which confers the potential to suppress growth of other organisms such as enterobacteria [33], or induce changes in large intestinal biochemistry [34]. Again, the presence of bifidobacteria in the colon before any dietary intervention is essential for the successful prophylactic use of prebiotics against intestinal infection or bacterial overgrowth.

The genus *Clostridium* contains numerous pathogenic species. Total numbers of these bacteria increased by $>\log_{10} 1.5$ in CDAD with high numbers of *C. difficile*, *C. ramosum*, *C. innocuum* and *C. sporosphaeroides*. This concurs with the concept that altered component(s) of the anaerobic microbiota allow proliferation of *C. difficile*, and changes at species, or indeed strain level, could be a reason why CDAD does not ensue in all individuals exposed to antibiotics and environmental challenges [35].

C. malenominatum does not utilise conventional carbohydrate substrates but ferments threonine to propionate and, like *C. sporosphaeroides*, is able to utilise pyruvate and lactate [19, 36]. The narrow nutritional requirements of these metabolic specialists suggests their involvement in biochemical interactions between different groups of bacteria in the gut. The large population of *C. sporosphaeroides* in one of the CDAD patients may have been related to increased lactobacilli numbers and lactate production.

Like clostridia, eubacteria have complex nutritional requirements and some members of these genera are relatively close in phylogenetic terms. Eubacteria have been reported to be the second most numerous bacterial group in the large intestine after the bacteroides [20], although results from this study do not confirm this. Such discrepancies are possibly due to the fastidious

nature of some members of this genus and differences in identification protocols. Molecular analyses based upon 16S rRNA sequences are confused by the genetic similarity of eubacteria to some clostridia. A probe for the *C. coccoides* group targets some eubacteria and studies have shown that the amount of RNA of this phylogenetic group did not account for more than 16% of the total bacterial RNA isolated from human faeces compared with 37% for bacteroides [37]. More recent data from whole-cell hybridisations showed slightly lower mean eubacterial counts than described by Finegold *et al.* [38], although these cannot be compared directly to the results of the present study, because they expressed them per gram dry weight of faeces [39]. The genus *Eubacterium* comprises a nutritionally diverse group of organisms that have been implicated in steroid and bile transformation, creating potentially toxic metabolites in the gut, and yet the genus remains poorly characterised and their role in the gut ecosystem is unclear.

In summary, the altered composition of metabolically active groups in the large bowel, such as bacteroides and eubacteria, could lead to profound changes in the biochemical capacity of the gut microbiota with age, and such alterations would not be obvious from studies that are usually made at genus level. In particular, this altered species diversity could have important implications for prebiotic therapy where the choice of carbohydrate would need to target a bifidobacterial species that is likely to be present in members of the subject group. The reduced number of bifidobacterial isolates from elderly subjects suggests that a combination of pre- and probiotic (synbiotic) therapy may be more likely to succeed as a treatment for *C. difficile* infection in the elderly. Furthermore, it is important to realise the limitations of such culture-based studies because large bacterial communities can obscure less dominant populations if suitable selective culture media are not available. Molecular analyses are useful alternatives and can provide ecological information on extremely fastidious organisms, but are themselves limited by oligonucleotide probe design and cellular access. Thus, information from as many sources as possible should be considered when studying bacterial community structure in the large intestine.

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