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Effect of supplements with lactic acid bacteria and oligofructose on the intestinal microflora during administration of cefpodoxime proxetil

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Thirty healthy volunteers in three groups participated in a study of the effect on the intestinal microflora of oral supplementation with *Bifidobacterium longum*, *Lactobacillus acidophilus* and oligofructose, an indigestible oligosaccharide, during oral administration of cefpodoxime proxetil bd for 7 days. Those in group A also received an oral supplement with *c.*10^{11} cfu of *B. longum* BB 536 and *L. acidophilus* NCFB 1748 and 15 g oligofructose daily, those in group B received a supplement with oligofructose only and those in group C received placebo, for 21 days. In all three groups there was a marked decrease in aerobic microorganisms, involving mainly a rapid and almost complete disappearance of *Escherichia coli* ($P < 0.05$) during antimicrobial administration and, thereafter, an overgrowth of enterococci ($P < 0.05$). The number of intestinal yeasts also increased significantly ($P < 0.05$) in groups A and B over the same period. There was a dramatic decrease in anaerobic microorganisms on day 4 of administration, mainly caused by loss of bifidobacteria ($P < 0.05$) in all groups. The number of lactobacilli also decreased but was significantly higher in group A than in group C at the end of cefpodoxime proxetil administration. *Clostridium difficile* was found in only one person from group A, but six persons each in groups B and C. Of the bifidobacterial strains isolated from the faecal samples in group A, one was similar to the strain of *B. longum* administered, but most volunteers were colonized by several different strains of *B. longum* during the investigation period. The administered strain of *L. acidophilus* was recovered from six patients in group A.

Introduction

The human intestinal microflora is a complex ecosystem where microorganisms normally live in a stable relationship with their host. The equilibrium can, however, be disrupted. Treatment with antimicrobial agents can seriously disrupt the gastrointestinal microflora.[1] Suppression of indigenous microorganisms can cause overgrowth of potential pathogens, diarrhoea and pseudomembranous colitis.

Cefpodoxime proxetil is an orally administered cephalosporin that is highly effective against a wide range of Gram-positive and Gram-negative bacteria. It is used to treat upper and lower respiratory tract infections. It causes side effects in *c.*10% of treated patients. Gastrointestinal symtoms, with increased gas production and diarrhoea, are most common. Overgrowth of enterococci and yeasts and reduced numbers of enterobacteria are the most pronounced ecological effects in the intestinal microflora.[2] Numbers of lactobacilli and bifidobacteria decrease and half of volunteers given cefpodoxime proxetil are colonized by *Clostridium difficile*.

Interest in using ecological methods to re-establish the balance of the intestinal microflora has increased recently. Supplements, so-called probiotics, containing viable bacteria (especially bifidobacteria and lactobacilli) producing human lactic acid, have been used for this purpose.[3]

Bifidobacteria and lactobacilli constitute a major part of the normal intestinal microflora in humans and probably play an important role in the host's resistance to colonization by exogenous microorganisms. *Bifidobacterium longum* and other species of bifidobacteria have been used as oral supplements to maintain or re-establish the balance of the intestinal microflora during and after antibiotic therapy.[4,5] The effects of giving *Lactobacillus acidophilus* to humans and animals have also been studied. *L. acidophilus*, given as a fermented milk product to volunteers, decreased the

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number of *Escherichia coli* and increased the number of lactobacilli.[6] Lactobacilli have also been used as dietary supplements to re-establish the balance of the intestinal microflora during or after antimicrobial therapy, alone[7,8] or in combination with bifidobacteria.[9,10] Supplementation with a combination of *B. longum* and *L. acidophilus* during administration of clindamycin significantly reduced the ecological changes in the intestinal microflora caused by clindamycin alone.[11]

Interest in the use of substances that promote the growth of certain endogenous microorganisms has increased recently. These food items, sometimes called prebiotics, are indigestible food ingredients that benefit the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species in the colon.[12] Oligofructose, which occurs naturally in inulin-rich plants like Jerusalem artichoke, onion and asparagus, is poorly digested by the human body but has been shown to stimulate selectively the growth of bifidobacteria *in vitro* and *in vivo*.[13,14]

The present investigation was performed to study the intestinal microflora, faecal pH and clinical status in healthy volunteers before, during and after administration of cefpodoxime proxetil, with or without supplements of *B. longum*, *L. acidophilus* and oligofructose.

Materials and methods

Subjects

Thirty healthy volunteers with no history of gastrointestinal, hepatic or renal diseases participated in the study. They were divided into three groups, each consisting of six women and four men. The mean age was 28 years (range 21–50 years). None of the volunteers had been treated with antibiotics in the 3 months before the study. No other medication except cefpodoxime proxetil was allowed during the investigation period. The investigation was performed as a randomized double-blind parallel group study and was approved by the Local Ethics Committee of Huddinge University Hospital, Karolinska Institute, Stockholm, Sweden.

Clinical examination

The subjects visited the hospital before the investigation (day 0) and on day 21 of the investigation. A routine physical examination was performed. Bowel habits and possible adverse events were monitored by asking volunteers to keep a diary and by questioning at the second hospital visit.

Administration of cefpodoxime proxetil

All subjects received two 100 mg cefpodoxime proxetil tablets (Orelox; Roussel Uclaf SA, France) orally bd for 7 days.

Administration of microorganisms and oligofructose

Ten of the volunteers receiving cefpodoxime proxetil were given 250 mL of a fermented milk supplement containing 5×10^7 to 2×10^8 cfu/mL of *B. longum* BB 536 and 2×10^8 to 3×10^8 cfu/mL of *L. acidophilus* NCFB 1748 bd for 21 days together with 15 g of oligofructose (Raftilose; Orafti, Belgium), starting on the same day as the cefpodoxime proxetil administration (group A). Ten other volunteers were given a placebo milk supplement with 15 g of oligofructose for 21 days (group B). The remaining 10 volunteers received a placebo milk supplement without oligofructose (group C). All milk products contained the yoghurt culture bacteria *Lactobacillus delbrueckii* subsp. *bulgaricus* LBU 108 (10^7–10^8 cfu/mL) and *Streptococcus salivarius* subsp. *thermophilus* STH 482 (10^8–10^9 cfu/mL) and were freshly prepared every week. No other fermented milk products or other probiotic products were allowed during the investigation.

Collection of faecal specimens

Stool specimens were collected before administration, on days 2, 4 and 7 of the antimicrobial administration period and 2, 4, 7, 14 and 21 days after stopping the antimicrobial agent. The specimens were collected in sterile plastic containers and immediately sent to the laboratory where they were stored at –70°C until analysis. It has been shown that this freezing procedure does not affect the results of the study.[15]

Microbiological procedures

The faecal samples were suspended in prereduced peptone–yeast extract medium, diluted, inoculated on non-selective or selective medium and processed as described by Heimdahl & Nord.[16] Selective media were used for anaerobic cultivation of bifidobacteria (BL agar without addition of horse blood[17]) and lactobacilli (Rogosa SL agar; Difco, Detroit, MI, USA).

Aerobic and anaerobic microorganisms were identified using morphological, serological and biochemical tests and gas–liquid chromatography. The lower limit of detection was 100 microorganisms/g faeces. Strains of bifidobacteria, lactobacilli and *C. difficile* were isolated and stored for further analyses.

Clostridium difficile cytotoxin test

The cytotoxin test was performed according to Aronsson *et al.*[18] Strains of *C. difficile*, isolated from samples growing on selective *C. difficile* agar plates, were grown under anaerobic conditions in chopped meat medium, centrifuged at 5000**g** for 15 min and passed through a sterile Millipore filter (0.2 μm). The positive control strain was *C. difficile* ATCC 9689. Twenty microlitres of the supernatants were

incubated for 20 h at 37°C with mouse fibroblast cells in microtitre plates. Cytopathogenic activity was assessed by light microscopy. A positive finding was confirmed by neutralization of a 20 μL sample with 20 μL antiserum against *C. difficile*, produced in goats (TechLab; Blacksburg, VA, USA).

Antimicrobial susceptibility tests

The MICs of cefpodoxime for *B. longum* BB 536, *L. acidophilus* NCFB 1748 and the isolated strains of *C. difficile* were determined by the agar dilution method on PDM agar (AB Biodisk, Solna, Sweden) with the addition of 5% defibrinated horse blood. The inoculum was *c.*10^6 cfu/mL, applied using a Steers replicator and incubated for 48 h at 37°C in anaerobic jars (GasPak; BBL Microbiology Systems, Cockeysville, MD, USA). The MBC was defined as the lowest concentration of cefpodoxime that killed ⩾99.9% of the initial inoculum.

Identification of strains by pulsed-field gel electrophoresis (PFGE)

Strains of lactobacilli and bifidobacteria were collected and compared by PFGE to identify changes in the composition of strains of bifidobacteria in the volunteers during the study period and to differentiate the administered strains of *B. longum* and *L. acidophilus* from endogenous bifidobacteria and lactobacilli.

Bifidobacteria were cultured in PYG broth at 37°C under anaerobic shaking conditions until an optical density at 650 nm of 1.0 was attained. The cells were harvested by centrifugation, washed twice in 500 μL buffer (10 mM Tris pH 8.0, 1 M NaCl) and resuspended in 200 μL of the same buffer. The suspension was embedded 1:1 in agarose plugs of 20 μL (1.5% agarose (SeaPlaque; FMC Bioproducts, Rockland, ME, USA) in buffer). The discs were allowed to solidify for 5 min at –20°C and were then lysed by incubation at 37°C with lysozyme (1–4 mg) and Mutanolysin (50 μg) in 1 mL of EC buffer (6 mM Tris pH 8.0, 1 M NaCl, 0.1 M EDTA pH 8.0, 0.2% sodium deoxycholate, 0.5% Sarcosyl) overnight. The discs were then incubated in proteinase K 1 mg/mL in ES buffer (0.5 M EDTA pH 9.0, 1% Sarcosyl) at 50°C for 18 h. The agarose discs were washed four times in 12 mL TE buffer (1 mM Tris pH 7.5, 0.1 mM EDTA pH 8.0) for 30 min with gentle agitation and stored in 1 mL of TE buffer at 4°C.

Restriction digestion. Bifidobacterial DNA was digested with *Xba*I (20 U/disc) and *Spe*I (20 U/disc) at 37°C overnight according to the manufacturer's recommendations.

Pulsed-field gel electrophoresis. The gels were prepared with 1% agarose (SeaKem LE; FMC Bioproducts) in 0.5 × TBE buffer (50 mM Tris, 50 mM boric acid, 0.2 mM EDTA (acid form), pH 8.0). Discs with restriction fragments were placed in the gel and separated by PFGE. The gels were run for 18.5 h at 14°C in a GenePath System (Bio-Rad, Hercules, CA, USA) in 0.5 × TBE buffer. A mid-range PFGE marker (15–291 kb) was used as standard. PFGE was also used to differentiate strains of lactobacilli. The method differed from that used for bifidobacteria in that lactobacilli were cultured on and harvested from Rogosa SL agar plates. DNA was cleaved using *Sma*I (30 U/disc) at 25°C and *Sgr*AI (15 U/disc) at 37°C and run for 22 h at 14°C.

Measurement of pH in faeces

The pH of the faecal samples was measured using a Methrom 744 pH meter (Methrom, Herisau, Switzerland) with a combined glass electrode (Methrom 6.0226.100).

Measurement of dry weight of faeces

Faecal specimens were weighed and dried in a freeze dryer until no further weight loss was recorded.

Collection of serum

Fasting serum samples for analysis of triglycerides and total cholesterol were taken before the start of the investigation and after 3 weeks of administration.

Statistical analysis

The quantitative culture results were compared within treatment groups (data from day 0 compared with day 7 or 9) using the Wilcoxon signed rank test, and between treatment groups using the Mann–Whitney *U*-test after logarithmic transformation of the numbers. P values of <0.05 were considered significant.

Results

Effect of cefpodoxime proxetil, administered microorganisms and oligofructose on aerobic intestinal microflora

The initial numbers of aerobic and anaerobic microorganisms tended to be lower in group A than in groups B and C (Figure 1). For aerobic microorganisms, this difference was statistically significant between groups A and B.

Figure 1 shows the numbers of total aerobic microorganisms in the three groups of volunteers during the investigation period. During administration of cefpodoxime proxetil and oral suppplements, the number of aerobic microorganisms decreased initially in groups B and C and then increased in all groups. The decrease was mainly caused

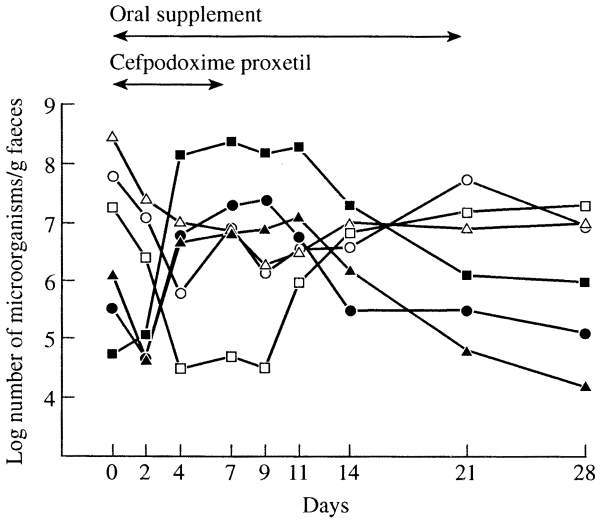

Figure 1. Effect of cefpodoxime proxetil and oral supplements with *B. longum* BB 536 and *L. acidophilus* NCFB 1748 and oligofructose (group A)/(■, □), oligofructose (group B)/(◆, ◇) or placebo (group C)/(●, ○) on the mean number of total intestinal microorganisms in nine, 10 and 10 subjects, respectively. Filled symbols represent aerobic microorganisms, open symbols anaerobic microorganisms.

by rapid and almost complete disappearance of *E. coli* ($P < 0.05$) in all groups; an overgrowth of enterococci ($P < 0.05$) was observed thereafter (Figure 2). The number of intestinal yeasts increased slightly in all groups, but the difference was significant only in groups A and B ($P < 0.05$) during administration of cefpodoxime proxetil and decreased thereafter to initial values.

Effect of cefpodoxime proxetil, administered microorganisms and oligofructose on anaerobic intestinal microflora

There was a dramatic decrease in anaerobic microorganisms on day 4 of administration; this was most pronounced in group A (Figure 1). The decrease resulted mainly from loss of bifidobacteria, which was significant in all groups at the end of administration, compared with initial values ($P < 0.05$; Figure 3). The number of lactobacilli also decreased in all three groups but was significantly higher in group A than in group C at the end of cefpodoxime proxetil administration. The mean number of *Bacteroides* spp. was also affected in all groups during the investigation.

C. difficile was isolated from one subject in group A on one occasion (day 21). In groups B and C, six persons each were colonized by the microorganism during the investigation period. One, three and four of the *C. difficile* strains isolated from groups A, B and C, respectively, produced toxin.

Antimicrobial susceptibility

The MIC of cefpodoxime was 0.25 mg/L for *L. acidophilus* NCFB 1748, 8 mg/L for *B. longum* BB 536 and 0.125 mg/L for *S. salivarius* subsp. *thermophilus* STH 482 and *L. delbrueckii* subsp. *bulgaricus* LBU 108. The MBC of cefpodoxime was 2.0 mg/L for *L. acidophilus* NCFB 1748, 32 mg/L for *B. longum* 536, 2.0 mg/L for *S. salivarius* STH 482 and 2.0 mg/L for *L. delbrueckii* LBU 108. The isolated strains of *C. difficile* were resistant to cefpodoxime, with MICs of 128–256 mg/L.

Identification of strains by PFGE

Comparison of the collected strains of bifidobacteria with the administered strain of *B. longum* showed that one isolate from day 14 from one volunteer in group A was *B. longum* BB 536 (Figure 4, lane 2). In this volunteer the bifidobacteria had disappeared on day 4 but reappeared on day 14, at 7×10^5 cfu/mL. The strain was not recovered in subsequent samples. A strain isolated on day 11 from another volunteer was very similar, but not identical, to *B. longum* BB 536 (Figure 4, lane 3). This strain also appeared at 6×10^5 cfu/mL after total depletion of bifidobacteria after day 4.

Most volunteers in group A were colonized by several different strains of bifidobacteria during the investigation period, some of these strains disappearing or decreasing to undetectable levels during the administration of the antimicrobial agent and increasing thereafter (data not shown).

In six of nine volunteers the administered strain of *L. acidophilus* NCFB 1748 was recovered occasionally during the supplementation period (Figure 5). The strain was not recovered in any of the samples taken 1 week after the end of administration of probiotics.

pH in faeces

The faecal pH decreased slightly in all groups during the first 4 days; the pH in group A was significantly lower ($P < 0.05$) than that in group C (data not shown). On day 14, the pH had increased to more than the initial pH, and then it decreased again in groups B and C by day 28 ($P < 0.05$). The fluctuations during the investigation period were most pronounced in groups A and B.

Dry weight of faeces

The dry part of faecal mass decreased significantly in all groups from a mean value of 27% to 17% (group A), from 28% to 21% (group B) and from 28% to 24% (group C) during the administration of the antimicrobial agent. Thereafter there was a significantly slower increase in groups A and B compared with group C; by the end of the investigation, values had returned to near normal. The measurements correlated well with the subjective assess-

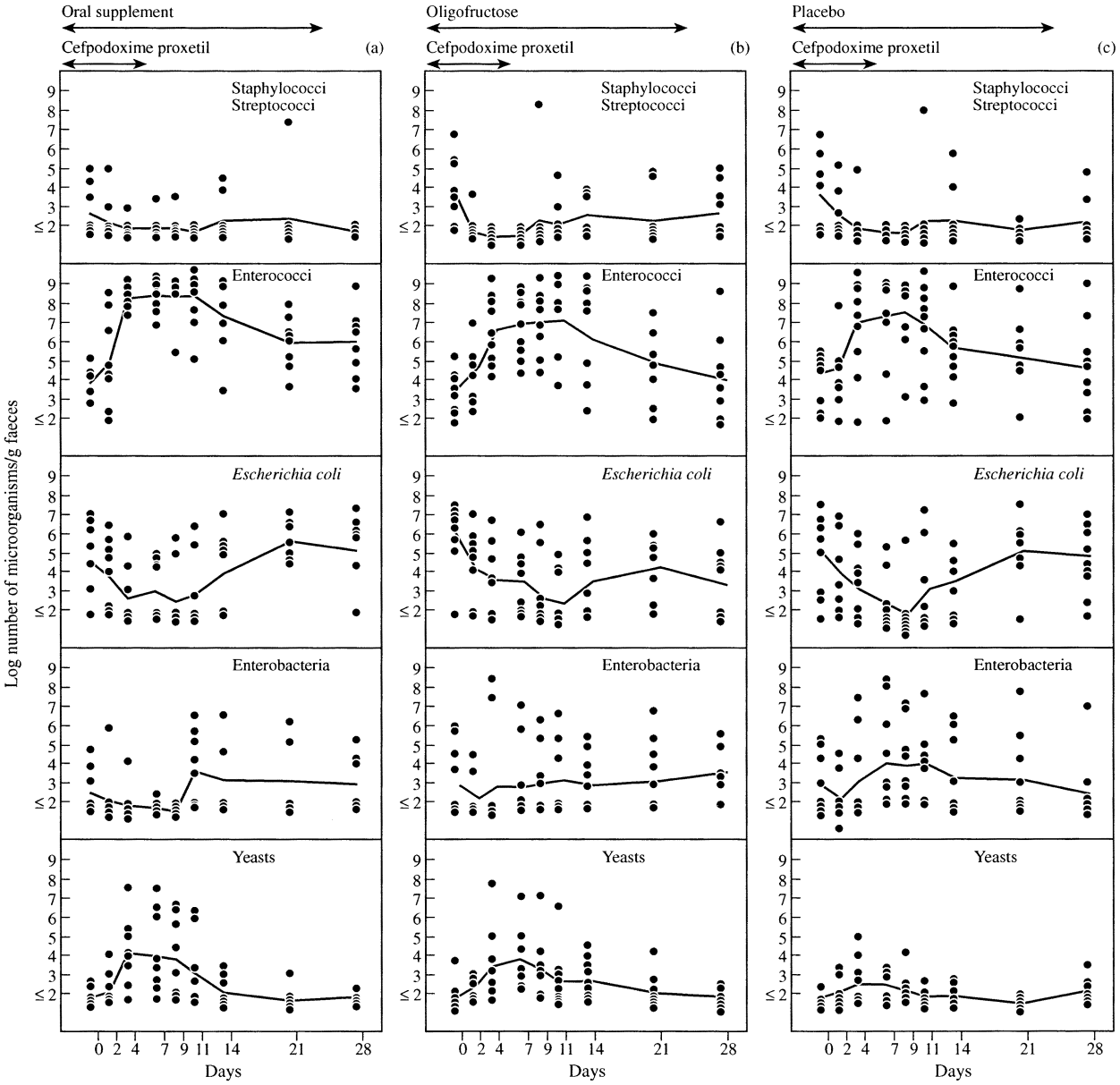

Figure 2. Effect of cefpodoxime proxetil and supplements on genera of aerobic microorganisms in the intestinal microflora in (a) group A ($n = 9$), (b) group B ($n = 10$) and (c) group C ($n = 10$). ——, mean values of the group

ment of consistency of stools reported by the volunteers during the study period.

Triglycerides and cholesterol

Concentrations of serum triglycerides and total serum cholesterol did not change in any of the groups during the investigation.

Clinical findings

One subject in group A was excluded because of treatment with another antimicrobial agent. All other volunteers completed the study without any serious side effects. The overall gastrointestinal symptoms, shown in the Table, tended to be more frequent in groups A and B than in group C. The total consumption of milk products before and during the study period increased from a mean of 0.39 L to 0.66 L daily.

Discussion

After administration of the antimicrobial agent and the oral supplements began, the number of *E. coli* strains decreased and there was a major overgrowth of entero-

cocci, as reported previously during cefpodoxime proxetil administration.[2,19] In the two groups given oligofructose, there was a significant increase in the number of yeasts, mainly *Candida albicans*, during the administration period. Enhanced colonization by yeasts is common during treatment with cefpodoxime proxetil and other cephalosporins.[19,20] Overgrowth by enterococci, yeasts and *C. difficile* is a sign of disturbance in the ecological balance of the microflora.

There was also a dramatic decrease in the anaerobic

Table. Number of subjects reporting gastrointestinal symptoms during the investigation period, recorded on eight occasions (days 2, 4, 7, 9, 11, 14, 21 and 28)

	Group A (n = 9)		Group B (n = 10)		Group C (n = 10)	
	n^a	mean (range)^b	n	mean (range)	n	mean (range)
Flatulence	6	2.2 (0–8)	8	2.7 (0–7)	5	1.1 (0–3)
Loose stools	5	2.4 (0–5)	6	2.3 (0–7)	2	0.6 (0–6)
Abdominal pain	3	0.4 (0–2)	2	0.2 (0–1)	2	0.3 (0–2)
Constipation	0	0 (0–0)	2	0.7 (0–4)	3	0.4 (0–2)
No symptoms	0	0 (0)	1	0.8 (0–8)	4	3.2 (0–8)

[a]Number of symptomatic subjects.
[b]Mean (range) of symptomatic sampling days during the investigation.

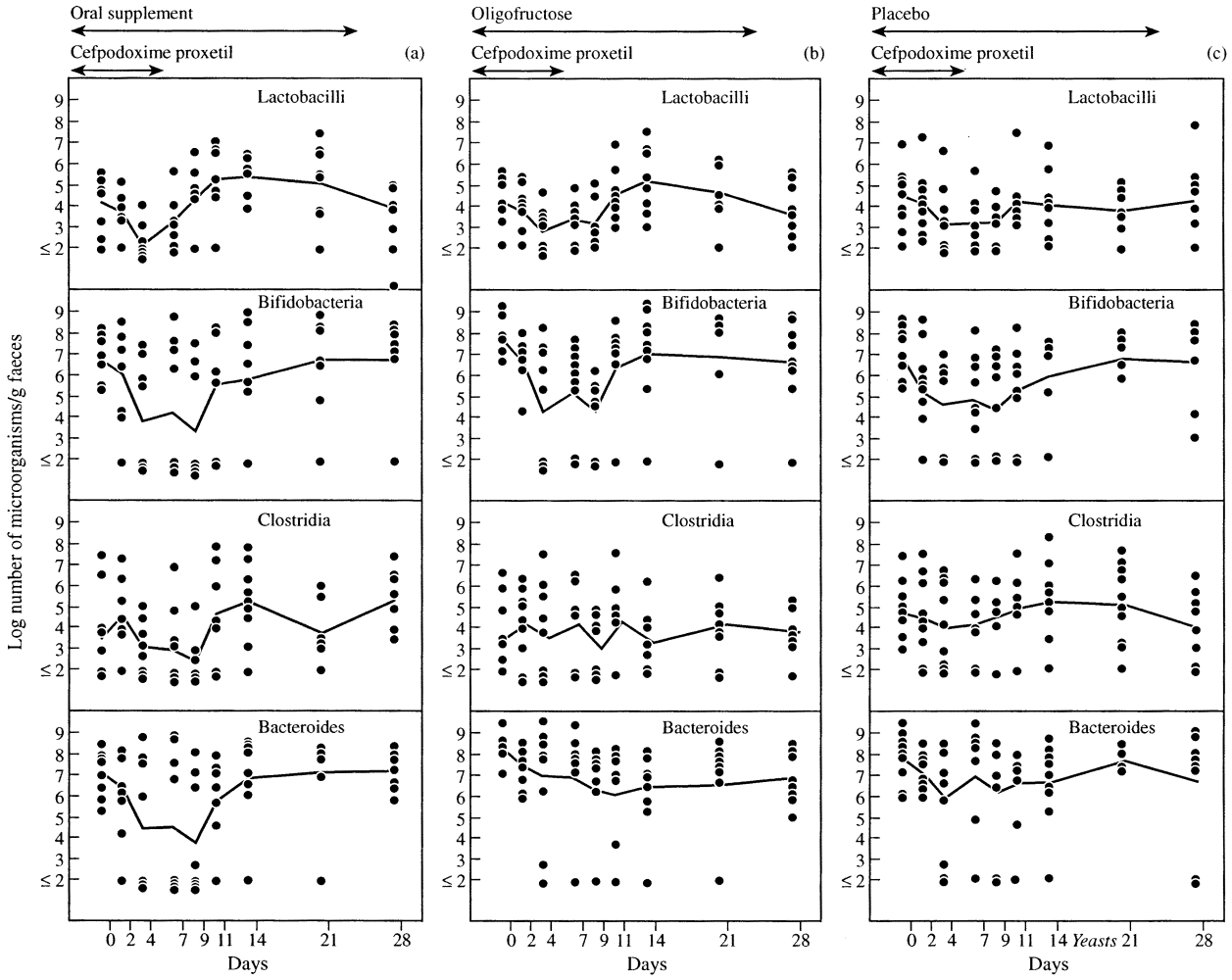

Figure 3. Effect of cefpodoxime proxetil and supplements on genera of anaerobic microorganisms in the intestinal microflora in (a) group A (n = 9), (b) group B (n = 10) and (c) group C (n = 10). ——, mean values of the group.

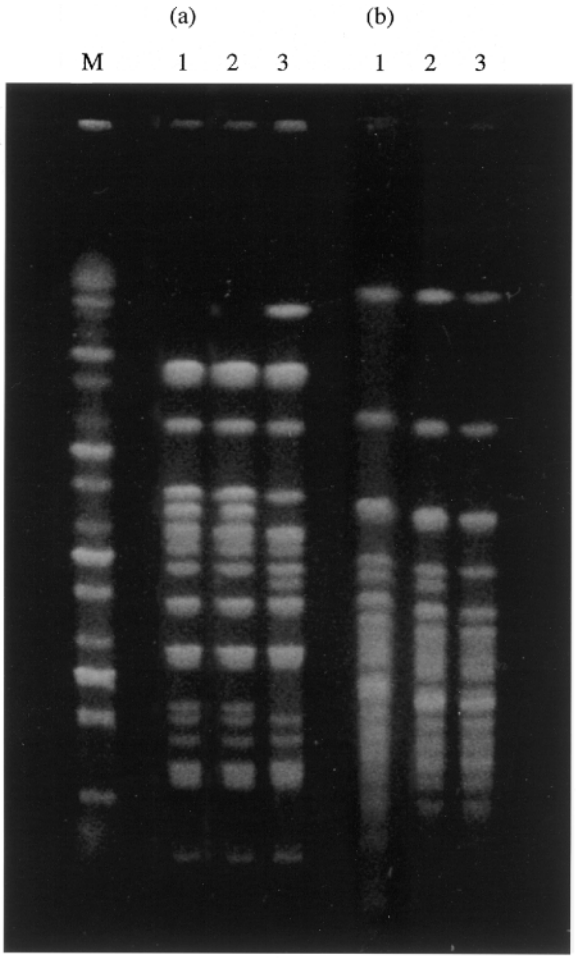

Figure 4. PFGE patterns of (a) *Xba*I and (b) *Spe*I digests of genomic DNA of bifidobacteria. The isolates are: lane 1: *B. longum* BB 536; lane 2: strain from volunteer 13; lane 3: strain from volunteer 24.

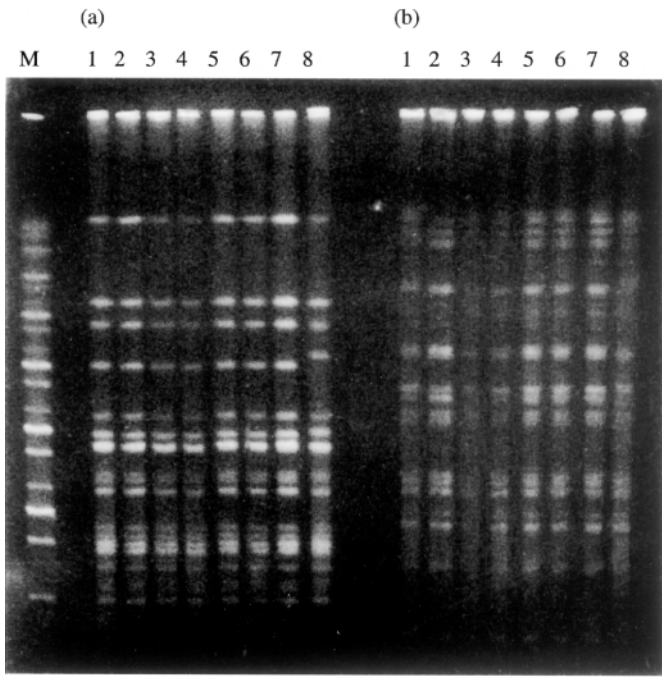

Figure 5. PFGE patterns of (a) *Sma*I and (b) *Sgr*AI digests of lactobacillus genomic DNA. Lane 1: *L. acidophilus* NCFB 1748; lane 2, isolate from volunteer 13; lane 3, isolate from volunteer 15; lane 4, isolate from volunteer 24; lane 5, isolate from volunteer 25; lane 6, isolate from volunteer 28; lane 7, isolate from volunteer 29.

microflora, mainly resulting from loss of bifidobacteria, lactobacilli and bacteroides; this was similar in all groups. The strains of lactobacilli and bifidobacteria administered were sensitive to cefpodoxime *in vitro*. However, by the end of administration of cefpodoxime and probiotics (group A), significantly higher numbers of lactobacilli were found. An increase in the number of lactobacilli during administration of *L. acidophilus* NCFB 1748 after administration of enoxacin or clindamycin was also observed by Lidbeck *et al.*,[21] but the total number of lactobacilli was not significantly altered during administration of *Lactobacillus rhamnosus* strain GG with erythromycin.[8]

Colonization by *C. difficile* is common during treatment with antimicrobial agents that markedly disturb the balance of the microflora, such as cephalosporins, clindamycin and ampicillin.[22] *C. difficile* has been shown to cause *c.*30% of cases of antimicrobial-associated non-specific colitis and *c.*20% of antimicrobial-associated diarrhoea without colitis.[23] Nosocomial infections with *C. difficile* are frequently reported and constitute an increasing problem.[24] In the present study, six of 10 volunteers in each of groups B and C were colonized by strains of *C. difficile* and half of them were cytotoxin-positive, a frequency comparable to that reported earlier.[2,18] However, in people in group A, who were given lactobacilli and bifidobacteria, the isolation of *C. difficile* was markedly reduced: only one subject had detectable numbers of *C. difficile* at one sampling occasion (day 21), 2 weeks after administration of the antimicrobial agent ended. A tendency to decreased frequency of isolation of *C. difficile* was also reported by Nord *et al.*[10] when a probiotic supplement containing *L. acidophilus* La-CH5 and *Bifidobacterium bifidum* Bb-12 was given to healthy volunteers during administration of clindamycin. Simultaneous intake of a strain of *B. longum* with erythromycin decreased the amount of detectable faecal clostridial spores in healthy volunteers compared with controls.[4] Certain strains of *Lactobacillus* and *Bifidobacterium* spp. inhibit the multiplication of *C. difficile in vitro*.[25] Unfortunately, there have so far been very few well-conducted clinical studies of the effects of supplementation with probiotic microorganisms on the intestinal microflora during administration of antimicrobial agents. Some of the few studies performed have focused on the treatment or prevention of antimicrobial diarrhoea. In several studies, patients with relapsing *C. difficile* diarrhoea that did not respond to courses of vancomycin and/or metronidazole have been

given *L. rhamnosus* strain GG.[26–28] The majority of patients in these studies were cured by the treatment. A significant effect on recurrence of *C. difficile* disease by treatment with *Saccharomyces boulardii* was shown in a placebo-controlled investigation where the organism was given in combination with standard antibiotics.[29]

It has been reported that oligofructose and inulin promote the growth of bifidobacteria *in vitro*[13] and in human supplementation.[14,30,31] This effect was not observed in the present study, possibly because the effects of cefpodoxime proxetil on the intestinal microflora were so pronounced that they may have swamped any effects of probiotic or carbohydrate administration on the endogenous bifidobacteria.

The administered strain of *B. longum* was isolated from one faecal sample on day 14 in a volunteer depleted of bifidobacteria from day 4 to the day of appearance of this strain. The strains of bifidobacteria were analysed by colony morphology on agar plates. The strain administered may also have been present in other samples, but in lower numbers, masked by the other, more numerous, bifidobacterial strains on the plates.

In six of the nine subjects given lactic acid bacteria, *L. acidophilus* NCFB 1748 was recovered. This species has a distinctive morphology on Rogosa agar plates, making it easy to identify. The number of endogenous lactobacilli, relative to the number administered, is lower than for bifidobacteria, making it easier to recover administered lactobacilli than bifidobacteria from the faecal samples. Lactobacilli, such as strains *L. rhamnosus* GG, *Lactobacillus gasseri* (ADH) and *Lactobacillus reuteri*, have been found in faeces after oral intake.[32–34]

Most of the volunteers who participated in this study experienced gastrointestinal symptoms during the investigation period. The most common symptoms were flatulence, loose stools and an increased frequency of defaecation. Increased flatulence during intake of oligofructose and inulin in approximately the same doses as in the present investigation has been reported by other investigators.[14,35] The symptoms were most pronounced in groups A and B, and the time taken for faecal dry weight/wet weight ratios to return to initial values after administration of cefpodoxime proxetil ended was longer in these groups, indicating that oligofructose was the cause. Van Dokkum *et al.*[36] recorded a non-significant trend to lower faecal dry weight percentage after intake of oligofructose or inulin 15 g daily. An increased stool frequency after intake of oligofructose and inulin has been shown by Gibson *et al.*[30] and Kleessen *et al.*[31]

It has been reported that intake of oligofructose and inulin reduces the serum concentrations of some lipids in animal and human studies.[37,38] In the present investigation, we did not find any changes in serum triglycerides or total serum cholesterol. In agreement, van Dokkum *et al.*,[36] in the study reported above, did not find any significant differences in blood lipids in young males. Pedersen *et al.*[35] did not find any effect on fasting cholesterol or serum triglycerides of inulin 14 g/day for 4 weeks in healthy women.

In conclusion, supplementation with *B. longum* BB 536 and *L. acidophilus* NCFB 1748 and/or oligofructose during administration of cefpodoxime proxetil was well tolerated by the volunteers. The recovery of the two given bacterial strains in faecal samples shows that these microorganisms can survive passage through the intestinal tract. In the group given *B. longum*, *L. acidophilus* and oligofructose, *C. difficile* was isolated at a lower frequency than in the other two groups. This observation may be of clinical value but has to be investigated further in clinical studies, mainly of patients at risk of developing *C. difficile* disease.

Acknowledgement

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