Alimentary Pharmacology & Therapeutics

Clinical trial: a multistrain probiotic preparation significantly reduces symptoms of irritable bowel syndrome in a double-blind placebo-controlled study

E. A. WILLIAMS*, J. STIMPSON*, D. WANG†, S. PLUMMER‡, I. GARAIOVA‡, M. E. BARKER* & B. M. CORFE*

*Human Nutrition Unit, School of Medicine, The University of Sheffield, Sheffield, UK; †Medical Statistics Unit, London School of Hygiene and Tropical Medicine, London, UK; ‡Obsidian Research Ltd, Baglan, Port Talbot, UK

Correspondence to:
Dr E. A. Williams, Human Nutrition Unit, School of Medicine, University of Sheffield, Royal Hallamshire Hospital, Sheffield S10 2RX, UK.
E-mail: e.a.williams@sheffield.ac.uk

Publication data
Submitted 29 May 2008
First decision 23 June 2008
Resubmitted 28 August 2008
Resubmitted 1 September 2008
Accepted 5 September 2008
Epub Accepted Article 9 September 2008

SUMMARY

Background
The efficacy of probiotics in alleviating the symptoms of irritable bowel syndrome (IBS) appears to be both strain- and dose-related.

Aim
To investigate the effect of LAB4, a multistrain probiotic preparation on symptoms of IBS. This probiotic preparation has not previously been assessed in IBS.

Methods
Fifty-two participants with IBS, as defined by the Rome II criteria, participated in this double blind, randomized, placebo-controlled study. Participants were randomized to receive either a probiotic preparation comprising two strains of *Lactobacillus acidophilus* CUL60 (NCIMB 30157) and CUL21 (NCIMB 30156), *Bifidobacterium lactis* CUL34 (NCIMB 30172) and *Bifidobacterium bifidum* CUL20 (NCIMB 30153) at a total of 2.5×10^{10} cfu/capsule or a placebo for 8 weeks. Participants reported their IBS symptoms using a questionnaire fortnightly during the intervention and at 2 weeks post-intervention.

Results
A significantly greater improvement in the Symptom Severity Score of IBS and in scores for quality of life, days with pain and satisfaction with bowel habit was observed over the 8-week intervention period in the volunteers receiving the probiotic preparation than in the placebo group.

Conclusion
LAB4 multistrain probiotic supplement may benefit subjects with IBS.

doi:10.1111/j.1365-2036.2008.03848.x

INTRODUCTION

Irritable bowel syndrome (IBS) is a chronic relapsing gastrointestinal condition characterized by abdominal discomfort, bloating and changes in bowel habit. It has a significant negative impact on quality of life and social functioning, but does not lead to the development of serious disease and associated mortality. Nevertheless, IBS does generate significant direct and indirect healthcare costs.[1] IBS symptom pathogenesis is far from clearly defined and most hypotheses focus on one or more of the following: altered intraluminal milieu, immune activation, enteric neuromuscular dysfunction and/or brain–gut axis dysregulation. It has been proposed that IBS may result from a dysfunctional interaction between the indigenous flora and the intestinal mucosa leading to immune activation in the colonic mucosa.[2] Changes in the colonic microbiota could result in the proliferation of gas-producing organisms or in organisms that facilitate deconjugation of bile acids thereby impacting upon water and electrolyte transport within the colon. Alleviation of the symptoms of the bacterial overgrowth (small intestinal bacterial overgrowth) observed in some IBS sufferers by the use of antibiotics also provides evidence for the contribution of microbial abnormalities to IBS symptoms.[3]

Dysregulation of the microbiota is also linked to the growing evidence for the onset of IBS following an attack of acute gastroenteritis, which is associated with on-going inflammation induced by the infecting organisms.[4]

Pharmacological therapy for IBS has primarily targeted individual symptoms by means of antidiarrhoeals, laxatives and antispasmodics with some successes using antidepressants and serotonergic agents, but the latter are associated with some safety issues.[5, 6] Success with these drugs has been limited and although potential therapeutic targets have been identified, new drugs are not available, which has led many IBS sufferers to seek alternative remedies.

Data are accumulating to suggest that the use of probiotic-based products may be beneficial for the control of IBS symptoms. Probiotics are live microorganisms which, when administered in adequate amounts, confer health benefits on the host.[6]

Quigley and Flourie[7] reviewed the use and efficacy of probiotics in IBS and suggested a clear rationale for probiotic usage in response to a dysfunctional relationship between the indigenous microbiota and the host. The authors further suggested the feasibility of probiotics for bacterial displacement and alteration of luminal content. However, clarification is required regarding the need for clear definition of strains, dosage and viability of the probiotic organisms in use.

The probiotic product used in this study comprises a consortium of lactobacillus and bifidobacterial organisms. Kassinen *et al.*[8] have shown that both the lactobacillus and the bifidobacterial components of the microbiota of IBS sufferers were present in lower numbers than in the controls suggesting a value for intervention strategies comprising both organisms. The consortium and dose used in this study had previously proved effective in both prevention of *Clostridium difficile in vivo*[8, 9] and the modulation of the composition of the intestinal re-growth population following antibiotic therapy.[10, 11]

The aim of this randomized, double-blind placebo-controlled trial was to assess the potential of the LAB4 multistrain probiotic (comprising two strains of *Lactobacillus acidophilus* CUL60 and CUL21 together with *Bifidobacterium lactis* CUL34 and *Bifidobacterium bifidum* CUL20) to attenuate the symptoms of IBS.

MATERIALS AND METHODS

Participants

Ethical approval for this study was granted by The University of Sheffield Research Ethics Committee (Ref.: SMBRER 13). Volunteers reporting active IBS symptoms were recruited to the study through advertisements in a local newspaper and posters placed around The University of Sheffield. Volunteers were informed at recruitment that the study was to investigate the effect of a probiotic on the symptoms of IBS in accordance with ethical requirements. Subjects were excluded if they had a history of abdominal surgery, were pregnant or lactating, had other gastrointestinal disorders, were already taking prebiotic or probiotic products or were receiving medication for symptoms of IBS. All volunteers reported a previous diagnosis of IBS by their general practitioner (GP), but GP records were not checked. Self-reported symptoms of IBS were used to confirm the presence of IBS according to the Rome II criteria.[12] Volunteers provided written, informed consent.

Study design

This was a double-blind placebo-controlled study to evaluate the efficacy of a multistrain probiotic

preparation in the treatment of IBS. The study was conducted over a 10-week period. Subjects were asked to complete a validated questionnaire to assess IBS symptoms[13] at baseline (0) and fortnightly throughout an 8-week intervention (2, 4, 6 and 8). IBS symptoms were again assessed at week 10 to investigate if there was an effect beyond the period of supplementation. The questionnaire assesses severity and duration of abdominal pain (abdominal pain, days with pain), abdominal distension (bloating), satisfaction with bowel habits (bowel habit) and quality of life. Volunteers were asked to record the number of days they had experienced abdominal pain over the previous 2 weeks and then this was calculated as a percentage. All other components were assessed using a visual analogue scale and scored out of 100. Individual scores were combined to give the total Symptom Severity Score with a maximum score of 500. This score classifies subjects as having no symptoms (<75), mild (75–175), moderate (175–300) or severe IBS (>300). The questionnaire has been shown to be reproducible, sensitive to change and is easy to complete.[13] The primary endpoint was the IBS Symptom Severity Score during the intervention and follow up and its components were the secondary endpoints.

Sample size and randomization

Fifty-six subjects were recruited to the study and randomized (stratified by age and gender) to receive probiotic treatment (n = 28) and placebo treatment (n = 28). The sample size was calculated based on a 15% reduction in severity of symptoms. It was calculated that 50 subjects (25 in each group of the study) were needed to detect a difference between the two groups with a power of 80% at the 5% level of statistical significance. The sample size was increased to 56 subjects to allow for just over 10% drop out rate.

Probiotic intervention

The probiotic and the placebo preparations were prepared as identically packaged, cellulose capsules by Cultech Ltd, Port Talbot, UK. The probiotic preparation contained two strains of *L. acidophilus*, CUL-60 (NCIMB 30157), CUL-21(NCIMB 30156), *B. bifidum* CUL-20 (NCIMB 30153) and *B. lactis* CUL-34 (NCIMB 30172) at a total of 2.5×10^{10} colony forming units (cfu) per capsule. The placebo contained 300 mg maltodextrin. Volunteers were instructed to ingest one capsule per day with water for 8 weeks. Compliance was assessed by counting the number of capsules remaining at the end of the intervention and checked against self-reported capsule diaries.

Compliance

Of the fifty-six volunteers recruited, four subjects in the placebo arm withdrew from the study (one due to ill health, two for deviation from protocol and one for unknown reasons) (Figure 1). Fifty-two subjects participated in the intervention comprising 28 subjects in the treatment group and 24 subjects in the control group (Table 1). Four subjects (two in each treatment arm) failed to return all the questionnaires.

Side effects

One subject in the treatment group reported an increase in flatulence throughout the duration of the study. No other side-effects were reported.

Statistical analysis

The primary and secondary endpoints were analysed by an ANOVA model with repeated measurements. In this model, the baseline measurement of an endpoint, treatment, time and interaction between time and treatment were treated as fixed effects whereas the subject was treated as a random effect. During the trial, four subjects failed to complete all questionnaires, resulting in some incomplete observations. These incomplete

Table 1. Baseline characteristics of the study population.

	Probiotic	Placebo
No. of subjects	28	24
Female % (n)	89 (25)	83 (20)
Mean age in years ± s.d.	40 (12)	38 (11)
Predominant bowel habit % (n)		
Alternating	61 (17)	62.5 (15)
Constipation	29 (8)	25 (6)
Diarrhoea	11 (3)	12.5 (3)
Mean IBS severity score ± s.d.	283 ± 61	252 ± 60
IBS classification % (n)		
Mild	7 (2)	12.5 (3)
Moderate	57 (16)	75 (18)
Severe	36 (10)	12.5 (3)

IBS, irritable bowel syndrome.

observations are not imputed but are assumed to be missing at random in the ANOVA model analysis. The estimated treatment differences from the ANOVA model are therefore reported together with their 95% confidence intervals. Reported *P*-values are two-sided and a *P*-value of <0.05 was considered statistically significant and all statistical analyses were carried out by using the Statistical Analysis System (SAS) version 9.1 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

The demographic and baseline characteristics of the subjects are shown in Table 1. The two groups of subjects were similar in terms of age, gender, type of bowel habit and symptoms. Subjects receiving the probiotic preparation had a higher IBS severity score at baseline than the subjects receiving placebo (mean ± s.d. 283 ± 61 vs. 252 ± 60 respectively).

Significant improvements from baseline for Symptom Severity Score were seen throughout the intervention period in both active and placebo groups from weeks 2 to 10 (Table 2). The overall *P*-value for the ANOVA analysis evaluating all time points was 0.0008. More detailed analysis of these symptoms showed that significant improvements from the baseline were reported in quality of life and satisfaction with bowel habit in both groups throughout the study. Abdominal pain/bloating symptoms did not show significant improvements from the baseline in the probiotic group until week 4 ($P = 0.0002$; LS mean −16.10; 95% CI: −24.64 to −7.56) of intervention and that in the placebo group, significant improvements in these symptoms were only recorded at week 6 ($P = 0.0218$; LS mean −11.04; 95% CI: −20.46 to −1.62) and week 8 ($P = 0.0028$; LS mean −14.74; 95% CI: −24.38 to −5.11). The number of days with pain improved significantly by week 2 in the probiotic group ($P = 0.0176$; LS mean −8.46; 95% CI: −15.44 to −1.49), but did not reduce significantly for the placebo group until 4 weeks into the study ($P = 0.0058$; LS mean −10.64; 95% CI: −18.17 to −3.11). The severity of abdominal pain reduced significantly in both groups from week 4 onwards (probiotic: $P < 0.0001$, LS mean −19.05, 95% CI: −27.41 to −10.70; placebo: $P = 0.0185$, LS mean −10.88, 95% CI: −19.93 to −1.84). The overall placebo effect for the Symptom Severity Score in this study was 33% ranging from 23% to 45% for the individual symptoms.

Comparison of the effectiveness of the probiotic in the presence of significant placebo effect in this study showed a significant difference in the Symptom Severity Score in favour of the probiotic at 6 weeks ($P = 0.0347$; difference between groups: −47.82; 95% CI: −92.18 to −3.4) and 8 weeks ($P = 0.0217$; difference between groups: −52.73; 95% CI: −97.67 to −7.78) but by 2 weeks postintervention, no significant differences could be detected between the probiotic and placebo groups (Figure 2).

Figure 3 shows that greater improvements were recorded for all symptoms for the probiotic group than for the placebo group throughout the study.

Table 2. Symptom severity at baseline and change in symptom severity in treatment and placebo group at weeks 8 and 10 (2 weeks postintervention)

	Baseline (mean ± s.d.)	Week 8 (mean ± s.d.)	Change at week 8	*P*-value	Week 10 (mean ± s.d.)	Change at week 10	*P*-value
Placebo group							
Symptom Severity Score	252.08 ± 59.92	172.00 ± 99.51	−80.66	**<0.0001**	193.41 ± 75.49	−59.25	**0.0005**
Abdominal distension/bloating	46.71 ± 21.83	32.05 ± 29.64	−14.74	**0.0028**	39.27 ± 25.00	−7.52	0.1259
Satisfaction with bowel habit	68.04 ± 20.08	44.36 ± 21.60	−24.41	**<0.0001**	48.68 ± 17.15	−20.09	**<0.0001**
Number of days with pain	42.67 ± 23.74	27.68 ± 23.31	−14.27	**0.0004**	32.14 ± 22.52	−9.82	**0.0148**
Quality of life	61.88 ± 11.64	47.41 ± 17.58	−16.07	**<0.0001**	48.59 ± 14.40	−14.89	**0.0001**
Abdominal pain	32.79 ± 15.04	20.50 ± 26.05	−16.16	**0.0009**	24.73 ± 23.59	−11.94	**0.0134**
Active group							
Symptom Severity Score	282.68 ± 60.59	150.23 ± 101.96	−133.39	**<0.0001**	189.19 ± 84.28	−94.43	**<0.0001**
Abdominal distension/bloating	48.54 ± 25.77	25.88 ± 25.05	−22.80	**<0.0001**	36.65 ± 23.51	−12.04	**0.0080**
Satisfaction with bowel habit	73.39 ± 17.73	39.65 ± 23.83	−32.34	**<0.0001**	48.38 ± 20.01	−23.61	**<0.0001**
Number of days with pain	48.64 ± 21.81	26.12 ± 24.29	−22.94	**<0.0001**	28.27 ± 21.09	−20.79	**<0.0001**
Quality of life	67.61 ± 15.70	37.50 ± 22.40	−29.65	**<0.0001**	48.58 ± 19.81	−18.57	**<0.0001**
Abdominal pain	44.50 ± 18.03	21.08 ± 24.06	−21.20	**<0.0001**	27.31 ± 21.09	−14.97	**0.0008**

Significant improvements in quality of life (Figure 3a) were recorded for those receiving the probiotic at the end of the intervention period ($P = 0.0068$; difference between groups: −13.58; 95% CI: −23.38 to −3.78 at week 8) and this was associated with significantly improved satisfaction with bowel habit (Figure 3c) for the probiotic subjects over the placebo group at 6 weeks ($P = 0.0422$; difference between groups: −11.05; 95% CI: −21.70 to −0.39).

The number of days with pain (Figure 3d) recorded was significantly lower in the probiotic group at week 10 than in the placebo group ($P = 0.0448$; difference between groups: −10.97; 95% CI: −21.69 to −0.26).

DISCUSSION

Significant differences in the Symptom Severity Score were recorded between the probiotic and placebo groups correlating with improved quality of life and bowel habit together with fewer numbers of days with pain for the probiotic group. No differences in abdominal pain or bloating were discernible between the two groups. The use of the probiotic was well tolerated and free from significant adverse effects. The effect of the probiotic on the different groups of bowel habit could not be ascertained in this study because of lack of numbers in each group.

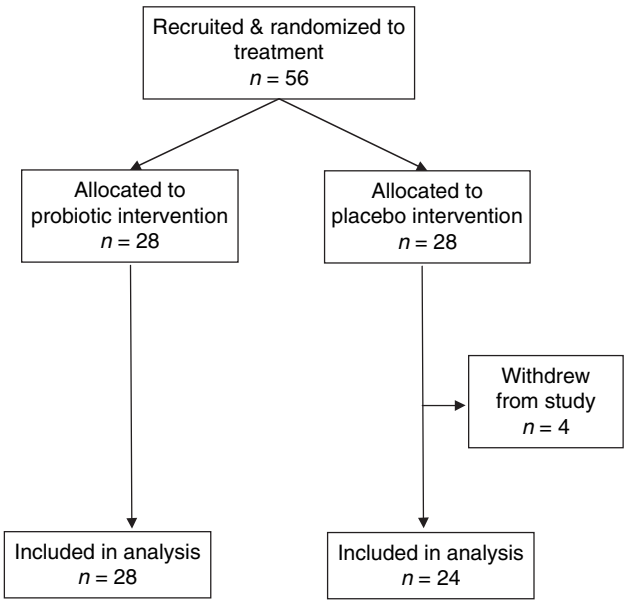

Figure 1. Flow chart of subject progression through the study.

The overall placebo response rate observed in this study (33%) is comparable with that seen in many other IBS studies. Patel *et al.*[14] concluded that placebo effects in IBS clinical trials measuring global outcome were highly variable ranging from 16 to 71%, whereas Dorn *et al.*[15] found a placebo response rate of 42.6% in complementary and alternative medicine IBS trials. Several factors are thought to contribute towards the placebo effect including Pavlovian conditioning and the expectation of a positive outcome.[16] In this trial all, volunteers had been informed that the purpose of the study was to investigate the possible benefits of a probiotic preparation, although they knew that they may be receiving a placebo. Owing to the nature of the intervention the volunteers may have been anticipating an improvement in their IBS symptoms which is likely to have contributed towards the placebo effect.

Several randomized controlled trials (RCTs) have been set up with IBS sufferers to assess the efficacy of multistrain probiotic preparations containing a variety of organisms at different doses and for different study periods and the responses have been variable. Most of the products provided daily doses in the range of $5–9 \times 10^9$ cfu and, in most cases, reductions in symptom severity score were observed[17–21] and some, but not all, of the products significantly reduced abdominal pain symptoms. Guyonnet *et al.*[22] demonstrated improvements in symptoms among a constipation-predominant group of IBS sufferers receiving a daily dose of 2.5×10^{10} cfu of *Bifidobacterium animalis*

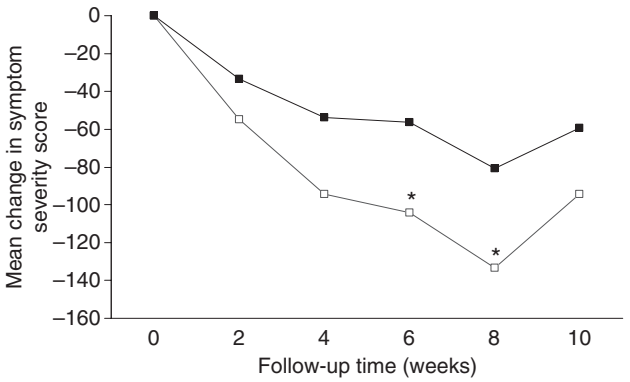

Figure 2. Effects of LAB4 multistrain probiotic on Symptom Severity Score in subjects with irritable bowel syndrome. There was a reduction in total symptom severity (mean) after the probiotic intervention (□) and in control (■) groups from baseline. Repeat measures analysis showed that there was a significant difference between the treatment groups (*$P < 0.05$).

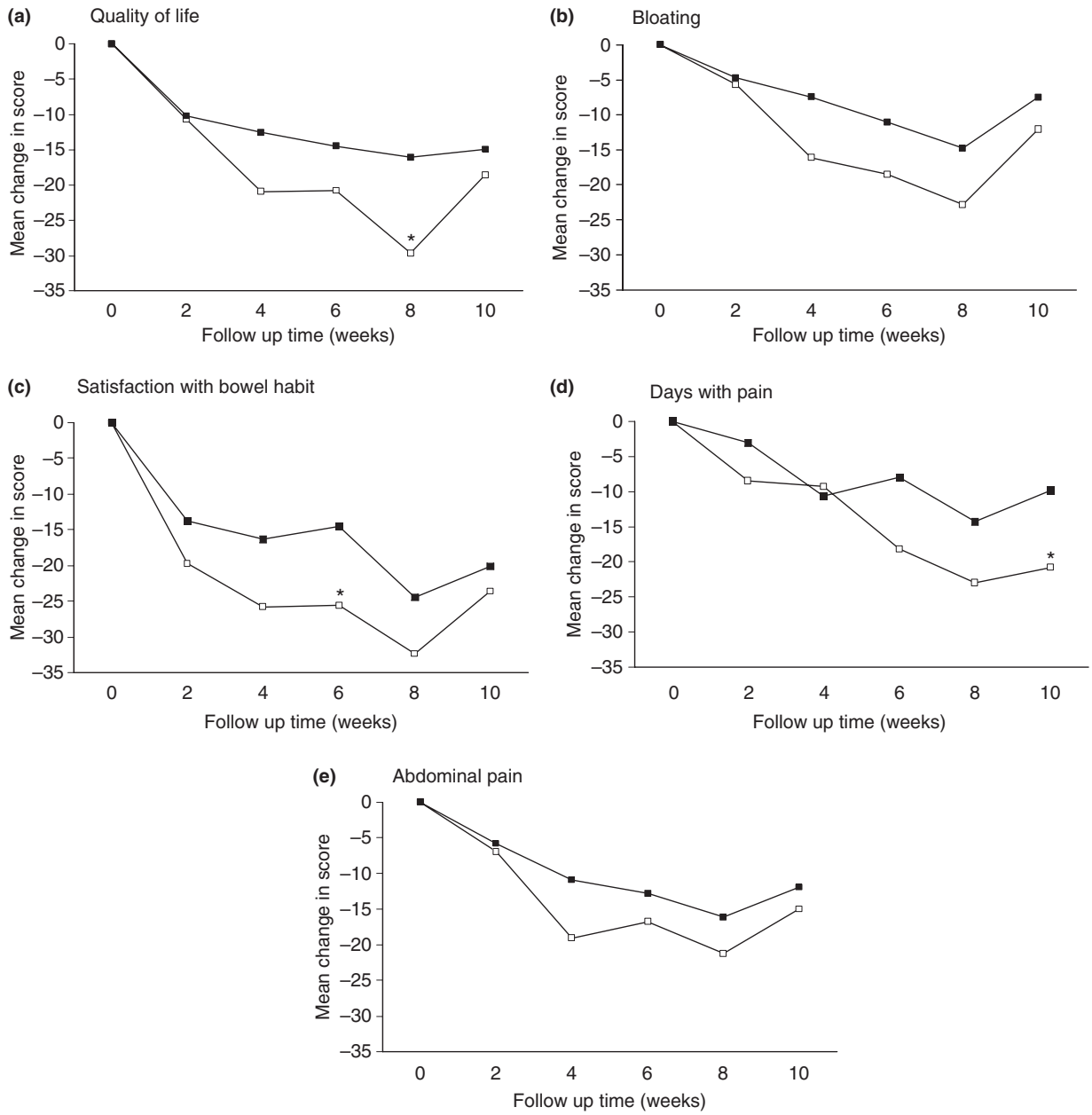

Figure 3. Change (mean) in scores for (a) quality of life, (b) bloating, (c) satisfaction with bowel habit, (d) days with pain and (e) abdominal pain during the 10-week study (*$P < 0.05$).

DN-173010, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* for a period of 6 weeks.

O'Mahoney *et al.*[23] demonstrated greater IBS symptom relief with the administration of *Bifidobacterium infantis* rather than *Lactobacillus salivarius* as single strain products and the results of Whorwell *et al.*[24] indicated that there may be a dose responsiveness to the administration of *B. infantis* (but formulation issues with the higher dosage in this study necessitate further clarification). Reduction in abdominal pain was demonstrated during an RCT with *L. acidophilus*-SDC 2012, 2013 at a daily dosage of 2×10^9 cfu by Sinn *et al.*[25], whereas in the current study with the LAB4 consortium, there was a significant reduction observed in the number of days with pain for the probiotic group. Rousseaux *et al.*[26] demonstrated that *L. acidophilus* NCFM induced MOR1 and CB2 expression through the NF-κB pathway when in contact with epithelial cells,

which contributes to the modulation of visceral pain. This potential of *L. acidophilus* to alleviate pain supports the reduction in days of pain observed for the IBS sufferers receiving the LAB4 product.

In conclusion, this study shows the potential benefit of the LAB4 multistrain probiotic supplement at a daily dosage of 2.5×10^{10} cfu in the management of IBS. Future studies will aim to identify the mechanism of the probiotics' potential beneficial effect.

ACKNOWLEDGEMENTS

Declaration of personal interests: E. A. Williams, J. Stimpson, D. Wang, M. E. Barker & B. M. Corfe: None. S. Plummer is the Managing Director and I. Garaiova is an employee of Obsidian Ltd. *Declaration of funding interests*: This study was funded in part by Obsidian Ltd, West Glamorgan, UK and by Cultech Ltd, West Glamorgan.

REFERENCES

1 Spiller R, Aziz Q, Creed F, *et al.* Guidelines on the irritable bowel syndrome: mechanisms and practical management. *Gut* 2007; **56**: 1770–98.
2 Quigley EM. Probiotics in irritable bowel syndrome: an immunomodulatory strategy? *J Am Coll Nutr* 2007; **26**: 684S–690S.
3 Pimental M. Do antibiotics influence IBS? *Am J Gastroenterol* 2002; **97**: 2681.
4 Dupont A. Post-infectious irritable bowel syndrome. *Current Gastroenterology Reports* 2007; **9**: 378–84.
5 Saad RJ, Chey WD. Recent developments in the therapy of irritable bowel syndrome. *Expert Opin Investig Drugs* 2008; **17**: 117–30.
6 FAO/WHO. *Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria.* ftp://ftp.fao.org/es/esn/food/probio_report_en.pdf: Food and Agriculture Organization of the United Nations and World Health Organization, October 2001.
7 Quigley E, Flourie B. Probiotics and irritable bowel syndrome: a rationale for their use and an assessment of the evidence to date. *Neurogastroenterol Motil* 2007; **19**: 166–72.
8 Kassinen A, Krogius-Kurikka L, Mäkivuokko H, *et al.* The fecal microbiota of irritable bowel syndrome patients differs significantly from that of healthy subjects. *Gastroenterology* 2007; **133**: 24–33.
9 Plummer S, Weaver M, Harris J, Dee P, Hunter J. *Clostridium difficile* pilot study: effects of probiotic supplementation on the incidence of *C. difficile* diarrhoea. *Int Microbiol* 2004; **7**: 59–62.
10 Plummer SF, Garaiova I, Sarvotham T, *et al.* Effects of probiotics on the composition of the intestinal microbiota following antibiotic therapy. *Int J Antimicrob Agents* 2005; **26**: 69–74.
11 Madden J, Plummer S, Tang J, *et al.* Effect of probiotics on preventing disruption of the intestinal microflora following antibiotic therapy: a double-blind, placebo-controlled pilot study. *Int Immunopharmacol* 2005; **5**: 1091–7.
12 Thompson WG, Longstreth GF, Drossman DA, *et al.* Functional bowel disorders and functional abdominal pain. *Gut* 1999; **45**: ii43–7.
13 Francis C, Morris J, Whorwell P. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. *Aliment Pharmacol Ther* 1997; **11**: 395–402.
14 Patel S, Stason W, Legedza A, *et al.* The placebo effect in irritable bowel syndrome trials: a meta-analysis. *Neurogastroenterol Motil* 2005; **17**: 332–40.
15 Dorn S, Kaptchuk T, Park J, *et al.* A meta-analysis of the placebo response in complementary and alternative medicine trials of irritable bowel syndrome. *Neurogastroenterol Motil* 2007; **19**: 630–7.
16 Musial F, Klosterhalfen S, Enck P. Placebo responses in patients with gastrointestinal disorders. *World J Gastroenterol* 2007; **13**: 3425–9.
17 Kajander K, Myllyluoma E, Rajilíc-Stojanovíc M, *et al.* Clinical trial: multispecies probiotic supplementation alleviates the symptoms of irritable bowel syndrome and stabilizes intestinal microbiota. *Aliment Pharmacol Ther* 2008; **27**: 48–57.
18 Kim H, Vazquez Roque M, Camilleri M, *et al.* A randomized controlled trial of a probiotic combination VSL# 3 and placebo in irritable bowel syndrome with bloating. *Neurogastroenterol Motil* 2005; **17**: 687–96.
19 Kajander K, Hatakka K, Poussa T, Färkkilä M, Korpela R. A probiotic mixture alleviates symptoms in irritable bowel syndrome patients: a controlled 6-month intervention. *Aliment Pharmacol Ther* 2005; **22**: 387–94.
20 Saggioro A. Probiotics in the treatment of irritable bowel syndrome. *J Clin Gastroenterol* 2004; **38**(Suppl. 6): S104–6.
21 Tsuchiya J, Barreto R, Okura R, *et al.* Single-blind follow-up study on the effectiveness of a symbiotic preparation in irritable bowel syndrome. *Chin J Dig Dis* 2004; **5**: 169–74.
22 Guyonnet D, Chassany O, Ducrotte P, *et al.* Effect of a fermented milk containing *Bifidobacterium animalis* DN-173 010 on the health-related quality of life and symptoms in irritable bowel syndrome in adults in primary care: a multicentre, randomized, double-blind, controlled trial. *Aliment Pharmacol Ther* 2007; **26**: 475–86.
23 O'Mahony L, McCarthy J, Kelly P, *et al.* Lactobacillus and bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. *Gastroenterology* 2005; **128**: 541–51.
24 Whorwell P, Altringer L, Morel J, *et al.* Efficacy of an Encapsulated Probiotic *Bifidobacterium infantis* 35624 in Women with Irritable Bowel Syndrome. *Am J Gastroenterol* 2006; **101**: 1581–90.
25 Sinn D, Song J, Kim H, *et al.* Therapeutic Effect of *Lactobacillus acidophilus* -SDC 2012, 2013 in Patients with Irritable Bowel Syndrome. *Dig Dis Sci* 2008; **53**: 2714–18, doi: 10.1007/s10620-007-0196-4.
26 Rousseaux C, Thuru X, Gelot A, *et al.* Lactobacillus acidophilus modulates intestinal pain and induces opioid and cannabinoid receptors. *Nat Med* 2007; **13**: 35–7.

ELSEVIER

International Journal of Antimicrobial Agents 26 (2005) 69–74

INTERNATIONAL JOURNAL OF Antimicrobial Agents

www.ischemo.org

Effects of probiotics on the composition of the intestinal microbiota following antibiotic therapy

Susan F. Plummer[a,*], Iveta Garaiova[a], Tinnu Sarvotham[b], Simon L. Cottrell[c], Stephanie Le Scouiller[d], Mark A. Weaver[a], James Tang[a], Philippa Dee[e], John Hunter[e]

[a] *Cultech Biospeciality Products, Research Department, York Chambers, York Street, Swansea SA1 3NJ, UK*
[b] *University of Wales, College of Medicine, Cardiff CF14 4XN, UK*
[c] *Cardiff School of Biosciences, Cardiff University, Cardiff CF10 3XQ, UK*
[d] *School of Construction Management and Engineering, The University of Reading, Reading RG6 6AH, UK*
[e] *Addenbrooke's Hospital, Cambridge CB2 2QQ, UK*

Received 14 February 2005; accepted 6 April 2005

Abstract

The effects of probiotic supplementation on the intestinal re-growth microbiota following antibiotic therapy were studied in a double-blind placebo-controlled study. In the placebo group, numbers of facultative anaerobes and enterobacteria increased significantly, and at day 35 the numbers were significantly higher in the placebo group than in the active group; in the active group, the numbers of bacteroides increased significantly. Although the numbers of enterococci in both groups did not change, in the placebo group the number of patients harbouring antibiotic-resistant enterococci post therapy increased significantly. There was no change in the incidence rate of antibiotic resistance among the patients in the probiotic group.

Keywords: Intestinal microbiota; Antibiotics; Probiotics; Antibiotic resistance

1. Introduction

The bacterial flora of the gastrointestinal tract play a major role in human physiology, modulating metabolic and immunological processes and providing colonisation resistance, which is the prevention of overgrowth of opportunistic microorganisms. Administration of antimicrobial agents, whether therapeutically or prophylactically, disturbs the ecological balance between the host and the normal microbiota [1]. The extent of the disturbance depends on the nature of the antimicrobial agent, the absorption, the route of elimination and any potential enzymatic degradation and/or binding to faecal material. However, predicting the effects of an antibiotic on the microbiota can be difficult due to the complex relationships among the components of the microbiota [2].

Disturbance of the microbiota is frequently associated with diarrhoea, gastritis, glossitis and pruritus [3] as well as fungal infections. In addition, altered sensitivity to secondary infection can occur. A single oral dose of streptomycin can enhance susceptibility of laboratory animals to challenge by *Salmonella* spp. by at least 100 000-fold [4]. Another important and growing area of concern is the effect of antibiotics on the colonisation resistance properties of the indigenous microbiota resulting in the emergence and spread of resistant strains between patients and the dissemination of resistance determinants between microorganisms [1]. Reid and Friendship [5] state that in 1998 the World Health Organization cited diarrhoeal diseases as the second most common cause of disability-adjusted life-years lost and of death (2.2 million). However, in many instances there is an essential requirement for the administration of antibiotics, and hence it is necessary to identify means of minimising the adverse effects of antibiotics whilst maximising their potential benefits. One method is to select for antimicrobial agents

* Corresponding author. Tel.: +44 1639 825100; fax: +44 1792 472466.
E-mail address: nsjl.plummers@virgin.net (S.F. Plummer).

0924-8579/$ – see front matter © 2005 Elsevier B.V. and the International Society of Chemotherapy. All rights reserved.
doi:10.1016/j.ijantimicag.2005.04.004

that do not disturb the microbial colonisation resistance, but this is not always possible.

Beneficial effects have been observed when probiotics have been used for the prevention and treatment of gastrointestinal disturbance [6,7]. Trials have shown the potential for the use of probiotics in the treatment of rotavirus infections, antibiotic-associated diarrhoea, traveller's diarrhoea, infantile diarrhoea, relapsing *Clostridium difficile* colitis, inflammatory bowel disease, irritable bowel syndrome, atopy in at-risk infants and chronic sinusitis [8–15].

For the purposes of this study, a cohort of *Helicobacter pylori*-infected patients receiving the triple therapy antibiotic treatment regimen was selected for investigation.

The aim was to determine the effects of probiotic supplementation during triple therapy on the composition of the intestinal re-growth population, looking both at numbers and types of microorganisms and on the incidence of antibiotic resistance in the intestinal microbiota.

2. Materials and methods

2.1. Subjects

One hundred and sixty-two patients infected with *H. pylori* were enrolled into a study at Addenbrooke's Hospital, Cambridge, UK. The *H. pylori* infection was verified by positive serology and histology by the Public Health Laboratory Service at Addenbrooke's Hospital. Patients provided written consent and had no other gastrointestinal disorders apart from peptic ulcers thought to be related to their *H. pylori* infection. None had received any antibiotics or been subject to any dietary intervention in 6 weeks prior to the study. Ethical approval was obtained from Cambridge Local Research Ethics Committee.

2.2. Trial design

The trial was a double-blind placebo-controlled study with all patients receiving antibiotics from days 1 to 7. One group of patients received the probiotic product (active group) from days 1 to 21 and the second group received the placebo product (placebo group) from days 1 to 21. Two consecutive faecal samples were tested prior to antibiotic therapy and statistical analysis indicated that the data could be pooled to provide day 1 results. A further sample was collected on day 7. Two consecutive faecal samples were obtained 4 weeks after completion of antibiotic therapy and these were pooled to provide day 35 results. The faecal samples were sealed in anaerobic bags and stored at −70 °C until tested.

2.3. Treatment

The patients received standard eradication therapy: amoxicillin (1 g twice a day (bd)), clarithromycin (500 mg bd) and lansoprazole (30 mg bd) for 7 days. For penicillin allergy, 400 mg metronidazole three times a day was substituted. The probiotic product (Cultech Ltd., Port Talbot, UK) comprised two strains of *Lactobacillus acidophilus* (CUL60 and CUL21) and two strains of *Bifidobacterium* spp. at a total of 2.5×10^{10} colony-forming units (CFU)/capsule, and the placebo comprised an inactive carrier (maltodextrin). Patients received one capsule daily. The probiotic strains used were sensitive to test antibiotics using the disk diffusion assay according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines [16].

2.4. Compliance

Of the 162 patients recruited, 7 were excluded for failing to provide the samples. The 155 remaining patients were randomly divided between the placebo group (79 patients) and the active group (76 patients).

2.5. General microbiological screen

Traditional microbiological methods were used to analyse the samples. On the basis of pilot screening studies (unpublished data), selective media and Gram staining of colony types were used to enable enumeration and differentiation of the faecal microbiotas. An anaerobic dilution series of the faecal samples was set up in pre-reduced Maximum Recovery Diluent (MRD; Oxoid Ltd., Basingstoke, UK). A modification of the Miles and Misra plate count technique [17] was used to plate $10 \times 10\,\mu L$ of appropriate dilutions onto the pre-reduced selective agars (all agars were obtained from Oxoid Ltd. unless otherwise stated): anaerobic blood (total anaerobes; bioMerieux, Basingstoke, UK); blood agar (total facultative anaerobes; bioMerieux); Wilkins–Chalgren agar (*Bacteroides* spp.); MacConkey No. 3 agar (MAC; enterobacteriaceae); Kanamycin Aesculin Azide agar (KAA; enterococci); Baird Parker agar (staphylococci); de Mann Rogosa Sharpe agar (MRS; *Lactobacillus* spp.); modified MRS agar (0.3% (w/v) sodium propionate, 0.2% (w/v) lithium chloride, 0.05% (w/v) cysteine hydrochloride and 5% (v/v) defibrinated sheep blood included; *Bifidobacterium* spp.); Rose Bengal Agar (yeasts); and ID2 Agar (*Candida albicans*; bioMerieux).

Anaerobic plates were incubated at 37 °C for 72 h and aerobic plates were incubated at 37 °C for 48 h. Organisms were identified by anaerobic/aerobic growth colony, Gram stain and API biochemical identification strips (bioMerieux). The results were expressed as the CFU per gram of dry weight of faecal material.

2.6. Antibiotic resistance analysis

The effects of the antibiotic therapy on the numbers of antibiotic-resistant enterococci and enterobacteriaceae in the faecal microbiotas pre and post antibiotic treatment were chosen for assessment in this study. KAA agar or MAC

agar containing a range of concentrations of amoxicillin or clarithromycin (0, 0.015, 0.06, 0.5, 1.0, 4.0, 8.0, 16.0, 32.0, 128.0 or 512.0 μg/mL) were used for enumeration of enterococci and enterobacteriaceae, respectively.

The samples were plated out using the modified Miles and Misra technique [17] (10 × 10 μL drops) and plates were incubated aerobically at 37 °C for 48 h (KAA agar) or 24 h (MAC agar). The breakpoints for enterococci/amoxicillin at a minimum inhibitory concentration (MIC) >8 μg/mL, for enterobacteriaceae/amoxicillin at a MIC >32 μg/mL, for enterococci/clarithromycin at a MIC >1 μg/mL and for enterobacteriaceae/clarithromycin at a MIC >8 μg/mL represented antibiotic resistance [16,18].

2.7. Statistical analysis

Statistics were performed using the SPSS v11.5 program (SPSS, Chicago, IL, USA). Within the same treatment group, two related samples from days 1/2 and days 35/36 were compared using the Wilcoxon signed-rank test to ensure that pooling of the replicates was feasible. No significant differences were detected between these two sets of replicates (except staphylococci at days 1/2; Table 1). The Wilcoxon signed-rank test was also used to compare related samples in each microbial population between days 1 and 7, days 7 and 35, and days 1 and 35. The non-parametric Mann–Whitney *U*-test was used to compare the unrelated median values (active and placebo) for each microbial population. A *P*-value of less than 0.05 was considered statistically significant. The McNemar test was used to compare antibiotic resistance between any two time points (days 1, 7 and 35) and $P \leq 0.05$ indicates that the proportions are not equal.

3. Results

In the placebo and active groups, the total bacterial numbers decreased during antibiotic therapy, with a small clinical significance ($P < 0.05$), and increased post therapy (Tables 1 and 2). There were no significant differences between the numbers at days 1 and 35.

3.1. Changes in the numbers of enterobacteria and enterococci

In the placebo group, the numbers of facultative anaerobes increased significantly between days 7 and 35, and the numbers of enterobacteria were significantly higher at day 35 than at day 1 ($P < 0.05$). No significant changes occurred in the numbers of enterococci and staphylococci in this group, although the numbers of enterococci decreased during antibiotic therapy (Table 1).

In the probiotic-supplemented group, the enterobacterial population decreased during therapy but then increased so that at day 35 the numbers were not significantly different from those at day 1 (Table 2).

3.2. Effects on the bacteroides population

The numbers of bacteroides in the placebo group decreased significantly between days 1 and 7 ($P < 0.05$), followed by a significant increase during the re-growth period so that at day 35 the numbers were not significantly different from day 1. In contrast, in the active group the numbers of bacteroides increased from days 7 to 35 ($P < 0.01$) so that the final numbers at day 35 were significantly higher than at day 1 ($P < 0.05$).

3.3. Changes in lactobacilli and bifidobacteria

The bifidobacterial population in both groups decreased in response to antibiotic therapy ($P < 0.0001$) and, despite increasing significantly between days 7 and 35, the numbers for both groups at day 35 were significantly lower than those at day 1 ($P < 0.0001$).

Table 1
Distribution of the intestinal microbiota in patients in the placebo group[a]

Population	*N*[b]	Day 1	Day 7	Day 35
Total bacterial count	55	10.4 (9.2–11.8)*	9.8 (7.2–11.8)**	10.4 (7.6–12.5)
Total facultative anaerobes	57	8.8 (<1.7–11.8)	8.1 (<1.7–11.8)**	9.0 (<1.7–11.7)
Enterobacteriaceae	58	7.7 (4.0–11.4)*	5.1 (<1.7–10.2)**	8.6 (<1.7–10.4)†
Enterococci	59	6.3 (<1.7–11.4)	4.0 (<1.7–10.3)	6.7 (<1.7–10.9)
Staphylococci[c]	54	4.7 (<1.7–10.3), 4.0 (<1.7–10.0)	4.5 (<1.7–9.4)	5.3 (<1.7–9.5)
Total anaerobes	48	10.3 (9.2–11.8)	9.8 (7.2–11.4)**	10.4 (7.3–12.5)
Bacteroides	60	9.9 (<1.7–11.0)*	9.0 (<1.7–11.2)**	10.0 (<1.7–10.9)
Bifidobacteria	63	9.1 (<1.7–11.6)*	1.7 (<1.7–11.6)**	4.7 (<1.7–12.5)†
Lactobacilli	54	8.3 (5.2–11.1)*	6.9 (<1.7–10.0)**	8.7 (5.9–11.8)
Yeasts	52	<1.7 (<1.7–7.4)*	4.8 (<1.7–8.2)**	2.3 (<1.7–7.2)
Candida albicans	61	<1.7 (<1.7–7.4)*	4.4 (<1.7–8.0)**	2.2 (<1.7–5.8)†

Statistical analysis using SPSS v11.5 statistical package: the Wilcoxon signed-rank test comparison between sample collection days: *$P \leq 0.05$, day 1 compared with day 7; **$P \leq 0.05$, day 7 compared with day 35; †$P \leq 0.05$, day 1 compared with day 35.

[a] Data given as median (minimum–maximum) $\log_{10}$ colony-forming units (CFU)/g dry weight of faeces.

[b] Number of patients harbouring microbial population.

[c] There were significant differences between the medians of two samples before therapy (day 1), therefore the results from these samples could not be merged to provide day 1 results.

Table 2
Distribution of the intestinal microbiota in patients in the active group[a]

Population	N[b]	Day 1	Day 7	Day 35
Total bacterial count	55	10.4 (9.5–13.2)*	10.1 (<1.7–12.0)**	10.4 (8.2–11.7)
Total facultative anaerobes	56	8.6 (4.0–11.6)	8.0 (<1.7–12.0)	8.6 (6.7–12.0)
Enterobacteriaceae	56	7.8 (<1.7–11.5)*	5.5 (<1.7–9.8)**	8.1 (<1.7–9.8)
Enterococci	57	6.3 (<1.7–9.9)	4.7 (<1.7–10.6)	7.0 (<1.7–9.0)
Staphylococci	58	4.9 (<1.7–11.6)	<1.7 (<1.7–11.6)	5.0 (1.6–9.3)
Total anaerobes	50	10.4 (9.4–13.2)	10.1 (7.7–11.2)**	10.4 (6.7–11.7)
Bacteroides	57	9.9 (5.7–11.6)	9.9 (<1.7–11.1)**	10.2 (<1.7–11.4)†
Bifidobacteria	62	9.5 (<1.7–12.4)*	<1.7 (<1.7–10.7)**	7.3 (<1.7–10.6)†
Lactobacilli	53	8.2 (5.9–10.6)	6.9 (<1.7–11.2)	8.5 (5.8–10.0)
Yeasts	51	<1.7 (<1.7–7.7)*	4.5 (<1.7–7.9)**	<1.7 (<1.7–6.2)
Candida albicans	62	<1.7 (<1.7–7.4)*	<1.7 (<1.7–7.5)	2.2 (<1.7–7.9)†

Statistical analysis using SPSS v11.5 statistical package: the Wilcoxon signed-rank test comparison between sample collection days: $^{*}P \leq 0.05$, day 1 compared with day 7; $^{**}P \leq 0.05$, day 7 compared with day 35; $^{\dagger}P \leq 0.05$, day 1 compared with day 35.
[a] Data given as median (minimum–maximum) $\log_{10}$ colony-forming units (CFU)/g dry weight of faeces.
[b] Number of patients harbouring microbial population.

The lactobacillus population of the placebo group decreased significantly between days 1 and 7, but then increased ($P < 0.01$) so that at day 35 the numbers were comparable with those at day 1 (Table 1). The numbers of lactobacilli in the probiotic group decreased during antibiotic therapy and increased again post treatment, but none of these changes was statistically significant (Table 2).

3.4. The effects of antibiotics on the yeast component of the microbiota

Although the numbers of yeast increased in both groups during antibiotic therapy, in the placebo group this was associated with a significant increase in the number of *C. albicans*; a similar increase did not occur among the patients receiving the probiotic supplement. At day 35, the numbers of *C. albicans* in both groups were significantly higher than at day 1 (Tables 1 and 2).

3.5. Comparison of the components of the microbiotas of the two groups

When the microbiotas of the two groups were compared (Table 3), the numbers of total facultative anaerobes at day 35 in the active group were significantly lower than in the placebo group ($U = 1648$; $P = 0.031$). Similarly, the numbers of enterobacteriaceae in the active group were significantly lower than in the placebo group ($U = 1608$; $P = 0.014$). The number of *C. albicans* after antibiotic therapy in the placebo group was significantly higher than in the active group ($U = 1891$; $P = 0.049$), but by day 35 the numbers of yeasts were comparable in both groups. There were no significant differences between the two treatment groups for any of the other microbial populations tested.

3.6. Antibiotic resistance

A very high level of indigenous antibiotic resistance was found among the enterobacteriaceae in this cohort of patients, which made it very difficult to make any assessment of changes in the antibiotic resistance profiles. There was no decrease in resistance in response to probiotic supplement action, but the indigenous resistance levels were too high to determine whether the probiotics had registered any impact.

The development of resistance to amoxicillin and clarithromycin between days 1 and 35 among the enterococcal population of patients in the two groups is shown in Tables 4 and 5, respectively. At day 35 in the placebo group, the number of patients expressing antibiotic resistance within the enterococcal population was significantly higher ($P \leq 0.05$) than the number in the initial population at all antibiotic concentrations up to 32.0 μg/mL (Table 4). At the highest concentrations of amoxicillin (128 and 512 μg/mL), comparison of days 1 and 35 showed no significant differences in the levels of antibiotic resistance in the enterococcal population.

However, for the probiotic-supplemented group at all amoxicillin concentrations, there was no significant increase in the number of patients carrying antibiotic-resistant enterococci between days 1 and 35.

Table 3
Comparison of microbial populations among the placebo and active groups

Population	Day 7		Day 35	
	CFU/g[a]	*P*-value[b]	CFU/g[a]	*P*-value[b]
Total facultative anaerobes				
Placebo	8.1	0.598	9.0	0.031
Active	8.0		8.6	
Enterobacteriaceae				
Placebo	5.1	0.983	8.6	0.014
Active	5.5		8.1	
Candida albicans				
Placebo	4.4	0.049	2.2	0.815
Active	<1.7		2.2	

[a] Data given as median $\log_{10}$ colony-forming units (CFU)/g dry weight of faeces.
[b] According to Mann–Whitney *U*-test.

Table 4
Number of patients developing amoxicillin resistance within the faecal enterococcal population between days 1 and 35

Amoxicillin (μg/mL)	Number of patients	*P*-value[a]
Placebo group		
4.0	13	0.049
8.0[b]	12	0.035
16.0	10	0.012
Active group		
4.0	9	N.S.
8.0[b]	11	N.S.
16.0	6	N.S.

N.S., no significant difference.
[a] According to McNemar test.
[b] The breakpoint for enterococci:amoxicillin at a minimum inhibitory concentration (MIC) >8 μg/mL represented antibiotic resistance.

Table 5
Number of patients developing clarithromycin resistance within the faecal enterococcal population between days 1 and 35

Clarithromycin (μg/mL)	Number of patients	*P*-value[a]
Placebo group		
0.5	17	<0.001
1.0[b]	20	<0.001
4.0	29	<0.001
Active group		
0.5	7	N.S.
1.0[b]	14	0.013
4.0	22	0.001

N.S., no significant difference.
[a] According to McNemar test.
[b] The breakpoint for enterococci:clarithromycin at a minimum inhibitory concentration (MIC) >1 μg/mL represented antibiotic resistance.

With clarithromycin, in the placebo group there was a significant development of resistance ($P \leq 0.001$) at concentrations near to the resistance breakpoint, which was not seen in the probiotic group. However, significant resistance ($P = 0.001$) developed in both groups to the same extent at higher clarithromycin concentrations (Table 5).

4. Discussion

Administration of antibiotics often causes disturbances in the normal intestinal microbiota [19,20]. In the present study, the total bacterial and total facultative anaerobe population results indicate that despite the probiotic supplement the microbiotas of both the placebo and active groups were susceptible to the effects of the antibiotics administered to eradicate *H. pylori*. It appeared that there was recovery of the majority of the components of the microbiota post antibiotic therapy, with no significant difference between days 1 and 35. However, the noticeable difference occurred with the enterobacterial component of the placebo group, which was subject to disturbance, suggesting that supplementation with probiotics had impacted on the intestinal microbiota, resulting in less disruption of the compositional balance for the active group.

Madden et al. [21] found a significant increase in the facultative anaerobe component of the microbiota between days 1 and 27 in placebo group with amoxicillin, metronidazole and lansoprazole treatment. When probiotics were given after antibiotics, numbers decreased significantly between days 7 and 27 back to the starting levels.

The eradication therapy did not significantly disrupt the total anaerobe population from days 1 to 35 (Tables 1 and 2), which contrasts with the results of other studies where anaerobes were suppressed [18,22]. Adamsson et al. [18,23] found that the total anaerobic microbiota was strongly suppressed in *H. pylori* patients (amoxicillin and metronidazole combination (OAM) group or clarithromycin and metronidazole (OCM) group), although the effect was most pronounced in the OCM group. Amoxicillin as a single agent causes only minor disturbances, but in some studies the anaerobic microbiota has been found to be disrupted due to metronidazole [23,24].

It is also interesting that despite the sensitivity of the probiotic organisms to antibiotics, no significant changes were observed for the total *Lactobacillus* numbers in the probiotic-supplemented group—an observation not recorded for the placebo group. However, the antibiotic sensitivity of the bifidobacteria was apparent in both groups, as also observed by Adamsson et al. [18] and Buhling et al. [25].

Although there was no significant change in total numbers of yeast between days 1 and 35 in the placebo group, the number of *C. albicans* increased significantly ($P < 0.01$). This finding contrasts with the study of Buhling et al. [25], who found that the numbers both of yeast and *C. albicans* in patients with *H. pylori* returned back to the starting levels after 4 weeks.

The very high levels of antibiotic resistance among the enterobacteriaceae in this cohort of patients made any assessment of changes (increases) in resistance post therapy very difficult, but the extent of antibiotic resistance might have been related to the significantly lower numbers of enterobacteria seen in the active group patients compared with the placebo group at day 35. Working with a similar cohort, Stark et al. [22] observed overgrowth by amoxicillin-resistant enterobacteria post antibiotic therapy.

Antibiotic resistance among the enterococci was significantly higher in the placebo group than in the probiotic group post therapy in this study, suggesting that the probiotics had in some way modulated the composition of the re-growth population. It is known that bacteria have an energy requirement to achieve antibiotic resistance [26], either owing to chromosomal alterations (e.g. target site alterations) or owing to the use of accessory elements (such as enzymes and antibiotic efflux pumps). Such energy requirements could affect the growth kinetics of the bacteria, but the antibiotic resistance provides a competitive advantage over the antibiotic-sensitive strains, enabling their survival. The energy costs involved in the mechanisms of resistance for the bacteria in this study are unknown, but it is possible that the additional challenge to these ‘energy-depleted’ bacteria

caused by the daily supplement of probiotic bacteria could be too great to enable their domination and hence this could account for the lower incidence of antibiotic resistance among the active group patients.

From this study, it would appear that daily supplementation with viable probiotic bacteria during and post antibiotic therapy reduces the extent of disruption to the intestinal microbiota as well as the incidence and total numbers of antibiotic-resistant strains in the re-growth population.

Acknowledgments

We would like to thank Dr Martin Day of Cardiff University, UK, and Dr Asa Sullivan of Karolinska University Hospital, Huddinge, Sweden, for their expert advice and knowledge of antibiotic resistance. We would like to express our sincere thanks posthumously to Prof. Denver Russell of Cardiff University, UK, for his support and guidance until his recent death.

References

[1] Sullivan A, Edlund C, Nord CE. Effect of antimicrobial agents on the ecological balance of human microflora. Lancet Infect Dis 2001;1:101–14.
[2] Levy J. The effects of antibiotic use on gastrointestinal function. Am J Gastroenterol 2000;95(Suppl. 1):S8-S10.
[3] Finegold SM. Interaction of antimicrobial therapy and intestinal flora. Am J Clin Nutr 1970;23:1466–71.
[4] Bohnhoff M, Miller CP, Martin WR. Resistance of the mouse's intestinal tract to experimental salmonella infection. I. Factors which interfere with the initiation of infection by oral inoculation. J Exp Med 1964;120:805–28.
[5] Reid G, Friendship R. Alternatives to antibiotic use: probiotics for the gut. Anim Biotechnol 2002;13:97–112.
[6] Sullivan A, Barkholt L, Nord CE. *Lactobacillus acidophilus*, *Bifidobacterium lactis* and *Lactobacillus* F19 prevent antibiotic-associated ecological disturbances of *Bacteroides fragilis* in the intestine. J Antimicrob Chemother 2003;52:308–11.
[7] Fernández MF, Boris S, Barbés C. Probiotic properties of human lactobacilli strains to be used in the gastrointestinal tract. J Appl Microbiol 2003;94:449–55.
[8] Frič P. Probiotics in gastroenterology. Z Gastroenterol 2002;40: 197–201.
[9] Hamilton-Miller JMT. The role of probiotics in the treatment and prevention of *Helicobacter pylori* infection. Int J Antimicrob Agents 2003;22:360–6.
[10] Cremonini F, Di Caro S, Nista EC, et al. Meta-analysis: the effect of probiotic administration on antibiotic-associated diarrhea. Aliment Pharmacol Ther 2002;16:1461–7.
[11] Ishikawa H, Akedo I, Umesaki Y, Tanaka R, Imaoka A, Otani T. Randomized controlled trial of the effect of bifidobacteria-fermented milk on ulcerative colitis. J Am Coll Nutr 2002;22:56–63.
[12] Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomized placebo-controlled trial. Lancet 2001;357:1076–9.
[13] Hilton E, Kolalowoski P, Singer C, Smith M. Efficacy of *Lactobacillus* GG as a diarrhoeal preventive in travellers. J Travel Med 1997;4:41–3.
[14] Reid G, Jass J, Sebulsky MT, McCormick JK. Potential uses of probiotics in clinical practice. Clin Microbiol Rev 2003;16:658–72.
[15] Marteau P, Lepage P, Mangin I, et al. Gut flora and inflammatory bowel disease. Aliment Pharmacol Ther 2004;20:18–23.
[16] National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests: approved standard, 8th ed. Wayne, PA: NCCLS; 2000.
[17] Miles AA, Misra SS. The estimation of the bactericidal power of the blood. J Hygeine 1938;38:732–49.
[18] Adamsson I, Nord CE, Lundquist P, Sjöstedt S, Edlund C. Comparative effects of omeprazole, amoxycillin plus metronidazole versus omeprazole, clarithromycin plus metronidazole on the oral, gastric and intestinal microflora in *Helicobacter pylori*-infected individuals. J Antimicrob Chemother 1999;44:629–40.
[19] van der Waaij D, Nord CE. Development and persistence of multi-resistance to antibiotics in bacteria; an analysis and a new approach to this urgent problem. Int J Antimicrob Agents 2000;16: 191–7.
[20] Lindberg E, Adlerberth I, Wold AE. Antibiotic resistance in *Staphylococcus aureus* colonising the intestines of Swedish infants. Clin Microbiol Infect 2004;10:890–4.
[21] Madden JAJ, Plummer S, Plummer NT, Herbison M, Hunter JO. A double-blind, placebo controlled pilot study to assess the effects of a probiotic on the response of the intestinal microflora to *Helicobacter pylori* eradication therapy. Gut 2002;50:A22.
[22] Stark CA, Adamsson I, Edlund C, et al. Effects of omeprazole and amoxycillin on the human oral and gastrointestinal microflora in patients with *Helicobacter pylori* infection. J Antimicrob Chemother 1996;38:927–39.
[23] Adamsson I, Edlund C, Nord CE. Microbial ecology and treatment of *Helicobacter pylori* infections. J Chemother 2000;12:5–16.
[24] Edlund C, Nord CE. Ecological impact of antimicrobial agents on human intestinal microflora. Alpe Adria Microbiol J 1993;2:137–64.
[25] Buhling A, Radun D, Muller WA, et al. Influence of anti-*Helicobacter* triple therapy with metronidazole, omeprazole and clarithromycin on intestinal microflora. Aliment Pharmacol Ther 2001;15:1445–52.
[26] Andersson DI, Levin BR. The biological cost of antibiotic resistance. Curr Opin Microbiol 1999;2:489–93.

ELSEVIER

International Immunopharmacology 5 (2005) 1091–1097

International Immunopharmacology

www.elsevier.com/locate/intimp

Preliminary report

Effect of probiotics on preventing disruption of the intestinal microflora following antibiotic therapy: A double-blind, placebo-controlled pilot study

Jennifer A.J. Madden[a,b], Susan F. Plummer[a,*], James Tang[a], Iveta Garaiova[a], Nigel T. Plummer[a], Mary Herbison[b], John O. Hunter[b], Takashi Shimada[c,d], Lei Cheng[d], Taro Shirakawa[d]

[a]*Research and Development Division, Cultech Limited, York Chambers, York Street, Swansea, SA1 3NJ, UK*
[b]*Department of Gastroenterology, Addenbrooke's Hospital, Cambridge, CB2 2QQ, UK*
[c]*Central Research Laboratories, Nichinichi Pharmaceutical Co. Ltd., Mie 518-1417, Japan*
[d]*Department of Health Promotion and Human Behaviour, Kyoto University Graduate School of Public Health, Kyoto 606-8501, Japan*

Received 15 September 2004; received in revised form 29 November 2004; accepted 9 February 2005

Abstract

In this pilot-scale, double-blind, placebo-controlled trial, 30 patients with *Helicobacter pylori* infection were randomised into three groups prior to their 7 days eradication therapy, to study the effects of probiotic supplement comprising *Lactobacillus acidophilus* and *Bifidobacterium bifidum* on the intestinal microflora in response to antibiotic therapy. Group I received the placebo product from day 1 to day 15, Group II received placebo from day 1 to day 7 and probiotics from day 8 to day 15 and Group III received probiotics from day 1 to day 15. Patients provided stool samples for analysis on days 1, 7, 12, 17 and 27. For patients in Groups I and II, significant increases in the facultative anaerobe component of the microflora occurred between days 1 and 7. In Group I, the numbers remained elevated to day 27 but in Group II, the numbers decreased significantly between days 7 and 27 back to the starting levels. In Group III, the facultative anaerobe population remained stable throughout. The total anaerobe numbers increased significantly at day 27 than at day 1 for Group I, were unchanged throughout for Group II and decreased significantly for the patients in Group III between days 1 and 7 before reverting to the starting levels by day 27. From these results, it can be seen that probiotic supplementation modulates the response of the intestinal microflora to the effects of antibiotic therapy.

Keywords: Probiotics; Antibiotics; Intestinal microflora; Lactic acid bacteria; *Helicobacter pylori*; Clinical trials

* Corresponding author. Tel.: +44 1792 472155; fax: +44 1792 472466.
E-mail address: nsjl.plummers@virgin.net (S.F. Plummer).

1567-5769/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.intimp.2005.02.006

1. Introduction

It is known that antibiotics can detrimentally affect the ecological balance of the intestinal microflora [1–3] allowing the proliferation of naturally opportunistic organisms, such as yeasts, and potentially pathogenic bacteria. The effects of an antibiotic on the indigenous population depend on several factors including the antimicrobial spectrum, pharmokinetics, dose, route of administration and intestinal concentration [3]. Disruption of the normal flora by antibiotics is usually as a result of incomplete absorption of orally administered antibiotics as well as their secretion by the salivary glands. In the case of poorly absorbed antibiotic agents, they may disturb the balance in the large intestine which can lead to the development of conditions such as irritable bowel syndrome (IBS) [2].

Probiotics are live microbial food supplements that change either the composition or metabolic activities of the microflora, or modulate immune system reactivity in a way that benefits health [4–7]. Probiotics have been given to healthy subjects receiving broad-spectrum antibiotics with only minor to moderate disturbances of the major bacterial groups [8–10]. In this study, the effects of probiotic supplementation both during and after broad-spectrum antibiotic therapy have been assessed in a clinical setting. *Helicobacter pylori* infected patients were selected for this study as a useful cohort of patients receiving a comparable antibiotic regime that could be timed to enable the collection of baseline specimens before the start of treatment. The use of eradication therapy for *H. pylori* has previously been shown to suppress the indigenous gastrointestinal anaerobic flora and increase numbers of aerobic organisms [11,12] and the aim of this study was to observe the effects of two programmes of probiotic supplementation on the response of the microflora to eradication therapy.

2. Materials and Methods

2.1. Patients

Thirty patients were recruited into the study at Addenbrooke's Hospital, Cambridge, UK. Patients recruited to the study had been found at endoscopy to be infected with *H. pylori* that was verified by positive serology and histology by the Public Health Laboratory Service at Addenbrooke's Hospital. The patients were otherwise healthy with no other gastrointestinal disorders apart from peptic ulcers, thought to be related to their *H. pylori* infection. They had not received any antibiotics or been subject to any dietary intervention in the 6 weeks prior to the study. All participants gave written informed consent to participate in the trial, according to the process approved by Cambridge Local Research Ethics Committee.

2.2. Study design

The trial was conducted as a double-blind, placebo-controlled pilot study. Patients were randomly split into three groups of ten patients. Patients were provided with written instructions on collection of faecal samples and use of anaerobic bags. Patients were also contacted on key days throughout the trial by a research nurse. Key days were deemed to be those where faecal samples required collecting, and start/end of triple-therapy, probiotic and placebo supplementation. All patients provided a control faecal specimen before commencement of antibiotic therapy, on day 1. Subsequent specimens were provided on days 7, 12, 17 and 27. Although, ideally, samples would be analysed immediately, distance between the hospital and laboratory facilities meant that this was not possible. Storage studies were conducted in triplicate using specimens collected from healthy volunteers to determine the best mode of storage and transport of specimens that would result in minimal loss of bacterial viability, and were designed to imitate possible sample storage conditions. On the basis of this viability testing, the faecal specimens were collected at patients' homes where they were stored at 4 °C for no more than 24 h and then frozen at −70 °C before analysis.

2.3. Regime

All participating patients received 7 days eradication triple-therapy with either placebo from day 1 to day 15 (Group I), placebo from day 1 to day 7 and probiotic supplementation from day 8 to day 15 (Group II) or probiotic supplementation from day 1 to day 15 (Group III). Eradication therapy comprised

amoxycillin 500 mg q.i.d., metronidazole 400 mg t.i.d. and lansoprazole 30 mg b.i.d. for 7 days. Patients took one probiotic or placebo capsule daily with food, according to their group. A commercially available probiotic product was provided by Cultech Ltd (Swansea, UK) and comprised two strains of *Lactobacillus acidophilus* (CLT60 and CUL21) and two strains of *Bifidobacterium bifidum* (CUL17 and *B. bifidum* Rhodia, New Jersey, USA) at a total of 2.5×10^{10} colony forming units (cfu)/capsule. The placebo comprised an inert carrier (maltodextrin, Cultech Ltd).

2.4. Compliance

Of the 30 patients recruited, eight were excluded for failing to comply with the written instructions provided. The twenty-two remaining were divided

Table 1
Microbial populations in different groups at key sampling days in patients received 7 days antibiotic therapy with or without probiotic supplementation

Microbial population	Group I ($n=7$)				
	Day 1	Day 7	Day 12	Day 17	Day 27
Total anaerobes	9.3 ± 0.3 (7)	8.8 ± 0.6 (7)	9.7 ± 0.1 (7)	9.2 ± 0.6 (7)	9.5 ± 0.6 (7)*,†
Bacteroides spp.	8.9 ± 0.4 (7)	7.6 ± 1.3 (6)	8.7 ± 0.4 (7)	6.7 ± 1.3 (6)	5.6 ± 1.8 (5)
Total lactic acid bacteria	8.5 ± 0.4 (7)	8.4 ± 0.8 (7)	9.0 ± 0.7 (7)	7.1 ± 1.3 (6)	6.7 ± 1.6 (6)
Bifidobacterium spp.	5.6 ± 1.5 (5)	6.2 ± 1.7 (5)	8.7 ± 0.8 (7)*,†	5.1 ± 1.5 (5)	6.6 ± 1.6 (6)
Lactobacillus spp.	6.8 ± 1.3 (6)	6.6 ± 1.3 (6)	8.0 ± 0.7 (7)	4.7 ± 1.7 (4)	4.0 ± 1.9 (4)
Total facultative anaerobes	6.1 ± 0.5 (7)	8.8 ± 0.6 (7)*	8.0 ± 0.5 (7)*,†	7.4 ± 0.8 (7)†	7.6 ± 0.7 (7)*
Enterobacteriaceae	4.8 ± 1.3 (5)	8.6 ± 0.6 (7)*	7.7 ± 0.5 (7)*,†	6.8 ± 1.2 (6)†	5.5 ± 1.2 (6)
Enterococcus/Streptococcus spp.	2.1 ± 1.0 (3)	5.5 ± 1.5 (5)	5.4 ± 1.1 (6)	6.4 ± 0.8 (7)*	5.8 ± 0.6 (7)
Staphylococcus spp.	<2.0 (2)	6.5 ± 1.4 (6)	5.0 ± 1.1 (6)†	4.8 ± 1.3 (6)	4.0 ± 1.2 (6)
Microbial population	Group II ($n=9$)				
	Day 1	Day 7	Day 12	Day 17	Day 27
Total anaerobes	9.1 ± 0.4 (9)	9.4 ± 0.5 (9)	9.7 ± 0.3 (9)	8.9 ± 0.3 (9)	8.9 ± 0.4 (9)
Bacteroides spp.	7.8 ± 1.1 (8)	6.4 ± 1.6 (6)	5.9 ± 1.5 (8)	6.4 ± 1.4 (7)	6.2 ± 1.2 (7)
Total lactic acid bacteria	7.7 ± 1.0 (8)	9.2 ± 0.3 (9)	8.6 ± 0.3 (8)†	8.4 ± 0.4 (9)†	8.1 ± 0.6 (9)
Bifidobacterium spp.	4.8 ± 1.5 (5)	7.0 ± 1.3 (7)	5.6 ± 1.4 (5)†	5.2 ± 1.5 (6)†	6.2 ± 1.3 (7)
Lactobacillus spp.	6.4 ± 1.3 (7)	5.9 ± 1.5 (6)	6.4 ± 1.2 (7)	6.7 ± 1.0 (8)	7.1 ± 0.4 (8)
Total facultative anaerobes	6.9 ± 0.9 (8)	9.2 ± 0.4 (9)**	8.6 ± 0.3 (8)*	7.8 ± 0.7 (9)*,†	7.0 ± 0.4 (9)‡
Enterobacteriaceae	6.8 ± 0.9 (8)	7.9 ± 1.1 (6)*	6.0 ± 1.2 (8)	4.6 ± 1.5 (6)†	4.1 ± 1.3 (6)†
Enterococcus/Streptococcus spp.	4.8 ± 1.0 (7)	7.7 ± 1.0 (9)*	7.8 ± 0.5 (7)*	6.7 ± 0.6 (9)†	6.0 ± 0.3 (9)‡
Staphylococcus spp.	3.5 ± 0.9 (6)	6.4 ± 1.2 (7)*	5.8 ± 1.2 (6)*	4.4 ± 0.8 (8)	3.9 ± 0.8 (7)†
Microbial population	Group III ($n=6$)				
	Day 1	Day 7	Day 12	Day 17	Day 27
Total anaerobes	10.0 ± 0.2 (6)	8.9 ± 0.5 (6)*	9.3 ± 0.5 (6)	9.0 ± 0.6 (6)	9.3 ± 0.7 (6)†
Bacteroides spp.	7.5 ± 1.9 (5)	2.9 ± 1.8 (2)	4.0 ± 1.9 (3)	4.5 ± 2.6 (3)	4.4 ± 2.0 (3)
Total lactic acid bacteria	8.8 ± 0.3 (6)	8.6 ± 0.4 (6)	9.3 ± 0.6 (6)	8.9 ± 0.5 (6)	8.1 ± 0.5 (6)
Bifidobacterium spp.	5.1 ± 0.9 (4)	4.2 ± 1.9 (3)	5.5 ± 1.8 (4)	6.3 ± 2.1 (5)	6.8 ± 1.4 (5)
Lactobacillus spp.	6.8 ± 1.7 (5)	6.7 ± 1.5 (5)	6.0 ± 2.0 (4)	6.4 ± 2.2 (5)	6.0 ± 1.9 (4)
Total facultative anaerobes	7.9 ± 0.9 (6)	6.6 ± 1.6 (5)	8.5 ± 1.0 (6)	8.4 ± 0.8 (6)	6.2 ± 0.5 (6)
Enterobacteriaceae	7.7 ± 0.9 (6)	5.5 ± 1.6 (5)	8.3 ± 1.0 (6)	8.1 ± 0.7 (6)	2.7 ± 1.4 (2)
Enterococcus/Streptococcus spp.	3.7 ± 1.6 (4)	6.2 ± 1.5 (5)	5.3 ± 1.8 (4)	6.4 ± 2.2 (5)	5.3 ± 0.2 (6)
Staphylococcus spp.	3.5 ± 1.5 (4)	5.6 ± 1.8 (4)	4.7 ± 1.6 (4)	6.2 ± 2.1 (5)	3.8 ± 1.3 (4)

Data given as mean count (log cfu/g dry weight of faeces) ± SEM, with carriage (number of patients harbouring a particular bacterial population) indicated in parentheses. Group I received the placebo product from days 1–15, Group II received placebo from days 1–7 and probiotics from days 8–15 and Group III received probiotics from days 1–15, with eradication therapy for *H. pylori* from days 1–7. *$P<0.05$, **$P<0.01$when compared to day 1. † $P<0.05$, ‡ $P<0.01$ when compared to day 7.

between Group I, seven patients (4 males and 3 females; mean age 49 years with range 33–66 years), Group II, nine patients (1 male and 8 females; mean age 54 years with range 35–66 years) and Group III, six patients (2 males and 4 females; mean age 60 years with range 46–70 years). There were no demographic differences between groups.

2.5. Sample analysis

Standard microbiological techniques were used to analyse the samples. Specimens (in anaerobic bags) were thawed at room temperature and transferred to an Electrotek Micro Anaerobic Workstation (Electrotek, Shipley, UK). Sub samples (1 g) were transferred to 9 ml pre-reduced Maximum Recovery Diluent (MRD; Oxoid, Basingstoke, UK) to produce a 10-fold dilution and an anaerobic dilution series was prepared. A modification of the Miles and Misra plate count technique (1938) was used to plate 10×10 μl of each dilution on to the following selective and non-selective agars (all agars were obtained from Oxoid, Basingstoke, UK, unless otherwise stated): Anaerobe Blood (total anaerobes; Biomerieux, Basingstoke, UK), Blood (total facultative anaerobes; Biomerieux), de Man Rogosa Sharpe (total lactic acid bacteria, lactobacilli and bifidobacteria), MacConkey (Enterobacteriaceae, incl. *E. coli*), Kanamycin Aesculin Azide (enterococci/streptococci), Baird Parker (staphylococci), and Wilkins Chalgren (bacteroides). Anaerobic plates were incubated at 37 °C for 72 h in an atmosphere of 80% N_2, 10% CO_2 and 10% H_2 and aerobic agars were incubated at 37 °C for 48 h. Organisms were identified by anaerobic/aerobic growth, colony morphology, Gram stain, light microscopy and API biochemical identification strips (API; Biomerieux, Basingstoke, UK). For the purposes of this study, bifidobacteria were classed as being members of the lactic acid bacteria (LAB).

2.6. Dry weight determination

Samples were dried in tared pre-dried/weighed tubes at 70 °C in a Thermocontrol oven (Status, UK) for 24 h. Specimens were dried to constant weight and the colony forming units per gram of dry weight (cfu/g DW) calculated.

2.7. Statistics

Statistical analyses were performed using the SPSS 9.0 statistics package (SPSS, Chicago, IL, USA). The Wilcoxon Signed Rank Test was used to compare paired, related samples at different sampling times. The Kruskal–Wallis test was used to determine any significance across the three groups for each organism type on different sampling days. Where significance ($P<0.05$) occurred, the Mann–Whitney test was used to determine between which two groups the significance lay. For Table 1, the data was logged and the mean and standard error of the mean (SEM) calculated from these data. The statistical methods and tests used were approved by the Centre for Applied Medical Statistics in Cambridge.

3. Results

The results of microbial populations in different groups at key sampling days are shown in Table 1. In the placebo group (Group I), numbers of total facultative anaerobes increased significantly during triple-therapy from day 1 to day 7 ($P<0.05$). The numbers of facultatives were significantly lower at days 12 and 17 than at day 7 ($P<0.05$), but the population at day 12 was still significantly higher than at day 1 ($P<0.05$). The re-growth populations at day 27 remained significantly elevated compared to the baseline value at day 1 ($P<0.05$). The increase in the numbers of the total facultative population after antibiotics was reflected in significant increases in numbers of Enterobacteriaceae when comparing days 1 and 7 as well as days 1 and 12 ($P<0.05$); the numbers were significantly lower at days 12 and 17 ($P<0.05$). By day 27, the re-growth populations were similar to those at day 1. The numbers of staphylococci at day 12 were higher than those at day 1 ($P<0.05$) but the numbers at days 1 and 27 were similar.

Although not significant, the numbers of total anaerobes in Group I subjects increased gradually in the period following antibiotic therapy so that, at day 27, the number of anaerobes was significantly higher than the day 1 starting population ($P<0.05$). The lactobacilli population remained unchanged in Group I throughout this study. As shown in Table 1, the

numbers of bifidobacteria were higher at day 12 than at days 1 and 7 ($P<0.05$), but there were no significant differences between the starting (day 1) and re-growth populations (day 27).

In the Group II patients (receiving probiotics post antibiotic therapy), the numbers of total facultative anaerobes increased significantly in response to antibiotics ($P<0.01$) and remained elevated at days 12 and 17 ($P<0.05$) compared to day 1. The numbers at day 7 were significantly higher than at day 27 ($P<0.01$) but for this group there were no significant differences between the starting and final facultative populations. The numbers of the Enterobacteriaceae population increased significantly from day 1 to day 7 ($P<0.05$) but numbers at days 17 and 27 were significantly lower than at day 7($P<0.05$).

Although Groups I and II received the same treatment (antibiotics–placebo) from day 1 to day 7, the enterococcal and staphylococcal populations in Group II responded slightly differently to treatment. Numbers of enterococci were significantly higher at days 7 and 12 ($P<0.05$) than at day 1, peaking at day 12. The numbers of enterococci were significantly lower at days 17 ($P<0.05$) and 27 ($P<0.01$) than at day 7. Staphylococci also increased and were significantly higher at days 7 and 12 ($P<0.05$), though numbers peaked at day 7. The numbers at days 17 ($P<0.05$) and 27 ($P<0.01$) were significantly lower than at day 7.

In Group II patients, the numbers of total anaerobes showed no significant alterations throughout the study. The numbers of total LAB and bifidobacteria were unaffected by the antibiotic therapy to day 7, but the total LAB numbers were significantly lower at days 12 and 17 than at day 7 ($P<0.05$). This was mirrored by similar decreases in bifidobacteria populations during the same two time periods ($P<0.05$). Lactobacilli populations remained stable throughout.

In the 'total treatment' group (Group III), probiotics were administered from day 1 to day 15 and, in this group, despite numerical increases there were no significant changes in the numbers of the facultative anaerobe populations. The numbers of total anaerobes for Group III patients decreased significantly between days 1 and 7 ($P<0.05$) but this disturbance was restored between days 7 and 27 ($P<0.05$). The lactobacilli and bifidobacteria populations remained stable throughout the study.

When the bacterial populations among the three groups were compared, as shown in Table 1, only numbers of bacteroides at day 7 in Group III were found to be significantly lower than the numbers in Group I ($P<0.05$) though there was a strong trend for them to be lower than in Group II ($P=0.06$). There were no inter-group differences for any of the other bacterial types tested.

4. Discussion

The role of triple-therapy in the eradication of *H. pylori* is well established with the most common eradication currently consisting of two antibiotics chosen from amoxycillin, tetracycline, metronidazole or clarithromycin and a proton pump inhibitor [12,13]. Increases in the numbers of the facultative anaerobic component of the microflora during triple-therapy are probably due to the activity of amoxycillin, which has previously been shown to cause proliferation of enterobacteria both in patients infected with *H. pylori* and in healthy volunteers [11,12,14]. In this study, the higher numbers of facultatives at day 27 in *H. pylori* patients following triple-therapy without probiotics suggested that permanent changes had possibly occurred in the composition of the intestinal microflora induced by the antibiotics. The timescale for the establishment of the re-growth population post antibiotics has not been standardised. In the present study, we used 27 days from the start of treatment to assess the climactic re-growth population; other workers have used 35 days [11,13].

Interestingly, we found that the number of anaerobes at day 27 in these patients (following eradication therapy without probiotic supplementation) was significantly higher than the day 1 starting population, which contrasts with the results of other studies where anaerobes were suppressed by eradication therapy [11,13]. Other workers have found that the eradication therapy did not significantly disrupt the total anaerobe population [12]. Metronidazole is known to have a greater effect on the anaerobic microflora when administered intravenously but has little effect when administered orally [2,15]. Also, it has been found that metronidazole has no effect on faecal bacteroides populations of healthy subjects [16]. One reason for this observation is that the nitroimidazoles (of which

metronidazole is a member) are excreted via liver metabolism and normally very low concentrations are found in faeces [1].

It is evident that in *H. pylori* patients receiving probiotics post triple-therapy the antibiotic treatment caused an immediate significant change in the facultative anaerobe populations but, in contrast to patients receiving triple-therapy only, there were no significant differences between day 1 and day 27. Although significant increases did not occur in all components of the aerobic microflora following antibiotics in this treated group, the significant decreases that occurred afterwards to re-growth suggest that probiotic supplementation accelerated the re-establishment of the re-growth populations. In a previous study by Lidbeck and Nord [17] where *L. acidophilus* was administered to patients after either enoxacin or clindamycin treatment there was no 'normalisation' of the facultative microflora but, in that case, the aerobic flora had been suppressed, rather than elevated, after antibiotic administration. Conversely, it has been found that administration of cefpodoxime proxetil, with or without probiotic organisms, to healthy volunteers results in an increase in the facultative anaerobe population in all groups [10].

An increase in numbers of facultative anaerobes following antibiotic therapy has potential consequences. Increased numbers of enterococci may be involved in the aetiology of antibiotic-associated diarrhoea while phenylethylamine, produced by enterococci, is thought to be a bowel irritant [18]. Alteration of the colonic flora may also increase the risk of infection with *Clostridium difficile* by affecting colonic adhesion, toxin production as well as the colonic flora [19]. IBS is a poorly-understood condition with no pathological basis and possibly linked to disturbances in the microflora caused by antibiotics [20]. The faecal microflora in IBS has been shown to be abnormal with higher numbers of facultative organisms and low numbers of lactobacilli and bifidobacteria [21]. It would be likely that any development of IBS in *H. pylori* patients would be due to antibiotic therapy, as there appears to be no direct correlation between *H. pylori* infection and IBS [22,23].

The efficacy of probiotics has been attributed to their possible immunomodulation effects or to their role in keeping the gut microbial ecosystem stable by restoring resident microflora [4–7]. Recent meta-analysis of placebo-controlled trials suggests that probiotics may be useful in the prevention of antibiotic-associated diarrhoea and that lactobacilli (including *L. acidophilus*) have the potential to be used in this situation [24,25]. In addition, a previous randomized, double-blind, crossover study using a heat-killed strain of *L. acidophilus* has shown a therapeutic benefit in patients with IBS [26]. In this study, a probiotic product comprising *L. acidophilus* and *B. bifidum* has been used to assess the potential to prevent disruption of the gut microbiota and so prevent associated problems such as antibiotic-associated diarrhoea and IBS. However, the evidence of the beneficial effects is unclear because despite the improvement in disruption of the microbiota during antibiotic therapy, data was not recorded with respect to the adverse events during antibiotic administration and the efficacy of probiotics in reducing the incidence of antibiotic gastrointestinal side-effects in our clinical pilot trial. Further study is needed to verify the role of our probiotic strains in combating gastrointestinal complications associated with antimicrobial treatment.

In addition, it is important that a commercial probiotic must be non-pathogenic and they should survive the challenges of gastric acid, bile, or concurrent antibiotics. As a matter of fact, in an acid tolerance study the preparation of *L. acidophilus* strains supplemented into artificial stomach medium provided excellent survival throughout the testing period, with very little reduction in numbers at 6 h (data not shown). Moreover, our strain of *L. acidophilus* is resistant to metronidazole but sensitive to amoxicillin, whilst the strain of *B. bifidum* is sensitive to amoxicillin and metronidazole (data not shown). These strains were chosen to limit possibility of resistance transfer from probiotic strains to indigenous microbiota. And we suggest that probiotic supplementation should be taken from start of antibiotic therapy to facilitate colonisation by the probiotics during and on completion of the antibiotic therapy.

In summary, it is apparent in this study that the facultative anaerobic component of the intestinal microflora was most prone to the effects of antibiotic therapy. The nature (size) of this study has meant that no final conclusions can be drawn from these

data but the trends indicate that further studies would be merited. It is also apparent that inclusion of the viable probiotic organisms in conjunction with the antibiotics elicits a noteworthy effect–suggesting that rather than the perceived notion that probiotics should be given post-antibiotic therapy, there are likely to be benefits from including the probiotics with the antibiotics.

Acknowledgements

This work was supported by a grant from the Teaching Company Scheme, UK. We would like to thank Vanessa Davies and Tinnu Sarvotham for technical assistance and Dr Sarah Shore for statistical advice.

References

[1] Sullivan A, Edlund C, Nord CE. Effect of antimicrobial agents on the ecological balance of human microflora. Lancet, Infect Dis 2001;1:101–14.
[2] Nord CE. The effect of antimicrobial agents on the ecology of the human intestinal microflora. Vet Microbiol 1993;35: 193–7.
[3] Hooker KD, DiPiro JT. Effect of antimicrobial therapy on bowel flora. Clin Pharm 1988;7:878–88.
[4] Bengmark S. Ecological control of the gastrointestinal tract. The role of probiotic flora. Gut 1998;42:2–7.
[5] Erickson KL, Hubbard NE. Probiotic immunomodulation in health and disease. J Nutr 2000;130:S403–9.
[6] Isolauri E, Sutas Y, Kankaanpaa P, Arvilommi H, Salminen S. Probiotics: effects on immunity. Am J Clin Nutr 2001;73: S444–50.
[7] Macfarlane GT, Cummings JH. Probiotics, infection and immunity. Curr Opin Infect Dis 2002;15:1–6.
[8] Lidbeck A, Edlund C, Gustafsson JA, Kager L, Nord CE. Impact of *Lactobacillus acidophilus* on the normal intestinal microflora after administration of two antimicrobial agents. Infection 1988;16:329–36.
[9] Black F, Einarsson K, Lidbeck A, Orrhage K, Nord CE. Effect of lactic acid producing bacteria on the human intestinal microflora during ampicillin treatment. Scand J Infect Dis 1991;23:247–54.
[10] Orrhage K, Sjostedt S, Nord CE. Effect of supplements with lactic acid bacteria and oligofructose on the intestinal microflora during administration of cefpodoxime proxetil. J Antimicrob Chemother 2000;46:603–12.
[11] Stark CA, Adamsson I, Edlund C, Sjosted S, Seensalu R, Wikstrom B, et al. Effects of omeprazole and amoxycillin on the human oral and gastrointestinal microflora in patients with *Helicobacter pylori* infection. J Antimicrob Chemother 1996;38:927–39.
[12] Adamsson I, Nord CE, Lundquist P, Sjostedt S, Edlund C. Comparative effects of omeprazole, amoxycillin plus metronidazole versus omeprazole, clarithromycin plus metronidazole on the oral, gastric and intestinal microflora in *Helicobacter-pylori*-infected individuals. J Antimicrob Chemother 1999;44:629–40.
[13] Buhling A, Radun D, Muller WA, Malfertheiner P. Influence of anti-*Helicobacter* triple therapy with metronidazole, omeprazole and clarithromycin on intestinal microflora. Aliment Pharmacol Ther 2001;15:1445–52.
[14] Brismar B, Edlund C, Nord CE. Impact of cefpodoxime proxetil and amoxycillin on the normal oral and intestinal microflora. Eur J Clin Microbiol Infect Dis 1993;12:714–9.
[15] Bergan T. Pharmacokinetic differentiation and consequences for normal microflora. Scand J Infect Dis, Suppl 1986;49: 91–9.
[16] Krook A, Kjellander J, Danielsson D. Susceptibility of *Bacteroides* species to metronidazole during treatment of patients with Crohn's disease and healthy individuals. Scand J Infect Dis 1982;14:45–8.
[17] Lidbeck A, Nord CE. Lactobacilli and the normal human anaerobic microflora. Clin Infect Dis 1993;16(Suppl 4): S181–7.
[18] Finegold SM. Interaction of antimicrobial therapy and intestinal flora. Am J Clin Nutr 1970;23:1466–71.
[19] Starr J, Impallomeni M. Risk of diarrhoea, *Clostridium difficile* and cefotaxime in the elderly. Biomed Pharmacother 1997;51:63–7.
[20] Mendall MA, Kumar D. Antibiotic use, childhood affluence and irritable bowel syndrome (IBS). Eur J Gastroenterol Hepatol 1998;10:59–62.
[21] Madden JA, Hunter JO. A review of the role of the gut microflora in irritable bowel syndrome and the effects of probiotics. Br J Nutr 2002;88:S67–2.
[22] Agreus L, Engstrand L, Svardsudd K, Nyren O, Tibblin G. *Helicobacter pylori* seropositivity among Swedish adults with and without abdominal symptoms. A population-based epidemiologic study. Scand J Gastroenterol 1995;30:752–7.
[23] Kawamura A, Adachi K, Takashima T, Yuki M, Ono M, Kinoshita Y. Prevalence of irritable bowel syndrome and its relationship with *Helicobacter pylori* infection in a Japanese population. Am J Gastroenterol 2001;96:1946.
[24] D'Souza AL, Rajkumar C, Cooke J, Bulpitt CJ. Probiotics in prevention of antibiotic associated diarrhoea: meta-analysis. BMJ 2002;324:1361–6.
[25] Cremonini F, Di Caro S, Nista EC, Bartolozzi F, Capelli G, Gasbarrini G, et al. Meta-analysis: the effect of probiotic administration on antibiotic-associated diarrhoea. Aliment Pharmacol Ther 2002;16:1461–7.
[26] Halpern GM, Prindiville T, Blankenburg M, Hsia T, Gershwin ME. Treatment of irritable bowel syndrome with Lacteol Fort: a randomized, double-blind, cross-over trial. Am J Gastroenterol 1996;91:1579–85.

RESEARCH NOTE

INTERNATIONAL MICROBIOLOGY (2004) 7:59–62

INTERNATIONAL MICROBIOLOGY

Sue Plummer[1*]
Mark A. Weaver[1]
Janine C. Harris[1]
Phillipa Dee[2]
John Hunter[2]

[1]Cultech Ltd., York Chambers, York Street, Swansea, United Kingdom
[2]Addenbrooke's Hospital, Department of Gastroenterology, Cambridge, United Kingdom

Clostridium difficile pilot study: effects of probiotic supplementation on the incidence of *C. difficile* diarrhoea

Summary. Colonic infection with *Clostridium difficile,* leading to pseudomembranous colitis, is a common complication of antibiotic therapy, especially in elderly patients. It has been suggested that non-pathogenic probiotic bacteria might prevent the development and recurrence of *C. difficile* infection. This double-blind, placebo-controlled study examines the role of probiotic administration in the prevention of *C. difficile*-associated diarrhoea (CDAD) in elderly patients receiving antibiotic therapy. Consecutive patients (150) receiving antibiotic therapy were randomised to receive either a probiotic containing both *Lactobacillus* and *Bifidobacterium* or placebo for 20 days. Upon admission to hospital, bowel habit was recorded and a faecal sample taken. Trial probiotic or placebo was taken within 72 h of prescription of antibiotics, and a second stool sample was taken in the event of development of diarrhoea during hospitalisation or after discharge. Of the randomised patients, 138 completed the study, 69 with probiotics in conjunction with antibiotics and 69 with antibiotics alone. On the basis of development of diarrhoea, the incidence of samples positive for *C. difficile*-associated toxins was 2.9% in the probiotic group compared with 7.25% in the placebo-control group. When samples from all patients were tested (rather than just those developing diarrhoea) 46% of probiotic patients were toxin-positive compared with 78% of the placebo group. [**Int Microbiol** 2004; 7(1):59–62]

Key words: *Clostridium difficile* · probiotic · antibiotic-therapy · diarrhoea

Received 22 May 2003
Accepted 7 September 2003

*Corresponding author:
Sue Plummer
Cultech Ltd., York Chambers
York Street
Swansea, SA1 3NJ United Kingdom
Tel. +44-1639825100. Fax +44-1639825101
E-mail: sue.cultech@btinternet.com

Introduction

Clostridium difficile is a gram-positive, anaerobic bacillus that colonises the human large intestine, and produces at least two exotoxins: toxin A, which is primarily an enterotoxin, and toxin B, a cytotoxin. Colonisation by this organism and subsequent infection occur in response to disruption of the stability of the indigenous microflora. The altered colonisation resistance frequently occurs following antibiotic therapy in hospitalised patients [12]. Finegold [4] claimed that all antimicrobial agents (with the exception of vancomycin and parenterally administered aminoglycosides) have been documented as pre-disposing patients to susceptibility to *C. difficile* infection. Responses to colonisation of the large intestine by *C. difficile* vary from asymptomatic, to mild diarrhoea, through to pseudomembranous colitis.

C. difficile is one of the most common causes of infectious diarrhoea in hospitals and nursing homes [10] and is the leading cause of nosocomially acquired intestinal infections in the USA [16]. Within hospitals, the extensive use of antibiotics, together with the inherent environmental contamination provides sources of cross-infection. Prevalence of *C. difficile* in the general environment is far lower than in healthcare facilities. A single case of *C. difficile*-associated diarrhoea per 15,000 out-patients has been recorded, but up to 20% of in-patients may be colonised by *C. difficile.*

The financial burden associated with *C. difficile* infections

is substantial for hospitals. Recurrence of symptoms following treatment of the infection is a particular problem with 5 to 66% of patients suffering from recurrences [16,18]. Jones and MacGowan [7] stated that, despite the issue of guidelines [DoH/PHLS, 1994 (Department of Health/Public Health Laboratory Service)] regarding the management and prevention of *C. difficile* infections, the problems continue and the authors suggested that the prophylactic use of biotherapies might be necessary to increase colonisation resistance.

Biotherapy (therapy involving probiotics) is emerging as a potential means of controlling *C. difficile* diarrhoeal recurrences, and promising results have been found when a stool sample was directly donated by colonoscopy [14] and when a non-pathogenic yeast (*Saccharomyces boulardii*) was used to treat *C. difficile*-infected rats and rabbits [2]. The role of the probiotic organisms is to restore the colonisation resistance of the normal flora, disrupted by the effects of antibiotic therapy, in order to prevent re-infection by *C. difficile* [9]. In this study, the emphasis is on the use of the probiotic product to prevent the initial infection and, thereby, minimise cross contamination and contain the spread of infection.

Materials and methods

Trial design. The trial was a double blind, placebo-controlled study in the departments of medicine and medicine for the elderly at Addenbrooke's Hospital, Cambridge. Patients with acute emergencies requiring treatment with antibiotics participated in the study. Ethical approval was obtained from the Cambridge Local Research Ethics Committee. Between March 1999 and July 2000, 150 patients were recruited and 138 patients fulfilled the inclusion criteria. For these patients, bowel habit on admission and prescribed medication were recorded.

Randomisation. Trial participants were randomised on arrival at hospital (probiotic group 69, placebo group 69) and each patient received one capsule/day for 20 days. The probiotic product (provided by Cultech, Swansea) comprised 2×10^{10} cfu *Lactobacillus acidophilus* and *Bifidobacterium bifidum*/capsule; the placebo comprised the inactive carrier. Trial treatments started within 36 h of antibiotic prescription (1.12 days for patients taking probiotics and 1.10 days for patients receiving placebo). Patients on a course of antibiotics lasting longer than 20 days were withdrawn from the trial, having had a final stool specimen collected.

Enumeration of *Clostridium difficile*. Faecal samples were enumerated following alcohol shock treatment. Faecal material was mixed with absolute alcohol (1:1, w/v), homogenised, and stored at 20°C for 60 min. Dilution series set up anoxically in pre-reduced Maximum Recovery Diluent (MRD, Oxoid, Basingstoke, UK) and appropriate dilutions plated onto *Clostridium difficile* Agar (Oxoid) using a modified version (10×10 µl) of the Miles and Misra plate count method [11]. Growth was recorded after 48 and 72 h incubation at 37°C. All presumptive *C. difficile* colonies were subcultured onto anoxic blood agar for Gram staining, and all obligate anaerobic gram-positive rods were tested to confirm that they were catalase-negative. Colonies were also tested using the Microscreen *C. difficile* Latex Slide Agglutination test (Microgen Bioproducts, Camberley, Surrey, UK) and/or API ID32A (Biomérieux, France). Samples positive for *C. difficile* were tested for the presence of *C. difficile* toxins A and B using an enzyme immunoassay kit (Ridascreen *C. difficile* toxin A/B, R-Biopharm, Darmstadt, Germany).

Statistical analysis. The trial was set up on the basis of a power calculation which estimated that, for an expected incidence rate of *C. difficile* infection of 10%, 400 patients would need to be recruited to show a 50% difference between the probiotic and the placebo in the prevention of *C. difficile* infection. The recruitment did not reach the required levels, which has made statistical analysis of the data limited. The data have been analysed using the methods of Newcombe [13] to determine confidence intervals for differences between proportions..

Results and Discussion

On arrival in hospital, patients were randomly allocated to receive probiotic or placebo in conjunction with their antibiotic therapy. Whilst on the ward, all episodes of diarrhoea were recorded, as is normal practice, and samples were sent to the hospital labs for typing. Unfortunately, it was not possible to achieve the required recruitment level during the course of this study so the final numbers were lower than had been indicated by the power calculation.

In addition to the standard hospital procedure of testing following the occurrence of diarrhoea, all participants in the trial provided faecal samples for testing at the start of the trial and following antibiotic therapy. On arrival at hospital, eight of the 138 participants (6%) were found to be carrying *C. difficile* asymptomatically (Table 1) with only one developing diarrhoea whilst in hospital (patient 16, who arrived with high numbers of *C. difficile* present). None of the patients tested positive for *C. difficile* toxin on arrival. This more detailed examination of the faecal samples from all patients (rather than exclusively for the diarrhoea patients) indicated that the numbers of patients carrying *C. difficile* was comparable in both groups, and such data (detailing presence of organisms) would not be detected using standard procedures in the hospital.

Finegold [4] suggested that up to 3% of healthy adults carry *C. difficile*, but many of the patients in this study may have received antibiotic therapy prior to hospitalisation or been hospitalised previously, which could have contributed to the elevated carrier status. Linevsky and Kelly [10] suggested that the asymptomatic carrier state may be due to toxin neutralisation rather than to the prevention of colonisation, as has been found in animals [8]. During this study, it appeared that there was an increase in *C. difficile*-associated problems following the admission of these asymptomatic carriers to hospital. In addition, the increase in the isolation rate of *C. difficile* from patients following antibiotic therapy clearly indicated the spread of this microorganism within the hospital environment.

Using the hospital-derived results to assess the 138 patients participating in the trial, 30 patients developed symptoms of diarrhoea (22% incidence rate), 15 patients in each treatment group. Analysis of the samples from these patients showed that five patients in the placebo group and

Table 1. Patients presenting with *Clostridium difficile* on arrival at hospital

Patient number	Date of admission	*C. difficile* viable number (cfu/g)
16	05/99	5.8×10^5
89	11/99	6.0×10^3
100	12/99	2.0×10^2
105	12/99	2.0×10^2
110	12/99	2.0×10^2
113	01/00	6.0×10^3
119	01/00	4.0×10^2
136	03/00	2.0×10^3

two of the patients in the probiotic group tested positive for the presence of *C. difficile* toxin. Statistical analysis of these results indicated that the proportion developing diarrhoea positive for *C. difficile*-associated toxins was 4.35% lower in the probiotic group (95% CI of –0.132 to 0.038).

There was a much greater proportion of patients positive for the toxins in the placebo group than in the probiotic group (which corresponds to the results obtained for diarrhoea patients from the hospital labs). In the placebo group, there appears to be a close relationship between the incidence of diarrhoea and the presence of toxin but this was less apparent among patients receiving probiotic. Thus, it would appear that many of the patients receiving the probiotic product were in the asymptomatic carrier state [10]. This may indicate the potential mode of action of the probiotic, i.e. by achieving some form of toxin neutralisation. Gorbach [6] found that administration of *Lactobacillus* GG resulted in an increase in the numbers of IgA- and other immunoglobulin-secreting cells in the intestinal mucosa, producing an enhanced immune response to the presence of *C. difficile* and/or its toxin. Such a response could account for the greater incidence of asymptomatic carriers observed among the probiotic group.

When the second faecal samples were analysed, *C. difficile* was detected in 20 of the 138 patients, four of whom had tested *C. difficile*-positive on arrival. Nine of the patients receiving placebo and 11 of the patients receiving probiotics were carrying this organism (Table 2). Toxin testing of the *C. difficile*-positive patients indicated that five of the 11 probiotic patients (46%) were toxin-positive with two of the five toxin-positive patients showing signs of diarrhoea. Seven of the nine placebo patients carrying *C. difficile* (78%) were toxin-positive, and six of these seven had diarrhoeal symptoms. Statistical analysis of the data obtained when all of the samples were analysed indicated a 32% difference between the detection of toxin among the *C. difficile*-positive placebo group patients and the probiotic group patients (95% CI of –0.096 to 0.61). There appeared to be an increased incidence of *C. difficile* detection corresponding to the arrival of the asymptomatic carriers at the hospital.

The fact that, more *C. difficile*-positive patients were detected in the probiotic group than in the placebo group again may support toxin neutralisation rather than prevention of colonisation as the role of the probiotic organisms.

The participants in the trial were contacted following discharge, and 14 of the patients reported incidences of diarrhoea at home (9 placebo, 5 probiotic). Of these patients, however, only one had tested positive for the presence of *C. difficile* when the second faecal samples were analysed. In a trial with *Lactobacillus* GG, Pochapin et al. [15] found that, for a group of patients receiving either placebo or probiotic in conjunction with their antibiotic therapy, 30% (3/10) of the patients in the placebo group developed recurrent *C. difficile*-associated diarrhoea (CDAD) while none of the patients (0/6) in the probiotic group suffered recurrent CDAD. However, for patients who had previously suffered an episode of CDAD, the probiotic product did not appear to exert any beneficial effect.

When the medical and financial implications of *C. difficile* diarrhoea were considered, Eriksson and Aronsson [3] found that the median time for hospitalisation of the *C. difficile* patients was 50 days, compared with 14 days for uninfected controls. The mortality rates were 21% for the infected group and 7% for the control group (morbidity 14% and 4% respectively).

From the financial aspect, Spencer [17] suggested that the major cost implications associated with *C. difficile* infection/outbreaks related to increased hospital stay, antibiotic treatment, possible ward closure and loss of bed days together with infection control requirements. Wilcox et al. [18] estimated that the average additional length of stay in hospital was 21.3 days longer for *C. difficile* patients, corresponding to additional treatment costs per patient of £4,000. On the basis of the hospital-derived results in this study, the five placebo-group positive patients would have incurred an additional £20,000 expenditure whereas the probiotic group costs would have been £8,000. If it is assumed that administration of probiotic to all the patients in the trial incurred an additional cost of £2,000, the overall savings achieved from the probiotic supplementation could have been £10,000, a 50% reduction in costs.

Table 2. Results from second faecal samples collected following antibiotic therapy

	Placebo group	Probiotic group
C. difficile positive	9	11
Toxin-positive	7	5
Diarrhoeal symptoms	6	2

In 1999, more than 15,000 cases of *C. difficile* were reported in the National Health Service (NHS), which would have cost more than £60 million to treat. If the findings of this pilot study can be confirmed by a more extensive study, treatment costs could be reduced by £30 million, and more than 300,000 hospital-bed days could be made available. If, with a larger study, the trends from this study are confirmed, the justification for the use of probiotic therapy for all patients receiving antibiotic therapy on admission to hospital would be clearly evident.

References

1. Biller JA, Katz AJ, Flores AF, Buie TM, Gorbach SL (1995) Treatment of recurrent *Clostridium difficile* colitis with *Lactobacillus* GG. J Pediatr Gastroenterol Nutr 21:224–226
2. Castagliuolo I, Riegler MF, Valenick L, LaMont JT, Pothoulakis C (1999) *Saccharomyces boulardii* protease inhibits the effects of *Clostridium difficile* toxins A and B in human colonic mucosa. Infect Immun 67:302–307
3. Eriksson S, Aronsson B (1989) Medical implications of nosocomial infection with *Clostridium difficile*. Scand J Infect Dis 21:733–734
4. Finegold SM (1986) Anaerobic infections and *Clostridium difficile* colitis emerging during antibiotic therapy. Scand J Infect Dis (Suppl) 49:160–164
5. Gorbach SL, Chang T-W, Goldin B (1987) Successful treatment of relapsing *Clostridium difficile* colitis with *Lactobacillus* GG. Lancet 2:1519
6. Gorbach SL (2000) Probiotics and gastrointestinal health. Am J Gastroenterol 95 (Suppl 1):2–4
7. Jones EM, MacGowan AP (1998) Back to basics in management of *Clostridium difficile* infections. Lancet 352:505–506
8. Kim PH, Iaconis JP, Rolfe RD (1987) Immunization of adult hamsters against *Clostridium difficile* associated ileocolitis and transfer of protection to infant hamsters. Infect Immun 55:2984–2992
9. Kyne L, Kelly CP (2001) Recurrent *Clostridium difficile* diarrhoea. Gut 49:152–153
10. Linevsky JK, Kelly CP (1997) *Clostridium difficile* colitis in gastrointestinal infections. In: LaMont, JT (ed) Gastrointestinal Infections: Diagnosis and Management. Marcel Dekker, New York, pp 293–325
11. Miles AA, Misra SS (1938) The estimation of the bactericidal power of the blood. J Hygiene 38:732–749
12. Naaber P, Mikelsaar RH, Salminen S, Mikelsaar M (1998) Bacterial translocation, intestinal microflora and morphological changes of intestinal mucosa in experimental models of *Clostridium difficile* infection. J Med Microbiol 47:591–598
13. Newcombe RG (1998) Interval estimation for the difference between independent proportions: comparison of eleven methods. Stat Med 17:873 – 890 [erratum in Stat Med 1999 May 30; 18:1293]
14. Persky SE, Brandt LJ (2000) Treatment of recurrent *Clostridium difficile*-associated diarrhea by administration of donated stool directly through a colonoscope. Am J Gastroenterol 95:3283–3285
15. Pochapin M, Oltikar A, Pringle-Smith R, Schreiber C (1998) A prospective randomised placebo-controlled trial of *Lactobacillus* GG in combination with standard antibiotics for the treatment of *Clostridium difficile* infection. Am J Gastroenterol 93:1697 (Abstract)
16. Pochapin M (2000) The effect of probiotics on *Clostridium difficile* diarrhea. Am J Gastroenterol 95(Suppl 1):S11–S13
17. Spencer RC (1998) Clinical impact and associated costs of *Clostridium difficile*-associated disease. J Antimicrob Chemother 41(Suppl C):5–12
18. Wilcox MH, Cunniffe JG, Trundle C, Redpath C (1996) Financial burden of hospital-acquired *Clostridium difficile* infection. J Hosp Infect 34:23–30

Estudio piloto de *Clostridium difficile*: efecto del aporte suplementario de probióticos sobre la incidencia de diarrea causada por *C. difficile*

Resumen. La infección de colon por *Clostridium difficile*, que produce colitis pseudomembranosa, es una complicación frecuente en las terapias con antibióticos, especialmente en pacientes de la tercera edad. Se ha sugerido que las bacterias probióticas no patógenas podrían prevenir el desarrollo de la infección por *C. difficile*. Este estudio de doble ciego con control mediante placebos examina la influencia de la administración de probióticos en la prevención de diarrea asociada a *C. difficile* (CDAD) en pacientes de la tercera edad sometidos a terapia con antibióticos. Se escogieron al azar 150 pacientes consecutivos sometidos a terapia con antibióticos y se les administró aleatoriamente durante 20 días un probiótico que contenía *Lactobacillus* y *Bifidobacterium* o un placebo. Tras su ingreso hospitalario, se anotó su régimen intestinal y se tomó una muestra fecal. El probiótico o el placebo se administró durante las 72 h primeras del tratamiento con antibióticos, y se tomó una segunda muestra de heces en el caso de aparecer diarrea durante la hospitalización o tras el alta médica. De los pacientes escogidos, 138 completaron el estudio, 69 tratados con antibióticos y probióticos y 69 solamente con antibióticos. Entre los pacientes que tuvieron diarrea, se encontró un 2,9% de muestras positivas para la toxina asociada a *C. difficile* en el grupo tratado con probióticos, en comparación con el 7,25% detectado en el grupo control tratado con placebo. Cuando se analizaron muestras de todos los pacientes (no solamente los que tuvieron diarrea), un 46% de los pacientes tratados con probióticos dieron positivo para la toxina, en comparación con el 78% del grupo tratado con placebo. [**Int Microbiol** 2004; 7(1):59–62]

Palabras clave: *Clostridium difficile* · probiótico · terapia antibiótica · diarrea

Estudo piloto de *Clostridium dificile*: efeito da suplementação com probióticos sobre a incidência de diarréia causada por *C. difficile*

Resumo. A infecção do cólon por *Clostridium difficile,* derivando em colite pseudomembranosa, é uma complicação comum nas terapias com antibióticos, especialmente em pacientes na terceira idade. Tem sido sugerido que as bactérias probióticas não patógenas poderiam ter um efeito preventivo sobre o desenvolvimento da infecção por *C.difficile*. Este estudo, duplamente cego, com controle mediante placebos, examina a influência da administração de probióticos sobre a prevenção de diarréia associada a *C. difficile* (CDAD) em pacientes da terceira idade submetidos à terapia com antibióticos. Foram escolhidos 150 pacientes submetidos à terapia com antibióticos e se administrou, aleatoriamente, durante 20 dias um probiótico que continha *Lactobacillus* e *Bifidobacterium* ou um placebo. Na admissão hospitalar foram anotados o regime intestinal do paciente e foram colhidas amostra fecal. Durante as primeiras 72 horas do tratamento com antibióticos foram administrados conjuntamente probiótico ou placebo e foi tomada uma segunda amostra de fezes, caso o paciente desenvolvesse diarréia durante ou após a alta médica. Dos pacientes escolhidos, 138 completaram o estudo, 69 tratados com antibióticos e probióticos e 69 somente com antibióticos. Dentre os pacientes que desenvolveram diarréia, foram encontradas 2,9% de amostras positivas para a toxina associada a *C. difficile* no grupo tratado com probióticos, em comparação com 7,25% detectado no grupo controle tratado com placebo. Quando foram analisadas as amostras de todos os pacientes (não somente os que desenvolveram diarréia), 46% dos pacientes tratados com probióticos apresentaram positividade para a toxina em comparação com 78% do grupo tratado com placebo. [**Int Microbiol** 2004; 7(1):59–62]

Palavras chave: *Clostridium difficile* · probiótico · terapia antibiótica · diarréia