Durable Alteration of the Colonic Microbiota by the Administration of Donor Fecal Flora

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Goals: To determine whether fecal bacteriotherapy results in a durable beneficial change in the colonic microbiota of patients with flora-related disorders.

Background: Earlier studies have implicated the colonic microbiota in a number of conditions. Administration of a fecal suspension from a healthy individual to an ill individual (fecal bacteriotherapy) can cure *Clostridium difficile* infection and potentially other diseases. Oral probiotics do not work in this condition, yet there has been no study to determine whether fecal bacteriotherapy results in prolonged implantation.

Study: Fecal samples were collected from 10 patients undergoing fecal bacteriotherapy. Patients completed an antibiotic schedule and bowel lavage before the infusion of healthy donor feces. Using a molecular approach, the bacterial populations in patient fecal samples were followed from pretreatment to 24 weeks post-initial infusion and compared with the initial infused donor fecal suspension.

Results: At intervals of 4, 8, and 24 weeks after the procedure, the bacterial populations in the patients' fecal samples consisted predominantly of bacteria derived from the healthy donor samples. Comparisons of similarity at 4, 8, and 24 week samples to the donor-infused sample were made and each recipient's baseline sample was statistically significant with Friedman test.

Conclusions: This study demonstrates a durable beneficial change in the patients' bacterial populations of the colon to represent those of the healthy donor's microbiota. Manipulation of the colonic microbiota to improve its protective and beneficial role represents a promising field of new therapeutic strategies for the treatment of gastrointestinal conditions.

Key Words: implantation, fecal bacteriotherapy, probiotic, *Clostridium*

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Thomas J. Borody has a pecuniary interest in the Centre for Digestive Diseases, where fecal bacteriotherapy is a treatment option for patients.

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Fecal bacteriotherapy, the administration of a suspension of feces from a healthy individual into the colon of an individual with colonic disease,[1–9] has been reported to be of therapeutic benefit, particularly in antibiotic-associated diarrhea,[1] *Clostridium difficile*-associated diarrhea,[2–7] and in ulcerative colitis.[8,9] These studies reported a high rate of response in patients with refractory *C. difficile* and antibiotic-associated diarrhea; however, the reports are small case series and have not addressed mechanisms of action, particularly bacterial implantation.

Part of the rationale for the favorable response of fecal bacteriotherapy in *C. difficile* infection stems from the suggestion that the infused donor microbiota permanently changes the bacterial population of the recipient colonic microbiota. To date, however, there has been no direct evidence to support this hypothesis. Currently marketed oral "probiotics" are generally mass produced from stock cultures and are known to transiently implant for 10 to 14 days after cessation of oral administration.[10,11] The only prolonged implantation of probiotics was reported by Tvede and Rask-Madsen (1989) where 6 individuals with relapsing *C. difficile* diarrhea were treated with either enemas of fecal suspension (1 of 6), a mixture of 10 freshly cultured bacteria (4 of 6), or both (1 of 6).[12] Before treatment, the patient's microbiota was shown by culture to consist predominantly of aerobic and facultative anaerobic bacteria. Earlier reports on fecal microflora have demonstrated that patients with diarrhea and ulcerative colitis have abnormally large numbers of aerobic and facultative anaerobic bacteria, with one of these, adhesive *Escherichia coli*, having been implicated in the pathogenesis of ulcerative colitis.[13,14] After bacteriotherapy, the authors demonstrated the restoration of previously absent or undetected anaerobic species. This change in bacterial populations seemed to represent a durable recovery of some of the missing components of the normal microbiota. Although reported to be highly successful in eradicating *C. difficile* infection, implantation durability of bacterial populations after fecal bacteriotherapy has yet to be investigated.

The aim of this study was to determine whether fecal bacteriotherapy results in successful, durable implantation of multiple colonic bacteria species derived from a healthy individual and infused into a patient with colonic symptoms.

MATERIALS AND METHODS

Participants

Sixty donor-recipient pairs planning to undergo human probiotic infusion at the Probiotic Therapy Research

Centre (PTRC) were invited to participate in the study. All eligible patients who were willing to participate in the study provided written informed consent. Donors or recipients aged 18 years or above who were attending the Center for human probiotic infusion, were able to provide written informed consent, able to communicate with the investigators, and able to comply with the requirements of the study were enrolled into the study. Patients were excluded if they were unable to comply with any requirements of the study; had a current drug or alcohol addiction; had participated in any other clinical trial within the preceding 30 days; had taken antibiotics in the 2 months before the commencement of the donor fecal enema protocol by the donor or the recipient; or were unlikely to remain off antibiotics for at least 2 months after donor human probiotic infusion (recipients only). Subjects wanting to withdraw or subjects who had to take antibiotics during the period after probiotic infusion (recipients only) were withdrawn without prejudicing their subsequent care by any of the investigators or staff at PTRC.

Ten patients (6 males and 4 females; aged 22 to 57 y) and 4 donors (males, aged 40 to 49 y) were recruited into the study between October 2001 and April 2003 at the PTRC in Sydney, Australia. Before donating their feces all donors were screened for gastrointestinal pathogens, including parasites, *C. difficile*, *Yersinia*, *Aeromonas*, *Klebsiella oxytoca*, *Campylobacter jejuni*, *Staphylococcus aureus*, and parenterally transmissible viruses. The donors had not taken antibiotics for 2 months before the study. Demographic and clinical information was collected for all test subjects (Table 1). The collection and analysis of the samples were approved by the University of New South Wales Human Research Ethics Committee (HREC01256) and the Centre for Digestive Diseases Human Research Ethics Committee.

Protocol

The clinical protocol for each patient and the allocation of a donor as the source of feces for infusion was empirically determined by the relevant c inic staff. Details of the regimens used to prepare each patient and administration of fecal suspensions are shown in Table 1. Patients were prepared with various combinations of oral vancomycin [500 mg twice daily (bd)], rifampicin (150 mg, bd), doxycycline (50 mg, bd), or metronidazole (400 mg, bd) for 5 to 10 days. This was immediately followed by bowel lavage using a polyethylene glycol solution (Glycoprep, Pharmatel Pty Ltd, Hornsby, NSW, Australia) according to the manufacturer's instructions. On the morning of the implantation, fecal suspensions were prepared from fresh stool samples collected from the donors, and mixed in a blender for 30 seconds with 250 mL of sterile normal saline. About 200 to 400 mL of this suspension was infused daily into the patients for a period of 5 to 15 days (mean time 9.1 ± 3.25 d). The first infusion was administered into the cecum through a colonoscope and subsequent infusions were given over a 60-minute period through a nasojejunal tube, enema, or combination of both (details in Table 1).

Fecal Sample Collection

To assess the bacterial ecology of the patients' colons preprocedure and postprocedure, fecal samples were collected from the patients at enrolment (T1); from the first stool after commencing polyethylene glycol solution

TABLE 1. Demographic Details and Treatment Protocol

Subject No.	Age (y)/ Sex	Diagnosis	Medication	Antibiotic Preparation	Mode of Suspension Administration	Donor No.
1	47/F	Diarrhea predominant IBS		5 d vancomycin and metronidazole	1 at colonoscopy then 4 enemas	1
2	38/M	Diarrhea predominant IBS		5 d vancomycin and metronidazole	1 at colonoscopy then 4 nasojejunal infusions then 5 enemas	2
3	34/F	Constipation	Colchicine, lactulose, and bisacodyl	5 d vancomycin and metronidazole	15 enemas	3
4	22/F	Constipation	Colchicine	5 d vancomycin and metronidazole	1 at colonoscopy then 9 enemas	4
5	57/M	Crohn's colitis	Mesalazine and azathioprine	10 d vancomycin and metronidazole	1 at colonoscopy then 9 enemas	2
6	38/M	IBS		5 d vancomycin and metronidazole	1 at colonoscopy then 5 combined nasojejunal infusions + enemas	4
7	51/M	IBS	Pantoprazole and bismuth	5 d doxycycline and metronidazole	1 at colonoscopy then 9 enemas	2
8	50/M	Constipation	Esomeprazole, aspirin, atorvastatin, and colchicine	5 d doxycycline and rifampicin	1 at colonoscopy then 5 combined nasojejunal infusions + enemas then 5 enemas	2
9	56/M	Diarrhea predominant IBS	Bismuth and lisinopril	5 d vancomycin and metronidazole	1 at colonoscopy then 4 enemas	4
10	41/F	Constipation	Beta-interferon	5 d vancomycin and metronidazole	1 at colonoscopy then 9 enemas	2

F indicates female; IBS, inflammatory bowel disease; M, male.

(T2), and then: at 4 weeks (T3); 8 weeks (T4), and 24 weeks (T5) after the infusions were completed.

Samples of the feces from each donor used in the first infusion (denoted S_1) were also collected. Fecal samples were collected in standard containers and frozen at −20°C in the patients' home freezers and were returned for analysis within 7 days of their collection.

Primer Development

Primer sets were developed to target the *Bacteroides-Prevotella* group (RDP 2.15.1.2), *Clostridium coccoides* group (RDP 2.30.4.1), and *Clostridium leptum* subgroups (RDP 2.30.9.1) of the Ribosomal Database Project (RDP-II).[15] Initial attempts to design nondegenerate primers using the published freeware program PRIMROSE[16] did not yield suitable sequences for denaturing gradient gel electrophoresis (DGGE), necessitating manual primer design. For manual design, named sequences within target and nontarget groups were downloaded using the HIERARCHY_BROWSER[15] and aligned with CLUSTALW.[17] The target sequences for the *Bacteroides-prevotella* group, *C. coccoides* group, and *C. leptum* subgroups were downloaded from the RDP (available upon request). Using this approach, 6 potential primer sites were identified for the *Bacteroides-prevotella* group, 5 for the *C. coccoides* group, and 4 for the *C. leptum* subgroup. The sequences of the potential primers were compared with the RDP database using PROBE_MATCH[15] to generate a list of matches and near matches with 1 or 2 mismatched bases at the 3′ end of the primer. For each of the primer sets, it was not possible

TABLE 2. Primers, PCR, and DGGE Conditions

Target	Primer	Sequence 5′ to 3′	16S rRNA Position (*E. coli* Numbering)	Thermal Cycling*	Denaturant Gradient†	Reference
Bacteria	L1401	CGG TGT GTA CAA GAC CC	1385-1401	94°C 5 min; 30 cycles of 94°C 30s, 56°C 30s, 72°C 60s; 72°C 4 min	43%-57%	Nubel et al[18]
	U968GC	CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAA CGC GAA GAA CCT TAC	968-984			
Bifidobacteria	Bif164f	GGG TGG TAA TGC CGG ATG	164-182	94°C 5 min; 30 cycles of 94°C 30s, 62°C 20s, 72°C 60s; 72°C 7 min	43%-65%	Satokari et al[19]
	Bif662G-Cr	CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC ACC GTT ACA CCG GGA A	645-662			
Bacteroides-prevotella group	Bac948F	ATGTGGTTTAATTCGAT-GATA	948-968	94°C 4 min; 30 cycles of 94°C 20s, 64°C 30 s, 72°C 60s; 72°C 4 min	40%-70%	This paper
	Bac1307-RGC	CGC CCC CCG CCG CCC CGC CGC CCG GCC CGC CGC CCC CGC CAT GCG CGA TTA CTA GCG AA	1289-1307			
Clostridium coccoides group	Ccoc447F	TGA CGG TAC CTG ACT AAG	447-464	94°C 4 min; 30 cycles of 94°C 20s, 63°C 30s, 72°C 60s; 72°C 4 min	43%-60%	This paper
	Ccoc986-RGC	CGC CCC CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC TTG AGT TTC ATT CTT GCG AA	967-986			
Clostridium leptum subgroup	Clept 751F	GTG CCG CAG TTA ACA CAA	751-768	94°C 4 min; 30 cycles of 94°C 20s, 63°C 30s, 72°C 90s; 72°C 7 min	38%-65%	This paper
	Clept1246 RGC	CGC CCC CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC GCG ATT ACT AGC AAT TCC GA	1227-1246			

*Hot Start PCR reactions were performed with reagents from Biotech International, Belmont, WA, Australia. The PCR for *Bifidobacteria* used 3 mM $MgCl_2$ whereas the remaining reactions used 2.5 mM.

†100% concentration of denaturants is 7 M urea and 40% formamide.

DGGE indicates denaturing gradient gel electrophoresis; *E. coli*, *Escherichia coli*; PCR, polymerase chain reaction.

to encompass all of the sequences within the target groups or exclude all nontarget groups with a single nondegenerate primer. Therefore, the potential primer sites that resulted in the greatest number of target and least nontarget matches suitable for polymerase chain reaction (PCR)-DGGE were selected and are shown in Table 2.

Optimization of PCR and DGGE

For each of the new GC-clamped primer sets, the annealing temperature for PCR was determined using the gradient PCR Express (Hybaid, Ashford, Middlesex, UK) and DNA template from bacterial strains from the University of New South Wales Culture Collection. These were *Bacteroides fragilis* and *Bacteroides vulgatus* for the *Bacteroides-prevotella* group primer set, *Clostridium nexile* for the *C. coccoides* group primer set, and *C. leptum* for the *C. leptum* subgroup primer set. The following bacterial strains served as positive and negative controls for PCR specificity testing at the optimized annealing temperature: *Bifidobacterium longum* (American Type Culture Collection 15707), *Fusobacterium mortiferum* (American Type Culture Collection 25557), *Lactobacillus salivarius* (American Type Culture Collection 11741), *C. leptum* (Deutsche Sammlung von Mikroorganismen und Zellkulturen 753) and University of New South Wales Culture Collection strains of: *Bacteroides vulgatus*, *Bacteroides fragilis*, *Clostridium histolyticum*, *C. nexile*, *Bifidobacterium adolescentis*, *Eubacterium limosum*, *Peptostreptococcus anaerobilus*, *E. coli*, *Enterococcus fecalis*, *Lactobacillus acidophilus*, *Propionbacterium acnes*, and *Bacillus cereus*. At the optimized annealing temperature, the new primers were specific for their positive controls with the exception of the *C. leptum* subgroup

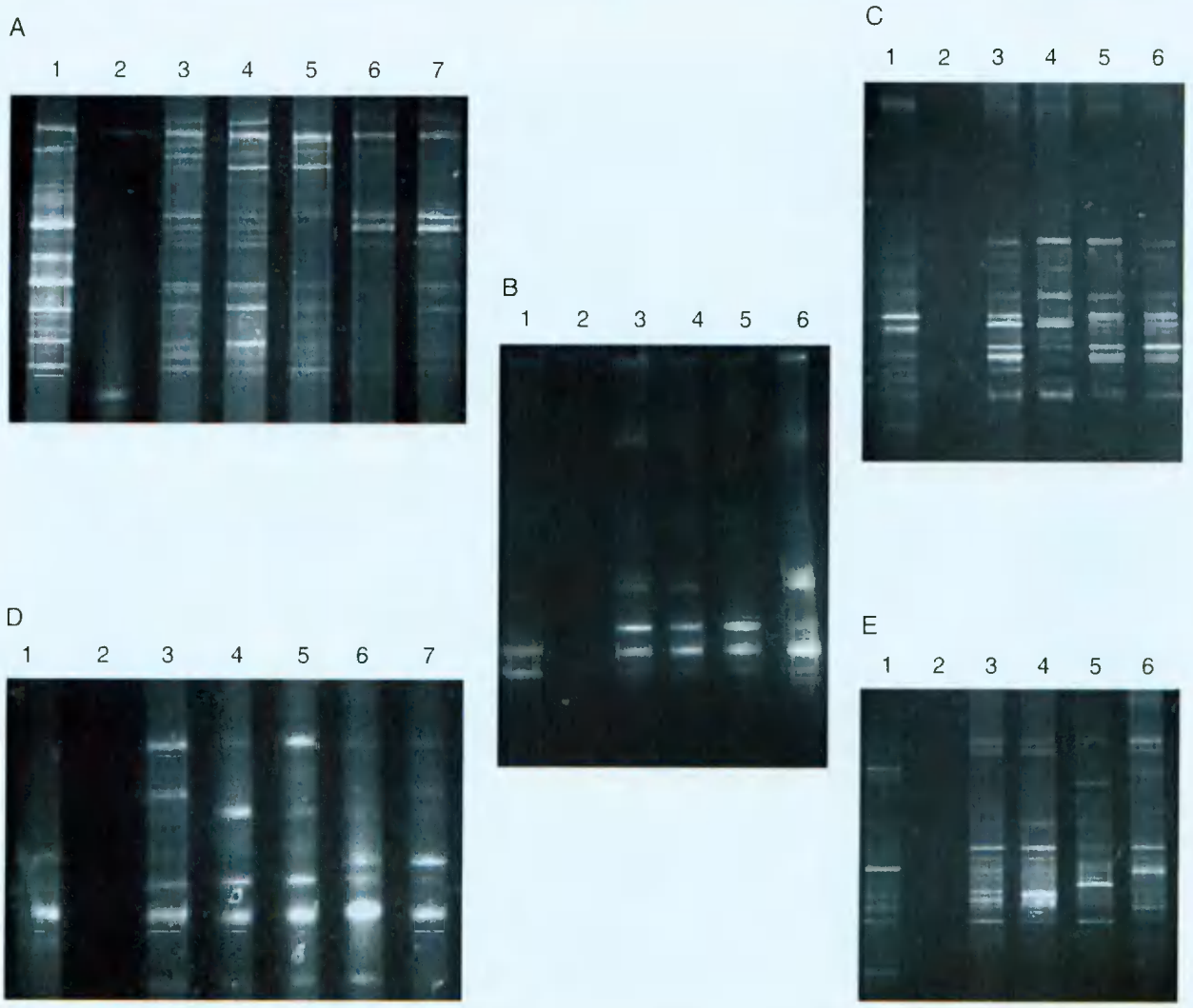

FIGURE 1. Examples of gels obtained with each primer set. Lanes 1 to 5 represent samples from the subject and lanes 6 and 7 are from the donor. Lane 1: baseline sample T1, Lane 2: day of lavage T2, Lane 3: 4 week sample T3, Lane 4: 8 week sample T4, Lane 5: 24 week sample T5, Lane 6: donor sample S_1, and where shown Lane 7: donor 4 week sample S_{4weeks}. A, DGGE gel with the domain *Bacteria* primer set obtained from the samples for subject number 1 and donor number 1. B, DGGE gel with the *Bifidobacteria* primer set obtained from the samples for subject number 10 and donor number 2. C, DGGE gel with the *Bacteroides-prevotella* primer set obtained from the samples for subject number10 and donor number 2. D, DGGE gel with the *Clostridium coccoides* group primer set obtained from the samples for subject number 3 and donor number 3. E. DGGE gel with the *Clostridium leptum* primer set obtained from the samples for subject number 10 and donor number 2. DGGE indicates denaturing gradient gel electrophoresis.

primers that also amplified template DNA from *E. limosum*. As the comparison using PROBE_MATCH suggested, the primers are not absolutely specific or completely inclusive of all of the species within their target groups.

PCR product derived from the positive control bacterial strains was used to optimize the gradient for DGGE. All electrophoresis was initially conducted with a 20% to 70% gradient of denaturants and this was gradually narrowed to maximize band separation.

Stool DNA Extraction and PCR-DGGE

DNA extraction was performed with the Puregene Kit (Gentra systems, Minneapolis, MN) according to the manufacturer's instructions. The template was amplified with primers targeting the *Bacteroides-prevotella* group, *C. coccoides* group, and *C. leptum* subgroup under the conditions described in Table 2. In addition, published assays for the domain *Bacteria* and *Bifidobacteria* were applied.[18,19] DGGE was performed in 6% polyacrylamide gels containing the gradients in Table 2 using the Dcode system (Bio-Rad, Hercules, CA) with electrophoresis for 16 hours at 75 V and 60°C. The gels were stained with ethidium bromide.

Gel Analysis

Each patient sample (T1 to T5) and respective donor sample (S_1) were electrophoresed on the same gel to facilitate analysis. The banding profiles on each gel were converted into a binary array in Microsoft Excel. Each lane became a column and each band position, a row. The presence of a band at a given gel position was recorded as 1 and absence as 0. The correlation between the banding patterns in each lane was calculated with Bray-Curtis similarity for binary data using the PRIMER 5 Software package (PRIMER-E Ltd, Plymouth, UK). Dendrograms showing the relatedness of the banding patterns were constructed by clustering using the arithmetic average algorithm of PRIMER 5.

Statistical Analysis

All statistical analyses were performed with Graphpad InStat (GraphPad Software, San Diego, CA). To determine whether the banding patterns of post-procedure samples were predominantly like T1 or S_1, the Bray-Curtis similarity measures for T3, T4, and T5 compared with T1 and S_1, were pooled for each primer set and assessed for statistical significance using the Friedman test. Analysis of the

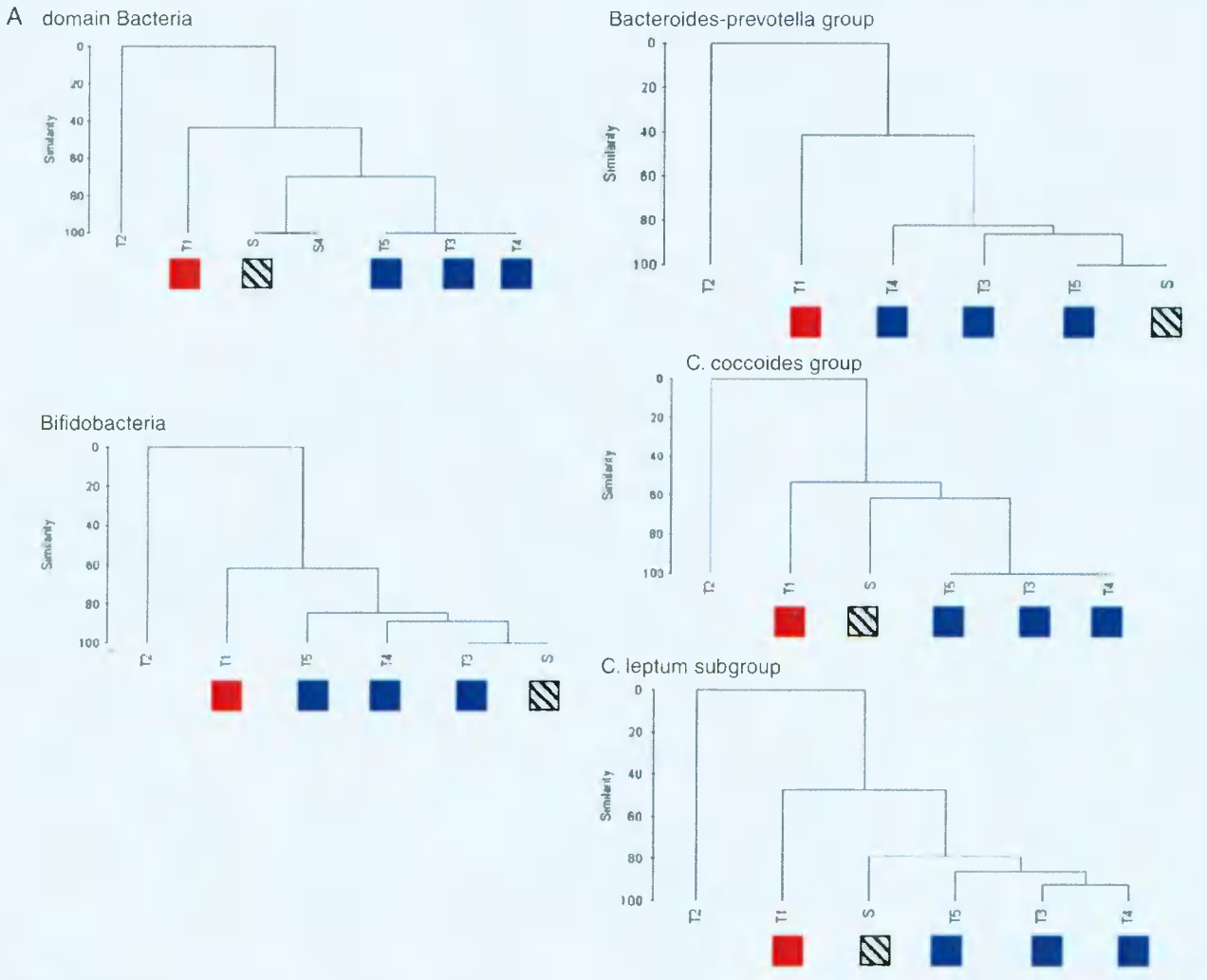

FIGURE 2. *Continued*

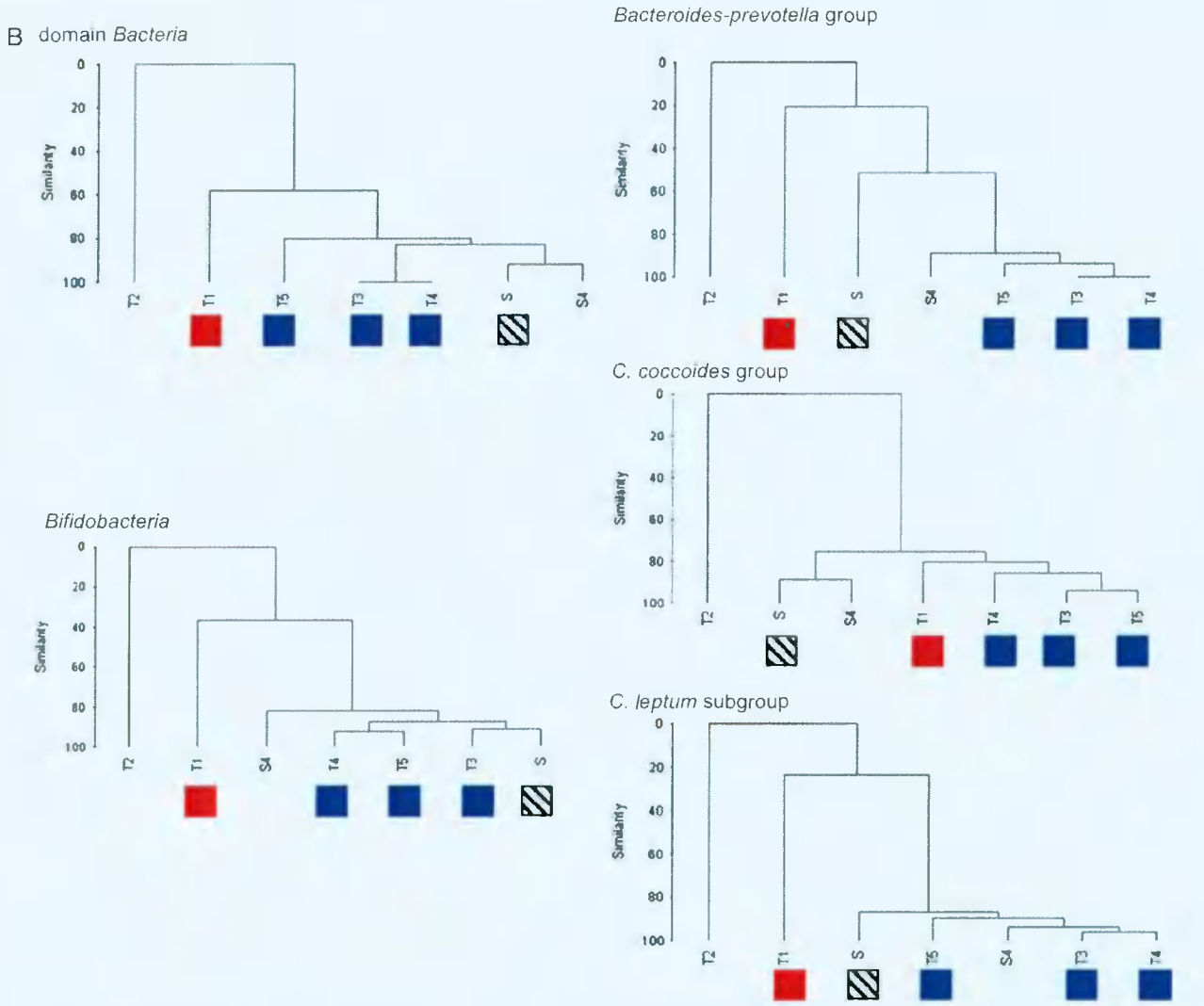

FIGURE 2. A, Dendrograms for each primer set using the samples from subject number 10 and the respective donor number 2. T1 is marked with a red box; S_1 with a black stripped box; and the post-procedure samples T3, T4, and T5 with blue boxes. B, Dendrograms for each primer set using the samples from subject number 1 and the respective donor number 1. T1 is marked with a red box; S_1 with a black stripped box; and the post-procedure samples T3, T4, and T5 with blue boxes. *C. coccoides* indicates *Clostridium coccoides*; *C. leptum*, *Clostridium leptum*.

significant results was performed with the Wilcoxon Matched Pairs Rank Sum test to make direct comparisons of the results for each time point (T3, T4, or T5) versus T1 and S_1. For example, the similarities for T3 versus T1 were compared with T3 versus S_1 for each primer set. The significance level for the comparisons was arbitrarily set at 0.01. To determine whether the microbiota changed in species composition at the follow-up time points T3, T4, and T5, the Bray-Curtis similarities for comparisons of each pair of time points (T3 vs. T4, T4 vs. T5, and T3 vs. T5) were pooled by primer set and assessed for significance with the Friedman test. Post-hoc testing of significant results was again performed with the Wilcoxon Matched Pairs Rank Sum test. Finally, the Bray-Curtis similarity for unrelated samples was calculated by comparing the banding patterns of samples from different patients, different donors, and subjects with unrelated donors, when these were represented on the same gel. Examples of gels produced with each primer set are shown in Figure 1.

RESULTS

Overall, post-procedure fecal analysis yielded banding patterns more like the infused donor fecal suspension than the patients' own baseline sample. Dendrograms depicting the Bray-Curtis similarity of banding patterns produced with each primer set for 2 of the subjects are shown in Figure 2. As demonstrated in Figure 2, the post-procedure samples comprising T3, T4, and T5 can be seen to predominantly cluster with the infused donor suspension (S_1) as opposed to their baseline sample (T1). The pooled Bray-Curtis similarities for each primer set for all of the subjects are shown in Table 3 and graphically depicted in Figure 3. Overall, the similarity measures for comparisons of samples T3, T4, and T5 to the infused fecal suspension

TABLE 3. Bray-Curtis Similarities by Primer Set

	Pooled Bray-Curtis Similarities for Sample Comparisons (Mean ± SD)								*P* of the Wilcoxon Matched-pairs Signed-ranks Test for Comparisons With T1 and S_1 of		
	T1			S_1							
Primer Set	T3	T4	T5	T3	T4	T5	Unrelated Samples	Friedman Test	T3	T4	T5
Bacteria	56.5 ± 11.3	55.8 ± 8.5	53.9 ± 6.6	70.5 ± 10.8	69.5 ± 11.3	72.1 ± 11	51 ± 11.7	$\chi^2 = 14.7$ $P = 0.012$*	0.027	0.027	0.02
Bacteroides-prevotella	44.8 ± 24.9	41.1 ± 22.1	39.3 ± 20.9	70.5 ± 24.1	77.1 ± 15.5	81.4 ± 13.9	37.1 ± 20	$\chi^2 = 38.1$ $P < 0.01$*	0.01	0.004**	0.004**
C. coccoides	59.8 ± 13.1	53.4 ± 12.3	54 ± 16.8	74.1 ± 11.4	77.3 ± 17.2	77.8 ± 15.5	53.6 ± 18.3	$\chi^2 = 17.6$ $P < 0.01$*	0.04	0.008**	0.03
C. leptum	49.4 ± 24.2	49.8 ± 24.1	44.2 ± 24.4	69.3 ± 20.9	67.9 ± 18.3	71.5 ± 18	39.1 ± 19.4	$\chi^2 = 21$ $P < 0.01$	0.08	0.048	0.07
Bifidobacteria	45.4 ± 28.3	45.2 ± 27.3	43.9 ± 26.2	85.9 ± 15.3	81.8 ± 11.9	76.8 ± 13.8	45 ± 24.4	$\chi^2 = 21.1$ $P < 0.01$*	0.03	0.01	0.03

*Significant at $P < 0.05$ level.
**Significant at $P < 0.01$ level.
C. coccoides indicates *Clostridium coccoides*; *C. leptum*, *Clostridium leptum*.

(S_1) are more pronounced than the similarity for comparisons to the baseline samples for each patient (T1). In addition, the similarities of the post-procedure samples to their own baseline sample (T1) were only marginally greater than the similarity of samples from unrelated subjects (Table 3). The results of the Friedman test confirmed that there were statistically significant differences within the data for each primer set (Table 3). Post-hoc analysis showed that comparisons of T4 to T1 and S_1 were significant with the *Bacteroides-prevotella* group and *C. coccoides* group primer sets and those comparisons of T5 to T1 and S_1 were significant with the *Bacteroides-prevotella* group primer set.

The stability of the microbiota within the groups defined by each of the primer sets after the procedure was assessed by comparing the similarity of the banding patterns at T3, T4, and T5. Figure 4 displays the Bray-Curtis similarities for comparisons of T3 versus T4, T3 versus T5, and T4 versus T5 by primer set. There was no significant difference in the similarity of T3 versus T4, T3 versus T5, and T4 versus T5 for all but one of the primer sets. The result of the Friedman test was χ^2 3.0, $P = 0.22$ for the *Bacteroides-prevotella* group primers; χ^2 3.0, $P = 0.22$ for *C. coccoides* group primers; χ^2 0.21, $P = 0.90$ for *C. leptum* subgroup primers; and χ^2 1.7, $P = 0.42$ for Bifidobacterial primers. However, there was a significant result for comparisons with the domain *Bacteria* primer set χ^2 9.9, $P = 0.007$. The similarity measures for comparisons of T3 versus T4, T4 versus T5, and T3 versus T5 with this primer set were 92.8 ± 5.4, 88.4 ± 7.9, and 88.4 ± 7.9, respectively. Post-hoc testing revealed a significant difference between T3 versus T4 compared with T3 versus T5 ($P < 0.01$) whereas the other comparisons were not significant.

DISCUSSION

A number of studies using PCR-DGGE-based methods have shown that a core group of bacterial species are present in fecal samples from any given adult over many months in the absence of antibiotic treatment.[19–21] The dramatic and curative effect of fecal bacteriotherapy in *C. difficile* infection has been reported in small studies. This begs the question as to how such dramatic treatment works and whether it could be used or modified to cure other conditions that may be infection-driven. Altered microbial composition is believed to be involved in the initiation and maintenance of a number of other gastrointestinal conditions including constipation, irritable bowel syndrome, and inflammatory bowel disease. Although the beneficial effects of live bacteria, termed probiotics, have gained increasing attention, the main disadvantage of oral probiotics is that they do not readily colonize the gastrointestinal tract.[22–24]

The human fecal flora is a complex mix of organisms, consisting of a compact mass of living bacterial cells, with almost 9 times more living cells than the entire body. Tvede and Rask-Madsen (1989) demonstrated in vitro how particular bacteria species can profoundly inhibit the growth of pathogenic strains. The accompanying mix of healthy microflora is capable of replacing lost or missing bacterial components such as *Bacteroides* species, restoring fecal physiology and deficient composition. Replacement of these core groups of bacterial species in individuals with gastrointestinal conditions presents a new therapeutic option aimed at ameliorating symptoms or potentially eliminating the condition.

In this study, feces from a healthy donor were infused into the cecum and colon of a recipient whose colon had been previously prepared with antibiotics and bowel lavage. After this procedure, a novel microbiota consisting primarily of bacterial species originating from the donor was present in the patient stool samples. This change involved all of the bacterial groups for which assays were performed including the domain *Bacteria*, *Bacteroides-prevotella* group, *C. coccoides* group, *C. leptum* subgroup, and *Bifidobacteria*. This novel microbiota remained largely stable in composition over the following 24 weeks albeit with a slight change over time as the microbiota stabilized.

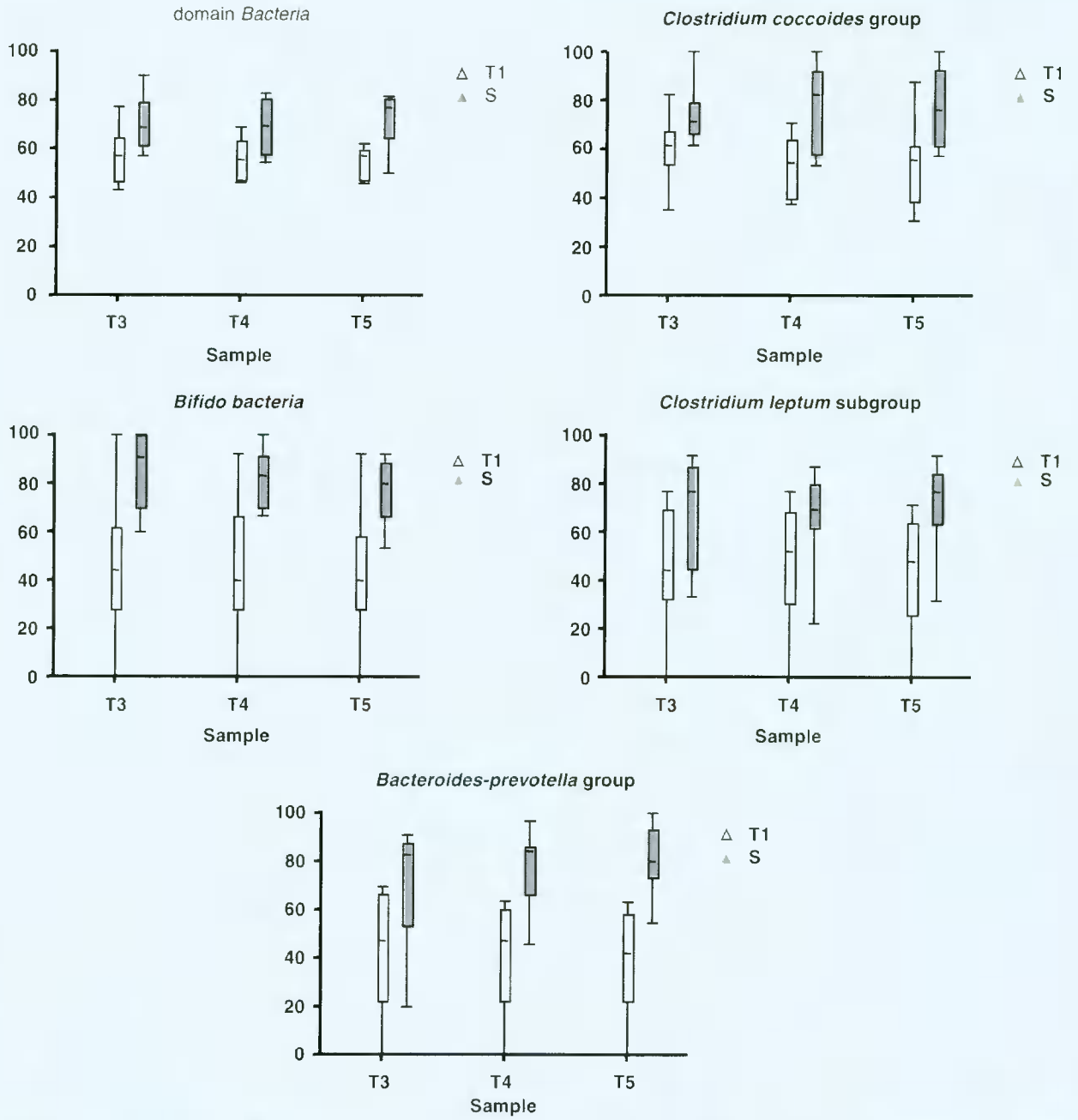

FIGURE 3. Box and whiskers plots of Bray-Curtis similarities by primer set for comparisons of post-procedure samples T3, T4, and T5 to T1 and S_1.

Although research into various aspects of oral probiotic use has grown considerably, there has yet to be conclusive evidence of predictable durable colonization after cessation of oral probiotic therapy.[22–24] Findings reported here document for the first time, the successful implantation of healthy donor microbiota which has significant therapeutic interest in these patient populations. Clearly, with our patients' well-being in mind, this area requires further study through funded research and a scientific approach to its practice.

A number of important questions are also raised by these findings. What is the minimum number of infusions that will produce a novel durable microbiota? The term "colonization resistance" is used to describe the ability of the colonic microbiota to resist exogenous bacterial species introduced into the gastrointestinal tract, which results in the introduced bacteria usually failing to persist for longer than 2 weeks.[23] Antibiotics are known to reduce the colonization resistance of both humans and mice.[25–29] The combination of antibiotics and colonic lavage used in the current protocol is likely to have dramatically reduced colonization resistance to allow for the implantation of healthy infused donor microbiota. Infusions of fresh fecal suspension given for 5 to 15 days changed the microbiota in this study, however, the minimum number of infusions required to achieve such change remains unclear.

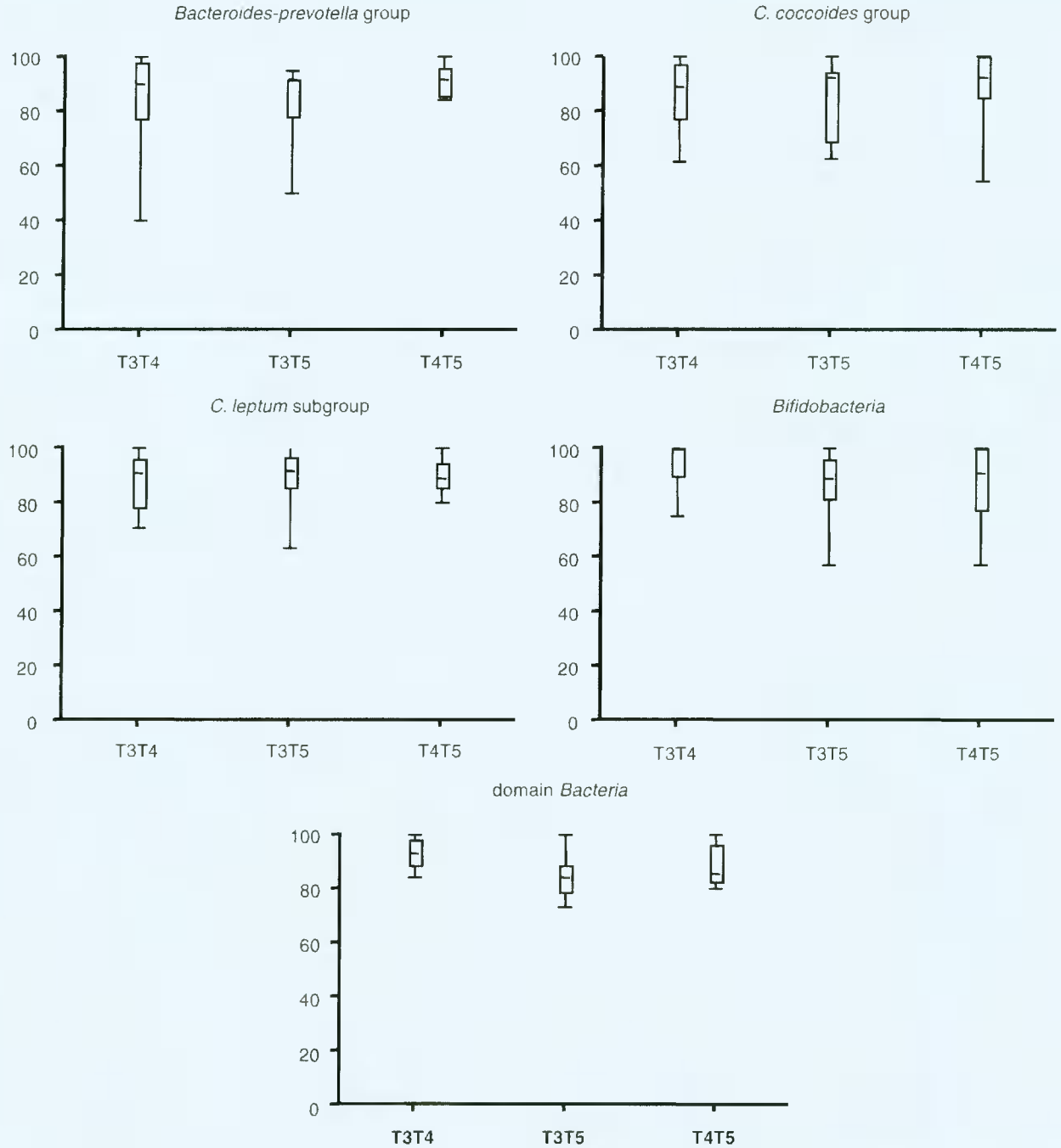

FIGURE 4. Box and whiskers plots of the Bray-Curtis similarities for comparisons of samples at post-procedure time points by primer set. In general, the banding profiles were highly similar. There is a trend for T3 versus T5 (middle bar) to be lower than the other comparisons.

Secondly, can a protocol like this be used to recolonize the human colon with cultured probiotic strains? Probiotics have been defined as "living microorganisms, which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition."[30] Specific bacterial strains have been suggested to be beneficial in a range of gastrointestinal disorders[31–33] including prevention and maintenance of remission in pouchitis.[34,35] However, studies have suggested that bacteria not adapted to the gut are far less likely to colonize, further complicating attempts to introduce new species.[27,36,37] Therefore, by introducing bacteria already adapted to the gastrointestinal tract and applying a regimen to reduce colonization resistance, such as the protocol used in this study, a more persistent change in the colonic microflora is witnessed with implantation measured up to 24 weeks post-procedure in comparison to oral probiotic formulations which transiently implant for a maximum of 10 to 14 days.

As with all such studies, patient safety remains of paramount importance. Although the risks associated with

use of antibiotics, bowel preparation, and colonoscopy are well known, no reported transmitted infections or significant adverse effects of fecal bacteriotherapy have been reported to date. Transmission of infection or the induction of colitis in patients because of an immune response to foreign flora has not been reported in earlier studies of fecal bacteriotherapy in studies of *C. difficile* colitis[2–7] and idiopathic ulcerative colitis,[8,9] however, these risks may be possible. Although the potential for transmitting pathogenic organisms to the recipient theoretically does exist, negative stool tests do minimize the risk. Selection of the donor is of crucial importance to avoid infecting the recipient with a separate disease. All donors are tested for human immunodeficiency virus; hepatitis A, B, and C; cytomegalovirus, and Epstein-Barr virus. Stool is tested for *Aeromonas* species, *Campylobacter jejuni*, *C. difficile*, *Plesiomonas* species, *Salmonella* species, *Shigella* species, *Yersinia* species, and parasitology testing of over 40 known species of human parasites.

Finally, the overriding question must be, will this type of treatment be of therapeutic benefit and if so under which conditions? The most up-to-date evidence suggests that fecal bacteriotherapy is of benefit in refractory or relapsing *C. difficile* diarrhea where conventional treatment has failed. At this present time, evidence also suggests a significant link between the microbiota of the bowel and gastrointestinal conditions such as irritable bowel syndrome and inflammatory bowel disease with patients responding favorably to oral probiotics, albeit briefly. This procedure could have some merit in changing the patients' bacterial populations to a "less pathogenic" microbiota.

Studies of this type of procedure in a clinical context have the potential to significantly increase our understanding of which bacterial species may impact on specific gastrointestinal disease states. The findings of this study demonstrate that it is possible to durably and substantially alter the bacterial populations residing in the human colon, thereby opening the door for further study of this new therapeutic option in combating debilitating gastrointestinal disorders. Future research measuring the host-immune response in response to fecal bacteriotherapy is now required to elucidate the precise mechanisms of action of this novel treatment.

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