Clinical Infectious Diseases

MAJOR ARTICLE

Fecal Microbial Transplants Reduce Antibiotic-resistant Genes in Patients With Recurrent *Clostridium difficile* Infection

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(See the Editorial Commentary by Halpin and McDonald on pages 1487–8.)

Background. Recurrent *Clostridium difficile* infection (RCDI) is associated with repeated antibiotic treatment and the enhanced growth of antibiotic-resistant microbes. This study tested the hypothesis that patients with RCDI would harbor large numbers of antibiotic-resistant microbes and that fecal microbiota transplantation (FMT) would reduce the number of antibiotic-resistant genes.

Methods. In a single center study, patients with RCDI (n = 20) received FMT from universal donors via colonoscopy. Stool samples were collected from donors (n = 3) and patients prior to and following FMT. DNA was extracted and shotgun metagenomics performed. Results as well as assembled libraries from a healthy cohort (n = 87) obtained from the Human Microbiome Project were aligned against the NCBI bacterial taxonomy database and the Comprehensive Antibiotic Resistance Database. Results were corroborated through a DNA microarray containing 354 antibiotic resistance (ABR) genes.

Results. RCDI patients had a greater number and diversity of ABR genes compared with donors and healthy controls. Beta-lactam, multidrug efflux pumps, fluoroquinolone, and antibiotic inactivation ABR genes were increased in RCDI patients, although donors primarily had tetracycline resistance. RCDI patients were dominated by Proteobacteria with *Escherichia coli* and *Klebsiella* most prevalent. FMT resulted in a resolution of symptoms that correlated directly with a decreased number and diversity of ABR genes and increased Bacteroidetes and Firmicutes with reduced Proteobacteria. ABR gene profiles were maintained in recipients for up to a year following FMT.

Conclusions. RCDI patients have increased numbers of antibiotic-resistant organisms. FMT is effective in the eradication of pathogenic antibiotic-resistant organisms and elimination of ABR genes.

Keywords. intestinal microbiome; antibiotics; colitis; antibiotic resistance; *C. difficile*.

The microbiota of the human gut is a complex ecosystem with the potential to be an enormous reservoir of antibiotic resistance (ABR) genes, known as the "gut resistome" [1]. ABR has become a major global clinical problem with the emergence of multidrug resistant organisms such as vancomycin-resistant enterococci (VRE), methicillin resistant *Staphylococcus aureus* (MRSA), and extended spectrum beta lactamase (ESBL) [2]. ABR may arise in a number of different ways, including the accumulation of point mutations and horizontal gene transfer from other bacterial populations through transformation, transduction and/or conjugation [1, 3, 4]. The increased use of antibiotics in agriculture and healthcare has led to a dramatic increase in the prevalence and incidence of antibiotic resistant bacteria, presenting a severe challenge in the treatment of patients infected with these multidrug resistant organisms.

A major complication associated with antibiotic use is *Clostridium difficile* (*C. difficile*) infection (CDI) [5]. *C. difficile* is an anaerobic, spore-forming, toxin producing bacteria that is present in 3% of the healthy adult population; however, up to 20%–50% of adults in hospitals and long-term care facilities become colonized [6]. CDI is treated with metronidazole or vancomycin, but the risk of the recurrence is 20%–30% within 30 days of initial treatment and increases further up to 50% after a second episode [7, 8]. Fecal microbiota transplantation (FMT) has emerged as an effective and safe therapy for recurrent *C. difficile* infection (RCDI), with over an 80% success rate [9]. This study was designed to analyze the gut resistome in patients with RCDI undergoing FMT and to examine how FMT influences the ABR profile of the recipients. We hypothesized that patients with RCDI would harbor large numbers of antibiotic-resistant microbes and that FMT would reduce the number of antibiotic-resistant genes.

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MATERIALS AND METHODS

Patient Cohorts

This study was conducted at University of Alberta Hospital in Edmonton, Alberta, between October of 2012 and November of 2014. Patients aged 35–85 with RCDI, defined as at least 3 episodes of CDI within 6 months were included. Active CDI was defined as diarrhea (>3 loose stools per day) with positive stool *C. difficile* toxin test. All participants provided written informed consent for FMT and to provide samples for analysis. This study was approved by the University of Alberta Health Research Ethics Board. Data from 87 healthy individuals between the ages of 18 and 40 were obtained from the Human Microbiome Project (HMP) Consortium (2012). Extensive medical history was not available for these healthy subjects; however, individuals were excluded from participating if they had been exposed to any form of antibiotics, antifungals, antivirals, or antiparasitics within the previous 6 months.

Donor Selection

Stool for FMT was obtained from 1 of 3 universal stool donors registered with the Edmonton FMT program. Each donation was matched to a single recipient and donations were stored at −80°C in a concentrated glycerol stock. All donors were screened by undergoing a full history and physical exam, specifically screening for gastrointestinal symptoms and risk factors for viral hepatitis or HIV. Donors were excluded if they had taken any antibiotics in the past 6 months. Donors were tested for HIV, hepatitis A, B, and C, syphilis, stool bacterial and feces culture, ova and parasite exam (C & S, O & P), *C difficile* toxin, and VRE and rescreened every 4 months.

Fecal Microbiota Transplantation (FMT)

Patients discontinued antibiotics for CDI 24 hours prior to FMT. FMT was performed using a preparation of fresh or frozen fecal slurry via colonoscopy. One day prior, patients took 4 L of polyethylene glycol-based bowel preparation (GoLYTELY). Fecal samples were collected by the patients at home prior to and following FMT. After collection, sample aliquots were placed into the −80°C freezer until DNA extraction.

DNA Extraction and Metagenomic Analysis

Stool samples were physically disrupted using a bead-beating kit and microbial DNA extracted using the Qiagen QIAamp DNA stool kit. Indexed paired-end DNA libraries were constructed using an Illumina Nextera XT DNA Sample Preparation Kit and sequenced on a MiSeq. Sequencing parameters consisted of paired-end 300 bp dual index sequencing chemistry using a MiSeq Reagent Kit-V3 (500 cycles) and the FASTQ Only workflow. There were 17 326 984 total reads from 29 donor and 73 patient samples. Any reads with a length <150 base pairs were removed so that the average Phred quality score was greater than 30 (>30; 0.1% error rate). Duplicate reads were collapsed using FASTX-Toolkit (version 0.0.13; http://hannonlab.cshl.edu/fastx_toolkit/index.html). Reads from individual samples were mapped to >5 kb assembled contigs using Bowtie2 against a custom database of bacterial genomes retrieved from NCBI RefSeq database [10]. Outputs were visualized in MEGAN (version 5) for taxonomic assignment and reads aligned using Bowtie2 against the Comprehensive Antibiotic Resistance Database (CARD; http://arpcard.mcmaster.ca). CARD contains 6020 different sequences from 4120 genes related to ABR, 3008 of which are tagged specifically for ABR, consisting of 31 different antibiotic classes [11]. Following alignment to CARD, the total read count was 5414 reads, with 228 different ABR genes detected as having at least a single read in one sample. In the HMP cohort there were 671 total reads, with 143 different ABR genes detected in at least one sample. Genes with different accession numbers but with ≥98% sequence similarity were grouped together and not considered distinct genes for the analysis. Positive detection of an ABR gene was considered if there was >0 read counts in just one of the samples for initial analysis.

DNA Microarray Analysis

DNA was extracted and analyzed on a custom oligonucleotide-based DNA microarray containing 370 probes targeting 354 ABR genes (Lallemand Health Solutions, Montreal, Canada). These were classified into antibiotic resistant gene types, including aminoglycosides (53), beta-lactams (49), tetracycline (44), amphenicols (24), erythromycin (21), vancomycin (20), multidrug resistance (19), trimethoprim (13), macrolides (13), lincosamides (10), integrons (7), and sulfonamides (5). In total, 200 ng of DNA was labelled using Cy-5 with the Bioprime DNA Labeling System (Life Technologies) and purified using the QIAquick polymerase chain reaction (PCR)

Table 1. Clinical Characteristics of Recurrent *Clostridium difficile* Infection Patients Receiving Fecal Microbiota Transplants

	Single FMT	Repeated FMT	*P* Value
Number of patients	11	9	
Age	67.7 (35.3–84.9)	71.4 (49.5–83.8)	.7039
Gender	7 male; 4 female	5 male; 4 female	1.000
Average duration of RCDI prior to FMT	150.4 d (71–275)	126.2 d (53–246)	.4237
CDI classification	7 community-acquired; 4 hospital-acquired	3 community-acquired; 6 hospital-acquired	.3698

Numbers are given as means with range in brackets.

Abbreviations: CDI, *C. difficile* infection; FMT, fecal microbiota transplantation; RCDI, recurrent *Clostridium difficile* infection.

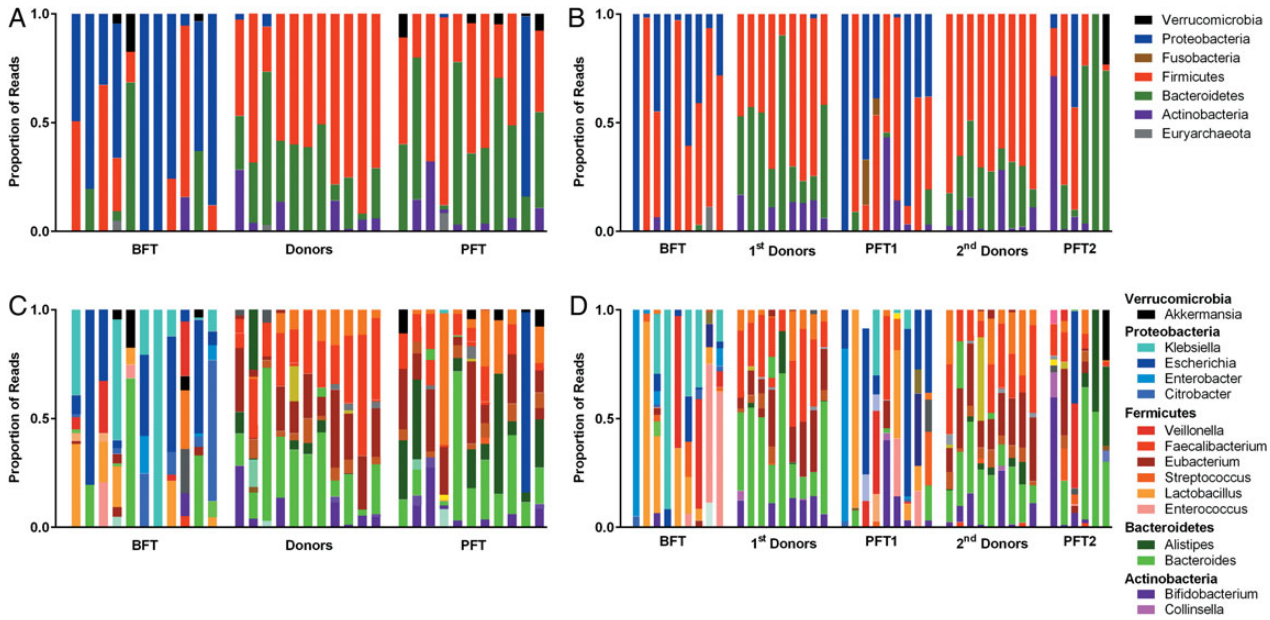

Figure 1. Microbial profile of stool samples from recurrent *Clostridium difficile* infection patients and donors. Microbial composition in stool samples from single successful fecal microbiota transplant (FMT) recipients shown at the phyla (*A*) and genus (*C*) levels before FMT (BFT; n = 11), donor samples (Donors; n = 11), and post FMT (PFT; n = 11). Microbial composition in stool samples from repeated FMT cohort shown at the phyla (*B*) and genus (*D*) level before FMT (BFT; n = 9), first donor samples (1st Donors; n = 9), patients following initial failed FMT (PFT 1; n = 9), second donor samples (2nd Donors; n = 9), and patients following successful second FMT (PFT2; n = 6). Data are presented as the fraction of total reads in each sample at the phyla level and 14 most prevalent genera.

purification kit. Slides were washed in 0.2% SDS and then incubated in prehybridization solution (5X SSC, 0.1% SDS and 0.1 mg/mL BSA). Labeled DNA was added to hybridization buffer (20 µL DIG Easy Hybridization Buffer, 1 µL 10 mg/mL Yeast tRNA and 1 µL 10 mg/mL Salmon Sperm DNA) and samples hybridized for 18 hours followed by 3X washing in 1X SSC/0.1% SDS at 42°C and drying. Slides were scanned and quantified using Quantarray.

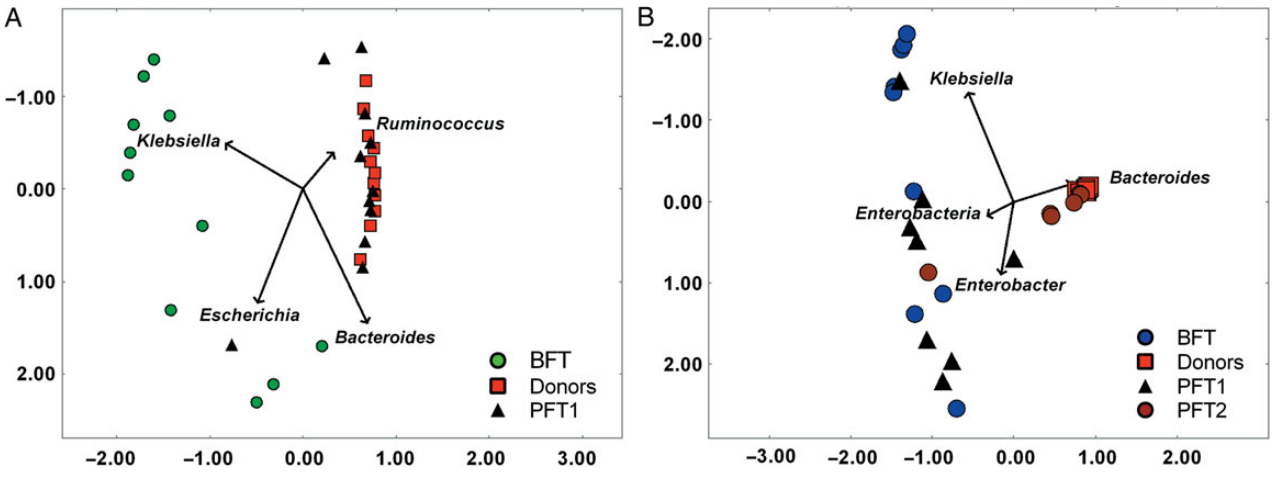

Figure 2. Principal coordinates analysis (PCoA) of taxonomic data from recurrent *Clostridium difficile* infection (RCDI) patients and donors. Biplot vectors show the 4 genera with the strongest magnitude that contributed to sample dissimilarity. *A*, Before FMT (BFT), RCDI patients were defined by higher proportions of *Klebsiella* and *Escherichia*, whereas donors were predominated by *Ruminococcus* and *Bacteroides*. Post FMT (PFT), all but one RCDI patient clustered together with donor samples. *B*, RCDI patients who failed the initial FMT were also defined by *Klebsiella, Enterobacteria*, and *Escherichia* BFT, and did not resemble the donors until after the second FMT. PCoA was performed using Bray-Curtis dissimilarity to quantify the compositional dissimilarity between samples. *A*, Green circles: BFT samples; Black triangles: PFT samples; Red squares: donors samples. *B*, Repeated FMT cohort: Blue circles: BFT samples; Red squares: 1st donor samples; Black triangles: post 1st FMT recipient samples; Red diamonds: 2nd Donors samples; Brown circle: post 2nd FMT recipient samples. Donor cohort: n = 31; Single FMT Cohort: n = 11; Repeated FMT Cohort n = 9. Abbreviation: FMT, fecal microbiota transplant.

Statistical Analysis

The Shapiro-Wilk test was used to determine if data were normally distributed. If normally distributed, a Student *t*-test was used, and if not the nonparametric Mann–Whitney U test was performed. A *P*-value <.05 was considered statistically significant. Principal coordinates analysis (PCoA) on the taxonomic data was performed using MEtaGenome ANalyzer (Megan, version 5) using Bray-Curtis dissimilarity which quantifies the compositional dissimilarity between different samples. Cluster analysis was performed on the microarray data using Bionumerics, where the similarity coefficient was determined by the ratio of the absolute value of the Pearson correlation coefficient. These values were then converted into a percentage value to be graphed.

RESULTS

Patient Cohorts

Twenty patients underwent FMT for RCDI between September 2012 and December 2014. All 20 patients were cured of RCDI following FMT, but while 11 patients were successfully treated with a single FMT, 9 patients failed the initial FMT and required a repeat FMT. Patient characteristics are shown in Table 1. There was no difference between the two groups in age, gender, or duration of RCDI. Data obtained from the HMP Consortium for 87 different healthy individuals aged 18–40 was analyzed to determine if the antibiotic profile of donors used in this study was representative of a healthy cohort.

Effects of FMT on Microbial Composition

Prior to FMT, RCDI patients had a larger proportion of Proteobacteria (Figure 1*A*, *B*), with *Klebsiella* and *Escherichia* being the most prevalent genera (Figure 1*C* and 1*D*). Donor samples exhibited higher levels of diversity compared with the RCDI patients (Supplementary Figure 1). In the RCDI cohort that responded following a single FMT, microbial profiles at the phyla (Figure 1*A*) and genus (Figure 1*C*) level increased in similarity to that of the donor (Figure 2*A*). In particular, the relative amount of Proteobacteria (*Klebsiella*, *Escherichia*) was reduced, and the relative amounts of Bacteroidetes (*Bacteroides*) and Firmicutes (*Ruminococcus*, *Faecalibacterium, Eubacterium*) were increased. In contrast, in the cohort that required a repeat FMT, the patients generally failed to resemble the donor at both the phyla (Figure 1*B*) and genus (Figure 1*D*) level following the initial FMT. However, following the second FMT, their intestinal microbial profile did become more similar to the donor and this correlated with a clinical response (Figure 2*B*). The number of raw reads aligned to Proteobacteria was much higher in the patients' before fecal transplant (BFT) samples in both cohorts compared with the healthy donors and decreased significantly following the FMT in the single FMT cohort, and following the second FMT in the cohort which required a repeat FMT (Supplementary Figure 4).

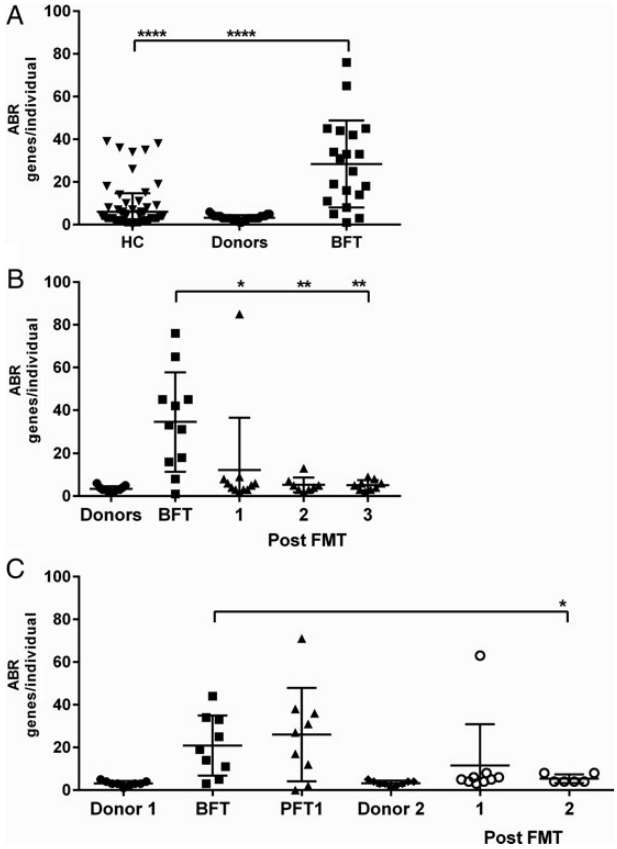

Figure 3. Number of antibiotic-resistance (ABR) genes in recurrent *Clostridium difficile* infection (RCDI) and donor stool samples pre and post fecal microbiota transplantation (FMT). Metagenomic data was aligned using Bowtie2 to the Comprehensive Antibiotic Resistance Database (http://arpcard.mcmaster.ca) to detect ABR genes. *A*, RCDI patients at baseline (before FMT [BFT]) had increased numbers of ABR genes compared with the donors and healthy controls (HC). *B*, Following successful FMT, the number of detected ABR genes decreased in the RCDI patients and this was maintained over time. All patients had a post-FMT (PFT) sample taken between 1–4 weeks following FMT (Group 1: n = 11); 9 patients had a sample taken between 4 and 8 weeks following FMT (Group 2: n = 9); 7 patients had a sample taken between 8 and 22 weeks following FMT and 2 patients had a 1 year PFT sample (Combined in Group 3). *C*, In the patients which did not respond to the initial FMT, there was no decrease seen in the number of ABR genes following the first FMT (PFT1). In this cohort, a decrease in the number of ABR genes was seen following the second FMT and this was maintained over time. All patients had a post-FMT (PFMT1) taken between 1–3 weeks following FMT2 (Group 1: n = 9); 4 patients had a sample taken between 3 and 12 weeks following FMT2 and 2 patients had a 28 week follow-up (Combined in Group 2: n = 6). Healthy cohort: n = 87; Donor cohort: n = 29; Single FMT Cohort: n = 11; Repeated FMT Cohort n = 9. Whiskers denote mean with the standard deviation. **P*-value <.05; ***P*-value <.005; ***P*-value <.0001.

RCDI Patients had Increased Numbers of Antibiotic-resistant Genes

Stool donors had a mean of 3.4 ± 0.4 and a range of 1–6 ABR genes in their samples. This was similar to healthy controls from the HMP, who had a mean of 6.0 ± 0.9 ABR genes with a range of 0–39 ABR genes. RCDI patients had increased numbers of ABR genes compared with donors (Figure 3*A*). There was no correlation between duration of disease or age of RCDI patients and number of ABR genes (Supplementary

Figure 2). Prior to FMT, the patients who responded to a single FMT had a mean of 34.5 ± 6.7 different ABR genes. At the first follow-up period (1–3 weeks post FMT [PFT]), the number of ABR genes in the RCDI patients who had a clinical response had significantly decreased to a mean of 12.2 ± 7.0. In subsequent follow-ups, the average number of ABR genes continued to drop to a mean of 5.1 ± 0.74 genes (Figure 3*B*). The RCDI cohort which failed to respond to the initial FMT and required a repeat FMT had a mean of 20.9 ± 4.4 ABR genes prior to FMT, and this did not decrease following the initial FMT. However, following the second successful FMT, the number of ABR genes per patient dropped to 10.0 ± 4.9 genes, suggesting that a clinical response was associated with a decrease in ABR genes (Figure 3*C*).

RCDI Patients had Increased Diversity of Antibiotic-resistant Genes

Figure 4*A* shows the main classes of ABR genes in the three cohorts. Beta-lactam, multidrug efflux pumps, fluoroquinolone, and antibiotic inactivation ABR gene classes were increased in the RCDI cohorts compared with donors. The RCDI patient cohort that responded to a single FMT had the highest number of ABR genes with 73 unique and a total of 151. Patients who required a repeat FMT had 25 unique and a total of 101 ABR genes (Figure 4*B*; Supplementary Figure 3). The donor cohort had 14 unique ABR genes and a total of 26, the majority of which were tetracycline antibiotic resistant genes. Although ABR genes were still detected in our donor cohort, 76% of those ABR genes detected were observed in fewer than 10% of the 29 donor samples and did not appear in the top 100 most detected ABR genes (Supplementary Figure 3). Three of the tetracycline genes were detected in 90% of the healthy donors; these included tet (W), tet (O), and tet (Q). In the healthy cohort from the HMP, tet (W) was also highly detected and was found in 45% of the individuals. The genes detected in the RCDI patients prior to FMT belonged to a total of 31 different ABR classes, where only one was unique to each of the RCDI cohorts and none to the donor cohorts (Figure 4*C*). Seventeen classes were shared among all 3 cohorts, whereas 11 were unique to the RCDI patient cohorts. These were most evident in the classes of glycopeptide resistance, polymyxin resistance, sulfonamide resistance, lipopeptide resistance, streptothricin resistance, and peptide ABR (Supplementary Figure 3). The sole class that was unique to the single FMT cohort was genes

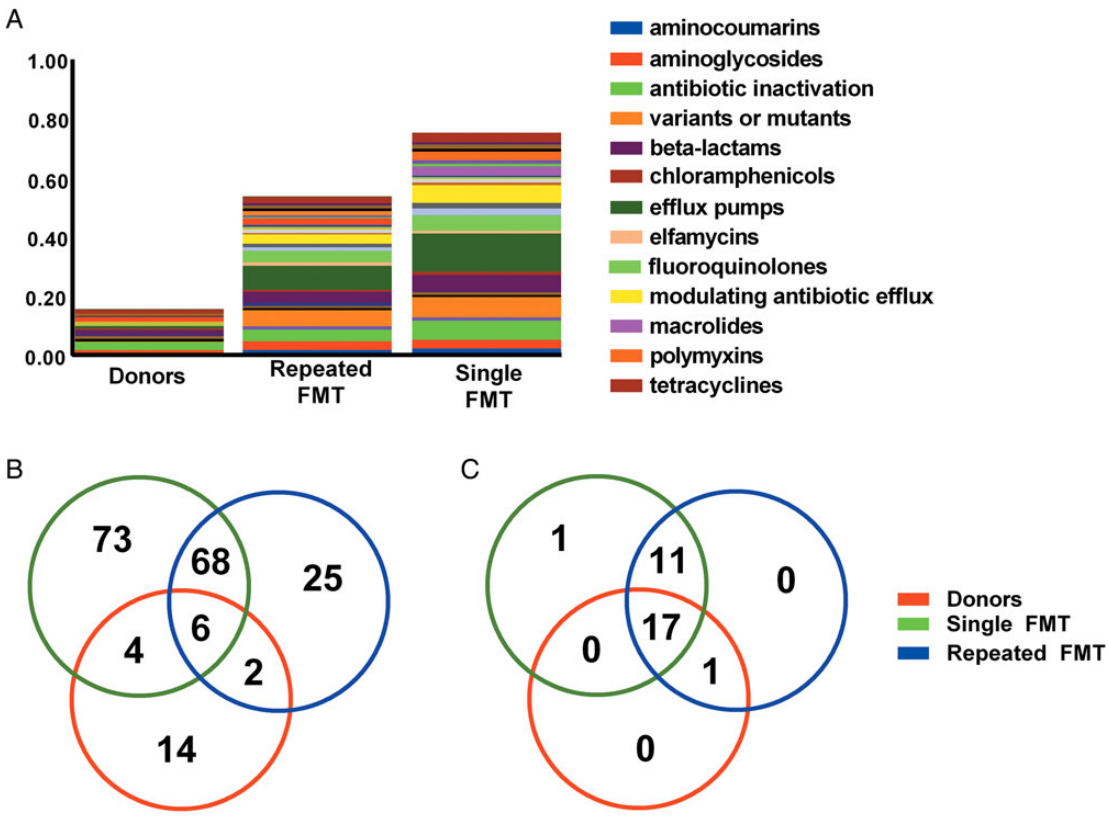

Figure 4. Relative abundance of antibiotic resistance (ABR) gene types assigned to antibiotic class resistance in recurrent *Clostridium difficile* infection (RCDI) patients and donors prior to fecal microbiota transplantation (FMT). Identified ABR genes were classified according to Comprehensive Antibiotic Resistance Database (http://arpcard.mcmaster.ca). Genes that confer resistance to multiple antibiotics were included in the analysis. *A*, The RCDI patients had a greater diversity of ABR gene classes compared with donors. *B*, Venn diagram showing the shared and unique ABR genes in the RCDI patients and donors. *C*, Venn diagram showing the shared and unique ABR gene classes in the RCDI patients and donors. Green: single FMT cohort; blue: repeated FMT cohort; red: donors. Donor cohort: n = 29; Single FMT Cohort: n = 11; Repeat FMT Cohort n = 9.

conferring fosfomycin resistance, whereas the class unique to the repeated FMT cohort was genes conferring trimethoprim resistance.

Antibiotic Resistant Profiles Remained Constant Over Time in Donors and in Recipients

Microarray analysis was performed to corroborate the metagenomic results and to examine stability over time in the donors and recipients. In total, 229 different genes were detected in at least one of the samples, with the RCDI patients showing positive detection of 178 genes and the donors having positive detection of 26. In sum, 90% of the donor samples had all 3 of the ABR genes, tet (O), tet (Q), and tet (W). Genes within the antibiotic classes of tetracycline, aminoglycosides, beta-lactams, macrolides, erythromycin, and multidrug efflux were the most prevalent antibiotic classes detected on the microarray, similar to what was seen in the metagenomic data. Samples taken from the donors were analyzed for stability over time on the microarray and RDCI recipients for similarity to the donor. Antibiotic resistant profiles in the donors remained relatively constant over the period of donation (Figure 5*A*). Figure 5*B* shows percent similarity of the single FMT RCDI patients to their respective donor before FMT and PFT. Following a successful FMT, the resistome of the recipient became more similar to the resistome of the donor and this was maintained up to a year following the FMT. In contrast, in the RCDI patients who failed the first FMT, the resistome did not become more similar to the donor (Figure 5*C*). However, following the second successful FMT, the resistome did become more similar to the donor, although there was more variability in this group compared with the single FMT group.

DISCUSSION

In this study we demonstrate that patients with RCDI harbor increased numbers and diversity of ABR genes compared with healthy stool donors. FMT was effective in reducing the load of ABR genes in conjunction with resolution of disease and this was maintained following the transplant. These findings suggest that FMT may have a significant role beyond that of treating RCDI and may be able to eradicate multidrug-resistant bacterial infections or alternatively restore antibiotic susceptibility to individual patients.

RCDI patients had approximately 30 more ABR genes compared with donors and the healthy cohort. Diversity of the antibiotic genes was also much higher in the RCDI patients, which had all 31 of the identified ABR gene classes, whereas only 19 were identified in healthy stool donors. In the donors, tetracycline and beta-lactam genes were the most prevalent. In congruence with our data, it has been previously shown that genes conferring resistance to the antibiotic tetracycline are present in the microbiota of the majority of individuals and are also the most abundant family of resistance genes [12, 13]. The majority of the ABR

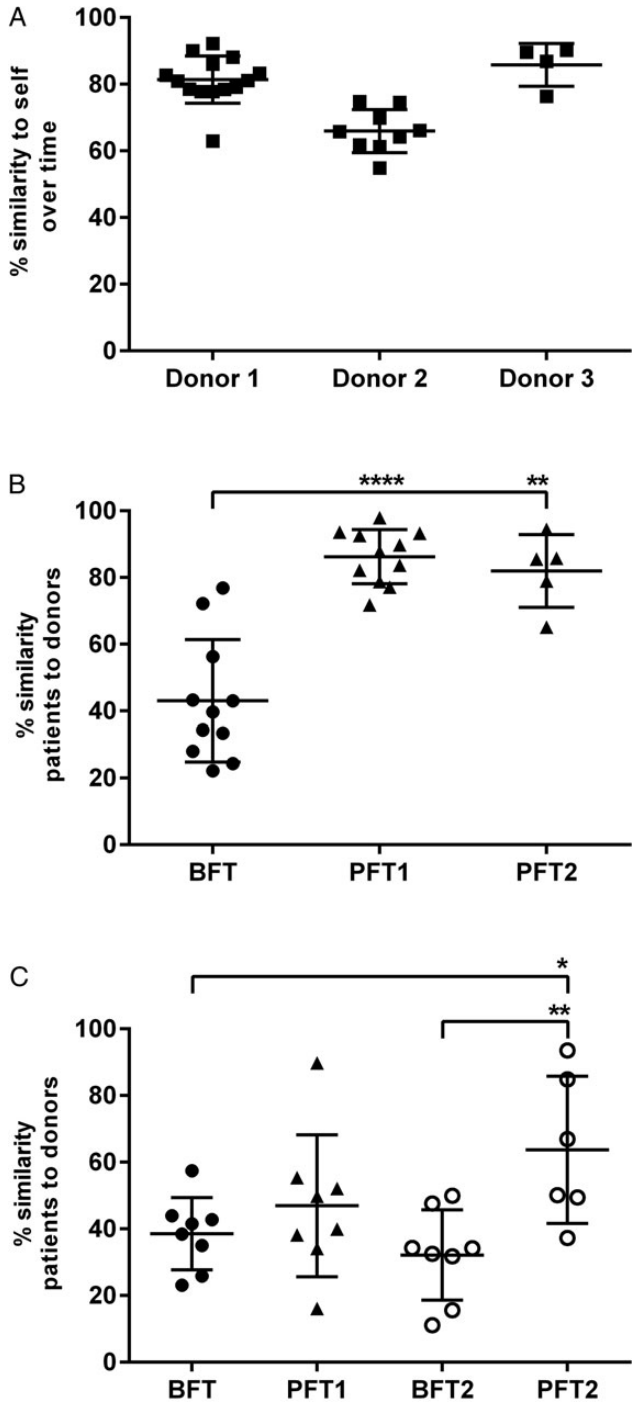

Figure 5. Pearson similarity index of donors over time and recurrent *Clostridium difficile* infection (RCDI) patients to donors. Samples were analyzed using a DNA microarray. *A*, Donors retained a high degree of similarity to their own samples over their donation time period. Donor 1 = 18 months; Donor 2 = 12 months; Donor 3 = 7 months (*B*) Percent similarity of the single fecal microbiota transplantation (FMT) recipient samples at those time points (1 = 1–4 weeks; 2 = 8–52 weeks post FMT [PFT]) to their respective donor. Following FMT, the similarity of the RCDI patients increased to more closely resemble the donors; ****P-value <.0001 calculated using paired t-test; ***P-value =.0005 calculated using Mann–Whitney test. *C*, Percent similarity of the repeated FMT recipient samples at those time points to the respective donor of the most recent FMT (PFT1: 1–2 weeks; PFT2: 3–28 weeks); *P-value <.05; **P-value <.005 both calculated using Mann–Whitney test. Whiskers denote mean with the standard deviation. Abbreviation: BFT, before FMT.

genes detected in the healthy stool donors are found predominately within Bacteroidetes and Actinobacteria, whereas ABR genes in the RCDI patients are found almost exclusively in Proteobacteria with a small number in Firmicutes [14]. This increased load and diversity of ABR genes likely reflects the significant dysbiosis that was seen in the RCDI patients with predominance of *Escherichia coli* and *Klebsiella*.

The number of ABR genes was dramatically reduced after the first FMT in the patients who showed a clinical response but not in the patients who failed the first FMT. This would suggest that the change in the ABR gene profile was likely due to the change in microbial composition, in particular the reduction in Proteobacteria induced by the FMT. However, although we did show a significant reduction in total Proteobacteria in patients following FMT, it is also possible that FMT reduced their abundance to below our limit of detection [15, 16]. Nonetheless, case reports showing eradication of multidrug-resistant organisms in patients following FMT together with evidence from a murine study suggesting that multidrug-resistant bacteria such as vancomycin-resistant *Enterococcus faecium* (VRE) and Carbapenem-resistant *Klebsiella penumoniae* (CRKP) can be eliminated from the gut following FMT supports the potential for this procedure [17, 18, 19]. The elimination of bacteria harboring ABR genes is thought to be a direct result of the ability of FMT to reestablish a healthy gut ecosystem, which provides colonization resistance against pathogens by removing favorable growth conditions [20].

Limited options are available for patients infected with multidrug-resistant organisms. Although selective digestive decontamination together with the intravenous administration of antibiotics has shown benefit as an infection prevention measure in critically ill patients [21–23], the problem arises in that this regimen is associated with dramatic increases in ABR genes [24]. In addition, once the treatment is discontinued, the patients remained colonized with these multidrug-resistant pathogens, and as the gut becomes recolonized following removal of antibiotics an increased horizontal transfer of resistance genes from the surviving organisms to opportunistic aerobic pathogens may occur [25]. Eradication of virulent organisms containing ABR genes from the gut may also help in alleviating systemic infections, as studies have shown pathogenic organisms to predominate in the gut prior to translocating to and infecting other body sites [26]. Thus, these findings have significant clinical implications and suggest that FMT may have a role beyond that in treating patients with RCDI.

Although we speculate that the increase in ABR genes in the RCDI patients was due to the extensive antibiotic treatment in this cohort, some studies have shown a greater number of ABR genes in the older population [27], which may be related to accumulated exposure over time to factors such as heavy metals [28–30] or to the spread of ABR organisms from animals to humans through the food chain [31]. In that the average age of our donors (36 years) was less than that of the patients (67 years), the increased ABR genes in the RCDI cohort could theoretically have arisen even without prolonged exposure to antibiotics. However, in that the linear regression of our data comparing age and number of ABR genes showed no significant correlation, it is more likely that the increase in ABR genes in this cohort was related to their extensive antibiotic usage.

In conclusion, these results demonstrate that patients with RCDI harbor large numbers of microbes that carry a great diversity of ABR genes. FMT is effective at both resolving RCDI and in reducing the carriage of multiple ABR genes in these patients.

Supplementary Data

Supplementary materials are available at http://cid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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Author contributions. K. L. M., T. A. T., and D. K. designed the study; B. M. and H. P. conducted the research; B. M., H. P., N. H., O. M., and P. B. carried out the data analysis; D. K. performed patient assessments and fecal microbial transplants; B. M., H. P., and K. L. M. wrote the manuscript. All authors read and approved the final manuscript.

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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