Loss of Vancomycin-Resistant *Enterococcus* Fecal Dominance in an Organ Transplant Patient With *Clostridium difficile* Colitis After Fecal Microbiota Transplant

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We report the use of fecal microbiota transplantation in a single heart-kidney transplant recipient with recurrent *Clostridium difficile*, vancomycin-resistant *Enterococcus* (VRE) fecal dominance, and recurrent VRE infections. Fecal microbiota transplantation resulted in the reconstruction of a diverse microbiota with (1) reduced relative abundance of *C difficile* and VRE and (2) positive clinical outcome.

Keywords. *C difficile*; fecal microbiota transplant; gut microbiome; vancomycin-resistant *Enterococcus*; VRE.

CASE REPORT

A 33-year-old white female with history of postpartum cardiomyopathy received orthotopic cardiac and single cadaveric kidney transplants in 2011. After the transplants, she had a complicated course including multiple episodes of bacteremia and urinary tract infections treated with multiple courses of antibiotics for varying periods. She had 2 diagnosed episodes of

Enterococcal bacteremia, 1 with *Enterococcus casseliflavus* before transplantation, and vancomycin-resistant *Enterococcus faecium* 2 months after transplant. She had numerous fecal cultures growing abundant amounts of vancomycin-resistant *Enterococcus* (VRE) and 2 episodes of symptomatic VRE urinary tract infection (*Enterococcus faecalis* and *E faecium*). Her course was also complicated by 6 episodes of *Clostridium difficile* colitis, and treatment with metronidazole, oral vancomycin, and extended oral vancomycin taper consistent with current guidelines [1]. The patient also was treated with a *Lactobacillus* probiotic. Due to the failure of standard therapies, fecal microbiota transplantation (FMT) was performed in 2013. In this study, we report the clinical and microbiological results of this procedure.

METHODS

Fecal Microbiota Transplantation

Before FMT, the patient was receiving oral vancomycin 125 mg every 6 hours, which was stopped the night before FMT. Her immunosuppressive regimen consisted of cyclosporine, sirolimus, and prednisone.

Donor fecal samples were obtained from the patient's spouse. The donor was healthy with negative serologies for hepatitis A, B, and C, *Helicobacter pylori*, rapid plasma reagin, human immunodeficiency virus, negative fecal culture, stool acid-fast bacilli staining, ova and parasites, and *C difficile* [2]. For transplantation, the donor's fecal sample was mixed with sterile normal saline as previously described and given via nasogastric tube [3] (Supplementary Methods). Consent was obtained from both patient and donor for FMT and microbiota analysis as part of an ongoing institutional review board-approved study at the University of Alabama at Birmingham.

Microbiota Analysis

The 16S rDNA V4 region analysis of fecal microbiota was performed as previously described [4]. Illumina sequencing of the 6 samples resulted in average 142 350 (range 94 170–168 596) paired end reads. After merging the paired reads and performing quality control steps, the samples were normalized at 70 409 single end reads (250 bases). The remainder of the analysis was performed with the Quantitative Insight into Microbial Ecology (QIIME), version 1.7 (Supplementary Methods). The alpha (Shannon and Simpson) and beta diversity (weighted UniFrac) were calculated using QIIME scripts.

RESULTS

Reconstruction of the Gut Microbial Community

Stool samples were collected from the donor (in replicate, called D and Dr) and recipient (called R) before FMT. Additional samples were taken from the recipient at weeks 1, and 3, and 7 months posttransplant (recipient posttransplant RpTw1, RpTw3, and RpTm7, respectively).

The donor sample revealed a composition of gut microbiota that was dominated by *Bacteroides*, *Blautia*, *Roseburia*, and *Faecalibacterium* (Figure 1A, Supplementary Table 1). In contrast, the recipient was found to have a gut microbiota dominated by *Enterococcus* (relative abundance 84%). A previous study has defined fecal dominance as the presence of an organism with relative abundance of >30% [5]. One week after transplant, the proportional abundance of *Enterococcus* had not significantly
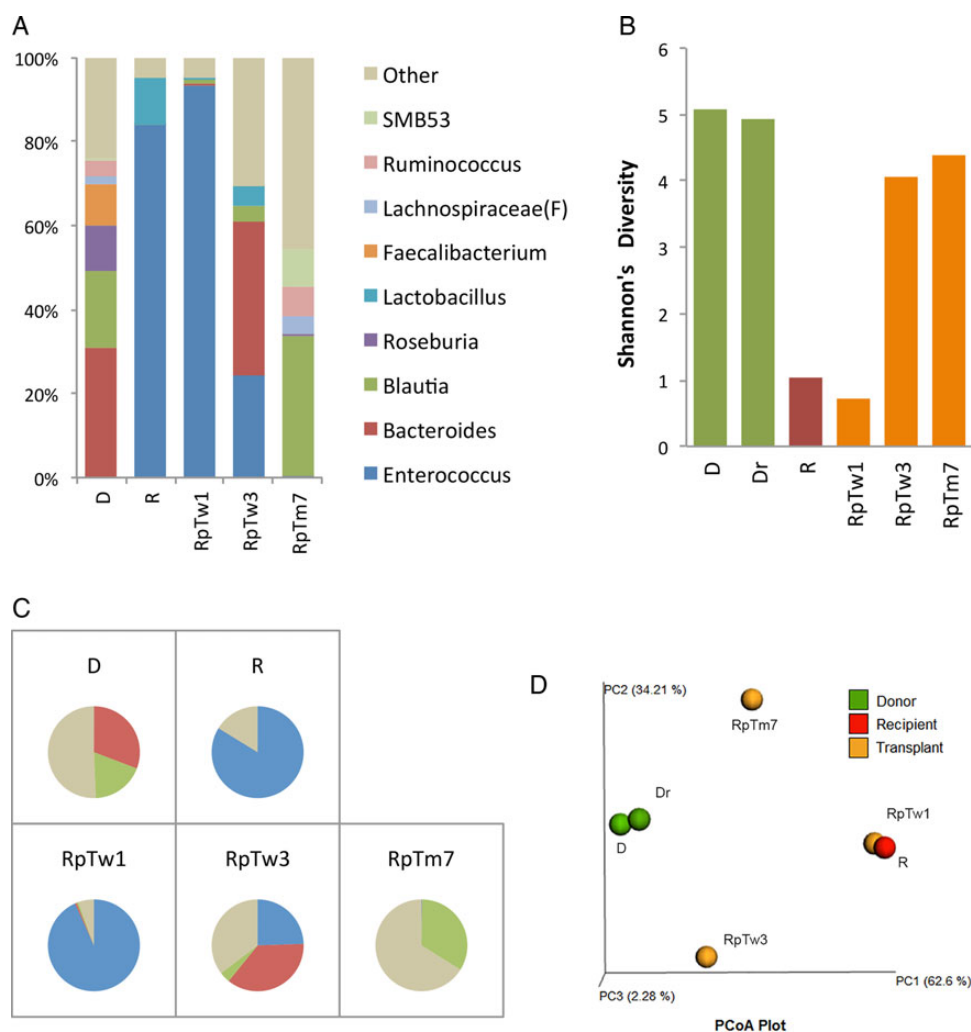

Figure 1. Analysis of microbial composition and diversity of donor, recipient, and recipient posttransplant. (A) Abundance of the fecal microbial taxa (genus level) of the donor, recipient, and recipient posttransplant at different times. The fecal dominance of the *Enterococcus* in the recipient (R) and RpTw1 (1 week after transplant) is denoted by the blue color. (B) Alpha diversity (Shannon's diversity) for donor, recipient, and recipient posttransplant at different times is depicted. Repeat sampling of the donor at 1-week intervals (D and Dr) is shown in green. The recipient is denoted in red, whereas the recipient posttransplant is shown in orange. Note that Shannon's diversity for RpTw3 and RpTm7 is similar to the donor. Additional diversity matrices can be found in Supplementary Table 2. (*C*) Pie chart depicting the abundance of the major taxa at the *genus* level of the donor, recipient, and recipient posttransplant at different times. Note the change in the percentage of the *Enterococcus* (blue) between the donor and recipient (top 2 pie charts) and the reduction of the *Enterococcus* in the recipient posttransplant samples at later times. Values for the abundance can be found in Supplementary Table 1. (D) Principal Coordinate Analysis (PCoA) plot of the donor, recipient, and recipient posttransplant samples. The distance matrix was created using weighted UniFrac metrics. The 2 sequential donor samples (D and Dr, green spheres) overlap, highlighting the consistency of the microbiome analysis. The R (red sphere) and RpT1w (orange) also overlap each other. The RpTw3 and RpTm7 are shown as orange spheres. Note that microbe composition of RpTw3 and RpTm7 differs from the recipient and donor. Distance matrix values used to generate the PCoA plot can be found in Supplementary Table 2.

changed (93%). However a significant decrease in genus *Lactobacillus* was observed (11%–0.7%), possibly due to the cessation of the *Lactobacillus* probiotic. Microbiological analysis of the recipient's sample revealed the presence of vancomycin resistance by growth on selective media. Analysis of several colonies by matrix-assisted laser desorption ionization time-of-flight (VITEK MS version 2.0 system; bioMerieux, Inc.) confirmed the predominance of *E faecium* [6].

Microbiota analysis of the recipient samples before and 1 week after transplant (RpTw1) confirmed significantly less diversity than the donor sample (Shannon's index of 5 for the donor compared with 0.8–1 for the recipient and RpTw1). Of note, we did not detect *C difficile* in the recipient pre- or post-transplant samples using 16S microbiota analysis or the nucleic acid amplification test (Meridian Illumigene) [2], likely because the patient was on oral vancomycin before FMT.

Analysis of the microbial diversity (Shannon's index) revealed that the week 3 and the 7-month samples diversity increased after transplant (Figure 1B, Supplementary Table 2). Coincidentally, we noted remarkable decline in the relative fecal abundance of *Enterococcus* at these later times from 24% (RpTw3) to 0.2% (RpTm7) (Figure 1C).

We next compared the microbiota composition of different samples using weighted UniFrac metrics and generated the principal coordinate analysis plot (Figure 1D, Supplementary Table 2). The microbiota of the donor and recipient clustered differently before transplant. The RpTw1 sample also clustered with the recipient's sample before transplant. By 3 weeks posttransplant, the composition of the gut microbiota in the recipient clustered differently from either the donor or recipient. By 7 months posttransplant, the composition of the gut microbiota was again different, clustering in a position between the donor and recipient. We noted in RpTw3 the presence of *Bacteroides* with a slight increase in the proportional abundance of *Firmicutes*; we also noted the presence of *Akkermansia*. In the RpTm7, we found increases in the abundance of *Firmicutes*, especially in the genus *Blautia* with a decrease in the abundance of *Bacteroides*; the abundance of the *Akkermansia* had also decreased compared with RpTw3. Taken together, these results demonstrate that although the microbial diversity of the recipient microbiota increases after FMT, the composition of the gut microbes still differs from that of the donor.

Clinical Outcome After Fecal Microbiota Transplantation

Between the time of the organ transplants and the FMT, the patient was hospitalized 18 times. The patient tolerated the FMT procedure without any complications and antibiotics were stopped the night before FMT. Although a potential limitation of our studies is that we did not quantitate the reduction of *Enterococcus* by enumeration of microbe growth on vancomycin containing agar plates, the patient has not had further *C difficile* episodes or VRE infections at 1 year of follow-up and has not required hospitalizations, indicating a positive clinical outcome.

DISCUSSION

Numerous studies have reported on the efficacy and safety of FMT for recurrent *C difficile* with success rates of 80%–90% [7]. Fecal microbiota transplantation has also been noted to be safe and effective in immune compromised patients [8], including transplant recipients [9]. In this study, we report the successful use of FMT for recurrent *C difficile* infection in a heart kidney transplant recipient, who also had VRE fecal dominance that was lost after the procedure, with an excellent clinical outcome.

There has been well documented risk of VRE infections in patients who receive organ transplants [10]. Clearance of VRE colonization occurs after variable periods of time with a median time of 26 weeks once antibiotics that promote VRE are stopped [11]. In our patient, one could argue that the interruption of vancomycin is what precipitated the changes in the flora over time. We cannot exclude the possibility that discontinuation of oral vancomycin played a role in the changes in the microbiome and VRE fecal dominance after FMT. However, in order for the cessation of antibiotics alone to result in the re-establishment of a normal gut microbiota, we would expect to have sufficient amounts of the commensal microbiota that survived the extensive use of antibiotics to restore the normal flora. From our microbiota analysis, we found that approximately 70% donor microbiota consisted of *Bacteroides*, *Blautia*, *Rosburia*, and *Faecalibacterium*, whereas the recipient had <0.1% of these commensal microbes before FMT (Supplementary Table 1). The loss of normal proportions of commensal microbes before FMT is likely the consequence of our patient's prior extensive antibiotic history. We have seen similar results in the analysis of our other FMT transplants used for recurrent *C difficile* infections (unpublished results). Further support for FMT contributing to the drastic reduction in fecal VRE abundance and the establishment of microbiota composition containing known gut commensal microbiota comes from prior studies in mice [5, 11]. In these studies, the abundance of the *Barnsiella*, *Coprobacillus*, *Akkermansia*, and *Blautia* was found to correlate with inhibition of dominance of *Enterococcus*. In contrast, we did not find *Barnsiella* or *Coprobacillus* in our analysis of the recipient or the donor fecal microbiota, but we did see an increase in the relative abundance of *Akkermansia* and *Blautia* post-FMT (Supplementary Table 2).

Our study represents the first analysis in humans of the capacity of FMT to reduce dominant VRE carriage in the colon. Fecal dominance of VRE within the colon has been shown to increase the risk of VRE bacteremia 9-fold [12]. The FMT in our patient provided a unique opportunity to study the efficacy of FMT for colonic VRE domination. Fecal microbiota transplantation is increasingly being used as an effective therapy for recurrent *C difficile* infection, and the frequent coexistence of *C difficile* and VRE infections will provide future opportunities for evaluation of FMT in patients similar to ours. Further

studies are needed to confirm our findings and to also explore the use of FMT for other difficult-to-treat pathogens present in the gut. With increasing antimicrobial resistance and limited options for management, alternatives to the traditional treatment approaches should be investigated.

Supplementary Material

Acknowledgments

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