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SHORT REPORT

Microbial composition analysis of *Clostridium difficile* infections in an ulcerative colitis patient treated with multiple fecal microbiota transplantations

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Gut microbiome

Abstract

Fecal microbiota transplantation (FMT) is a promising therapy for *Clostridium difficile* infection (CDI). However, questions remain regarding efficacy and safety in inflammatory bowel disease (IBD) patients, as well as longitudinal stability of donor stool composition. This report describes an IBD patient with two CDIs 18 months apart, each successfully treated with FMT with no IBD flares or complications. Microbiome composition analysis of patient samples during each infection revealed low-diversity microbiota patterns similar to those previously described in non-IBD patients with CDI and active IBD alone. Samples taken after each transplant demonstrated quick remodeling towards the donor's sample composition coinciding with symptom resolution. Of note, samples taken from the same donor 18 months apart reflected marked differences in microbiota abundances, suggesting that the use of single donors in FMT programs offers little benefit in ensuring predictability of donor stool composition over time. This report describes similar microbial composition patterns during CDI in IBD patients to those described previously in non-IBD patients, and supports FMT as safe and effective treatment for recurring CDI in this patient population.

Abbreviations: CDI, *Clostridium difficile* infection; FMT, fecal microbiota transplantation; IBD, inflammatory bowel disease; OTU, operational taxonomic unit; UC, ulcerative colitis.

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1. Introduction

Clostridium difficile infection (CDI) is a major cause of antibiotic-associated diarrhea and patients with inflammatory bowel disease (IBD) experience a disproportionate burden of disease. Colonization with toxigenic *C. difficile* is significantly higher in IBD than in the general population (8.2% vs. 1%)[1] and having ulcerative colitis raises risk of CDI by almost eight times from baseline.[2] Patients with concomitant IBD and CDI are more likely to have a longer hospital stay, higher colectomy rates, and increased case fatality rates.[3] No randomized controlled studies exist to guide management of CDI in patients with coexisting IBD, and high failure rates (>50%) have been reported with antibiotic treatment.[4]

Fecal microbiota transplantation (FMT) has emerged as a safe and effective management option for recurrent CDI when standard approaches have failed.[5–7] A systematic review reported FMT as being curative in 92% of 317 non-IBD patients with CDI,[5] and in 2013, a randomized controlled trial highlighted the effectiveness of FMT (81%) over Vancomycin (31%).[6] Evidence for FMT in IBD patients with CDI is limited; a systematic review identified just 12 cases, though *C. difficile* eradication was described in all patients.[8] Safety concerns have been raised with a recent report of an ulcerative colitis flare after transplant.[9] Additionally, outcomes of repeat FMT for CDI relapses have not been explored in this population, and 5% of the general population receiving FMT for CDI require retreatment after initial transplant.[5]

This is the first report of serial use of the same FMT donor to treat an IBD patient with CDI recurring months after initial transplant. Microbiome composition analysis of patient stool samples before and after each respective transplant was carried out to shed light on microbial patterns with coexisting CDI and IBD, and dynamic changes after each transplant. Analysis of donor samples was carried out to delineate the stability of single-donor stool composition over time, which is a significant product quality concern in the development of FMT protocols.

2. Case report

This patient presented at age 87 with bloody stool, abdominal pain, and weight loss for 6 months. A sigmoidoscopy revealed inflamed ulcerated mucosa with fibrinous exudates beginning in the distal rectum with a loss of vascular pattern extending to 30 cm. No infectious causes could be identified by stool culture and toxin immunoassay. Biopsies revealed a mixed cell infiltrate with neutrophils, lymphocytes and plasma cells with evidence of cryptitis and crypt branching. A diagnosis of inflammatory bowel disease (IBD) favoring ulcerative colitis (UC) was made based on histological findings. The patient entered clinical remission with mesalamine and budesonide enemas, and was maintained on mesalamine.

Three years later, the patient received Cephalexin for a cellulitis and subsequently presented with non-bloody diarrhea and tested positive for *C. difficile* A/B toxin (Fig. 1). Though symptoms improved with oral Vancomycin (125 mg po q6h), the diarrhea returned several days after completion of a 14-day course. Vancomycin was restarted in combination with oral Metronidazole (500 mg po q8h) for four weeks, with symptom resolution. A second recurrence occurred five days after antibiotic cessation. After this second recurrence, oral Vancomycin was restarted and the patient was evaluated for fecal microbial transplantation (FMT). All recurrences were confirmed by toxin immunoassay and required admission to hospital.

The patient was assessed by specialists in infectious disease and gastroenterology. The study protocol was approved by the Human Research Ethics Boards at Queen's University. Written informed consent for participation in research was obtained, and the patient identified her son as a suitable donor. The

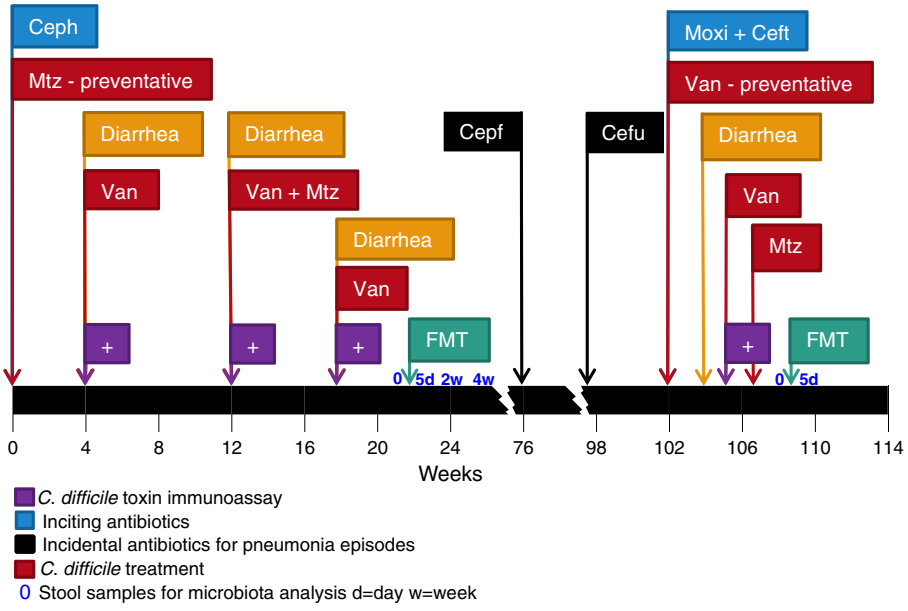

Figure 1 Clinical timeline of events. Abbreviations: CEPH — Cephalexin; MTZ — Metronidazole; VAN — Vancomycin; CEFP — Cefprozil; CEFU — Cefuroxime; CEFT — Ceftriaxone; MOXI — Moxifloxacin; FMT — Fecal microbiota transplantation.

donor was consented for participation and screened with standard questions and pathogen tests (see supplementary file).

Vancomycin was withheld 48 h prior to FMT and the patient underwent standard colon cleansing. The following morning, 50 g of fresh donor fecal material were re-suspended in 200 mL of sterile normal saline, pulse-homogenized, and filtered through sterile gauze to remove all particulate material. The homogenate was immediately delivered to the endoscopy unit and hooked up to the colonoscopy pump. During colonoscopy, one half (100 mL) of the fecal material was first deposited in the cecum/proximal ascending colon and the rest was distributed throughout the transverse colon. Total time elapsed from when the donor produced the sample to colonoscopy was less than 4 h. Post-procedure, the patient was maintained in Trendelenburg position for 60 min for observation. After discharge the patient was instructed not to consume probiotics and was followed by a study nurse to obtain stool samples and monitor clinical response. The patient reverted to normal bowel pattern within five days. No *C. difficile* toxin was detectable by immunoassay at six weeks post-procedure.

The patient remained free of CDI recurrence despite Cefprozil and Cefuroxime treatments for two pneumonias. A third pneumonia, 18 months after transplant 1, was treated with intravenous Ceftriaxone and Moxifloxacin, along with preventative oral Vancomycin. One month later the patient was admitted with non-bloody diarrhea and a *C. difficile* toxin positive result. Following a poor response to oral Vancomycin and intravenous Metronidazole, and a sigmoidoscopy revealing pseudomembranes, a second transplant was performed using the same donor and same methodology. Symptoms cleared within 48 h and normal bowel patterns returned one week post-procedure. She remained symptom-free four weeks after discharge. There were no UC flares after either FMT.

Patient fecal samples were taken before and after each transplant for microbial composition analysis; donor samples were also analyzed. Methods for microbial composition analysis are described in a supplemental file, and compositional similarity of samples was calculated with the Jaccard index using operational taxonomic units clustered at 97% sequence identity.

Preceding the first transplant, Proteobacteria (*Enterobacteriaceae* particularly — see supplemental figure) predominated in the patient microbiota, with deficiencies in Firmicutes and Bacteroidetes (Fig. 2A). The donor sample for transplant 1 was composed largely of Firmicutes. Five days after transplant, the patient sample closely resembled that of the donor, with strong representation of Firmicutes, Clostridia and Bacilli. As weeks progressed, the proportion of Actinobacteria increased, however, dominance of Firmicutes was maintained.

Immediately preceding the second transplant, the patient's sample contained dominant populations of Proteobacteria, especially *Enterobacteriaceae*, with very few Firmicutes. Five days after transplant 2 *Enterobacteriaceae* persisted, however, large groups of Bacteroidetes and Firmicutes were present, consistent with the donor sample composition.

Donor samples had large microbiota composition abundance differences (Fig. 2B), despite use of the same methods for screening and preparation and no donor use of antibiotics between transplants. Sample 1 reflected Firmicutes dominance while sample 2 had approximately equal populations of Bacteroidetes and Firmicutes. Analysis of the donor samples collected 18 months apart revealed large differences in bacterial abundance at the Family level but a Jaccard index at the OTU level of 0.91 (see supplemental file). Both transplants were successful in curing the infection.

3. Discussion

This is the first report to describe sequential FMT from a single donor for an IBD patient with CDI recurrences. Microbiota composition analysis indicated that the patient's pre-transplant samples exhibited reduced diversity, with deficiencies in the usually dominant populations of Firmicutes and Bacteroidetes and an overabundance of Proteobacteria. This microbiota pattern is consistent with microbial analyses of non-IBD patients during CDI[10,11] supporting the theory that predominating Firmicutes and Bacteroidetes groups may confer colonization resistance against *C. difficile*.[12] The persistence of these findings across IBD and non-IBD patients suggests a vulnerable microbial profile that may augment susceptibility to recurrent CDI. Determining what defines this "vulnerability profile" may help predict those at high risk for developing recurrent CDI.

Interestingly, a Proteobacteria-dominant pattern has also been described in non-infected patients experiencing an active UC flare, and FMT in these patients has been shown to reduce Proteobacteria dominance.[13,14] However, while rehabilitating our patient's microbiota towards that of her donor correlated to symptom resolution, and her UC remained quiescent, the clinical response to FMT for treatment of UC has been limited[13,14] and has even exacerbated symptoms of UC.[9] Therefore, further characterization of microbiota dynamics after FMT is warranted to elucidate microbiologic mechanisms and to further clarify its clinical applications in this patient population.

Our findings also demonstrate that significant changes may take place in microbiota abundances from the same donor over time, consistent with evidence that only 60% of the human microbiota is stable and durable.[15] Nevertheless, despite markedly different donor microbiota profiles both transplants resulted in complete CDI symptom resolution and remodeling of the patient's fecal microbiota towards that of the donor; studies in non-IBD patients with CDI describe similar observations coinciding with disease remission.[11,16] These data suggest that a wide range of microbiota profiles may effectively treat recurrent CDI. Our findings describe only one patient–donor set, which is a limitation of this study. Further studies and research in this area may lead to new insights and approaches that take into account both the donor profile and the vulnerability profile of those patients most at risk of relapse.

Conflict of interest

None of the authors have a conflict of interest.

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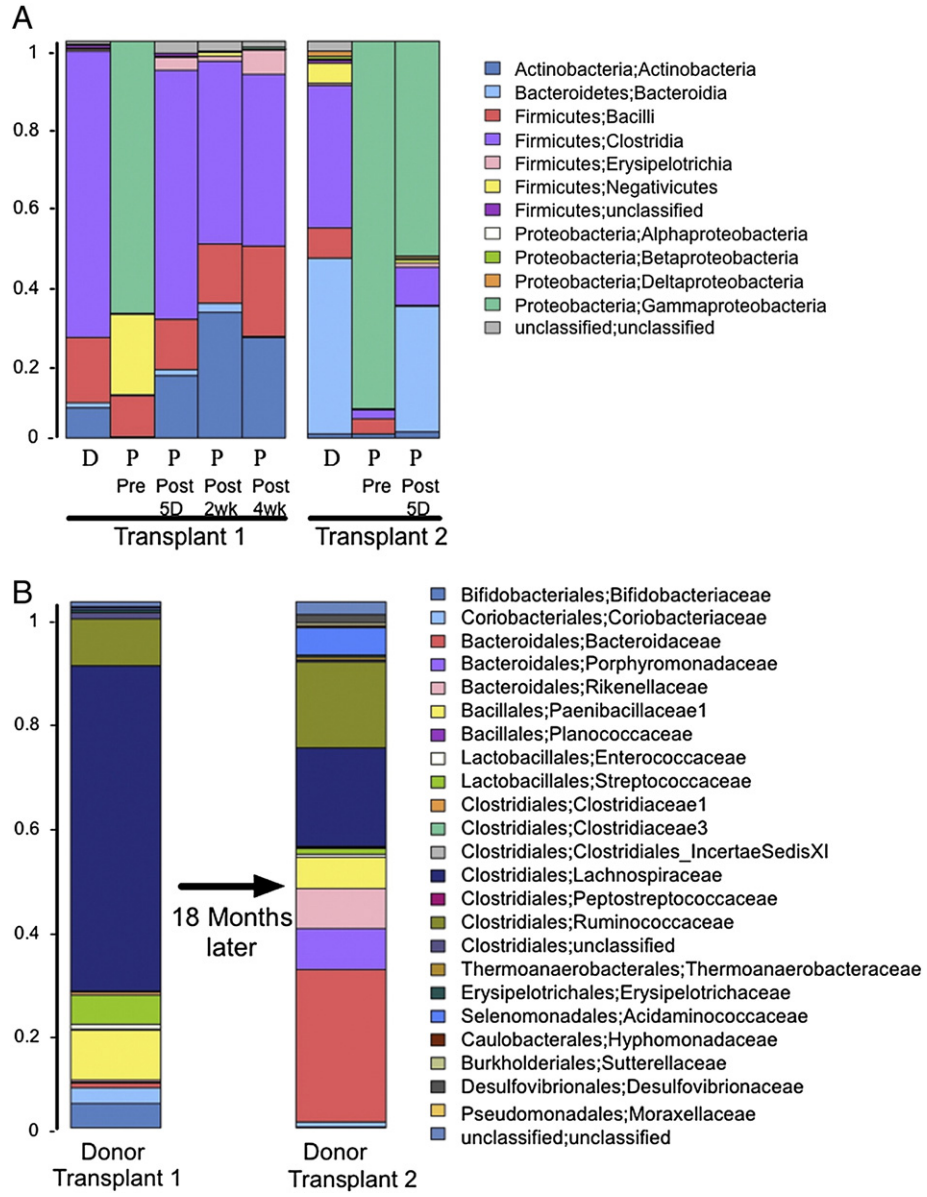

Figure 2 Simplified barplot representation of bacterial phylum composition of donor and patient samples (A) and of bacterial family composition of donor samples (B).

Statement of authorship:

1) Chantalle Brace: acquisition and interpretation of data; drafting of the manuscript,
2) Gregory Gloor: acquisition of data; analysis and interpretation of data; statistical analysis; critical revision of the manuscript for important intellectual content,
3) Mark Ropeleski: study supervision; critical revision of the manuscript for important intellectual content,
4) Emma Allen-Vercoe: acquisition and interpretation of data; critical revision of the manuscript for important intellectual content,
5) Elaine Petrof: study concept and design; study supervision; acquisition and interpretation of data; drafting of the manuscript; obtained funding.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.crohns.2014.01.020.

References

1. Clayton EM, Rea MC, Shanahan F, Quigley EM, Kiely B, Hill C, et al. The vexed relationship between *Clostridium difficile* and inflammatory bowel disease: an assessment of carriage in an outpatient setting among patients in remission. *Am J Gastroenterol* 2009;**104**:1162–9.
2. Nguyen GC, Kaplan GG, Harris ML, Brant SR. A national survey of the prevalence and impact of *Clostridium difficile* infection

among hospitalized inflammatory bowel disease patients. *Am J Gastroenterol* 2008;**103**:1443–50.
3. Berg AM, Kelly CP, Farraye FA. *Clostridium difficile* infection in the inflammatory bowel disease patient. *Inflamm Bowel Dis* 2013;**19**:194–204.
4. Reddy SS, Brandt LJ. *Clostridium difficile* infection and inflammatory bowel disease. *J Clin Gastroenterol* 2013;**47**:666–71.
5. Gough E, Shaikh H, Manges AR. Systematic review of intestinal microbiota transplantation (fecal bacteriotherapy) for recurrent *Clostridium difficile* infection. *Clin Infect Dis* 2011;**53**(10):994–1002.
6. van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoentendal G, de Vos WMK, et al. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. *N Engl J Med* 2013;**368**(5):407–15.
7. Kassam Z, Lee CH, Yuan Y, Hunt RH. Fecal microbiota transplantation for *Clostridium difficile* infection: systematic review and meta-analysis. *Am J Gastroenterol* 2013;**108**:500–8.
8. Anderson JL, Edney RJ, Whelan K. Systematic review: faecal microbiota transplantation in the management of inflammatory bowel disease. *Aliment Pharmacol Ther* 2012;**36**:503–16.
9. De Leon LM, Watson JB, Kelly CR. Transient flare of ulcerative colitis after fecal microbiota transplantation for recurrent *Clostridium difficile* infection. *Clin Gastroenterol Hepatol* 2013;**11**:1036–8.
10. Shahinas D, Silverman M, Sittler T, Chiu C, Kim P, Allen-Vercoe E, et al. Toward an understanding of changes in diversity associated with fecal microbiome transplantation based on 16S rRNA gene sequencing. *mBio* 2012;**3**(5):e00338-12.
11. Khoruts A, Dicksved J, Jansson JK, Sadowsky MJ. Changes in the composition of the human fecal microbiome after bacteriotherapy for recurrent *Clostridium difficile*-associated diarrhea. *J Clin Gastroenterol* 2009;**00**(00).
12. Bakken JS. Fecal bacteriotherapy for recurrent *Clostridium difficile* infection. *Anaerobe* 2009;**15**:285–9.
13. Petrof EO, Gloor GB, Vanner SJ, Weese SJ, Carter D, Daigneault MCA, et al. Stool substitute transplant therapy for the eradication of *Clostridium difficile* infection: 'rePOOPulating' the gut. *Microbiome* 2013;**1**(3).
14. Faith JJ, Guruge JL, Charbonneau M, Subramanian S, Seedorf H, Goodman ALG, et al. The long-term stability of the human gut microbiota. *Science* 2013;**341**(6141).
15. Kump PK, Grochenig HP, Lackner S, Trajanoski S, Reicht G, Hoffman KM, et al. Alteration of intestinal dysbiosis by fecal microbiota transplantation does not induce remission in patients with chronic active ulcerative colitis. *Inflamm Bowel Dis* 2013;**19**:2155–65.
16. Angelberger S, Reinisch W, Makristathis A, et al. Temporal bacterial community dynamics vary among ulcerative colitis patients after fecal microbiota transplantation. *Am J Gastroenterol* 2013;**108**:1620–30.