

SUPPLEMENTARY MATERIAL

Table 1. Primer sequences used in this study.

Primer Name	Sequence (5' - 3')
Forward Primer 1	CNACGCGAAGAACCTTANC
Forward Primer 2	CAACGCGAAAAACCTTACC
Forward Primer 3	CAACGCGCAGAACCTTACC
Forward Primer 4	ATACGCGARGAACCTTACC
Forward Primer 5	CTAACCGANGAACCTYACC
Reverse Primer 1	[6bp ID tag]CGACAGCCATGCANACCT
Reverse Primer 2	[6bp ID tag]CGACAACCATGCANACCT
Reverse Primer 3	[6bp ID tag]CGACGGCCATGCANACCT
Reverse Primer 4	[6bp ID tag]CGACGACCATGCANACCT

Case Histories

Patient 1. 66 year old woman was admitted to the University Hospital with six day history of progressive diarrhea following recent treatment of sinusitis with two courses of amoxicillin. Her past medical history was most notable for metastatic ovarian carcinoma diagnosed and surgically debulked seven years earlier. She had received multiple courses of different chemotherapy in the intervening years. Her fourth cycle of paclitaxel was administered four weeks prior to admission. The patient reported severe diarrhea, continuous through day and night, along with chills, fatigue, and anorexia. Notable findings on physical examinations included tender lower abdomen, which was otherwise soft and flat. Computerized tomography (CT) scan of her abdomen demonstrated diffuse colonic wall thickening without pneumatosis, pericolonic fat stranding, and small amount of ascites. She had slightly increased white blood cell (WBC) count of $12.6 \times 10^9/L$ and albumin of 3.4 g/dL. Her stool studies were positive for *C. difficile* toxin B by PCR. The patient was initially started on oral metronidazole, but changed to oral vancomycin in the

next two days as she failed to show any clinical improvement. On day 2 of hospitalization her abdomen was noted to be markedly distended, tympanitic, and diffusely tender. She developed fevers of 39.5°C, fluid bolus refractory hypotension with blood pressure of 70/40 mmHg and metabolic acidosis with bicarbonate level of 12. Antibiotic management at this time included oral and rectal vancomycin, and intravenous metronidazole. The patient received an infusion of intravenous immunoglobulin. On day 3 of hospitalization she was transferred to the medical intensive care unit where she was placed on ventilatory and vasopressor support. Surgical consultant concluded that subtotal colectomy was the surgical procedure of choice, but would carry high risk of mortality, and cited metastatic ovarian carcinoma and extremely low albumin of 1.3 to be complicating factors. The consultant recommended to consider FMT prior to committing to subtotal colectomy.

Antibiotics were discontinued at mid-day of hospitalization day 3 and two liters of polyethylene glycol electrolyte solution (GoLYTELY) were administered via nasogastric tube. FMT was performed via colonoscopy in the late evening of hospitalization day 3 using freshly prepared fecal material from a pre-screened volunteer donor. The colonoscope was gently advanced to the hepatic flexure and severe pseudomembranous colitis was noted (Fig 1A). Within several hours of the procedure there was hemodynamic improvement with lessening vasopressor support and defervescence. WBC count decreased from peak of $24.3 \times 10^9/L$ to $10.8 \times 10^9/L$ within 24 hours (Fig 1B). Over the next several days her clinical recovery was described as “remarkable”. However, on day 6 after the procedure clear signs of clinical deterioration were noted with increasing diarrhea, abdominal pain and distended abdomen. Unfortunately, the option to perform a second FMT was not available due to out of town absence of the performing endoscopist. The patient was taken to surgical subtotal colectomy. She was able to recover over the next six weeks and continues to be clinically stable.

Patient 2. 74 year old man was admitted to an outside hospital with abdominal pain, watery diarrhea, nausea and vomiting, about two and a half weeks following treatment of foot cellulitis with clindamycin. He had numerous medical problems, including severe emphysema with oxygen dependence, atherosclerotic heart disease, history of aortic aneurism repair, recurrent aspiration pneumonias, including an episode a year ago

requiring mechanical ventilation, narcotic dependent back pain, and legal blindness due to glaucoma. He was afebrile and hemodynamically stable, although his WBC count was $20.4 \times 10^9/L$ and albumin was 3.2 g/dL. He was admitted and started empirically on oral metronidazole. His stool studies were positive for *C. difficile* toxin B by PCR, and also grew out vancomycin resistant enterococcus. Over the next three days he showed little clinical improvement and started having fevers up to 39.5°C. Intravenous ertapenem was administered for suspected systemic infection and metronidazole was changed to intravenous route of administration. The patient was reported increasing abdominal pains and required fluid resuscitation for hypotensive episodes. CT scan of the abdomen showed thickening of the colon wall from cecum to sigmoid colon. There was moderate amount of ascites. The patient was transferred to the medical intensive care unit, started on oral vancomycin, and surgical consultation was obtained for consideration of colectomy. The surgeon determined that his multiple medical co-morbidities greatly increased the likely mortality of subtotal colectomy and recommended stopping intravenous antibiotics and transferring the patient to the University hospital for consideration of FMT.

On re-evaluation at the University the patient appeared very tired. His systolic blood pressure was in the 90s mmHg. Abdomen was distended, mildly tender diffusely, and bowel sounds were absent. WBC count was $40.0 \times 10^9/L$, albumin was 2.0 g/dL, and C-reactive protein (CRP) was 208 mg/L; these laboratory indicators were monitored daily through the remainder of his hospital course (Fig 2A). Vancomycin was held for 24 hours during which the patient received several liters of GoLYTELY via nasogastric tube. FMT was done the next day via colonoscopy, during which pseudomembranous colitis was seen to involve the entire colon (Fig 2A). The patient appeared clinically improved the following day and reported complete resolution of abdominal pain. On the second day following the procedure he had a solid bowel movement. However, his bowel movement was loose on day 3 after the procedure and he noted return of fatigue. He had several more loose bowel movements on day 4, and the worsened symptoms correlated with increased CRP and WBC counts (Fig 2A). On day 5 his stool was positive for *C. difficile* toxin B by PCR. The patient was restarted on oral vancomycin and was discharged to a rehabilitation facility. A second FMT was performed two weeks following discharge at which time signs of

pseudomembranous colitis were absent (Fig 2B). The patient had no relapse of *C. difficile* infection in the subsequent one year despite two episodes of aspiration pneumonia.

Patient 3. 68 year old woman was admitted to the University hospital with hematochezia, abdominal pain and increasing nausea five weeks following Nissen fundoplication surgery. She had a complex past medical history complicated by a cognitive disorder and a narcotic dependent chronic pain syndrome related to a motor vehicle accident five years previously, heavy tobacco dependence, hypertension, polyneuropathy among a number of other medical and surgical items. She was tachycardic on admission with heart rate of 131 beats per minute and blood pressure of 182/92 mmHg. She was febrile with a temperature of 38.1°C. Abdomen was diffusely tender, but not distended. WBC count was $24.1 \times 10^9/\text{L}$ and albumin was 4.1 g/dL. CT of the abdomen demonstrated marked colonic wall thickening extending from rectosigmoid to the mid-transverse colon with mild surrounding fat stranding and small amount of ascites. Extensive atherosclerosis was noted in the abdominal aorta and iliac arteries, although the proximal celiac axis and the superior mesenteric artery were noted to be patent. Despite these findings, the primary suspected diagnosis was ischemic colitis. The patient was started on intravenous piperacillin/taxobactam and metronidazole. There was little clinical improvement over the next three days as the patient required increasing amounts of narcotics for pain control and continued to have tachycardia and low grade fevers. *C. difficile* infection was considered, but fecal samples failed to be collected during this time. CT angiogram was performed demonstrating patent inferior mesenteric artery proximally and in its major branches. A flexible sigmoidoscopy performed on day 4 of hospitalization due to persistent rectal bleeding described dark, edematous mucosa with confluent large pseudomembranes. *C. difficile* toxin B was found to be positive on day 5 of hospitalization by PCR. The primary diagnosis was changed to that of severe *C. difficile* colitis, and non-CDI antibiotics were discontinued. Oral vancomycin was added to the intravenous metronidazole. Nevertheless, the patient continued to have diarrhea and complain of severe abdominal pain, concordant with diffuse abdominal tenderness seen on physical examination. This CDI antibiotic therapy continued for another five days without significant change in the clinical status. However, on day 11 she developed worsening abdominal distension with

cessation of stools. Abdominal X-ray showed dilatation of the transverse colon to 6 cm. Antibiotics were discontinued, and the patient received GoLYTELY in preparation for FMT; the primary service also opted to simultaneously administer intravenous globulin at 1g/kg. On hospitalization day 12 the patient was noted to be more lethargic than the previous day.

The FMT was performed via colonoscopy on hospitalization day 12, noting pseudomembranous colitis most severe in the transverse colon as well as 6 mm polyp in the rectum. There was little clinical improvement seen on day 13 as the patient became increasingly somnolent and spiked a fever of 38.6°C. Fidaxomicin was initiated despite resolution of colonic dilatation on hospitalization day 14, followed by abdominal CT scan that demonstrated decreased thickening of the colon and decreased amount of ascites. On hospitalization day 15 her abdominal examination was markedly improved compared to all preceding days. However, her mental status worsened further. She was extremely lethargic, and was no longer able to follow simple commands or identify her husband. Analysis of cerebrospinal fluid obtained by lumbar puncture showed moderate pleocytosis with 11 WBC/ μ L, protein 48 mg/dL, and glucose of 54 mg/dL. Stool testing for *C. difficile* was negative. Neurological assessment was that the patient experienced aseptic meningitis caused by intravenous immunoglobulin administered to the patient shortly before the FMT. She was also discovered to have cystitis with > 100,000 Gram negative rods cultured from the urine and received a three day course of trimethoprim/sulfamethoxazole starting on hospitalization day 19. The neurologic status gradually improved over five days following the lumbar puncture. Difucid was continued until day 24 and a repeat FMT was performed via colonoscopy on day 25. There were no signs of pseudomembranous colitis during this examination although some regenerative changes were still present in the transverse colon mucosal. The adenomatous polyp noted previously was resected and the patient was transferred to a rehabilitation unit on day 27. The patient continued to undergo rehabilitation for the next six months and her bowel function remained normal.

Patient 4. 83 year old woman was admitted with diarrhea and abdominal pain four days after completion of a 10 day course of amoxicillin/clavulanate, following hospitalization for gallstone induced pancreatitis and ascending cholangitis. The patient had refused

cholecystectomy and was not interested in ever having surgery for any reason. Her past medical history was notable for type II diabetes, major depression, and at least mild dementia. She was tachycardic, but afebrile. Abdomen was non-distended. CT scan of the abdomen demonstrated marked mural thickening with surrounding fat stranding involving the rectum, sigmoid colon, and descending colon. WBC count on admission was 17.2 and albumin was 3.2. In addition, she was found to have a urinary tract infection for which she was started on intravenous levofloxacin along with oral vancomycin. Stool studies were positive for *C. difficile* toxin B by PCR, and the patient was started on intravenous metronidazole. Over the next three days the patient developed increasing abdominal pain and distention, and increasing mental confusion. Abdominal X-ray demonstrated dilatation of the sigmoid colon to 6 cm. Levofloxacin was discontinued on day 3 of the hospitalization and the CDI antibiotics were stopped on day 4. WBC count at this time was $19.7 \times 10^9/L$ and CRP level was 71.3 mg/L. The patient was administered GoLYTELY prep and underwent FMT on day 5 by colonoscopy, during which she was noted to have pseudomembranous colitis greatest on the left side along with diverticulosis. Clinical improvement was noted the day after the procedure with lesser abdominal pain and distention. Both WBC count came down to $8.9 \times 10^9/L$ over two days, at which time CRP level was 24.8 mg/L. The patient remained off antibiotics for three days after FMT, after which she was started on fidaxomicin with plans to repeat her FMT after completion of the 10 day course of this antibiotic. The patient was discharged to a nursing home. However, she refused to have the second FMT and returned to the hospital approximately six weeks after finishing fidaxomicin with abdominal pain and nausea. Abdominal CT scan showed massive dilatation of the colon. Stool studies were positive for *C. difficile* toxin B. The patient refused further care, including antibiotics, and elected hospice care for comfort.