ADDENDUM

Treatment of recurrent Clostridium difficile infection using fecal microbiota transplantation in patients with inflammatory bowel disease

Krista M. Newman, Kevin M. Rank, Byron P. Vaughn, and Alexander Khoruts

Department of Medicine, University of Minnesota, Minneapolis, Minnesota, USA; Center for Immunology and BioTechnology Institute, University of Minnesota, Twin Cities Campus, Minneapolis, Minnesota, USA

ABSTRACT
We recently compared results of fecal microbiota transplantation (FMT) in patients with refractory, recurrent Clostridium difficile infection (rCDI), with and without underlying inflammatory bowel disease (IBD). Here we extend this cohort and analyze outcomes in greater detail by subtype of IBD. We find that FMT is generally effective in breaking the cycle of CDI recurrence, but its effects on overall IBD progression are much less predictable. We discuss several challenges intrinsic to this complex clinical situation and outline the next steps that can address these challenges going forward.

KEYWORDS
Crohn’s disease; clostridium difficile; inflammatory bowel disease; fecal microbiota transplantation; ulcerative colitis

Introduction

Concurrence of inflammatory bowel disease (IBD) and Clostridium difficile infection (CDI) presents several formidable diagnostic and therapeutic challenges. Diagnosis of CDI in IBD patients is complicated by a higher rate of asymptomatic carriage of C. difficile among individuals with IBD compared with the general population and absence of CDI-specific diagnostic features, such as presence of pseudomembranes. Therefore, determining whether C. difficile is driving the colitis or marks a progression of underlying disease is difficult in the absence of a clear clinical response to antibiotic therapy. More so, the clinical assessment of a therapeutic response can also be complicated by diarrheal symptoms caused by antibiotic-associated dysbiosis and post-infectious irritable bowel syndrome. Nevertheless, CDI can be a trigger of IBD activity and testing for presence of C. difficile is part of a standard evaluation of severe IBD colitis. Notably, several studies have found that IBD patients with CDI have worse outcomes, including prolonged length of hospitalization, intensive care unit admissions, rates of colectomy, post-surgical complications, post-colectomy pouch failure, and death.

One of the most common problems associated with CDI in all patients is recurrence of the infection following antibiotic therapy. The problem of CDI recurrence is even greater in patients with underlying IBD. Antibiotics fail to eradicate the spores of C. difficile, and can perpetuate the cycles of CDI recurrence by causing progressive suppression of indigenous gut microbiota. Thus, patients can develop recurrent CDI (rCDI) syndrome, which is characterized by indefinite cycles of antibiotic treatments and CDI recurrences. Fecal microbiota transplantation (FMT), a treatment that restores normal fecal microbial community composition and functionality, has emerged as a highly effective treatment of rCDI. However, outcomes of FMT performed for treatment of rCDI in the IBD population are more varied compared with the non-IBD population. Recently, we reported the experience of FMT in our clinical program, which compared outcomes in IBD and non-IBD rCDI recipients. We found that the success rate in preventing CDI recurrence with FMT was somewhat lower in IBD patients and there was also greater than 25% chance of triggering a flare of IBD activity with FMT despite conversion to a C. difficile negative status. Here we extend the experience from our clinical program further by including additional patients treated since that report and consider types and
severity of underlying IBD in greater detail. We then discuss the implications for FMT treatment and continued research.

**Methods**

Inclusion and exclusion criteria for FMT in our clinical program were described previously. Patients from our entire FMT cohort who had a diagnosis of IBD (either preceding FMT or diagnosed de novo at the time of FMT) were included in this analysis. The subtype of IBD, ulcerative colitis (UC) or Crohn’s disease, was assigned based on patient history, previous endoscopic findings, and colonscopic findings at the time of FMT. Patients with a past diagnosis of IBD, but lacking any evidence for the diagnosis during the FMT colonoscopy were not included in this case series. Colonscopic findings at the time of FMT were used to type UC as either left-sided or pancolitis and Crohn’s disease as ileal, ileocolonic, or colonic only. Severity of IBD was determined using the Mayo endoscopic score and the simplified endoscopic activity score for Crohn’s disease for UC and Crohn’s disease, respectively. Several instances of indeterminate colitis diagnosis were classified either as ulcerative colitis (UC) or Crohn’s disease based on the most prominent characteristics of each. All patients underwent a standardized clinic visit before FMT during which demographic information and disease history and characteristics were documented. Importantly, one of the eligibility criteria was a relapse of CDI following an extended course of antibiotics up until 2 d before FMT, followed without interruption by a chaser with rifaximin or fidaxomicin. All FMT procedures were performed via colonoscopy using microbiota material from standardized, unrelated donors. Baseline levels of C-reactive protein and erythrocyte sedimentation rate were measured on the day of the procedure. Microbiota material was the frozen/thawed preparation described previously. Success of FMT was defined as conversion to a *C. difficile* negative status within 2 months of the procedure or complete resolution of diarrhea (< 3 formed bowel movements per day). All subjects had a post FMT clinical visit at 2 months to document response and need for escalation of underlying IBD therapy.

**Results**

The overall demographics of the IBD patients who received FMT for rCDI is presented in Table 1. Unlike the general rCDI population, which disproportionately affects female patients, the IBD patients demonstrated no female predisposition to rCDI. As all patients were on anti-*C. difficile* antibiotics up until 2 d before FMT, any inflammation was presumed to be due to underlying IBD and not *C. difficile* colitis. All patients referred for consideration of FMT treatment were having poorly controlled diarrheal symptoms. Not surprisingly, these patients had gone through many cycles of antibiotics in attempts to eradicate CDI for a mean period exceeding one year (Table 1). As we reported previously, in some instances (7 patients) a *de novo* diagnosis of previously unsuspected IBD was made at the time of colonoscopic FMT.

**Outcomes of FMT in rCDI patients with underlying IBD**

A single FMT was successful (defined as conversion to a *C. difficile* negative status within 2 months of the procedure) in 48/56 (85.7%) of cases (Table 2). However, the treatment was also accompanied by non-trivial side effects. Greater than 50% of patients with UC experienced a flare of IBD activity despite disappearance of *C. difficile* toxin B (as measured by the PCR assay) in the stool, and required a brief course of corticosteroids. The risk of a flare was significantly higher for UC patients compared with Crohn’s disease patients (p = 0.002 Fisher’s exact test). Thre
Failure to resolved CDI is defined as persistent diarrhea and positive PCR test for *C. difficile* toxin B.

Flare of IBD activity is defined as worsened symptoms such as increased bowel movement frequency, abdominal pain, fever, rectal bleeding; increased systemic inflammatory markers (C-reactive protein and sedimentation rate); and documented negative PCR test for *C. difficile* toxin B.

Long-term (at least 4 months) was available in 47/56 (84%) of patients, and only these are shown in the denominator. The mean duration was 2.05 ± 1.7 years (median 1.75 years).

Late (>2 months) was available in 2 months1 and 12 months2 relapse of CDI without any new antibiotic provocations. These individuals had a clinical response to FMT and documented to be *C. difficile* toxin B negative within 2 months. However, they subsequently experienced symptomatic deterioration and were found to have *C. difficile* toxin B once again in their stool. Long-term follow-up was available in 47/56 (83.9%) patients and late *C. difficile* recurrence (or re-infection) was seen in approximately 23% (Table 2).

**Factors associated with FMT success/failure**

At the time of the initial evaluation of patients we tried to understand whether *C. difficile* was driving their diarrheal symptoms because a clear causal relationship between CDI and worsened IBD symptoms could be expected to result in better outcomes. One factor we thought could be helpful was history of a symptomatic response to anti-CDI antibiotics. Some clinical response to antibiotics was noted in 84% of patients that ultimately improved following FMT if it led to resolution of rCDI. However, such response to FMT was rarely complete, and the interpretation was often complicated by escalation of concurrent immunosuppressive medications. Notably, failure to observe any improvement of diarrheal symptoms with antibiotics before FMT (which would suggest that there was no causal relationship between CDI and IBD) was only 50% predictive of ultimate failure to demonstrate clinical improvement following FMT.

We tried to estimate the overall clinical benefit that may be attributable to FMT in our cohort by synthesising outcomes at the 2-month time point and long-term follow-up, although clearly such analysis is difficult without a placebo control. Patients that had colon-restricted Crohn’s disease appeared to have experienced the most benefit, although this was not statistically significant when compared with other
groups. However, some of these patients had the most dramatic overall improvements from the entire cohort. Two of them were ultimately able to stop all anti-inflammatory medications. The rest had complete normalization of bowel function and most were able to reduce immunosuppression. However, a significant fraction (11/31) of patients in other subgroups, including Crohn’s with small bowel involvement and both UC subgroups continued to struggle with their IBD in the long-term. Colectomy was ultimately required in 5 UC patients, although 2/5 also had primary sclerosing cholangitis (PSC) and the increased risk of colon cancer associated with PSC factored into the decision. One patient underwent a diverting colostomy.

Discussion

Our results with the expanded cohort of IBD patients from our clinical FMT program support our previous conclusions that FMT is generally effective in achieving resolution of CDI in IBD patients. However, there are important caveats that need to be considered by clinicians offering FMT to IBD patients and discussed during the consent process. These include a substantial possibility of an IBD flare and progression of IBD despite resolution of rCDI. Our results are in agreement with a recent multi-center retrospective analysis of a similar cohort of 54 IBD patients treated with FMT for rCDI by Fischer and colleagues. These investigators found that the initial FMT was successful against rCDI in 81% of patients, and this rate increased further with multiple FMTs. However, they found the overall improvement in IBD activity was seen in only a third of the patients.

One of the most challenging questions in evaluating CDI in patients with IBD is whether C. difficile contributes to their IBD activity or not. If patients are merely colonized with C. difficile bacteria, but are protected against its toxins, disappearance of C. difficile from the intestine should not contribute to clinical improvement. In fact, Clayton and colleagues reported that asymptomatic carriage of toxigenic C. difficile was more common in patients with IBD compared with healthy controls. However, the IBD population in that study consisted of patients in long-standing clinical remission and no immunosuppression, which is rather different from the patient population in our cohort and most IBD patients in practice. The ability to identify carriers at the time of the initial clinical evaluation is limited. Clinical improvement during treatment with anti-CDI antibiotics could be a discriminating characteristic; however, there are numerous confounding factors including escalation of immunosuppressive drugs, antibiotics-associated diarrhea, and post-infectious IBS. In addition, antibiotics can transiently diminish IBD activity even in absence of CDI. Similarly, absence of a clear antibiotic trigger temporally associated with the initial diagnosis of CDI is not a reliable factor because IBD alone can be associated with marked dysbiosis, sufficient to allow CDI.

Late relapse of CDI in IBD patients without any new antibiotic provocation was a considerably more common event compared with our non-IBD FMT recipients in the program. The most likely explanation for this phenomenon is instability of the engrafted microbial communities in the inflamed intestines. However, it is possible that these represented new infections in the setting of ongoing dysbiosis from underlying IBD. Previously, Hourigan and colleagues described microbiome changes over 6 months following FMT in a small case series of children rCDI with and without underlying IBD. All patients maintained negative C. difficile cultures through the study period. However, the fecal diversity of IBD patients decreased to pre-FMT levels at 6 months, and certain taxa associated with IBD-associated dysbiosis, such as Proteobacteria and Fusobacteria, increased in relative abundance. These observations suggest that the IBD intestinal environment drives dysbiosis, which was sufficient to allow recurrence of CDI in some of our patients. Nevertheless, achievement of a sustained period without C. difficile also suggests that FMT can lead to at least transient correction of dysbiosis in these patients.

Of course, IBD is a highly heterogeneous disease that is driven by different genetic risk factors and has a spectrum of distinct phenotypes. Therefore, it should be expected that the results of FMT should vary in patients based on IBD subtype, severity, and ongoing inflammatory activity. The numbers of patients in our cohort remain too small to yield a clear pattern of outcome differences among IBD subtypes. In addition to limited patient numbers other limitations of our study include its mostly retrospective design, lack of a placebo control, single center experience, and lack of systematic characterization of microbiota composition and functionality at different time points. Although
endoscopic assessment at the time of FMT was available in all patients, there were also no structured follow-up evaluations with standardized clinical activity scores and endoscopic examinations.

In conclusion, there is an emerging consensus that FMT is an effective and safe approach in IBD patients in breaking the cycle of rCDI, even though the success rate of the initial FMT is somewhat lower and there is a non-trivial chance of a late relapse. However, given the variable course of IBD following FMT, it is important to establish best timing for the treatment. Since ongoing inflammation may unfavorably impact stability of new microbiota, we currently strive to optimize IBD therapy with appropriate immunosuppressive and anti-inflammatory agents while maintaining the patient on suppressive vancomycin before offering FMT.

Moving forward and challenges ahead

Differentiation of C. difficile carriage versus symptomatic infection has the potential to improve appropriate best FMT candidates. The phenomenon of asymptomatic carriage of toxigenic C. difficile bacteria has long baffled investigators and clinicians. Approximately one-third of infants are colonized by toxigenic C. difficile bacteria up to 6 months of age, after which the carriage rate gradually decreases.23 By 3 y of age, carriage rates decrease to 0–3%, similar to the healthy adult population.23,24 Clinical symptoms attributable to CDI are rare in children before 12–24 months of age, a phenomenon hypothesized to be due to absence of the C. difficile toxin receptor in the infant colon.25 However, identity of such receptors has been unknown, although recently members of the Wnt receptor frizzled family (FZDs) were shown to be physiologically relevant receptors for C. difficile toxin B.26 Naturally, it would be of great interest to test whether asymptomatic C. difficile carriers have altered expression or functionality of FZDs in the colon. Asymptomatic adult patients may also have protective humoral immunity against C. difficile toxins,27 an observation that is supported by beneficial effects of bezlotoxumab, a monoclonal antibody against C. difficile toxin B.28 This new biologic may become a useful therapeutic tool in distinguishing carriers vs. symptomatic patients and may fit into an overall treatment algorithm of IBD patients with CDI. We anticipate that carriers would not experience any symptomatic benefit from bezlotoxumab treatment, whereas patients with C. difficile toxin-driven symptoms would experience symptomatic improvement.

Mechanistic understanding of FMT in rCDI is essential in teasing out which elements are compromised in presence of IBD, and consequently allow for design of more effective FMT protocols. The topic of FMT mechanisms has been a subject of previous detailed reviews.29 Briefly, restoration of indigenous microbiota may allow (1) competition for limiting nutrients, (2) direct targeting of C. difficile by bacteriocins or phage, (3) engagement of host immune mechanisms, and (4) alteration of the bile acid composition in the colon to create an inhibitory environment to C. difficile spore germination and vegetative growth. We are yet to identify to key members of microbiota that contribute to the therapeutic effects of FMT in rCDI, although some of the most obvious candidates are bacteria that mediate 7α-dehydroxylation of primary bile acids.30 Interestingly, active IBD is associated with a decrease in all reactions associated with microbiota-driven secondary bile acid metabolism,31 which can allow for a more permissive environment for C. difficile bacteria. Therefore, longitudinal monitoring of the fecal bile acid composition following FMT has the potential to provide clinically useful information in patients with IBD as the results may anticipate vulnerability to CDI relapse.

The preliminary experience assembled thus far from pragmatic cohort studies provides important background for design of randomized, placebo-controlled studies. Such studies should include stratification of IBD subtypes, long-term follow-up, careful monitoring of IBD activity using standardized clinical instruments and endoscopic assessments, and thorough characterization of changes in the microbiota composition (ideally to include non-bacterial components such as the virome and mycome) and functionality.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

ORCID

Byron P. Vaughn https://orcid.org/0000-0001-5081-3761
References

[1] Clayton EM, Rea MC, Shanahan F, Quigley EM, Kiely B, Hill C, Ross RP. 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; PMID: 19319128; http://dx.doi.org/10.1038/ajg.2009.4

[2] Ben-Horin S, Margalit M, Bossuyt P, Mau J, Shapira Y, Bojic D, Chermesh I, Al-Rifai A, Schoepfer A, Bosani M, et al. Prevalence and clinical impact of endoscopic pseudomembranes in patients with inflammatory bowel disease and Clostridium difficile infection. J Crohns Colitis 2010; 4:194-8; PMID: 21122505; http://dx.doi.org/10.1016/j.crohns.2009.11.001

[3] Wadhwa A, Al Nahhas MF, Dierkhising RA, Patel R, Kashyap P, Pardi DS, Khamna S, Grover M. High risk of post-infectious irritable bowel syndrome in patients with Clostridium difficile infection. Aliment Pharmacol Ther 2016; 44:576-82; PMID: 27444134; http://dx.doi.org/10.1111/apt.13737

[4] Mowat C, Cole A, Windsor A, Ahmad T, Arnott I, Wadhwa A, Al Nahhas MF, Dierkhising RA, Patel R, Kashyap P, Pardi DS, Khamna S, Grover M. High risk of post-infectious irritable bowel syndrome in patients with Clostridium difficile infection. Aliment Pharmacol Ther 2016; 44:576-82; PMID: 27444134; http://dx.doi.org/10.1111/apt.13737

[5] Kelsen JR, Kim J, Latta D, Smathers S, McGowan KL, Zaatuis T, Mamula P, Baldassano RN. Recurrence rate of clostridium difficile infection in hospitalized pediatric patients with inflammatory bowel disease. Inflamm Bowel Dis 2011; 17:50-5; PMID: 20722068; http://dx.doi.org/10.1002/ibd.21421

[6] Issa M, Vijayapal A, Graham MB, Beaulieu DB, Otterson MF, Lundeen S, Skaras S, Weber LR, Komorowski RA, Knox JF, et al. Impact of Clostridium difficile on inflammatory bowel disease. Clin Gastroenterol Hepatol 2007; 5:345-51; PMID: 17368234; http://dx.doi.org/10.1016/j.cgh.2006.12.028

[7] Murthy SK, Steinhart AH, Timmough J, Austin PC, Daneman N, Nguyen GC. Impact of Clostridium difficile colitis on 5-year health outcomes in patients with ulcerative colitis. Aliment Pharmacol Ther 2012; 36:1032-9; PMID: 23061526; http://dx.doi.org/10.1111/apt.12073

[8] 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; PMID: 18513271; http://dx.doi.org/10.1111/j.1572-0241.2007.01780.x

[9] Skowron KB, Lapin B, Rubin M, Hurst RD, Rubin DT, Hyman NH, Umanskiy K. Clostridium Difficile Infection in Ulcerative Colitis: Can Alteration of the Gut-associated Microbiome Contribute to Pouch Failure? Inflamm Bowel Dis 2016; 22:902-11; PMID: 26891259; http://dx.doi.org/10.1097/MIB.0000000000000710

[10] Negron ME, Rezaie A, Barkema HW, Rioux K, De Buck J, Checkley S, Beck PL, Carroll M, Fedorak RN, Dieleman L, et al. Ulcerative Colitis Patients With Clostridium difficile Are at Increased Risk of Death, Colectomy, and Postoperative Complications: A Population-Based Inception Cohort Study. Am J Gastroenterol 2016; 111:691-704; PMID: 27091322; http://dx.doi.org/10.1038/ajg.2016.106

[11] Razik R, Rumman A, Bahreini Z, McGeer A, Nguyen GC. Recurrence of Clostridium difficile Infection in Patients with Inflammatory Bowel Disease: The RECIDIVISM Study. Am J Gastroenterol 2016; 111:1141-6; PMID: 27215924; http://dx.doi.org/10.1038/ajg.2016.187

[12] Borody TJ, Khoruts A. Fecal microbiota transplantation and emerging applications. Nat Rev Gastroenterol Hepatol 2012; 9:88-96; http://dx.doi.org/10.1038/nrgastro.2011.244

[13] 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 2010; 44:354-60; PMID: 20048681

[14] van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, Visser CE, Kuijper EJ, Bartelsman JF, Tijssen JG. Duodenal infusion of donor feces for recurrent Clostridium difficile. N Engl J Med 2013; 368:407-15; PMID: 23323867; http://dx.doi.org/10.1056/NEJMoa1205037

[15] Weingarden AR, Chen C, Bobr A, Yao D, Lu Y, Nelson VM, Sadowsky MJ, Khoruts A. Microbiota transplantation restores normal fecal bile acid composition in recurrent Clostridium difficile infection. Am J Physiol Gastrointest Liver Physiol 2015; 308:G310-9; PMID: 24284963; http://dx.doi.org/10.1152/ajpgi.00282.2013

[16] Khoruts A, Rank KM, Newmann KM, Viskocil K, Vaughn BP, Hamilton MJ, Sadowsky MJ. Inflammatory Bowel Disease Affects the Outcome of Fecal Microbiota Transplantation for Recurrent Clostridium difficile Infection. Clin Gastroenterol Hepatol 2016; 14(10):1433-8; PMID: 26905904

[17] Hamilton MJ, Weingarden AR, Sadowsky MJ, Khoruts A. Standardized frozen preparation for transplantation of fecal microbiota for recurrent Clostridium difficile infection. Am J Gastroenterol 2012; 107:761-7; PMID: 22290405; http://dx.doi.org/10.1038/ajg.2011.482

[18] Fischer M, Kao D, Kelly C, Kuchipudi A, Jafri SM, Blumenkohl M, Rex D, Mellow M, Kaur N, Sokol H, et al. Fecal Microbiota Transplantation is Safe and Efficacious for Recurrent or Refractory Clostridium difficile Infection in Patients with Inflammatory Bowel Disease. Inflamm Bowel Dis 2016; 22:2402-9; PMID: 27580384; http://dx.doi.org/10.1097/MIB.0000000000000908

[19] Ohkusa T, Kato K, Terao S, Chiba T, Mabe K, Murakami K, Mizokami Y, Sugiyama T, Yanaka A, Takeuchi Y, et al. Newly developed antibiotic combination therapy for ulcerative colitis: a double-blind placebo-controlled multicenter trial. Am J Gastroenterol 2010; 105:1820-9; PMID: 20216533; http://dx.doi.org/10.1038/ajg.2010.84
[20] Burke DA, Axon AT, Clayden SA, Dixon MF, Johnston D, Lacey RW. The efficacy of tobramycin in the treatment of ulcerative colitis. Aliment Pharmacol Ther 1990; 4:123-9; PMID: 2104079; http://dx.doi.org/10.1111/j.1365-2036.1990.tb00456.x

[21] Ursing B, Alm T, Barany F, Bergelin I, Ganrot-Norlin K, Hoevels J, Huitfeldt B, Järnerot G, Krause U, Krook A, et al. A comparative study of metronidazole and sulfasalazine for active Crohn's disease: the cooperative Crohn's disease study in Sweden. II. Result. Gastroenterology 1982; 83:550-62.

[22] Hourigan SK, Chen LA, Grigoryan Z, Laroche G, Weidner M, Sears CL, Oliva-Hemker M. Microbiome changes associated with sustained eradication of Clostridium difficile after single faecal microbiota transplantation in children with and without inflammatory bowel disease. Aliment Pharmacol Ther 2015; 42:741-52; PMID: 26198180; http://dx.doi.org/10.1111/apt.13326

[23] Jangi S, Lamont JT. Asymptomatic colonization by Clostridium difficile in infants: implications for disease in later life. J Pediatr Gastroenterol Nutr 2010; 51:2-7; PMID: 20512057; http://dx.doi.org/10.1097/MPG.0b013e318d29767

[24] Hafiz S, Oakley CL. Clostridium difficile: isolation and characteristics. J Med Microbiol 1976; 9:129-36; PMID: 933146; http://dx.doi.org/10.1099/00222615-9-2-129

[25] Pothoulakis C, Lamont JT. Microbes and microbial toxins: paradigms for microbial-mucosal interactions II. The integrated response of the intestine to Clostridium difficile toxins. Am J Physiol Gastrointest Liver Physiol 2001; 280:G178-83.

[26] Tao L, Zhang J, Meraner P, Tovaglieri A, Wu X, Gerhard R, Zhang X, Stallcup WB, Miao J, He X, et al. Frizzled proteins are colonic epithelial receptors for C. difficile toxin B. Nature 2016; 538:350-355.

[27] Kyne L, Warna M, Qamar A, Kelly CP. Asymptomatic carriage of Clostridium difficile and serum levels of IgG antibody against toxin A. N Engl J Med 2000; 342:390-7; PMID: 10666429; http://dx.doi.org/10.1056/NEJM200002103420604

[28] Yang Z, Ramsey J, Hamza T, Zhang Y, Li S, Yfantis HG, Lee D, Hernandez LD, Seghezzi W, Furneisen JM, et al. Mechanisms of protection against Clostridium difficile infection by the monoclonal antitoxin antibodies actoxumab and bezlotoxumab. Infect Immun 2015; 83:822-31; PMID: 25486992; http://dx.doi.org/10.1128/IAI.02897-14

[29] Khoruts A, Sadowsky MJ. Understanding the mechanisms of faecal microbiota transplantation. Nat Rev Gastroenterol Hepatol 2016; 13(9):508-16; PMID: 27329806

[30] Buffie CG, Bucci V, Stein RR, McKenney PT, Ling L, Gobourne A, No D, Liu H, Kinnebrew M, Viale A, et al. Precision microbiome reconstitution restores bile acid mediated resistance to Clostridium difficile. Nature 2014; 517(7533):205-8; PMID: 25337874

[31] Duboc H, Rajca S, Rainteau D, Benarous D, Maubert MA, Quervain E, Thomas G, Barbou V, Humbert L, Despras G, et al. Connecting dysbiosis, bile-acid dysmetabolism and gut inflammation in inflammatory bowel diseases. Gut 2013; 62:531-9; PMID: 22993202; http://dx.doi.org/10.1136/gutjn1-2012-302578