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Short Report

Longitudinal Bile Acid Composition Changes Following Faecal Microbiota Transplantation for *Clostridioides difficile* Infection in Children With and Without Underlying Inflammatory Bowel Disease

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Abstract

Background and Aims: Faecal microbiota transplant [FMT] is effective in treating recurrent *Clostridioides difficile* infection [CDI] and restores gut microbiota composition. This is unlikely to account for its entire mechanism of efficacy, as studies have shown that factors such as bile acids influence the risk of infection by affecting *Clostridioides difficile* germination. We therefore aimed to investigate longitudinal changes in the gut bile acid composition after FMT performed for recurrent CDI, in children with and without inflammatory bowel disease [IBD].

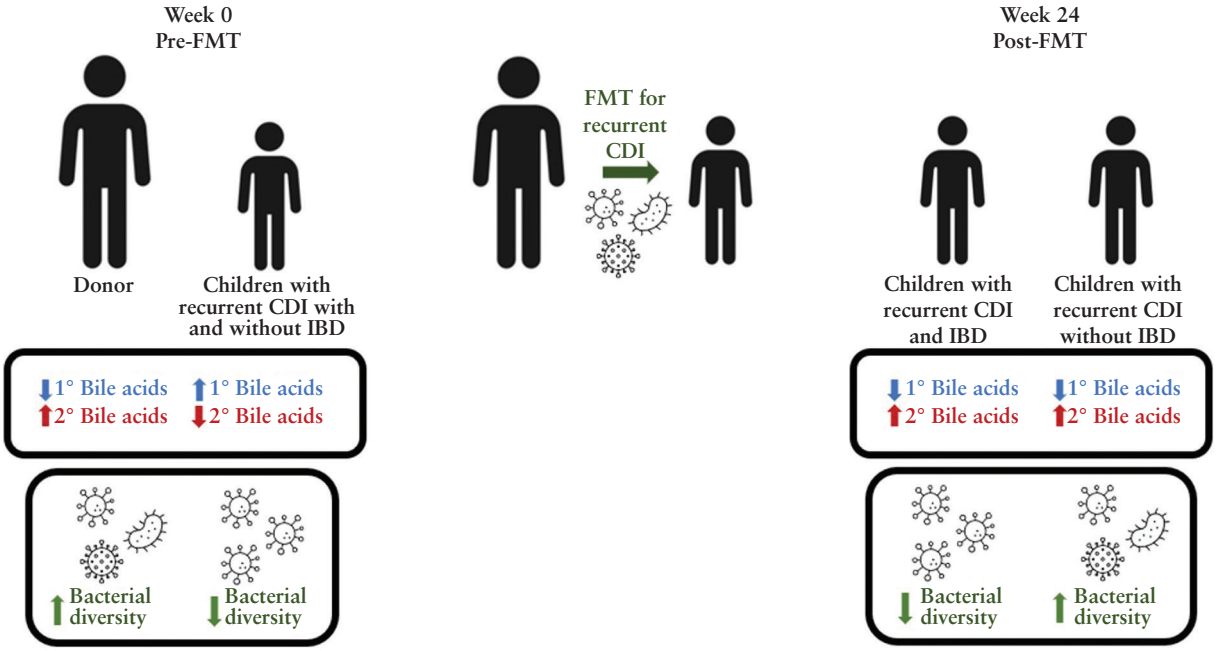
Methods: Eight children received FMT; five had underlying IBD. Primary and secondary faecal bile acids were measured by liquid chromatography–mass spectrometry in recipients [pre-FMT and longitudinally post-FMT for up to 6 months] and donors.

Results: Pre-FMT, recipients had higher primary and lower secondary bile acid proportions compared with donors. Post-FMT, there was a gradual increase of secondary and decrease of primary bile acids. Whereas gut bacterial diversity had been shown to be restored in all children shortly after FMT, normalisation of bile acids to donor levels occurred only by 6 months. In children with IBD, although microbiota diversity returned to pre-FMT levels within 6 months, secondary bile acids remained at donor levels.

Conclusions: The differences in bile acid profiles compared with gut bacterial diversity post-FMT suggests that interactions between the two may be more complex than previously appreciated and may contribute to FMT efficacy in different ways. This initial finding demonstrates the need to further investigate gut metabolites in larger cohorts, with longitudinal sampling to understand the mechanisms of FMT effectiveness.

Key Words: Clostridioides difficile; paediatric; metabolites

Graphical Abstract



Schematic diagram demonstrating bile acid composition changes in comparison with microbiome diversity changes in children with and without inflammatory bowel disease, receiving faecal microbiota transplantation for recurrent *C. difficile* infection.

1. Introduction

Clostridioides difficile [*C. difficile*] infection [CDI] is the leading cause of nosocomial diarrhoea in the USA and causes significant morbidity and mortality.[1] The risk of CDI recurrence ranges from 20% after an initial episode to up to 60% after multiple prior recurrences.[2] Faecal microbiota transplantation [FMT], the transfer of stool from one or more donors to a recipient, is an effective treatment for recurrent CDI. A recent meta-analysis of 45 studies including randomised controlled trials showed clinical improvement of 84% in those treated with a single FMT.[3] Studies have shown similar efficacy in paediatric patients, and FMT is part of the practice guidelines for the treatment of recurrent CDI.[4,5] FMT for recurrent CDI shifts the microbiome composition of recipients towards that of their respective donors while increasing bacterial diversity, but this is unlikely to explain the entire mechanism by which FMT works.[6–9] For instance, there are mixed results on whether alpha diversity, either before FMT or immediately afterwards, correlates with FMT effectiveness.[10–12]

Furthermore, studies of intestinal metabolites have shown their ability to alter the germination and growth of *C. difficile*. Clinical and *in vivo* animal studies have demonstrated that secondary bile acids can promote CDI resistance,[13–15] potentially mediating the durability of FMT effects. In particular the primary bile acid, taurocholic acid, is a known germinant of *C. difficile* and is often used as a used in its growth media.[16,17] Conversely secondary bile acids, lithocholic and ursodeoxycholic acids, have been shown to inhibit *C. difficile* germination *in vitro*.[18,19] Pre-FMT stool samples in adults have been shown to have higher levels of primary bile acids and lower levels of secondary bile acidsm whereas post-FMT samples show increased secondary bile acids to varying degrees, most often assessed at a single time point after FMT.[13,20,21] Parallel studies in children are lacking but are important to consider, as children differ from adults in their underlying microbiome composition and function and related metabolites.[22] For instance in young children, there is a predominance of primary bile acids, which could relate to the increased rates of *C. difficile* carriage at a young age.[23] Additionally the gut microbiome is dynamic with a rapid increase in gut microbial diversity in early childhood.[22,23] Furthermore, CDI is common in children with underlying IBD.[24] Whereas FMT is effective for CDI in children with or without IBD, the long-term microbiota dynamics after FMT are different in those with IBD compared with their non- IBD counterparts.[5,8,25] We therefore aimed to investigate longitudinal changes in the gut bile acid composition after FMT for CDI in children with and without IBD.

2. Case Report

Eight children, aged 6–17 years, received FMT as a treatment for recurrent CDI [Table 1]. All children received vancomycin, which was stopped 2 days prior to the FMT. Five of these children had underlying IBD, including four with Crohn's disease and one with ulcerative colitis [Table 1]. Stool samples were collected from patients as well as donors prior to the FMT. Donor stool was delivered via colonoscopy as previously described.[8] Following FMT, stool was collected from patients between Weeks 2–10, Weeks 10–20, and at 6 months [all three time points were available in three patients with IBD and in three patients without IBD]. After FMT, all eight patients had resolution of symptoms. There was eradication of *C. difficile*, tested by polymerase chain reaction [PCR] for Toxin B, in all eight patients at 10–20 weeks post-FMT and again for all subjects who provided samples at 6 months [$n = 6$]. No children received any antibiotics during the study period.

After acetonitrile extraction, faecal bile acids were assessed as their methyl ester derivatives by liquid chromatography–mass spectrometry using a Simultaneous

Ion Monitoring program. Proportions of primary [cholic and chenodeoxycholic] and secondary [deoxycholic and lithocholic] bile acids were determined for each sample. Differences in bile acid profiles between groups were compared using Student's t test. Patients had lower amounts of secondary bile acids at baseline [pre-FMT] compared with their donor counterparts [mean percentage for patients 31%, mean percentage for donors 95%, p <0.001, Figure 1A]. Overall, levels of secondary bile acids increased post-FMT [patient mean at baseline 35%, patient mean at 6 months 85%, p <0.01], reaching donor levels by 6 months [donor mean at baseline 95%, patient mean at 6 months 85%, Figure 1A].

After 6 months, although secondary bile acids increased in all children overall, this reached donor levels only in IBD patients [mean secondary bile acid percentage for IBD patients at 6 months 96% vs donors 95%, p = 0.77, Figure 1B] but not in non-IBD patients [mean secondary bile acid percentage for

Table 1. Subject demographics

				Donor	
Patient	**Comorbidities**	**IBD location**	**Medical therapy at FMT**	**Age [years]**	**Relation**
10	Crohn's disease	Pancolitis	Adalimumab Methotrexate Oral corticosteroids Mesalamine enemas	45	Father
15	Crohn's disease	Ileocolonic	Oral mesalamine	42	Mother
16	Crohn's disease	Ileocaecal	Oral corticosteroids	56	Father
17	Ulcerative colitis	Pancolitis	Oral mesalamine	50	Father
13	Crohn's disease	Ileocolonic	Infliximab	44	Mother
16	Postural orthostatic tachycardia syndrome	N/A	Fludrocortisone	24	Brother-in-law
6	Mitochondrial disease, caecostomy	N/A	None	36	Father
3	None	N/A	None	Unknown	OpenBiome

IBD, inflammatory bowel disease; FMT, faecal microbiota transplantation; N/A, not available.

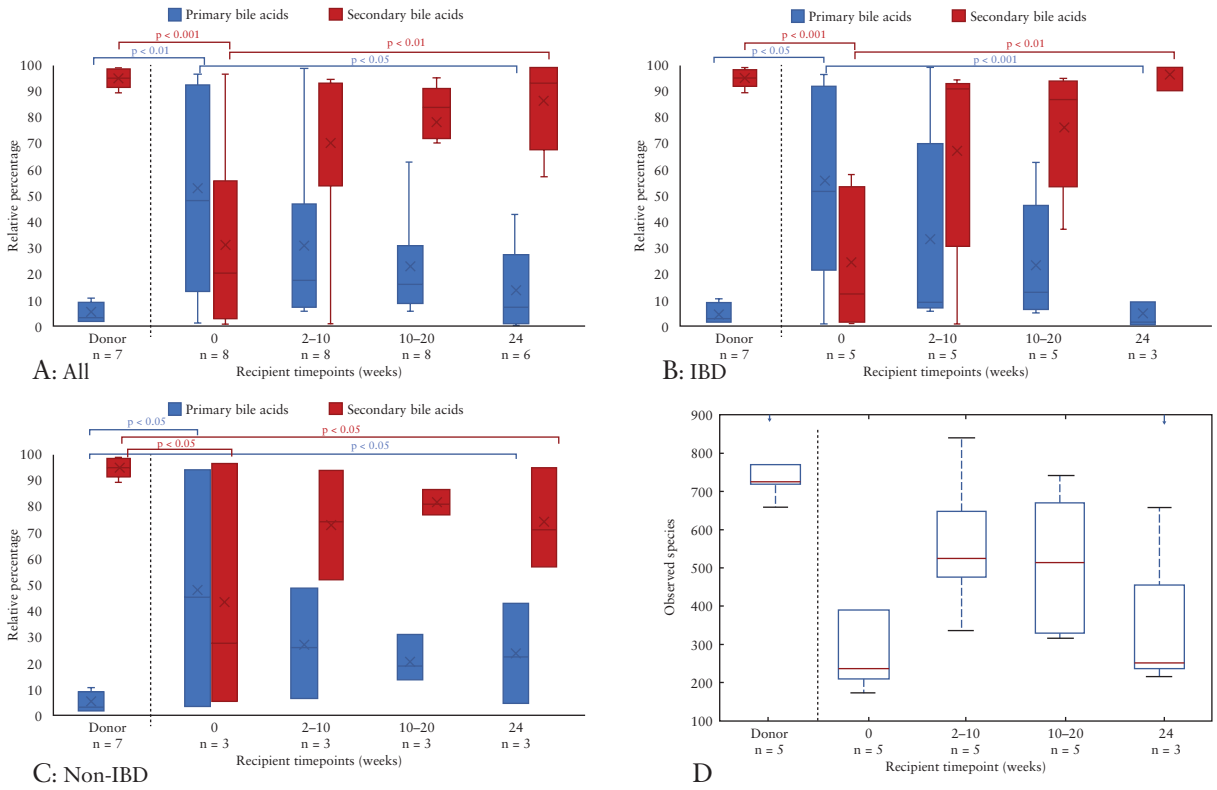


Figure 1 Box and whisker plots showing primary and secondary bile acid profiles following faecal microbiota transplantation [FMT] in children with recurrent *C. difficile* infection and healthy FMT donors in: A] all children, B] children with IBD and C] children without IBD. Statistically significant differences are labelled. D] Bacterial diversity [alpha diversity, observed species] following FMT in children with IBD with recurrent *C. difficile* infection and healthy FMT donors [adapted from Hourigan *et al.*[8]]. Pre-FMT, children with IBD had a lower diversity than healthy donors [p = 0.02]. 2–10 weeks post-FMT, diversity had increased to donor levels [no statistically significant difference between recipients and donors]. At 6 months post-FMT, diversity had decreased again to the pre-FMT baseline. IBD, inflammatory bowel disease.

non-IBD patients at 6 months 74% vs donors 95%, $p = 0.02$. Figure 1C]. Bile acid proportions did not differ by IBD status at baseline pre-FMT.

Interestingly, even though bile acid profiles normalised in patients with IBD, they had only transient restoration of their gut bacterial diversity and had reverted to their pre-FMT, low alpha diversity status at 6 months [Figure 1D; alpha diversity was measured by observed species, microbiome results as previously published.[8] Of note, in patients without IBD, gut bacterial diversity remained restored at 6 months. In comparison with bacterial diversity which normalised by 2–10 weeks in those with and without IBD, bile acid profiles took longer to normalise and remained significantly different from the donor's until the 6-month time point. This observation is somewhat limited by the small sample size at the 6-month time point.

3. Discussion

Over 6 months after FMT for recurrent CDI, paediatric patients had a progressive decrease in primary bile acids and correlating increase in secondary bile acids post-FMT, resulting in a bile acid pattern reflective of their healthy donors. Similar findings have been shown in adult studies after FMT, although few studies followed samples longitudinally.[13,20,21] Furthermore, this has not previously been examined in children. In the current study, despite only a transient change in bacterial diversity in children with IBD, their bile acid profiles became similar to their donors' profiles, and patients had continued eradication of CDI. This emphasises the importance of longitudinal follow-up with repeat sampling to understand the true dynamics of the microbiome and metabolome after FMT. The difference in the bile acid profile compared with that of the microbiota shows that the efficacy of FMT may go beyond restoration of gut microbiota.

Bile acids have a role in germination and growth of *C. difficile* with the primary bile acid, taurocholic acid, being a known germinant of the *C. difficile*.[16,17] We hypothesise that the presence of secondary bile acids, which are known to suppress *C. difficile* spore germination, may be a key factor in breaking the recurrent CDI cycle in patients following FMT.[26] Furthermore, bile acids may play a large role in the host's immune response to FMT, given their own antimicrobial and immunological effects, including bile acid receptor expression in innate immune cells.[27]

In patients with IBD, intestinal microbiome dysbiosis is a known issue related to underlying inflammation, and likely a risk factor for *C. difficile* infections.[28] Our findings of gradual normalisation of bile acids up to 6 months after FMT in IBD patients are particularly compelling, given that secondary bile acid metabolites, which are reduced in patients with IBD, appear to be directly involved with mucosal immune function.[29] Therefore, restoration of secondary bile acids may be a key mechanism of efficacy of FMT in treating *C. difficile* infection and even IBD, an ongoing line of research.[30]

Our study was limited by a small sample size. Two of the eight patients did not have samples at the 6 month follow-up, but samples were analysed from all patients at 10 weeks and 20 weeks post-FMT. Moreover, the patient population was somewhat heterogeneous, although bile acid changes were similar in all patients. All children were on vancomycin treatment until 2 days prior to FMT as per standard practice, and so it cannot be determined whether initial bile acid profiles were related to the disease or the antibiotics. Whereas this study focused on bile acids, it is recognised that other metabolites, such as short chain fatty acids, may also play an important role in the effectiveness of FMT, and this should be examined in future studies.[20]

In conclusion, the temporal difference in bile acid profile normalisation compared with microbial diversity restoration in children with IBD treated with FMT, suggests the efficacy of faecal microbiota transplant for CDI may possibly be an ongoing and dynamic process beyond just restoration of gut microbiota. This observation in our small patient cohort should be further investigated in a larger cohort, with longitudinal sampling, to better understand the mechanisms of FMT effectiveness.

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Conflict of Interest

CLS reports participating in a Ferring Advisory Board meeting. No authors have any conflicts of interest to disclose.

Author Contributions

Conceptualization: LAC, SKH, MO-H, CLS. Methodology: LAC, AR, NBJ. Investigation: all authhors. Data curation: LAC, AR, NBJ. Visualisation: LAC, AR, BDO'L. Supervision: LAC, SKH. Writing original draft: BDO'L, LAC, SKH. Writing review and editing: all authors.

References

1. Lessa FC, Mu Y, Bamberg WM, *et al*. Burden of Clostridium difficile infection in the United States. *N Engl J Med* 2015;**372**:825–34.
2. Alrahmany D, Ereshefsky BJ, El Nekidy WS, Harb G, Pontiggia L, Ghazi IM. Risk factors for recurrence of clostridioides difficile in hospitalized patients. *J Infect Public Health* 2021;**14**:1642–9.
3. Baunwall SMD, Lee MM, Eriksen MK, *et al*. Faecal microbiota transplantation for recurrent clostridioides difficile infection: an updated systematic review and meta-analysis. *EClinicalMedicine* 2020;**29–30**:100642.
4. McDonald LC, Gerding DN, Johnson S, *et al*. Clinical practice guidelines for Clostridium difficile infection in adults and children: 2017 update by the Infectious Diseases Society of America [IDSA] and Society for Healthcare Epidemiology of America [SHEA]. *Clin Infect Dis* 2018;**66**:987–94.
5. Nicholson MR, Mitchell PD, Alexander E, *et al*. Efficacy of fecal microbiota transplantation for Clostridium difficile infection in children. *Clin Gastroenterol Hepatol* 2020;**18**:612–9.e1.
6. Song Y, Garg S, Girotra M, *et al*. Microbiota dynamics in patients treated with fecal microbiota transplantation for recurrent Clostridium difficile infection. *PLoS One* 2013;**8**:e81330e81330.
7. Seekatz AM, Aas J, Gessert CE, *et al*. Recovery of the gut microbiome following fecal microbiota transplantation. *mBio* 2014;**5**:e00893–14.
8. Hourigan SK, Chen LA, Grigoryan Z, *et al*. 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.
9. Mullish BH, McDonald JAK, Pechlivanis A, *et al*. Microbial bile salt hydrolases mediate the efficacy of faecal microbiota transplant in the treatment of recurrent Clostridioides difficile infection. *Gut* 2019;**68**:1791–800.
10. Staley C, Kaiser T, Vaughn BP, *et al*. Predicting recurrence of Clostridium difficile infection following encapsulated fecal microbiota transplantation. *Microbiome* 2018;**6**:166.
11. Wei S, Bahl MI, Baunwall SMD, Dahlerup JF, Hvas CL, Licht TR. Gut microbiota differs between treatment outcomes early after fecal microbiota transplantation against recurrent Clostridioides difficile infection. *Gut Microbes* 2022;**14**:2084306.
12. Khanna S, Montassier E, Schmidt B, *et al*. Gut microbiome predictors of treatment response and recurrence in primary Clostridium difficile infection. *Aliment Pharmacol Ther* 2016;**44**:715–27.
13. Weingarden AR, Chen C, Bobr A, *et al*. Microbiota transplantation restores normal fecal bile acid composition in recurrent Clostridium difficile infection. *Am J Physiol Gastrointest Liver Physiol* 2014;**306**:G310–9.
14. Buffie CG, Bucci V, Stein RR, *et al*. Precision microbiome reconstitution restores bile acid mediated resistance to Clostridium difficile. *Nature* 2015;**517**:205–8.
15. Winston JA, Theriot CM. Impact of microbial derived secondary bile acids on colonization resistance against Clostridium difficile in the gastrointestinal tract. *Anaerobe* 2016;**41**:44–50.
16. Sorg JA, Sonenshein AL. Bile salts and glycine as cogerminants for Clostridium difficile spores. *J Bacteriol* 2008;**190**:2505–12.
17. Wilson KH. Efficiency of various bile salt preparations for stimulation of Clostridium difficile spore germination. *J Clin Microbiol* 1983;**18**:1017–9.
18. Sorg JA, Sonenshein AL. Inhibiting the initiation of Clostridium difficile spore germination using analogs of chenodeoxycholic acid, a bile acid. *J Bacteriol* 2010;**192**:4983–90.
19. Thanissery R, Winston JA, Theriot CM. Inhibition of spore germination, growth, and toxin activity of clinically relevant C. difficile strains by gut microbiota derived secondary bile acids. *Anaerobe* 2017;**45**:86–100.
20. Brown JR, Flemer B, Joyce SA, *et al*. Changes in microbiota composition, bile and fatty acid metabolism, in successful faecal microbiota transplantation for Clostridioides difficile infection. *BMC Gastroenterol* 2018;**18**:131.
21. Seekatz AM, Theriot CM, Rao K, *et al*. Restoration of short chain fatty acid and bile acid metabolism following fecal microbiota transplantation in patients with recurrent Clostridium difficile infection. *Anaerobe* 2018;**53**:64–73.
22. Yatsunenko T, Rey FE, Manary MJ, *et al*. Human gut microbiome viewed across age and geography. *Nature* 2012;**486**:222–7.
23. Cheng S, Zhu L, Faden HS. Interactions of bile acids and the gut microbiota: learning from the differences in Clostridium difficile infection between children and adults. *Physiol Genomics* 2019;**51**:218–23.
24. Martinelli M, Strisciuglio C, Veres G, *et al*.; Porto IBD Working Group of European Society for Pediatric Gastroenterology, Hepatology and Nutrition [ESPGHAN]. Clostridium difficile and pediatric inflammatory bowel disease: a prospective, comparative, multicenter, ESPGHAN study. *Inflamm Bowel Dis* 2014;**20**:2219–25.
25. Nicholson MR, Alexander E, Ballal S, *et al*.; North American Society of Pediatric Gastroenterology and Nutrition Faecal Microbiota Transplantation Special Interest Group. Efficacy and outcomes of faecal microbiota transplantation for recurrent *Clostridioides difficile* infection in children with inflammatory bowel disease. *J Crohns Colitis* 2022;**16**:768–77.
26. Theriot CM, Bowman AA, Young VB. Antibiotic-induced alterations of the gut microbiota alter secondary bile acid production and allow for clostridium difficile spore germination and outgrowth in the large intestine. *mSphere* 2016;**1**:e00045-15–e00045-15.
27. Fiorucci S, Biagioli M, Zampella A, Distrutti E. Bile acids activated receptors regulate innate immunity. *Front Immunol* 2018;**9**:1853.
28. Aldars-Garcia L, Marin AC, Chaparro M, Gisbert JP. The interplay between immune system and microbiota in inflammatory bowel disease: a narrative review. *Int J Mol Sci* 2021;**22**:3076.
29. Bromke MA, Krzystek-Korpacka M. Bile acid signaling in inflammatory bowel disease. *Int J Mol Sci* 2021;**22**:9096.
30. El Hage Chehade N, Ghoneim S, Shah S, *et al*. Efficacy of fecal microbiota transplantation in the treatment of active ulcerative colitis: a systematic review and meta-analysis of double-blind randomized controlled trials. *Inflamm Bowel Dis* 2022. Online ahead of print.