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Citation for final published version:

Mullish, B. H., Marchesi, Julian Roberto , Thursz, M. R. and Williams, H. R. T. 2015. Microbiome manipulation with faecal microbiome transplantation as a therapeutic strategy in *Clostridium difficile* infection. *QJM: An International Journal of Medicine* 108 (5) , pp. 355-359. 10.1093/qjmed/hcu182

Publishers page: <http://dx.doi.org/10.1093/qjmed/hcu182>

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The Potential of Microbiome Manipulation as a Therapeutic Strategy in *Clostridium difficile* Infection.

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Summary:

Faecal microbiome transplantation (FMT) has generated huge recent interest, as it presents a potential solution to a significant clinical problem - the increasing incidence of *Clostridium difficile* infection. In the short term, however, there remain many practical questions regarding its use, including the optimal selection of donors, material preparation and the mechanics of delivery. In the longer term, enhanced understanding of the mechanisms of action of FMT may potentiate novel therapies, such as targeted manipulation of the microbiome in *C. difficile* infection and beyond.

Introduction:

The concept of 'colonisation resistance' - the ability of the healthy gut microbiome to inhibit colonisation and overgrowth by invading microorganisms – has been recognised for over forty years¹. It is similarly well-established that perturbation of the gut microbiome, or 'dysbiosis' (as may occur in response to antibiotics, along with other triggers) disrupts colonisation resistance, with *Clostridium difficile* infection (CDI) with associated diarrhoea being the archetypal clinical manifestation.

Limitations of the current antibiotic treatment for CDI have driven the search for novel treatments, with one option being faecal microbiome transplantation (FMT), i.e. generation of a liquidised bacterial suspension from the faeces of healthy donors, and delivery of this into the gastrointestinal (GI) tract of affected patients. Assessment of FMT in the setting of CDI has demonstrated that this is a viable treatment option.

The recognition that dysregulation of the gut microbiome is characteristic not just of CDI but a wide variety of human diseases² raises the possibility that manipulation of the composition or function of the gut microbiome could develop beyond CDI to be used more broadly as a therapeutic strategy.

***Clostridium difficile* infection: a global problem:**

Clostridium difficile infection ranges in clinical severity from mild diarrhoea to the life-threatening states of pseudomembranous colitis and toxic megacolon. Whilst the increasing impact of CDI over the past 15 years has been felt globally (with antibiotic use **being** the predominant risk factor), the burden has been greatest in Europe and North America³. One major factor contributing to this has been the arrival of newer, more virulent and increasingly antibiotic-resistant strains, such as NAP1/ribotype 027. Whilst CDI acquisition still occurs most commonly in healthcare facilities, there has been increasing recognition of community-associated CDI, even amongst conventionally low risk groups such as children⁴.

Standard therapy for CDI involves metronidazole for mild disease, and vancomycin for severe or recurrent CDI (with pulsed/ tapered regimens typically being used in recurrent disease⁵). Worryingly, however, the response to metronidazole has declined from approximately 90% to 70% over the past decade⁶. A further serious concern has been the increasing recognition of recurrent CDI. Recurrence occurs in approximately 20% of patients treated initially with either metronidazole or vancomycin⁷; the risk of further recurrence increases to 40% after a first recurrence, rising to 60-70% after more than two recurrences⁸. The presence of just three clinical criteria (age > 65 years, severe disease and

continued use of antibiotics after treating the initial CDI episode) are predictive of an almost 90% relapse rate⁹.

A number of different approaches have been proposed to address this problem, including intravenous immunoglobulin, probiotics, toxin binding and new antibiotics. An example of the latter is fidaxomicin, a macrocyclic antibiotic of narrow spectrum that is now approved for the treatment of CDI in Europe and North America following the outcomes of two randomised controlled trials. However, studies to date have not investigated the efficacy of fidaxomicin in cases of recurrent CDI, and alternative therapeutic strategies have been proposed.

Faecal microbiome transplantation (FMT):

i. Efficacy:

The recognition of CDI as a condition representing the loss of colonisation resistance through antibiotic-associated gut dysbiosis prompted the hypothesis that reconstitution of the normal gut microbiota with FMT could be an effective therapeutic strategy. Many different techniques for the provision of FMT have been described, all with similar principles: collection of stool from a healthy donor (who has undergone screening for transmissible infections and has not recently used antibiotics); homogenisation of stool (often in a domestic blender) and filtration of large particulate matter; and administration of the slurry into either the upper GI tract (via nasogastric or nasoduodenal tube), or the lower GI tract (via enema or colonoscopy).

At present, FMT to treat CDI has been described for over 500 patients in the literature, with efficacy rates of over 90%. The time from receiving FMT until response is variable; a median time to resolution of one day was reported in a cohort receiving colonoscopic FMT¹⁰. Although uncontrolled case series of FMT for CDI have been reported for over a decade, the first randomised controlled trial comparing FMT to standard therapy was published only recently¹¹. Patients with recurrent CDI were randomised to one of three treatment arms: vancomycin 500mg four times daily for four or five days followed by bowel lavage and then FMT; standard vancomycin therapy (500mg four times daily for 14

days); or standard vancomycin therapy with bowel lavage. The primary outcome was resolution of diarrhoea without relapse at 10 weeks. FMT consisted of at least 50g of fresh stool from donors unrelated to the recipient that was blended with 500 millilitres of normal saline and filtered before immediate administration via a nasoduodenal tube. Trial participants who failed to respond in their initial treatment arm were offered FMT “off protocol”. The trial was stopped early (after randomising 42 patients) following an interim analysis which demonstrated significantly improved outcomes from FMT compared to other treatment arms, with cure rates of 89% in the FMT group (94% after two infusions), 31% in the standard vancomycin group, and 23% in the vancomycin-bowel lavage group.

ii. Safety and acceptability:

One major concern regarding FMT has been the potential for transmission of infectious diseases from donor to recipient, **although no such cases have been reported**. As such, donor risk assessment through clinical, social and travel assessment – along with blood and stool screening for transmissible diseases - has been recommended¹² and widely-instigated (**Table 1**).

FMT appears to be well-tolerated with few significant side effects. In the trial of van Nood *et al*, the most common side effects included diarrhoea, cramping and belching, consistent with other studies¹¹. Symptoms tended to resolve quickly without specific intervention. Aspiration was not observed when 500 millilitres of solution was infused over approximately 20 minutes into these patients¹¹. No significant adverse sequelae have been reported in FMT recipients over longer term follow up¹⁰.

Poor acceptability of FMT to patients has been a concern, but this is not borne out in practice. Many patients with recurrent CDI (and other conditions for which FMT has been proposed as therapy¹³) actively seek out FMT providers, often via online forums. Furthermore, those who received FMT generally found the procedure acceptable; 97% of patients who had undergone FMT for recurrent CDI reported willingness to undergo further FMT if required, with 53% stating that they would choose FMT as first-line treatment before antibiotics¹⁰.

iii. Practical aspects of FMT administration:

Recruitment and screening of stool donors can be a difficult and expensive process. Therefore, the ability to use pre-screened 'universal donors' - who have provided stool that can be processed into FMT in advance, then frozen prior to thawing on the day of use - is attractive. A standardised protocol for frozen FMT preparation (using glycerol as a cryopreservative) has been recently reported¹⁴, with efficacy against recurrent CDI described of at least 90% from colonoscopic FMT using both fresh and frozen stool.

The mechanics of preparing the solution have varied between centres. Typically 50 – 60g of stool is homogenised with 250 – 300ml of diluent. Saline, water and even milk have all been used successfully as diluents. Most centres administer large-volume bowel lavage prior to FMT, often regardless of the route of administration (to remove residual clostridial organisms and any antibiotic remnants). Recipients typically stop antibiotics anywhere between one and three days prior to the transplant, although this has not been compared in a trial setting to continuing antibiotics up to or even after the procedure. It has become conventional practice to administer loperamide prior to colonoscopic administration (to aid retention), and a proton pump inhibitor prior to upper GI administration (to minimise gastric acidity). Whether upper or lower GI administration of FMT is more efficacious has been recently addressed in an open-label randomised controlled trial¹⁵; there was no significant difference in outcome between administration colonoscopically or via a nasogastric tube.

iv. A viable treatment option:

Consensus guidelines with regards to the role of FMT in CDI treatment have recently been published and broadly adopted¹² (**Table 2**). Some authorities argue that FMT should be considered early in the clinical course of CDI, and even as first-line therapy¹⁶. Experience of the use of FMT in severe CDI is more limited, but it appears effective in this setting¹⁷. Recent data suggest that FMT is of similar efficacy in immunocompromised patients, with no additional risk of infectious complications⁶.

FMT has been shown to be more cost-effective than other treatment modalities for recurrent CDI¹⁸. Based on the available evidence, FMT is now recommended as treatment for recurrent CDI in professional guidelines both from the USA⁵ and the UK, where FMT for recurrent CDI was recently advocated for use by NICE¹⁹.

Gut microbiome in human disease: from FMT to novel therapies:

Recent research using molecular techniques (including sequencing of 16S rRNA genes and metabolic profiling platforms) has identified distinctive alterations in the composition and function of the gut microbiome accompanying a wide range of human diseases^{2, 20}. Whilst many of these are primary GI/liver conditions (including inflammatory bowel disease, colorectal cancer and non-alcoholic fatty liver disease), many are not, including obesity, diabetes, and even neurological conditions. Whether such dysregulation of the gut microbiome in disease states is causal or consequential remains largely unclear, although there is certainly now some evidence for the former: for instance, obese and lean phenotypes can be induced in germ-free mice by transfer of faecal microbiota from human twins discordant for obesity²¹. Similarly, men with metabolic syndrome who received FMT from lean male donors demonstrated an increase in gut microbial diversity and improvement in peripheral insulin sensitivity, when neither of these changes were seen when these individuals received FMT of their own processed stool²².

Such findings have clear implications for the screening of FMT donors; potential donors with any of the conditions in which gut microbiome dysregulation have been consistently linked are typically excluded. Additionally, given that FMT has demonstrated that sustainable alterations in the gut microbiome are achievable, the manipulation of the gut microbiome's structure (or modification of its functional activity) has been highlighted as a potential new mechanism of therapeutic intervention for a broader range of diseases²³.

If 'microbiome therapeutics' are truly to represent a novel treatment modality, then the means by which such therapy may be optimally administered must be established. FMT has already been used

as an experimental treatment for a number of conditions other than CDI, although the results to date have been highly variable²⁴. Furthermore, FMT in itself clearly has significant drawbacks (not least its unpalatable nature), and other techniques of achieving manipulation of the microbiome merit exploration. One favoured idea is the administration of a 'defined microbiome ecosystem' of selectively-cultured bacterial strains (ideally either as a drink or an oral capsule containing a mixture of lyophilised bacteria) that target specific dysregulated components of the gut microbiome²⁵. An alternative strategy may be to design drugs that modulate microbial signalling or enzymatic activities and alter host metabolism²³.

Conclusion:

FMT is gaining widespread acceptance as a viable treatment option for CDI. Ongoing trials will help to clarify the uncertainties that still exist regarding the optimal means of administration. The recent identification of gut microbiome dysregulation as a feature of a broad range of diseases has raised the possibility that the success of FMT for CDI may be transferrable to other conditions, although the potential contribution of the microbiome to the pathogenesis of many of these diseases is much less well characterised than in CDI. Mechanisms of manipulating the gut microbiome in a more targeted way than FMT are clearly of great potential interest. The key next step is to understand the mechanism by which FMT exerts its efficacy in CDI, and further to explore the interaction between the gut microbiome and host metabolism in both health and disease states (and the factors that influence this, including diet and antibiotic use). The true potential of 'microbiome therapeutics' may then begin to be realised.

Conflicts of interest: None.

Funding: This work was supported by the National Institute of Health Research (NIHR) Biomedical Research Centre based at Imperial College Healthcare NHS Trust and Imperial College London, and by the Imperial College Healthcare Charity [grant number 141516].

Figures:

Table 1: Screening protocol for transmissible diseases for potential donors to an FMT programme.

This is the screening protocol currently used in the programme at Imperial College London; assays are repeated six monthly. In addition, donors complete detailed questionnaires regarding their medical, family, and medication history when being considered as donors, and questionnaires regarding recent symptoms suggestive of GI disease or infection (as well as recent travel) when donating stool for processing into FMT.

Table 2: Conventional indications and contraindications for use of FMT in the treatment of

***Clostridium difficile* infection:** (Adapted from ¹²). As described in the text, arguments have been made for using FMT earlier in the clinical course of CDI¹⁶, and that the concerns regarding the risk of FMT to treat CDI in immunosuppressed states may be overestimated⁶.

References:

1. Lawley TD, Walker AW. Intestinal colonization resistance. *Immunology* 2013; 138(1): 1 – 11.
2. Holmes E, Li JV, Athanasiou T, Ashrafian H, Nicholson JK. Understanding the role of gut microbiome-host metabolic signal disruption in health and disease. *Trends Microbiol* 2011; 19(7): 349 – 359.
3. Freeman J, Bauer MP, Baines SD, Corver J, Fawley WN, Goorhuis B, Kuijper EJ, Wilcox MH. The changing epidemiology of *Clostridium difficile* infections. *Clin Microbiol Rev* 2010; 23(3): 529 - 549.
4. Khanna S, Pardi DS, Aronson SL, Kammer PP, Orenstein R, St Sauver JL, Harmsen WS, Zinsmeister AR. The epidemiology of community-acquired *Clostridium difficile* infection: a population-based study. *Am J Gastroenterol* 2012; 107(1): 89 – 95.

5. Surawicz CM, Brandt LJ, Binion DG, Ananthakrishnan AN, Curry SR, Gilligan PH, McFarland LV, Mellow M, Zuckerbraun BS. Guidelines for diagnosis, treatment, and prevention of *Clostridium difficile* infections. *Am J Gastroenterol* 2013; 108(4): 478 – 498.
6. Kelly CR, Ihunnah C, Fischer M, Khoruts A, Surawicz C, Afzali A, Aroniadis O, Barto A, Borody T, Giovannelli A, Gordon S, Gluck M, Hohmann EL, Kao D, Kao JY, McQuillen DP, Mellow M, Rank KM, Rao K, Ray A, Schwartz MA, Singh N, Stollman N, Suskind DL, Vindigni SM, Youngster I, Brandt L. Fecal microbiota transplant for treatment of *Clostridium difficile* infection in immunocompromised patients. *Am J Gastroenterol* 2014; 109(7): 1065 – 1071.
7. Pépin J, Routhier S, Gagnon S, Brazeau I. Management and outcomes of a first recurrence of *Clostridium difficile*-associated disease in Quebec, Canada. *Clin Infect Dis* 2006; 42(6): 758 – 764.
8. McFarland LV, Surawicz CM, Rubin M, Fekety R, Elmer GW, Greenberg RN. Recurrent *Clostridium difficile* disease: epidemiology and clinical characteristics. *Infect Control Hosp Epidemiol* 1999; 20(1): 43 – 50.
9. Hu MY, Katchar K, Kyne L, Maroo S, Tummala S, Dreisbach V, Xu H, Leffler DA, Kelly CP. Prospective derivation and validation of a clinical prediction rule for recurrent *Clostridium difficile* infection. *Gastroenterology* 2009; 136(4): 1206 – 1214.
10. Brandt LJ, Aroniadis OC, Mellow M, Kanatzar A, Kelly C, Park T, Stollman N, Rohlke F, Surawicz C. Long-term follow-up of colonoscopic fecal microbiota transplant for recurrent *Clostridium difficile* infection. *Am J Gastroenterol* 2012; 107(7): 1079 – 1087.
11. van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, Visser CE, Kuisper EJ, Bartelsman JF, Tijssen JG, Speelman P, Dijkgraaf MG, Keller JJ. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. *N Engl J Med* 2013; 368(5): 407 – 415.

12. Bakken JS, Borody T, Brandt LJ, Brill JV, Demarco DC, Franzos MA, Kelly C, Khoruts A, Louie T, Martinelli LP, Moore TA, Russell G, Surawicz C; Fecal Microbiota Transplantation Workgroup. Treating *Clostridium difficile* infection with fecal microbiota transplantation. *Clin Gastroenterol Hepatol* 2011; 9(12): 1044 – 1049.
13. Swaminath A. The power of poop: patients getting ahead of their doctors using self-administered fecal transplants. *Am J Gastroenterol* 2014; 109(5): 777 – 778.
14. 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(5): 761 – 767.
15. Youngster I, Sauk J, Pindar C, Wilson RG, Kaplan JL, Smith MB, Alm EJ, Gevers D, Russell GH, Hohmann EL. Fecal microbiota transplant for relapsing *Clostridium difficile* infection using a frozen inoculum from unrelated donors: a randomized, open-label, controlled pilot study. *Clin Infect Dis* 2014; 58(11): 1515 – 1522.
16. Brandt LJ, Borody TJ, Campbell J. Endoscopic fecal microbiota transplantation: “first-line” treatment for severe *Clostridium difficile* infection? *J Clin Gastroenterol* 2011; 45(8): 655 – 657.
17. Weingarden AR, Hamilton MJ, Sadowsky MJ, Khoruts A. Resolution of severe *Clostridium difficile* infection following sequential fecal microbiota transplantation. *J Clin Gastroenterol* 2013; 47(8): 735 – 737.
18. Konijeti GG, Sauk J, Shrime MG, Gupta M, Ananthakrishnan AN. Cost-effectiveness of competing strategies for management of recurrent *Clostridium difficile* infection: a decision analysis. *Clin Infect Dis* 2014; 58(11): 1507 – 1514.

19. NICE. Faecal microbiota transplant for recurrent clostridium difficile infection, interventional procedure guidance IPG 485, 2014, <http://guidance.nice.org.uk/IPG485>.
20. Nicholson JK, Holmes E, Kinross J, Burcellin R, Gibson G, Jia W, Pettersson S. Host-gut microbiota metabolic interactions. *Science* 2012; 336(6086): 1262 – 1267.
21. Ridura VK, Fiath JJ, Rey FE, Cheng J, Duncan AE, Kau AL, Griffin NW, Lombard V, Henrissat B, Bain JR, Muehlbauer MJ, Ilkayeva O, Semenkovich CF, Funai K, Hayashi DK, Lyle BJ, Martini MC, Ursell LK, Clemente JC, Van Treuren W, Walters WA, Knight R, Newgard CB, Heath AC, Gordon JI. Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science* 2013; 341(6150): 1241214.
22. Vrieze A, van Nood E, Holleman F, Salojärvi J, Kootte RS, Bartelsman JFWM, Dallinga-Thie GM, Ackermans MT, Serlie MJ, Oozeer R, Derrien M, Druesne A, Van Hylckama Vlieg JET, Bloks VW, Groen AK, Heilig HGJ, Zoetendal EG, Stoes ES, de Vos WM, Hoekstra JBL, Nieuwdorp M. Insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* 2012; 143(4): 913 – 916.
23. Holmes E, Li JV, Marchesi JR, Nicholson JK. Gut microbiota composition and activity in relation to host metabolic phenotype and disease risk. *Cell Metab* 2012; 16(5): 559 – 564.
24. Borody TJ, Brandt LJ, Paramsothy S. Therapeutic faecal microbiota transplantation: current status and future developments. *Curr Opin Gastroenterol* 2014; 30(1): 97 – 105.
25. Petrof EO, Khoruts A. From stool transplants to next-generation microbiota therapeutics. *Gastroenterology* 2014; 146(6): 1573 – 1582.