Fecal transplantation: passing fashion or here to stay?

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ABSTRACT

Several trillions of bacteria, distributed among more than 1,000 species, are natural inhabitants of the human intestinal tract and constitute what is now known as the gut microbiota. Although its composition varies within and between individuals with age, diet, and health status, it is becoming increasingly recognized that imbalances in the bacterial microbiota (dysbiosis) are linked to a number of conditions such as antibiotic-associated diarrhea, inflammatory bowel disease, and obesity, among others. Fecal transplantation where a preparation of stool from a microbiologically screened donor is administered into the colon of an affected recipient has been shown to be highly effective for the treatment of recurrent *Clostridium difficile* infection. Several trials of this therapy are now underway for gut dysbiosis in a number of patient disease groups raising concerns on the risk of transmission of infectious agents from donor to recipient, possible long-term adverse consequences of treatment, and effective regulation of the stool material used for the procedure. A worrying aspect is the emergence of private stool banks providing samples to the general public for self-administration.

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THE GUT MICROBIOME

Bacterial cells outnumber human cells in the healthy individual by at least ten-fold and reside in complex communities (microbiomes) on skin, and internally within the oral, respiratory, genital, and gastrointestinal (GI) tracts. The vast majority play a defensive role and contribute to maintaining health by a variety of means chiefly, the occupation of anatomical niches to deter overgrowth of pathogenic species, and involvement in immunity, metabolism, and synthesis of essential nutrients[1].

The link between health and diet has long been recognized but it is in the last 50 years that our understanding of the complexity of the human intestinal microbiome and its relationship with health and disease has become clearer. Apart from natural physiological roles including immune system maturation and energy metabolism, evidence continues to accumulate directly implicating changes in gut microbiota with several pathological conditions such as colorectal cancer, inflammatory bowel disease, and obesity[2]. Indeed, in colon cancer, apart from their role as possible infectious agents, there is evidence that microbial metabolites of diet can also act as epigenetic activators of gene expression that may influence cancer risk in humans[3]. Moreover, perhaps counterintuitively, there is an increasing number of reports linking the gut microbiome with several other conditions (table 1) ranging from depression and anxiety[4], to increased risk of cardiovascular disease as a consequence of phosphatidylcholine metabolism by gut bacteria[5].

Table 1: Conditions linked to imbalance of fecal microbiota

C. difficile diarrhoea and pseudomembranous colitis
Irritable bowel
Ulcerative colitis
Crohn's
Coeliac
Colorectal cancer
Cardiovascular
Neurological (Parkinson's, multiple sclerosis)
Diabetes
Obesity

Relatively few bacterial species are able to live in the acid environment of the stomach but numbers increase by several orders of magnitude further along the GI tract with the result that bacterial cells constitute approximately 60% of normal fecal mass (figure 1). This is a complex community of archaeal and primarily bacterial phyla (evolutionary related taxonomic groups), representative of an estimated 1,150 unique bacterial species, and each individual may harbor >160 of these species[6], but with considerable variation between them. The great majority of the bacteria are strict anaerobes, which require specialized techniques for their isolation and identification. However, the recent advent of high throughput deep sequencing-based methods targeting the phylogenetically-informative 16S rRNA gene has revolutionized our understanding of the diversity, complexity and dynamics of the gut microbiota and provided fundamental insights into their role in health and disease[7].

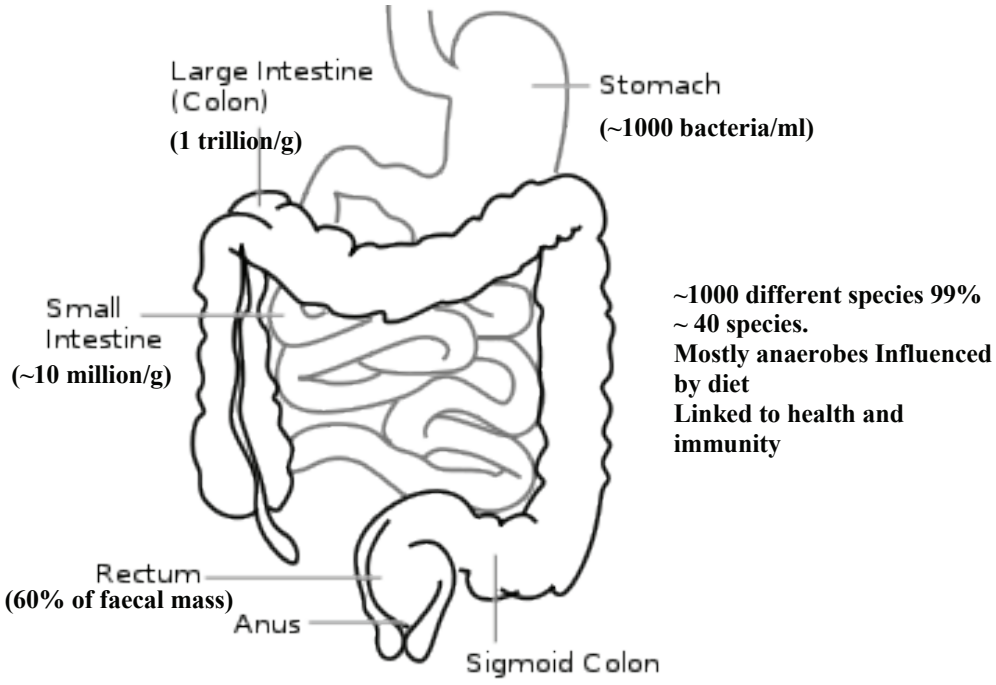

Figure 1. Distribution of bacteria in the normal human gut.

Four phyla predominate in the GI tract. Firmicutes (genera including *Ruminococcus, Clostridium, Lactobacillus*) and Bacteroidetes (*Bacteroides, Prevotella, Xylanibacter*) account for about 90% of the flora, with Actinobacteria (*Bifidobacterium*), and Proteobacteria (coliforms) in the minority – although the latter group constitute the majority of organisms readily grown from fecal *specimens* in diagnostic microbiology laboratories. The myriad of species exhibit a broad range of substrate utilization of carbohydrates, amino acids, and short chain fatty acids, and are essential for vitamin metabolism as well as harvesting of energy from diet[8]. At this taxonomic level, the gut microbiome exhibits extensive conservation but differs more markedly at genus and species level in individuals which may not only be linked with diet but also with age and underlying health. Disturbance of the microbiota as a result of antimicrobial therapy or other factors may lead to dysbiosis (imbalance) of the microbiota and an associated risk of intestinal infection with *Clostridium difficile*[9], and other inflammatory diseases.

C. difficile infection

C. difficile is a Gram-positive, anaerobic, spore-forming bacillus which is a normal resident of the large intestine in about 3% of individuals. *C. difficile* infection (CDI) is manifest as a profuse and sometimes life-threatening diarrhea typically in elderly patients on broad-spectrum antibiotic therapy[10], but also on occasion in young healthy individuals without prior exposure to antibiotics. Spores of the organism contaminate the near environment of patients and are able to persist for long periods and thereby spread infection in nosocomial outbreaks. There is evidence of widely prevalent hypervirulent clonal types (NAP 1/ ribotype O27 and NAP7/ribotype O78) in hospitals in several countries[11] which is associated with increased mortality but community- acquired CDI caused by diverse strain genotypes appears to have arisen owing to increased exposure of the public to antibiotics.

Surveillance reports indicate that CDI was responsible for about 1600 and 14,000 deaths in the UK and USA respectively in 2012 (Health Protection Agency, UK; Centers for Disease Control and Prevention, USA). In the UK, mortality rates currently appear to be decreasing from their previous 5 years peak, which may be due to better infection control practice and more effective prescribing of antibiotics. CDI appears to be less frequent in Latin America[12] though this might be either a consequence of a lack of diagnostic testing or the absence of epidemic clonal types in hospitals and community, or both.

Conventional treatment for severe CDI is a combination of oral vancomycin and metronidazole[13], and bowel lavage, but relapse occurs in about 1 in 4 cases, with repeated relapses in some individuals. Other less studied approaches to treatment include novel antibiotics, probiotics, intravenous immunoglobulin, and more recently, fecal transplantation. Fidaxomicin is a novel narrow-spectrum macrocyclic antibiotic, which is approved in the USA and Europe for the treatment of CDI. It lacks activity against other major anaerobes in the gut such as *Bacteroides* spp. and *Prevotella* spp. and is less disruptive of the microbiota than vancomycin[14], but clinical outcomes with fidaxomicin are not better than those with vancomycin treatment.

Fecal microbiota transplantation

CDI

Disruption of the cycle of overgrowth of C. difficile in the gut of patients with symptomatic infections has been attempted by the oral administration of probiotics with a single bacterial species, but with little success[15]. An alternative approach to restore the balance and diversity of the gut microbiota and so attempt to reverse the dysbiosis is to introduce fecal material taken from a healthy person directly into the gut of the patient. The concept of administering fecal preparations as therapy dates back to the 4th century in China where it was used as a treatment for diarrhea in humans[16]. It was subsequently used in the 17th century for the treatment of ruminants and more lately for *Salmonella* infection in commercially reared poultry[17]. However, it was not until 1958 that a fecal enema was used successfully to treat four patients with pseudomembranous colitis, predating the recognition of *C. difficile* as the cause of this disease[18]. Since then, several sporadic case reports of fecal microbiota therapy (FMT) began to appear in the literature but it was the cardinal study of van Nood et al. in 2013[19] which provided strong evidence of its efficacy for the treatment of CDI. This study randomized 43 patients with recurrent CDI, the test group received antibiotics along with a filtered fecal extract from a volunteer donor via a nasogastric tube and the control group received antibiotics alone. FMT was found to be more

than twice as effective in resolving symptoms as antibiotics alone and this finding resulted in early cessation of the trial. However, the statistical validity of their conclusions has been questioned by others chiefly on the grounds of unintended ascertainment bias through inequalities among the treatment groups[20], and the relatively small sample size which may obscure the effects of serious adverse events in a minority of the patients[21]. Several other non- randomized studies have reported similarly high (~ 90%) success rates for CDI treatment[22].

Other conditions

It is well established that many patients with Crohn's disease and ulcerative colitis have significant imbalances in their gut microbiota composition – particularly a deficiency of the phyla Bacteroidetes and Firmicutes[23]. Several case reports in the literature affirm that FMT is of potential benefit for the treatment of ulcerative colitis (UC) where standard treatments have failed[24]; see also, http://clinicaltrials.gov for registered clinical FMT trials for UC in progress (June 2014) in the USA. A review of 21 UC cases over 25 years treated with FMT by Borody[25], a pioneer in the field, reported that 57% exhibited normal histologically uninflamed mucosa followed up by repeat colonoscopy for a mean of 33 months. However, in Borody's experience, Crohn's patients generally showed little or no sustained response to FMT.

To date, there is a lack of supportive evidence beyond case anecdotes for the benefit of FMT in inflammatory bowel syndrome, metabolic syndrome, diabetes, and obesity. However, with regard to obesity, it has recently been demonstrated that gut bacteria from lean or obese humans induce similar phenotypes when introduced into the gut of mice; bacteria from lean donors reduced formation of adipose fat in the obese recipient if the mice were given an appropriate diet[26]. These findings taken together with the apparent correlation of increased blood insulin sensitivity and colonic fermentation products, mainly short chain fatty acids, and gut bacterial composition of some obese individuals[27,28] will no doubt serve to stimulate wider interest in the potential of FMT as a treatment for this condition.

Safety and other issues

Given the increasing interest in the use of FMT to correct perceived imbalances in gut microbiota several key safety and practical issues need to be addressed by the scientific and medical community [29]. Chief among these are i) transmission of recognized, and more importantly, hitherto unrecognized infectious agents, ii) association of certain species with syndromes linked to gut microbiota, iii) nature of fecal sample, anaerobic storage and bacterial viability iv) mode of administration to recipient, v) monitoring of efficacy, and vi) effective regulation and control of use.

Donors - The composition of the gut microbiota of an individual is strongly linked to diet, hygiene, underlying health, and possibly genetic factors, but a common microbial core comprising three basic enterotypes characterized by the proportions of the genera *Bacteroides, Prevotella or Ruminococcus* has been proposed[30]. As some syndromes appear to be associated with specific imbalances in composition, it follows therefore that not all donor samples would be equally as effective as therapy. Some FMT trials have attempted to decrease the risk of a recipient acquiring an infection by using material from immediate family members. However, there is little evidence to suggest that genetic relatedness or age matching of donors to recipients significantly improves efficacy[31].

From a safety standpoint, screening of FMT donors has generally followed the framework widely used for blood and tissue donors covering medical history, high risk behaviors, recent travel, carriage of enteric pathogens, parasites, blood borne viruses, and syphilis serology (quadro 2). Some centers in addition screen donors for methicillin-resistant *Staphylococcus aureus* and vancomycin resistant enterococci; the association of the apparently commensal gut species *Fusobacterium nucleatum* and *Streptococcus infantarius* with colorectal cancer should also be noted[32,33]. Although two cases of norovirus gastroenteritis have been documented 2 and 12 days post FMT for CDI, there was no evidence of direct transfer of the agent from donors to recipient[34]. Nevertheless, screening of fecal samples by routine diagnostic methods is limited by the fact that these detect only currently known bacterial pathogens and the viral content remains unknown. FMT therefore has the potential to introduce occult infection in the recipient and thus compromise their future suitability as blood or organ donors. Non- bacterial bioactive compounds in stool could also be damaging to the recipient. These risks, particularly the lack of data on possible long-term adverse effects, of the treatment should be fully explained to prospective subjects.

Quadro 2: Minimum FMT Donor screen

Medical history (GI)

Excluded hospitalisation or antibiotic treatment (3 months); previous transfusion, transplant

Serology: Hepatitis A,B,C (?E), HIV, HTLV, CMV, EBV; syphilis, H. pylori

Culture: *Salmonella, Shigella, Campylobacter, Aeromonas, Vibrio, E. coli O157, C. difficile*

C. difficile toxins

Microscopy for ova cysts and parasites

Sample - Several ways of preparing the stool sample have been suggested but generally involve the use of freshly voided stool blended with saline from which particulate material has been removed by graded filtration. Some users advocate centrifugation of the bacteria and resuspension in glycerol/saline, which can be frozen at -80oC for up to 6 weeks in order to limit loss of viability[35]. This is diluted and up to 250 ml is delivered by nasojejunal tube or via a colonoscope. Prior to treatment, gut decontamination (vancomycin, metronidazole, rifampicin) and bowel lavage is recommended as well as the use of loperamide to maximize contact time of the infusate in the colon. It is noteworthy that there are several reports of "do-it-yourself" treatments using enemas at home for inflammatory bowel diseases and ulcerative colitis (see, YouTube and http://www.bbc.co.uk/news/magazine-27503660) and most disconcertingly, even with the use of animal stool!

Stool substitutes - Despite the apparently increasing acceptability by patients of fecal therapy, the use of synthetic stool substitutes containing mixtures of gut bacteria appears to be gaining ground over fresh stool. A recent study reported the successful treatment of two patients with recurrent CDI, who received a laboratory prepared cocktail of 33 different intestinal bacterial species isolated from a single donor[36]. This approach theoretically facilitates tailoring the bacterial cocktail to imbalances of the gut microbiota in conditions other than CDI, with the exclusion of different members of the microbiota, which have been linked with specific syndromes. Also, safety could be enhanced by excluding potential pathogens and through screens for carriage of antibiotic, toxin and virulence genes. Such synthetic preparations would also be free of human cells and cell products thus reducing the risk of immune reactions. These preparations can be delivered as lyophilized material in time-release capsules or suppositories and this may help to reduce the natural antipathy of some individuals towards this form of therapy. A commercial synthetic stool preparation of defined microbial composition manufactured by Rebiotix was approved by the FDA for a phase II trial in July 2013, and other stool derived pills are in development in the US (Symbiotic Health, New York, and Seres Health, Massachusetts).

However, Borody et al.[37] make a compelling case for the advantages of whole stool over selectively cultured bacteria. They point out that the majority of gut microbes cannot be grown *in vitro* and that culturing of isolated strains leads to loss of adherence ability, and hence clinical efficacy in comparative studies. This view is supported by studies of the structure and assembly of the complex microbial community, which found that bacterial gene function correlates more with the community as a whole than with the profile of individual species[38]. This suggests that the whole microbial community may contain additional bioactive molecules relevant to the curative process.

Efficacy -. The primary outcome measure of FMT for CDI is the resolution of symptoms and secondarily, prevention of relapse. A systematic review of 27 reports (including single cases) involving 317 CDI patients concluded that FMT resolved symptoms in 92% of subjects[39]. A more recent meta analysis which considered only those studies involving more than 10 patients (total 273), also found a high (89%) resolution rate; better outcomes were evident with lower gastrointestinal administration, but samples from related donors were not associated with increased efficacy[40]. Some studies have shown that following FMT the species profile of the recipient's stool closely resembles that of the donor and this may persist for about a month. Indeed, with the stool substitute used by Petrof et al.[36], DNA sequences identical to those in the synthetic sample constituted over 25% of the sequences identified in the recipient's gut up to 6 months after treatment indicating that the donor microbiota stably colonize the colon. However, owing to the limitations of conventional culture, the complexity of the gut microbiota can only be demonstrated with confidence by the use of sophisticated metagenomic and metatranscriptomic techniques which allow determination of the relative abundance of different bacterial species

and their functional capabilities, and their relation to clinical outcome. In addition, there is a lack of information on gene expression, proteomic and metabolic behavior within and between members of the microbiota, and their impact on outcome. The National Institute for Health and Care Excellence of the UK concluded in 2014 that on current evidence FMT is safe and effective for the treatment of recurrent CDI [www.nice.org.uk/nicemedia/live/14154/67040/67040.pdf].

Regulation - Regulation of fecal transplants has been problematic owing to the difficulty of classification of the material used for the procedure. Debate has centered on whether human stool should be considered a tissue or a drug[41]. Initially the US Food and Drug Administration opted to classify it as a drug on the basis that it most fitted their definition of "articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease", and physicians were required to file an investigational new drug application to use donor stool for the treatment of CDI. Following extensive pressure from patient and doctor's groups, this requirement was relaxed in July 2013 but to date, the issue remains unresolved. However, owing to the unforeseen increase in demand from patients and physicians for FMT, revised draft guidelines published in March 2014 by the FDA now require that the donor be known either to the patient or the physician/provider treating the patient with CDI, and that the donor and stool are screened under the direction of the physician. No guidance has been given for treatment conditions other than CDI. This ruling will clearly have a significant impact on the sourcing of stools for transplant in the US, and effectively curtail the activities of recent start up companies banking stools. One of these, 'Openbiome', a non profit company (http://www.openbiome.org/) provides material at about 250 dollars per screened stool; donors are screened for a wide range of infectious agents, metabolic syndromes, autoimmune disorders and digestive problems.

Several arguments have been made against the ruling to ban stool banks by Internet groups (www.thepowerofpoop.com/fda-ban-stool-banks/) on the basis that it severely restricts availability of material as many donors prove to be unsuitable on screening and the high cost of testing (500-1000 dollars). Stool banks also have the advantage of the necessary infrastructure for extensive screening of a pool of anonymized regular donors, and the capacity to freeze and transport material safely and speedily.

Drugs are also generally characterized by defined consistent formulations in contrast to the variable microbial, metabolic and cellular complexity of stools. Clearly the usual drug parameters (pharmacokinetics, bioavailability, toxicity, etc.) are not applicable to fecal preparations. An alternative option would be to classify human stool in its own category in much the same way as used for blood, cartilage, bone, and skin etc. The European Medicines Agency has yet to rule on classification of FMT with the natural stool product but readers are referred to the discussion of regulatory aspects of FMT for CDI from a Health Canada perspective which calls for rigorous donor screening and better standardization of fecal material and its delivery to the patient[31].

In conclusion, although a growing number of reports support the clinical efficacy of FMT for the treatment of CDI and possibly ulcerative colitis, there are several currently unresolved issues and concerns that need to be addressed if its use is to become more widespread. Key amongst these are, the establishment of effective standards for donor screens, optimal preparation of natural stool, influence of nonbacterial factors on efficacy, the most beneficial bacterial species profile for different dysbiotic conditions, and the wider use of metagenomic analysis to document and monitor changes in gut microbiota of the recipient. For natural stool, rigorous donor screening is essential to enhance safety but given the complexity of the material there will always be a low risk of occult infection and unintended long-term consequences for the recipient. Some risks may be circumvented by the use of synthetic stool preparations but there is an increasing awareness and interest among the general public and some may be encouraged by uninformed Internet sources to view this as a panacea treatment for the widely prevalent life style associated conditions (irritable bowel, type 2 diabetes, obesity, etc.). This concern is compounded by the entry of the commercial market and the lack of clear regulation by appropriate state bodies. One can readily envisage the clinical consequences of sepsis in an individual arising from injudicious, uncontrolled and self-administered use of such materials and the ensuing media interest.

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