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REVIEW ARTICLE
Challenges and opportunities for faecal microbiota transplantation therapy

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SUMMARY

The incidence, morbidity, and mortality associated with *Clostridium difficile* gastrointestinal infections has increased greatly over recent years, reaching epidemic proportions; a trend due, in part, to the emergence of hypervirulent and antibiotic-resistant strains. The need to identify alternative, non-antibiotic, treatment strategies is therefore urgent. The ability of bacteria in faecal matter transplanted from healthy individuals to displace pathogen populations is well recognized. Further, there is growing evidence that such faecal microbiota transplantation can be of benefit in a wide range of conditions associated with gut dysbiosis. Recent technical advances have greatly increased our ability to understand the processes that underpin the beneficial changes in bacterial community composition, as well as to characterize their extent and duration. However, while much of the research into faecal microbiota transplantation focuses currently on achieving clinical efficacy, the potential for such therapies to contribute to the transmission of infective agents also requires careful consideration.

Key words: *Clostridium difficile*, diarrhoea, gastrointestinal infections, microbiology.

Our understanding of the gut microbiome

The human intestinal tract contains more than 10^{14} bacterial cells, outnumbering human cells within our bodies by tenfold [1]. Efforts to understand the complexity of the microbial communities present in the gastrointestinal (GI) tract have a long history, certainly dating back to the late 19th century [2]. However, research in this field, and the resulting gains in understanding, increased substantially during the 1960s (for a detailed review of work during this era, see [3]). An important contribution to these advances was the recognition that a substantial proportion of the species that comprise the GI microbiota require an absence of atmospheric oxygen in order to grow, and the development of anaerobic culture techniques that allowed the isolation of such species. For example, until the mid-1960s, *Escherichia coli* was considered commonly to be the chief inhabitant of the bowel. However, with improvements in anaerobic culture methods, the gut microbiota was revealed to be dominated by strict anaerobes, typically outnumbering the facultative microbes such as *E. coli* by as many as 1000:1 [4].

The advancement of culture techniques led to characterizations of the gut bacterial communities that were far more comprehensive than was possible previously [5, 6]. Such studies revealed that individual humans had hundreds of bacterial species detectable

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within their GI tract, with the genera *Bacteroides*, *Bifidobacterium*, *Eubacterium*, *Clostridium*, *Peptococcus*, *Peptostreptococcus*, and *Ruminococcus* often predominant [7]. Facultative anaerobes were also detected, including members of genera such as *Enterobacter, Enterococcus, Klebsiella, Lactobacillus, Proteus* and, as above, *Escherichia*, but at lower relative levels [7].

Despite this increased ability to culture gut microbes, a significant disparity between the bacteria that could be visualized microscopically and those that could be grown in culture remained [8]. The true scale of the GI microbiota complexity only began to emerge with the development of culture-independent analytical techniques. Here, rather than relying on the isolation of individual species through *in vitro* growth, the presence of bacteria can be determined through the detection of specific signatures in nucleic acids extracted directly from samples. Importantly, by avoiding the need for *in vitro* cultivation, such approaches are able to report on the totality of bacteria present, including those that can be visualized microscopically but not readily cultured.

Molecular approaches to determining bacterial community composition are based most commonly on the 16S rRNA gene, which contains both the highly conserved and highly variable regions required for amplification and differentiation of bacterial species present [9]. Initially, investigations relied on dideoxynucleotide sequencing and bacterial community profiling techniques, such as terminal-restriction fragment length polymorphism (T-RFLP) profiling [10] and denaturing gradient gel electrophoresis (DGGE) [11] to ascertain community structure in the human gut. While informative, the level of detail obtainable with such approaches was limited to broad scale descriptions. However, more recently, the development of next generation sequencing (NGS) approaches has greatly expanded the detail of characterization achievable [12, 13]. Such approaches provide analogous information to earlier clone library-based analyses. That is, each sequence obtained can be compared to extensive publicly held databases to allow the identification of the bacteria from which it was derived. However, in being greatly more processive than these earlier approaches, the number of such species identities that can be readily determined for an individual sample is many orders greater. By allowing the rapid and relatively cheap profiling of the bacterial communities present in high detail, such analysis allows us to extend our understanding of key issues, such as the extent to which the GI microbiota varies between and within individuals over time, in both health and disease (reviewed in detail in [14]).

What does the gut microbiota do?

The presence of a complex microbiota in the gut has a number of important functions. These range from supplying nutrients to the host [15], to immune system development and function [16], and to angiogenesis [17]. In addition, the gut microbiota can help to protect against infection. This protection can be by occupying metabolic niches within the gut, and thus excluding pathogenic species [1], through the production of metabolites that can inhibit pathogenesis [18], or by protecting enterocytes from acute inflammatory responses that might occur in response to infection [19].

Given the increasing list of positive roles played by the gut microbiota under normal circumstances, it is unsurprising that its disruption has been associated with a wide range of health issues, including obesity [20] malnutrition [21], inflammatory bowel disease (IBD) [22], neurological disorders [22] and cancer [23].

What happens when the gut microbiome is disrupted?

The composition of the gut microbiota appears to be relatively stable in most individuals over time [24]. However, its composition and function can be disrupted by external factors. One of the principal ways in which this can happen is through antibiotic therapy. Antibiotics are used commonly and, even when targeting infections in non-GI regions of the body, have typically a marked impact on certain populations of bacteria in the gut [25, 26]. The extent of this impact will vary depending on the characteristics of the antibiotic used; however, it is likely to include both a reduction in the number of viable bacterial cells and, due to differences in antibiotic susceptibility, an alteration in the relative abundance of the types of bacteria present.

In many cases, bacterial populations may return to their pre-treatment levels on cessation of antibiotic therapy without the occurrence of complication. However, until the gut microbiota is re-established, opportunities exist for species that are excluded or suppressed under normal circumstances to expand to significant levels [1]. Arguably the most clinically important example of this process involves *Clostridium difficile*, a toxin-producing, Gram-positive, anaerobic, spore-forming bacillus. The production of *C. difficile*

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toxins can cause pseudomembranous colitis; the destruction of colonic epithelial cells and inflammation with resultant disease symptoms. *C. difficile* infection (CDI) is implicated in 15–25% of antibiotic-associated diarrhoea [27].

The incidence, morbidity, and mortality associated with CDIs have increased greatly over recent years, reaching epidemic proportions [28]. This trend is, in part, related to the emergence of certain strains that are hypervirulent and antibiotic resistant [29]. The demographic of those susceptible to CDI has also expanded to increasingly include young, healthy individuals without prior exposure to antibiotics or hospitalization [29]. Patients with IBD, compromised immune systems, and peripartum women are also recognized increasingly to represent at-risk groups [29, 30].

With increased antibiotic exposure, so the efficacy of the antibiotics available has decreased. The first-line treatments for CDI are metronidazole and vancomycin. However, the efficacy of metronidazole appears to be waning [31]. Further, the antibiotic treatments for CDI can ultimately exacerbate the situation. By reducing populations of resident gut species along with *C. difficile*, antibiotic therapy creates further niche space for this pathogen; a vacuum that can be exploited by a re-expansion of *C. difficile* populations, either seeded by their recalcitrant and largely antibiotic-resistant spores, or through *de novo* infection [32]. Recurrence has been documented to occur in as many as 15–30% of patients after an initial bout of CDI, and up to 65% of patients who experience one such episode will have subsequent episodes after antibiotic therapy is stopped [32]. There are few effective drug-based treatments for patients experiencing multiple recurrences of CDI. The diminishing efficacy of available antibiotics [28], coupled with a growing awareness of the importance of limiting antibiotic use generally [33], mean that the need to identify alternative approaches to therapy has become urgent.

Faecal microbiota transplantation

One way in which the cycle of antibiotic therapy followed by overgrowth by pathogens such as *C. difficile* can be broken is to re-establish the balance of bacterial species in the gut. Attempts have been made to achieve this through the oral administration of probiotics containing a single bacterial species [34]. However, such approaches have two key limitations; first, it is difficult to administer sufficient and sustained levels of probiotic bacteria to make a significant impact of gut microbiota composition, and second, the small number of species that can be administered in this way do not represent the complex bacterial mixtures characteristic of the healthy gut microbiota. These factors may to have contributed to the relatively low reported success of such interventions.

An alternative approach is to introduce faecal material obtained from a symptomless individual directly into the gut. This practice, referred to as faecal microbiota transplantation (FMT), bacteriotherapy, or stool transplant, has a long history, with reports of its use for example in 4th century China to treat diarrhoea in humans, and later in Italy in the 17th century in the treatment of ruminants [35].

In the modern era, FMT has been performed since 1958 [36], when it was used in the successful treatment of four patients with pseudomembranous colitis, a time before the causative role played by *C. difficile* was known. Of note, three of the four patients reported in the 1958 study were in a critical state when faecal enemas were administered, and in all patients symptoms were found to resolve within hours of treatment.

The first documented case of confirmed CDI treated with FMT was reported in 1983 by Schwan *et al.* In this case, therapy resulted in the 'prompt and complete normalization of bowel function' in the 65-year-old woman to whom it was administered [37]. At follow-up 9 months later, the patient remained asymptomatic. In 1989, Tvede & Rask-Madsen reported the treatment of patients with chronic relapsing diarrhoea caused by *C. difficile* were treated with rectal instillation of homologous faeces (one patient) or a mixture of ten different facultative or obligate anaerobic bacterial species diluted in sterile saline (five patients) [38]. Both therapeutic approaches resulted in the prompt loss of *C. difficile* and its toxin from the stools, restoration of normal bowel function within 24 h, and disappearance of abdominal symptoms. Following these initial demonstrations of efficacy, the use of FMT has continued to expand ever since [39].

The problems/challenges to implementing FMT as a more routine therapy

While FMT appears to have the potential to revolutionize the treatment of CDI and other GI disorders, a number of significant challenges must be overcome

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for its routine deployment. The first is the potential of FMT to result in the transmission of pathogens, a factor that has led to the recommendation of screening processes to identify key viral, bacterial, and parasitic infections in donors [28].

It has been suggested that considerations of a person's suitability to act as an FMT donor could be similar to those applied more widely in organ transplantation [40]. This would involve the screening of donors for a panel of viral pathogens. In addition, stool would be screened for a range of bacterial pathogens and helminths (for a detailed discussion of these considerations, see [41]). However, despite such screening, the highly complex nature of the microbial content of the gut means that the clinical significance of many of the microbes present is not yet known. This raises the possibility that species for which there is no current reason to screen, may be identified later as causative agents of disease.

The risk of an individual acquiring a *de novo* infection as a result of FMT could be reduced by using a donor with whom the recipient is sexually intimate. The selection of a spouse, for example, is less likely to be a source of novel infection, and in some such circumstances, screening has been deemed unnecessary [42]. In other studies, screening has been foregone where immediate family members are acting as donors [37, 43]. However, even in these cases, the failure to screen is difficult to justify in retrospect given the potential for transmission of occult infections.

The use of close relatives as FMT donors has both potential advantages and drawbacks. Adaptive immune elements in the mucosal immune system (e.g. antigen-specific antibodies) may result in a greater tolerance of microbiota derived from close donors [28]. However, similarities in microbiota composition between relatives might also mean that they too are predisposed to certain types of infection. Further, 'natural antipathy' towards FMT might be reduced through the use of anonymous, screened donors [44].

Challenges of handling, processing and administering donated material

Once a suitable donor has been identified and screened, there are a number of logistical issues that require consideration. In some cases, material will be used immediately. However, in others, storage might be required. This is particularly the case where anonymous donation and banking of material is to be performed.

It is important that care is taken both to maintain the viability of bacteria within the donated material, and to prevent bacterial growth that would lead to changes in the relative species abundance. The most appropriate procedures for handling and storing material for FMT are yet to be identified. However, it is important to note that the species that are most able to survive the stresses of sample processing, such as spore-forming bacteria, may not be those that one would want to promote.

With the majority of faecal bacteria being obligate anaerobes, care must be taken to prevent loss of viability as a result of exposure to atmospheric oxygen. Refrigeration of material over the short term, or freezing over longer periods, may help to stabilize samples, but again, these processes are likely to result in some loss of bacterial viability. Further research is therefore required to inform the design of appropriate protocols.

There are a number of ways in which donated material can be introduced to the patient's gut, with associated implications for the preparation of the material to be used. FMT delivery methods have included nasogastric and nasoduodenal tubes, colonoscope, and retention enema [45] although no clear superiority of one method has yet been demonstrated [28]. Currently, selection of an administration route is largely dependent on the clinical situation, although transcolonoscopic infusion has been favoured for the majority of patients [46]. Regardless of which of these delivery strategies is to be employed, a slurry must be created from donated stool. Here, homogenization, liquefaction and bulking may all aid the successful delivery of the material [28]. Filtration to remove particulate matter can then be performed, with material re-suspended to an appropriate volume prior to delivery [44].

Implications of FMT for organ donation by recipients

An important consideration beyond the immediate issues surrounding the screening of FMT donors is the implications that receiving donated stool material has for the recipient. As above, the complexity of faecal material means that it is only practical to screen for those pathogens considered currently to represent a significant risk. However, relatively little is known about many of the species present, including the extent to which particular strains may harbour antibiotic resistance or virulence genes. Perhaps of even more concern is the transmission of viral infections.

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Administration of FMT will increase the likelihood of occult infection in the recipient. Therefore, the risks associated with their subsequent donation of any material, including blood or solid organs, must be considered carefully. Given the potentially widespread deployment of FMT, a decision to exclude FMT recipients from the pool of potential organ donors could have serious and far-reaching implications.

Assessing efficacy – the potential of emerging technology

The primary measure of FMT success is the resolution of symptoms. In addition, in the case of treatment for conditions such as CDI, the absence of a relapse can be a secondary endpoint. The detection of specific pathogens, such as *C. difficile* in the case of CDI, can be unhelpful due to the fact that patients can be colonized without developing disease [47]. However, when attempts are being made to achieve beneficial outcomes through the alteration of the gut microbiota composition, the direct characterization of the nature and extent of these changes would clearly be informative.

Here, molecular techniques that allow the characterization of faecal bacterial composition, as above, are invaluable. Such techniques have already been applied to assess the extent and duration of the impact of FMT on the residual gut microbiota, allowing changes in the relative abundance of different bacterial species to be linked with clinical outcomes [48–50].

It is important to note that, rather than the establishment of a specific gut microbiota composition, what is being sought through FMT and analogous therapies is the re-establishment of gut microbiota function, whether metabolic, immunological, or through the ability to exclude pathogenic species. An assessment of gut microbiota behaviour and function may therefore be important in determining treatment efficacy.

Here, it can be useful to consider the gut microbiota as a distinct entity. For example, meta-genomic analysis can be used to assess the genetic composition of the gut microbiome as a whole [51]. Further, meta-transcriptomic, meta-proteomic and metabolomic approaches allow assessment of microbiota behaviour and its impact on the gut environment [14, 52, 53]. The data derived from each of these approaches can be mapped onto microbial composition profiles, as determined through 16S rRNA sequencing. Such parallel application of approaches that provide information on microbiome function with those that detail microbiota composition may be particularly important in identifying where bacterial functions are conserved between phylogenetically distant members, or are particular to certain species or strains. In turn, these data will help to identify components within the gut microbiota whose restoration would confer the greatest clinical benefit.

Augmenting FMT efficacy through parallel therapies

The primary basis for FMT efficacy is believed to be the ability of introduced bacterial populations to displace gut pathogens. The likelihood of achieving this could be augmented by careful selection of medium in which the introduced material is to be suspended. Previously, osmotically appropriate bulking agents, such as buffered saline or 4% milk [28], have been used. However, a more sophisticated approach might promote bacterial retention within the gut, or provide substrates that encourage desirable growth strategies or bacterial behaviour.

Dietary substrates that selectively stimulate growth or activity of particular types of bacteria in the gut are referred to as prebiotics [54]; the ability of specific food supplements to influence the composition of the gut microbiota has been the subject of an increasing number of studies [55]. Further, prebiotics can be administered in parallel to probiotic treatments, with the aim of promoting bacterial survival; a practice known as synbiotics [56]. Where supported by experimental data, prebiotics could be administered at the time of FMT, either as food supplements, or as components of the delivery medium itself. The formulation of FMT slurries to maximize treatment outcomes is therefore an area that warrants further research.

The potential for 'artificial donor material'

Deploying FMT on a large scale presents substantial logistical challenges. The potential use of a synthetic material as an alternative to donated faecal material is therefore an attractive proposition. Bacterial cells represent 40–60% of the bulk of faeces [57, 58] and it is this bacterial content from which the beneficial effects of FMT are believed to be derived. Representative faecal bacteria could be grown *in vitro*, harvested, mixed in appropriate proportions, and suspended in a suitable medium for delivery. If care was taken to exclude virulent, pathogenic or antibiotic-resistant

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strains, such a strategy would remove both the need to screen potential FMT donors, and the implications for FMT recipients to act as organ donors subsequently. Further, the potential for immunogenic reactions would be removed since the synthetic stool would be free from human cells or cell products.

An additional and important advantage of using a synthetic material is that it would standardize therapy. Although the composition of intestinal microbiota can vary between individuals, functional gene profiles show greater similarity [59, 60]. This observation suggests that the use of a consistent synthetic faecal transplant material to treat broad patient groups could be possible, while maintaining microbiome functionality. Bacterial preparations could be generated on a large scale, and stabilized by freezing or lyophilization for transportation and reconstitution at the point of use. With the same material used in each treatment, a better platform for determining treatment efficacy would exist. Finally, the inclusion of marker sequences in the bacterial strains used could allow them to be tracked, providing both information on their retention, and their identification if implicated subsequently in opportunistic infections, such as peritonitis.

The use of defined bacterial mixtures as an alternative to FMT has been suggested previously [38, 61]. However, its development has been hampered by the challenges of characterizing the microbial composition of faecal matter accurately and in sufficient detail. With technological advances (as above) such characterization is now achievable.

Petrof *et al.* reported recently the use of a preparation of 33 different intestinal bacterial species isolated in pure culture from a single healthy donor to treat recurrent CDI that was unresponsive to conventional therapy in two patients [50]. Here extensive culture of stool bacteria and screening of isolates was used to exclude antibiotic-resistant strains. The relative abundance of the bacteria in the administered material was determined based on meta-analysis of data from previous studies of healthy donor stool. In both cases, resolution of symptoms was reported, with patients remaining symptom-free at 6 months post-therapy. While this study still relied on the isolation of bacteria from donated stool samples through culture, it represents a substantially more sophisticated approach compared to previous efforts.

There is no theoretical limit to the complexity of such artificial FMT preparations, and they could, for example, be formulated to reflect the characteristics of a particular individual's GI microbiome. Determination of the functional roles of different bacterial groups within the intestine, and the likely implications of their relative abundances is, however, required before such an approach becomes a realistic option.

Potential for FMT to be beneficial

The focus of this review has been the use of FMT in the treatment of CDI. However, there are many other clinical scenarios in which FMT could prove beneficial. For example, a recent, small-scale prospective study has reported FMT to be effective in the treatment of children and young adults with ulcerative colitis [62]. Other conditions associated with gut dysbiosis include IBD, obesity, anorexia nervosa, systemic autoimmunity, food allergies, eosinophilic disorders of the GI tract, as well as neurodegenerative and neurodevelopmental disorders [39]. However, unlike recalcitrant CDI, in which the native microbiota have been severely affected by repeated antibiotic exposure, microbial communities in patients with such conditions might require antibiotic conditioning to suppress or eliminate resident bacterial populations prior to FMT. The need for such conditioning, as well as identification of optimal protocols for transplant preparation and delivery, now require careful consideration. In turn, this will allow the potential of FMT to provide effective therapy in wider patient populations is to be assessed through systematic clinical trials.

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DECLARATION OF INTEREST

None.

REFERENCES

1. **Savage DC.** Microbial ecology of the gastrointestinal tract. *Annual Review of Microbiology* 1977; **31**: 107–133.
2. **Rosebury T.** *Microorganisms Indigenous to Man*. New York: McGraw-Hill, 1962, pp. 435.
3. **Savage DC.** Microbial biota of the human intestine: a tribute to some pioneering scientists. *Current Issues in Intestinal Microbiology* 2001; **2**: 1–15.

4. **Gordon JH, Dubos R.** The anaerobic bacterial flora of the mouse cecum. *Journal of Experimental Medicine* 1970; **132**: 251–260.
5. **Drasar BS, *et al.*** The influence of a diet rich in wheat fibre on the human faecal flora. *Journal of Medical Microbiology* 1976; **9**: 423–431.
6. **Holdeman LV, Good IJ, Moore WE.** Human faecal flora: variation in bacterial composition within individuals and a possible effect of emotional stress. *Applied and Environmental Microbiology* 1976; **31**: 359–375.
7. **Simon GL, Gorbach SL.** Intestinal flora in health and disease. *Gastroenterology* 1984; **86**: 174–193.
8. **Savage DC, Blumershine RV.** Surface-surface associations in microbial communities populating epithelial habitats in the murine gastrointestinal ecosystem: scanning electron microscopy. *Infection and Immunity* 1974; **10**: 240–250.
9. **Clarridge 3rd JE.** Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clinical Microbiology Reviews* 2004; **17**: 840–862.
10. **Jernberg C, *et al.*** Monitorig of antibiotic-induced alterations in the human intestinal microflora and detection of probiotic strains by use of terminal restriction fragment length polymorphism. *Applied and Environmental Microbiology* 2005; **71**: 501–506.
11. **Donskey CJ, *et al.*** Use of denaturing gradient gel electrophoresis for analysis of the stool microbiota of hospitalized patients. *Journal of Microbiological Methods* 2003; **54**: 249–256.
12. **Armougom F, Raoult D.** Use of pyrosequencing and DNA barcodes to monitor variations in *Firmicutes* and *Bacteroidetes* communities in the gut microbiota of obese humans. *BMC Genomics* 2008; **9**: 576.
13. **Dethlefsen L, *et al.*** The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biology* 2008; **6**: 280.
14. **Lozupone CA, *et al.*** Diversity, stability and resilience of the human gut microbiota. *Nature* 2012; **489**: 220–230.
15. **Salminen S, *et al.*** Functional food science and gastrointestinal physiology and function. *British Journal of Nutrition* 1998; **80** (Suppl. 1): S147–S171.
16. **Olszak T, *et al.*** Microbial exposure during early life has persistent effects on natural killer T cell function. *Science* 2012; **336**: 489–493.
17. **Stappenbeck TS, Hooper LV, Gordon JI.** Developmental regulation of intestinal angiogenesis by indigenous microbes via paneth cells. *Proceedings of the National Academy of Sciences USA* 2002; **99**: 15451–15455.
18. **Fukuda S, *et al.*** Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature* 2011; **469**: 543–547.
19. **Candela M, *et al.*** Interaction of probiotic *Lactobacillus* and *Bifidobacterium* strains with human intestinal epithelial cells: adhesion properties, competition against enteropathogens and modulation of IL-8 production. *International Journal of Food Microbiology* 2008; **125**: 286–292.
20. **Ley RE, *et al.*** Microbial ecology: human gut microbes associated with obesity. *Nature* 2006; **444**: 1022–1023.
21. **Kau AL, *et al.*** Human nutrition, the gut microbiome and the immune system. *Nature* 2011; **474**: 327–336.
22. **Frank DN, *et al.*** Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proceedings of the National Academy of Sciences USA* 2007; **104**: 13780–13785.
23. **Gonzalez A, *et al.*** The mind–body–microbial continuum. *Dialogues in Clinical Neuroscience* 2011; **13**: 55–62.
24. **Lupton JR.** Microbial degradation products influence colon cancer risk: the butyrate controversy. *Journal of Nutrition* 2004; **134**: 479–482.
25. **Duytschaever G, *et al.*** Cross-sectional and longitudinal comparisons of the predominant fecal microbiota compositions of a group of pediatric patients with cystic fibrosis and their healthy siblings. *Applied and Environmental Microbiology* 2011; **77**: 8015–8024.
26. **Rogers GB, *et al.*** Comparing the microbiota of the cystic fibrosis lung and human gut. *Gut Microbes* 2010; **1**: 85–93.
27. **Bartlett JG, Gerding DN.** Clinical recognition and diagnosis of *Clostridium difficile* infection. *Clinical Infectious Diseases* 2008; **46** (Suppl. 1): S12–S18.
28. **Bakken JS, *et al.*** Treating *Clostridium difficile* infection with faecal microbiota transplantation. *Clinical Gastroenterology and Hepatology* 2011; **9**: 1044–1049.
29. **Rupnik M, Wilcox MH, Gerding DN.** *Clostridium difficile* infection: new developments in epidemiology and pathogenesis. *Nature Reviews Microbiology* 2009; **7**: 526–536.
30. **Freeman J, *et al.*** The changing epidemiology of *Clostridium difficile* infections. *Clinical Microbiology Reviews* 2010; **23**: 529–549.
31. **Zar FA, *et al.*** A comparison of vancomycin and metronidazole for the treatment of *Clostridium difficile*-associated diarrhea, stratified by disease severity. *Clinical Infectious Diseases* 2007; **45**: 302–307.
32. **McFarland LV, Elmer GW, Surawicz CM.** Breaking the cycle: treatment strategies for 163 cases of recurrent *Clostridium difficile* disease. *American Journal of Gastroenterology* 2002; **97**: 1769–1775.
33. **Rogers GB, Carroll MP, Bruce KD.** Enhancing the utility of existing antibiotics by targeting bacterial behaviour? *British Journal of Pharmacology* 2012; **165**: 845–57.
34. **Lawrence SJ, Korzenik JR, Mundy LM.** Probiotics for recurrent *Clostridium difficile* disease. *Journal of Medical Microbiology* 2005; **54**: 905–906.
35. **Klein W, Müller R.** The minimum protein, the zymogenic symbiosis and generation of microbial protein in the rumen, from nitrous compounds not of proteinaceous origin (an article regarding the biology of the ruminant). *Journal of Animal Breeding Biology* 1941; **48**: 255–276.
36. **Eiseman B, *et al.*** Faecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. *Surgery* 1958; **44**: 854–859.

37. **Schwan A, *et al.*** Relapsing *Clostridium difficile* enterocolitis cured by rectal infusion of homologous faeces. *Lancet* 1983; **2**: 845.
38. **Tvede M, Rask-Madsen J.** Bacteriotherapy for chronic relapsing *Clostridium difficile* diarrhoea in six patients. *Lancet* 1989; **1**: 1156–1160.
39. **Borody TJ, Khoruts A.** Fecal microbiota transplantation and emerging applications. *Nature Reviews Gastroenterology & Hepatology* 2011; **9**: 88–96.
40. **Borody TJ, *et al.*** Bacteriotherapy using fecal flora: toying with human motions. *Journal of Clinical Gastroenterology* 2004; **38**: 475–483.
41. **Borody TJ, *et al.*** Treatment of ulcerative colitis using faecal bacteriotherapy. *Journal of Clinical Gastroenterology* 2003; **37**: 42–47.
42. **Persky SE, Brandt LJ.** Treatment of recurrent *Clostridium difficile*-associated diarrhea by administration of donated stool directly through a colonoscope. *American Journal of Gastroenterology* 2000; **95**: 3283–3285.
43. **Faust G, *et al.*** Treatment of recurrent pseudomembranous colitis with stool transplantation: report of six cases. *Canadian Journal of Gastroenterology* 2002; **16**: A43.
44. **Hamilton MJ, *et al.*** Standardized frozen preparation for transplantation of fecal microbiota for recurrent *Clostridium difficile* infection. *American Journal of Gastroenterology* 2012; **107**: 761–767.
45. **Bakken JS.** Faecal bacteriotherapy for recurrent *Clostridium difficile* infection. *Anaerobe* 2009; **15**: 285–289.
46. **Brandt LJ, Borody TJ, Campbell J.** Endoscopic fecal microbiota transplantation: 'first-line' treatment for severe *Clostridium difficile* infection? *Journal of Clinical Gastroenterology* 2011; **45**: 655–657.
47. **Cohen SH, *et al.*** Clinical practice guidelines for *Clostridium difficile* infection in adults: 2010 update by the society for healthcare epidemiology of America (SHEA) and the infectious diseases society of America (IDSA). *Infection Control and Hospital Epidemiology* 2010; **31**: 431–455.
48. **Khoruts A, *et al.*** Changes in the composition of the human fecal microbiome after bacteriotherapy for recurrent *Clostridium difficile*-associated diarrhea. *Journal of Clinical Gastroenterology* 2010; **44**: 354–360.
49. **Grehan MJ, *et al.*** Durable alteration of the colonic microbiota by the administration of donor fecal flora. *Journal of Clinical Gastroenterology* 2010; **44**: 551–561.
50. **Petrof EO, *et al.*** Stool substitute transplant therapy for the eradication of *Clostridium difficile* infection: 'RePOOPulating' the gut. *Microbiome* 2013; **1**: 3.
51. **Nelson KE, *et al.*** A catalog of reference genomes from the human microbiome. *Science* 2010; **328**: 994–999.
52. **Verberkmoes NC, *et al.*** Shotgun metaproteomics of the human distal gut microbiota. *International Society for Microbial Ecology Journal* 2009; **3**: 179–189.
53. **Jansson J, *et al.*** Metabolomics reveals metabolic biomarkers of Crohn's disease. *PLoS ONE* 2009; **4**: e6386.
54. **Gibson GR, Roberfroid MB.** Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *Journal of Nutrition* 1995; **125**: 1401–1412.
55. **Roberfroid M, *et al.*** Prebiotic effects: metabolic and health benefits. *British Journal of Nutrition* 2010; **104**: S1–S63.
56. **Gibson GR, *et al.*** Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutrition Research Reviews* 2004; **17**: 259–275.
57. **Moore WE, Holdeman LV.** Human faecal flora: the normal flora of 20 Japanese-Hawaiians. *Applied Microbiology* 1974; **27**: 961–979.
58. **Stephen AM, Cummings JH.** The microbial contribution to human faecal mass. *Journal of Medical Microbiology* 1980; **13**: 45–56.
59. **Qin J, *et al.*** A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010; **464**: 59–65.
60. **The Human Microbiome Project Consortium.** Structure, function and diversity of the healthy human microbiome. *Nature* 2012; **486**: 207–214.
61. **Kelly CP.** Fecal microbiota transplantation – an old therapy comes of age. *New England Journal of Medicine* 2013; **368**: 474–475.
62. **Kunde S, *et al.*** Safety, tolerability, and clinical response after fecal transplantation in children and young adults with ulcerative colitis. *Journal of Pediatriatric Gastroenterology and Nutrition* 2013. doi:10.1097/MPG.0b013e318292fa0d.