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Bacteriotherapy for the treatment of intestinal dysbiosis caused by *Clostridium difficile* infection

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Faecal microbiota transplantation (FMT) has been used for more than five decades to treat a variety of intestinal diseases associated with pathological imbalances within the resident microbiota, termed dysbiosis. FMT has been particularly effective for treating patients with recurrent *Clostridium difficile* infection who are left with few clinical options other than continued antibiotic therapy. Our increasing knowledge of the structure and function of the human intestinal microbiota and *C. difficile* pathogenesis has led to the understanding that FMT promotes intestinal ecological restoration and highlights the microbiota as a viable therapeutic target. However, the use of undefined faecal samples creates a barrier for widespread clinical use because of safety and aesthetic issues. An emerging concept of bacteriotherapy, the therapeutic use of a defined mixture of harmless, health-associated bacteria, holds promise for the treatment of patients with severe *C. difficile* infection, and possibly represents a paradigm shift for the treatment of diseases linked to intestinal dysbiosis.

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Introduction

The human intestine is inhabited by a diverse and abundant community of microorganisms, collectively termed the intestinal microbiota, that plays crucial roles in our development and sustenance [1]. Proper functioning and homeostasis of our intestine relies on an intimate and symbiotic relationship between our mucosal surface, the microbiota and its metabolic by-products. Homeostasis is characterized by a diverse microbiota that produces a variety of metabolites such as short-chain fatty acids

(SCFAs), and this is coupled to a lack of pathology associated with specific T-cell subsets [2].

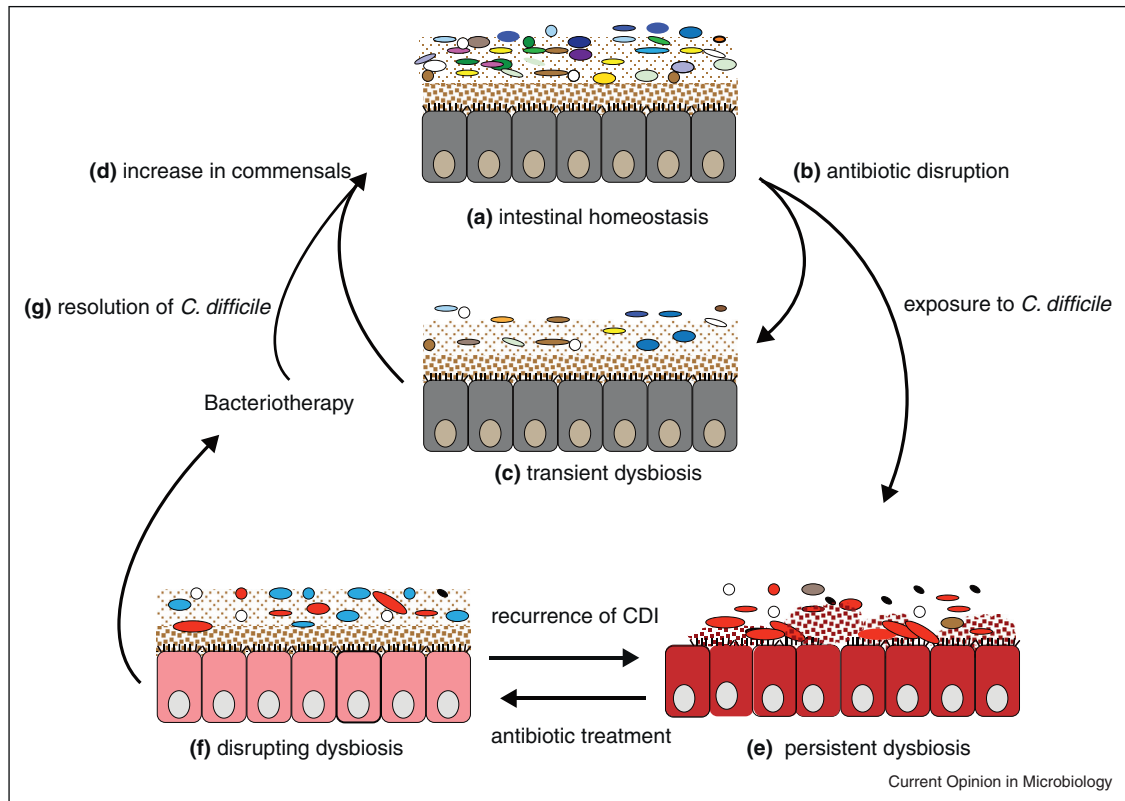
Pathological imbalances in the intestinal microbiota, referred to as dysbiosis, are increasingly linked to intestinal diseases. Disturbances in the intestinal microbiota caused by infections and antibiotics have profound effect on the microbiota's composition and function and can predispose the individual to antibiotic-associated diarrhoea (AAD). In the last decade, the incidence of morbidity and mortality from *Clostridium difficile* infection (CDI), the leading cause of AAD, increased largely due to the emergence and global spread of fluoroquinolone-resistant variant of *C. difficile* (characterized as genotype BI/NAPI/027) [3]. *C. difficile* is continuously evolving in response to antibiotic selection and other ribotypes (such as 017 and 078) have also been identified in humans and animal sources that could also spread globally [4,5]. First line treatment for severe *C. difficile* infections include metronidazole or vancomycin although in 15–35% of these cases, a recurrence (relapse or reinfection) follows the cessation of antibiotic therapy.

Faecal microbiota transplantation (FMT) has been mainly used as an alternative treatment for patients with persistent, recurrent *C. difficile* infection and involves the restoration of the intestinal microbiota through instillation or engraftment of homogenized faecal suspension from a healthy donor [6]. FMT has also been utilized in the treatment of diseases associated with intestinal dysbiosis such as inflammatory bowel diseases (IBD; manifested as Crohn's disease and ulcerative colitis), irritable bowel syndrome (IBS) and obesity [6]. This review focuses on the use of FMT for the treatment of recurrent CDI, the potential for designing a mixture of 'harmless' bacteria for the treatment of CDI infection and applications to other diseases. We refer to this clinical application as 'Bacteriotherapy'.

The human intestinal microbiota in health and disease

The human intestinal microbiota contains >1000 bacterial species, as well as other understudied organisms like Archaea, eukaryotic organisms and viruses, that collectively encode 150 times more unique genes than the human genome [7,8,9]. Intestinal bacteria consist mainly of strict anaerobes (>99%) and are involved in a variety of functions beneficial to the host, including immunological development and stimulation, SCFA production from the breakdown of dietary fibre, conversion of xenobiotics to

Figure 1



A proposed model for recurrent *C. difficile* infection and the restoration of the intestinal microbiota through FMT or bacteriotherapy. Intestinal homeostasis (a) is characterized by large microbial diversity in the microbiota and health-associated metabolites. Antibiotic exposure disrupts the microbiota (b–c) by destroying the microbial community leading to reduction in the diversity and loss of colonization resistance. The microbiota usually expands in diversity (d) after antibiotics are stopped to restore diversity. Antibiotic disruption makes individuals hyper-susceptible to *C. difficile* colonization potentially leading to chronic infection and persistent dysbiosis (e). After treatment of CDI with antibiotics such as vancomycin, further microbiota disruption (f) occurs potentially leading to recurrent CDI after discontinued use of the antibiotic. FMT or bacteriotherapy disrupts intestinal dysbiosis leading to resolution of CDI (g) and increase in species diversity (d) and restores intestinal homeostasis. Figure modified from [48].

less harmful substances, maintaining intestinal epithelial integrity and generation of nutrients and vitamins [10,11].

An important function of our microbiota is colonization resistance, through acting as a barrier against pathogen colonization or overgrowth of resident opportunistic bacteria present at low levels [2,10,12]. These processes are made possible due to the presence of abundant and diverse microorganisms competing with an invading bacterium directly for niches and nutrients or through production of antibacterial products like bacteriocins [2] (Figure 1). Other factors such as diet, hygiene, lifestyle, host genetics and immune status influence the bacterial groups present in the intestinal microbiota to promote intestinal homeostasis and colonization resistance [13].

Various diseases have been associated with dysbiosis in the intestinal microbiota such as AAD, IBD, IBS, asthma and obesity [14–17]. In addition, certain pathogens, such as *Salmonella* Typhimurium and *Citrobacter rodentium*, exploit

intestinal inflammation to subvert colonization resistance in mice [18,19]. Inflammation of the intestinal mucosa caused by enteric pathogens leads to dysbiosis and a decrease in the species diversity of the intestinal microbiota, allowing opportunistic pathogens to flourish at the expense of the commensal or beneficial microbes [7•].

Effects of antibiotics on the intestinal microbiota

Numerous studies in humans and animals have investigated the effects of various antibiotic classes on the intestinal microbiota [14,20]. Generally, antibiotics deplete the overall organismal abundance and drastically alter the composition leading to a number of metabolic shifts such as decreased production of SCFAs due to reduced carbohydrate fermentation [14,21,22]. After cessation of antibiotic treatment, the microbiota typically recovers in abundance and diversity but may not return to the original community structure as some species may be missing [20] (Figure 1).

The extent of perturbation and damage depends on the particular antibiotic used and the degree of resistance within the community [14]. For example, vancomycin is active against both commensal and pathogenic Gram-positive bacteria leading to intestinal dysbiosis [23–26]. Antibiotic usage can also lead to an increase in antibiotic resistant organisms such as vancomycin-resistant *enterococci*, methicillin-resistant *Staphylococcus aureus* and transfer of antibiotic resistance genes among the microbial community [23,25,27]. The long-term consequence of antibiotic exposure on host health is poorly understood. However, during the short-term (days to weeks), the host may become hyper-susceptible to certain infections and antibiotic-associated diarrhoea.

Clostridium difficile diarrhoea

Antibiotic perturbation of the microbiota predisposes the host to pathogen colonization and overgrowth and potentially diarrhoeal disease caused by bacteria such as *Klebsiella oxytoca*, *Clostridium perfringens*, pathogenic *Escherichia coli* and *C. difficile* [28]. *C. difficile*, an anaerobic, spore-forming Gram-positive bacterium, is the leading cause of AAD in hospitalized patients [3,29]. The incidence of CDI in the last decade has increased leading to a public health burden with estimated economic costs of at least \$1.5 billion per year in the USA alone [30].

Major risk factors for CDI include prolonged antimicrobial use, exposure to a healthcare environment and advanced age [29]. *C. difficile* produces 2 toxins encoded by *tdcA* and *tdcB* (though some variants also produce a third binary toxin) that are known to facilitate pathogenesis [31]. *C. difficile* colonization is associated with different outcomes ranging from asymptomatic carriage, mild/chronic diarrhoea, fulminant colitis to toxic megacolon and even death [29,32*] (Figure 1).

C. difficile produces highly resistant and transmissible spores that can potentially persist in the gut of infected individuals or contaminate skin or environmental surfaces. These spores are resistant to antibiotics and can re-colonize and germinate after antibiotic therapy leading to recurrent disease [29,33]. Asymptomatic carriers are also a source of spores that can promote transmission and persistence at both the hospital and global levels [5].

Standard treatments for CDI involve the use of antibiotics such as metronidazole and vancomycin [34,35]. However, 15–35% of these patients usually experience recurrent disease, leaving few treatment options [36] (Figure 1). Many patients become dependent on vancomycin (oral, tapered and/or pulsed) to maintain remission and this has implications for the intestinal microbiota. Chang *et al.* used 16S rRNA gene clone libraries to analyse the faecal microbiota of 7 patients with initial and recurrent CDI [21]. This study demonstrated that patients with recurrent CDI had decreased proportional abundance of Bacteroidetes and

increased Proteobacteria and Verrucomicrobia [21]. However, a new antibiotic, fidaxomicin was found to have lower rates of recurrence of *C. difficile* infection associated with the non-epidemic strain compared to vancomycin, an effect that is attributed to its lower activity against commensal and beneficial gut microbes [26].

FMT for treatment of recurrent CDI

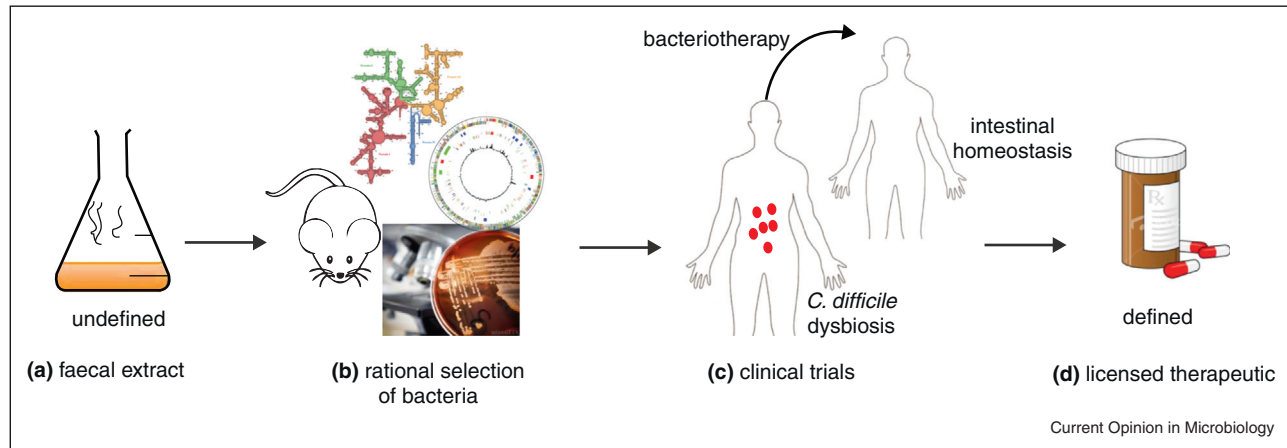
Because of the recent increase in the rates of recurrence and severity from epidemic *C. difficile* strains, there has been renewed interest in the use of FMT for the treatment of recurrent CDI. In this process, homogenized faeces from a healthy donor is infused through colonoscopy, enema or nasogastrically to an individual with *C. difficile* disease to restore the intestinal microbiota and thereby eradicate CDI [37–39] (Figure 1). The donor, usually a healthy individual/relative, is screened for contagious pathogenic agents such as *Salmonella* spp., *Staphylococcus aureus*, *C. difficile* and HIV and other infections or inflammatory conditions as previously described [32*].

The intestinal microbiota of *C. difficile* patients treated with FMT is characterized by expansions in species diversity characterized by an increase in *Bacteroides*, *Roseburia* and *Faecalibacterium* and a reduction in *Enterobacteriaceae* [40] (Figure 1). Recently, Hamilton and co-workers analysed the faecal microbiota of 3 patients treated by FMT using frozen donor sample from the same individual and an increase in Bacteroidetes and Firmicutes was observed [41]. The Bacteroidetes were represented by greater abundance of the families *Bacteroidaceae*, *Rikenellaceae* and *Porphyromonadaceae* and members of the Firmicutes were represented by *Lachnospiraceae*, *Ruminococcaceae* and unclassified Firmicutes [41]. These families are typically found in abundant numbers in the intestines of healthy individuals, suggesting that key groups of health-promoting bacteria are associated with the displacement of CDI and restoration of homeostasis in treated patients.

Several studies that employed FMT for the treatment of recurrent CDI have reported success rates of 86–100% [32*,40–42]. van Nood and colleagues recently published the first clinical trial to directly compare FMT with vancomycin only or vancomycin with bowel lavage for the treatment of recurrent CDI [43**]. Remarkably, FMT resolved 13 of the 16 cases (81%) after first infusion compared to only 31% for vancomycin and 23% for vancomycin with bowel lavage [43**]. On second infusion, 2 of the 3 failed cases had resolution of CDI leading to an overall success rate of >90% in this trial [43**]. The success rates observed in these studies highlight the importance of having a healthy and diverse intestinal microbiota and should establish FMT as a viable clinical option for diseases associated with intestinal dysbiosis.

Presently, there is no global standardized protocol for FMT as the process is largely dependent on the centre

Figure 2



Generic model to create a standardized, defined bacteriotherapy mixture for treatment of patients with severe CDI. Culturing and genomic profiling of faecal samples from healthy donors and CDI patients (a) could potentially identify candidate bacteria that can be tested *in vivo* for safety and efficacy. Whole genome sequencing will define the bacteria and provide a basis to determine phylogenetic position within the microbiota community (b). Clinical trials (c) will be required to test efficacy of bacteriotherapy mixture in diseased humans with severe CDI before widespread clinical use (d).

performing the FMT. However, it is pertinent to note that in 2011, the Faecal Microbiota Transplantation Workgroup proposed standard guidelines for treatment of CDI with FMT such as patient inclusion/exclusion criteria, donor selection and screening, preparation and administering of faecal samples [32*].

Rational design of a defined bacteriotherapy

The use of undefined faecal samples for FMT creates a barrier for widespread clinical use, mainly because of the amount of time needed to prepare and screen donor samples, patient safety issues, non-standardization of the treatment procedure and general doctor and patient aversion [6,44]. Therefore, there is an unmet clinical need to design a combination of harmless, health associated bacteria as a viable therapeutic option (Figure 2). Over two decades ago, Tvede and Rask-Madsen demonstrated that simple mixtures of 10 bacteria isolated from healthy faecal samples can resolve recurrent *C. difficile* as effectively as whole faecal transplants [45**]. More recently, a mixture of 33 bacterial species isolated from a healthy donor was used to eradicate CDI in two patients [46**]. These studies have pioneered the concept of 'Bacteriotherapy' in humans but have yet to meet the scrutiny of regulatory agencies during the development a pharmaceutical product [47].

Experimental studies are also providing insight into the mechanisms of successful FMT. A recently developed murine infection model for *C. difficile* used a mixture of 6 defined bacteria to cure mice infected with the epidemic *C. difficile* 027 strain [48]. During the resolution of infection, at least 4 out of 6 bacterial strains colonized the mice post-transplant and many low level commensal bacteria present during disease expanded to increase

the microbiota diversity during disease resolution [48]. Whole genome sequencing was used to establish the phylogeny of these therapeutic bacteria within the broader microbiota and also rule out the possibility that they code for known virulence factors. An important study, Reeves *et al.* have used novel culturing methods to identify a single *Lachnospiraceae* strain that can suppress *C. difficile* infection in mice [49]. Studies into the basic mechanisms of *C. difficile* suppression should guide the rational selection of candidate bacteria for bacteriotherapy development (Figure 2).

Furthermore, it will be important to identify the microbial differences between healthy individuals and patients with severe *C. difficile* disease, as this could guide the selection of the bacterial species from the healthy donors (Figure 2). It may be necessary to conduct a retrospective study to identify the shifts in the intestinal microbiota of patients' post-FMT from several studies that have been published in order to identify what bacterial groups are present after FMT. Though, it is worth noting that personalized responses to FMT have been previously observed [50] and this could be due to factors such as diet, genetics and lifestyle that influence the microbiota structure and function.

Emerging applications of bacteriotherapy

Bacteriotherapy has potential applications for other diseases associated with intestinal dysbiosis such as IBD and IBS. Borody and colleagues used FMT to treat 6 patients with refractory ulcerative colitis and follow up at 1–13 years post-FMT showed no clinical evidence of ulcerative colitis [51]. Similarly, Duplessis *et al.* utilized FMT to treat a patient with severe Crohn's disease complicated

by refractory CDI [52]. Obesity is another condition that could potentially benefit from FMT [17,53]. FMT from lean to obese individuals led to increased insulin sensitivity in the patients compared to the control group [54].

Conclusion

The diversity of the human intestinal microbiota is key to a number of biological processes that ensure the well-being of an individual. Alterations caused by long-term antibiotic use and infections are detrimental to the host as seen in CDI. FMT is increasingly being accepted as a treatment for recurrent CDI, but large, randomized double-blinded studies are needed. However, beyond FMT, bacteriotherapy using standardized mixtures of beneficial bacteria could potentially be used in the future for the treatment of recurrent CDI and other diseases associated with dysbiosis in the intestinal microbiota such as IBD, IBS and obesity.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Morgan XC, Segata N, Huttenhower C: **Biodiversity and functional genomics in the human microbiome.** *Trends Genet* 2012, **29**:51-58.
2. Lawley TD, Walker AW: **Intestinal colonization resistance.** *Immunology* 2013, **138**:1-11.
3. He M, Miyajima F, Roberts P, Ellison L, Pickard DJ, Martin MJ, Connor TR, Harris SR, Fairley D, Bamford KB, D'Arc S *et al.*: **Emergence and global spread of epidemic healthcare-associated *Clostridium difficile*.** *Nat Genet* 2013, **45**:109-113.
4. 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**:529-549.
5. Clements ACA, Magalhães RJS, Tatem AJ, Paterson DL, Riley TV: ***Clostridium difficile* PCR ribotype 027: assessing the risks of further worldwide spread.** *Lancet Infect Dis* 2010, **10**:395-404.
6. Borody TJ, Khoruts A: **Fecal microbiota transplantation and emerging applications.** *Nat Rev Gastroenterol Hepatol* 2012, **9**:88-96.
7. Sekirov I, Russell SL, Antunes LCM, Finlay BB: **Gut microbiota in health and disease.** *Physiol Rev* 2010, **90**:859-904.
8. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR *et al.*: **A human gut microbial gene catalogue established by metagenomic sequencing.** *Nature* 2010, **464**:59-65.
9. Fraher MH, O'Toole PW, Quigley EMM: **Techniques used to characterize the gut microbiota: a guide for the clinician.** *Nat Rev Gastroenterol Hepatol* 2012, **9**:312-322.
10. Dave M, Higgins PD, Middha S, Rioux KP: **The human gut microbiome: current knowledge, challenges, and future directions.** *Transl Res* 2012, **160**:246-257.
11. Maurice CF, Haiser HJ, Turnbaugh PJ: **Xenobiotics shape the physiology and gene expression of the active human gut microbiome.** *Cell* 2013, **152**:39-50.
12. Kau AL, Ahern PP, Griffin NW, Goodman AL, Gordon JL: **Human nutrition, the gut microbiome and the immune system.** *Nature* 2011, **474**:327-336.
13. Lozupone CA, Stombaugh JI, Gordon JL, Jansson JK, Knight R: **Diversity, stability and resilience of the human gut microbiota.** *Nature* 2012, **489**:220-230.
14. Jernberg C, Löfmark S, Edlund C, Jansson JK: **Long-term impacts of antibiotic exposure on the human intestinal microbiota.** *Microbiology* 2010, **156**:3216-3223.
15. Manichanh C, Borruel N, Casellas F, Guarner F: **The gut microbiota in IBD.** *Nat Rev Gastroenterol Hepatol* 2012, **9**:599-608.
16. Azad MB, Kozyrskyj AL: **Perinatal programming of asthma: the role of gut microbiota.** *Clin Dev Immunol* 2012, **2012**:9.
17. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M *et al.*: **A core gut microbiome in obese and lean twins.** *Nature* 2009, **457**:480-484.
18. Stecher B, Robbiani R, Walker AW, Westendorf AM, Barthel M, Kremer M, Chaffron S, Macpherson AJ, Buer J, Parkhill J, Dougan G *et al.*: ***Salmonella enterica* serovar typhimurium exploits inflammation to compete with the intestinal microbiota.** *PLoS Biol* 2007, **5**:2177-2189.
19. Lupp C, Robertson ML, Wickham ME, Sekirov I, Champion OL, Gaynor EC, Finlay BB: **Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of enterobacteriaceae.** *Cell Host Microbe* 2007, **2**:119-129.
20. Dethlefsen L, Relman DA: **Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation.** *Proc Natl Acad Sci U S A* 2011, **108**:4554-4561.
21. Chang JY, Antonopoulos DA, Kalra A, Tonelli A, Khalife WT, Schmidt TM, Young VB: **Decreased diversity of the fecal microbiome in recurrent *Clostridium difficile*-associated diarrhea.** *J Infect Dis* 2008, **197**:435-438.
22. Binder HJ: **Role of colonic short-chain fatty acid transport in diarrhea.** *Annu Rev Physiol* 2010, **72**:297-313.
23. Robinson CJ, Young VB: **Antibiotic administration alters the community structure of the gastrointestinal microbiota.** *Gut Microbes* 2010, **1**:279-284.
24. Rea MC, Dobson A, O'Sullivan O, Crispie F, Fouhy F, Cotter PD, Shanahan F, Kiely B, Hill C, Ross RP: **Effect of broad- and narrow-spectrum antimicrobials on *Clostridium difficile* and microbial diversity in a model of the distal colon.** *Proc Natl Acad Sci U S A* 2011, **108**:4639-4644.
25. Ubeda C, Taur Y, Jenq RR, Equinda MJ, Son T, Samstein M, Viale A, Socoli ND, van den Brink MRM, Kamboj M, Pamer EG: **Vancomycin-resistant enterococcus domination of intestinal microbiota is enabled by antibiotic treatment in mice and precedes bloodstream invasion in humans.** *J Clin Invest* 2010, **120**:4332-4341.
26. Louie TJ, Miller MA, Mullane KM, Weiss K, Lentnek A, Golan Y, Gorbach S, Sears P, Shue YK: **Fidaxomicin versus vancomycin for *Clostridium difficile* infection.** *N Engl J Med* 2011, **364**:422-431.
27. Lode HM: **Clinical impact of antibiotic-resistant gram-positive pathogens.** *Clin Microbiol Infect* 2009, **15**:212-217.
28. Cochetière MF, Durand T, Lalande V, Petit JC, Potel G, Beaugerie L: **Effect of antibiotic therapy on human fecal microbiota and the relation to the development of *Clostridium difficile*.** *Microb Ecol* 2008, **56**:395-402.

29. Deakin LJ, Clare S, Fagan RP, Dawson LF, Pickard DJ, West MR, Wren BW, Fairweather NF, Dougan G, Lawley TD: **The *Clostridium difficile* spo0A gene is a persistence and transmission factor.** *Infect Immun* 2012, **80**:2704-2711.
30. Ghantouji SS, Sail K, Lairson DR, DuPont HL, Garey KW: **Economic healthcare costs of *Clostridium difficile* infection: a systematic review.** *J Hosp Infect* 2010, **74**:309-318.
31. Carter GP, Rood JI, Lyras D: **The role of toxin a and toxin b in *Clostridium difficile*-associated disease: past and present perspectives.** *Gut Microbes* 2010, **1**:58-64.
32. Bakken JS, Borody T, Brandt LJ, Brill JV, Demarco DC, Franzos MA, Kelly C, Khoruts A, Louie T, Martinelli LP, Moore TA et al.: **Treating *Clostridium difficile* infection with fecal microbiota transplantation.** *Clin Gastroenterol Hepatol* 2011, **9**:1044-1049.
- This article focuses on the standard practice guidelines for FMT including the criteria for donor and recipient selection.
33. Underwood S, Guan S, Vijayasubhash V, Baines SD, Graham L, Lewis RJ, Wilcox MH, Stephenson K: **Characterization of the sporulation initiation pathway of *Clostridium difficile* and its role in toxin production.** *J Bacteriol* 2009, **191**:7296-7305.
34. Rea MC, O'Sullivan O, Shanahan F, O'Toole PW, Stanton C, Ross RP, Hill C: ***Clostridium difficile* carriage in elderly subjects and associated changes in the intestinal microbiota.** *J Clin Microbiol* 2012, **50**:867-875.
35. Cornely OA, Crook DW, Esposito R, Poirier A, Somero MS, Weiss K, Sears P, Gorbach S: **Fidaxomicin versus vancomycin for infection with *Clostridium difficile* in Europe, Canada, and the USA: a double-blind, non-inferiority, randomised controlled trial.** *Lancet Infect Dis* 2012, **12**:281-289.
36. van Nood E, Speelman P, Kuijper EJ, Keller JJ: **Struggling with recurrent *Clostridium difficile* infections: is donor faeces the solution?** *Euro Surveill* 2009, **14**:19316.
37. Khoruts A, Dicksved J, Jansson JK, Sadowsky MJ: **Changes in the composition of the human fecal microbiome after bacteriotherapy for recurrent *Clostridium difficile*-associated diarrhea.** *J Clin Gastroenterol* 2010, **44**:354-360.
38. Rubin TA, Gessert CE, Aas J, Bakken JS: **Fecal microbiome transplantation for recurrent *Clostridium difficile* infection: report on a case series.** *Anaerobe* 2013, **19**:22-26.
39. Bakken JS: **Fecal bacteriotherapy for recurrent *Clostridium difficile* infection.** *Anaerobe* 2009, **15**:285-289.
40. Shahinas D, Silverman M, Sittler T, Chiu C, Kim P, Allen-Vercoe E, Weese S, Wong A, Low DE, Pillai DR: **Toward an understanding of changes in diversity associated with fecal microbiome transplantation based on 16S rRNA gene deep sequencing.** *mBio* 2012, **3**.
41. Hamilton MJ, Weingarden AR, Unno T, Khoruts A, Sadowsky MJ: **High-throughput DNA sequence analysis reveals stable engraftment of gut microbiota following transplantation of previously frozen fecal bacteria.** *Gut Microbes* 2013, **4**:125-135.
42. Yoon SS, Brandt LJ: **Treatment of refractory/recurrent *C. difficile*-associated disease by donated stool transplanted via colonoscopy: a case series of 12 patients.** *J Clin Gastroenterol* 2010, **44**:562-566.
43. van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, Visser CE, Kuijper EJ, Bartelsman JFWM, Tijssen JGP, Speelman P et al.: **Duodenal infusion of donor feces for recurrent *Clostridium difficile*.** *N Engl J Med* 2013, **368**:407-415.
- First clinical trial comparing FMT with vancomycin for treatment of recurrent CDI. FMT led to overall resolution in >90% of patients compared to 31% with vancomycin.
44. Zipursky JS, Sidorosky TI, Freedman CA, Sidorosky MN, Kirkland KB: **Patient attitudes toward the use of fecal microbiota transplantation in the treatment of recurrent *Clostridium difficile* infection.** *Clin Infect Dis* 2012, **55**:1652-1658.
45. Tvede M, Rask-Madsen J: **Bacteriotherapy for chronic relapsing *Clostridium difficile* diarrhoea in six patients.** *Lancet* 1989, **333**:1156-1160.
- The first study on bacteriotherapy using a mixture of 10 bacterial strains to treat patients with relapsing *Clostridium difficile* diarrhoea.
46. Petrof E, Gloor G, Vanner S, Weese S, Carter D, Daigneault M, Brown E, Schroeter K, Allen-Vercoe E: **Stool substitute transplant therapy for the eradication of *Clostridium difficile* infection: 'RePOOPulating' the gut.** *Microbiome* 2013, **1**:3.
- Second study describing the use of designed mixture of bacterial strains (33) isolated from a healthy donor to treat two individuals with recurrent CDI.
47. Relman DA: **Restoration of the gut microbial habitat as a disease therapy.** *Nat Biotechnol* 2013, **31**:35-37.
48. Lawley TD, Clare S, Walker AW, Stares MD, Connor TR, Raisen C, Goulding D, Rad R, Schreiber F, Brandt C, Deakin LJ et al.: **Targeted restoration of the intestinal microbiota with a simple, defined bacteriotherapy resolves relapsing *Clostridium difficile* disease in mice.** *PLoS Pathog* 2012, **8**:e1002995.
49. Reeves AE, Koenigsnecht MJ, Bergin IL, Young VB: **Suppression of *Clostridium difficile* in the gastrointestinal tracts of germfree mice inoculated with a murine isolate from the family *lachnospiraceae*.** *Infect Immun* 2012, **80**:3786-3794.
50. Goodman AL, Kallstrom G, Faith JJ, Reyes A, Moore A, Dantas G, Gordon JI: **Extensive personal human gut microbiota culture collections characterized and manipulated in gnotobiotic mice.** *Proc Natl Acad Sci U S A* 2011, **108**:6252-6257.
51. Borody TJ, Campbell J: **Fecal microbiota transplantation: techniques, applications, and issues.** *Gastroenterol Clin N Am* 2012, **41**:781-803.
52. Duplessis CA, You D, Johnson M, Speziale A: **Efficacious outcome employing fecal bacteriotherapy in severe Crohn's colitis complicated by refractory *Clostridium difficile* infection.** *Infection* 2012, **40**:469-472.
53. Schwartz A, Taras D, Schäfer K, Beijer S, Bos NA, Donus C, Hardt PD: **Microbiota and SCFA in lean and overweight healthy subjects.** *Obesity* 2010, **18**:190-195.
54. Vrieze A, Van Nood E, Holleman F, Salojärvi J, Kootte RS, Bartelsman JFWM, Dallinga-Thie GM, Ackermans MT, Serlie MJ, Oozeer R, Derrien M et al.: **Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome.** *Gastroenterology* 2012, **143**:913.e7-916.e7.