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Review

Fecal Microbiota-based Therapeutics for Recurrent *Clostridium difficile* Infection, Ulcerative Colitis and Obesity

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ABSTRACT

The human gut microbiome is a complex ecosystem of fundamental importance to human health. Our increased understanding of gut microbial composition and functional interactions in health and disease states has spurred research efforts examining the gut microbiome as a valuable target for therapeutic intervention. This review provides updated insight into the state of the gut microbiome in recurrent *Clostridium difficile* infection (CDI), ulcerative colitis (UC), and obesity while addressing the rationale for the modulation of the gut microbiome using fecal microbiota transplant (FMT)-based therapies. Current microbiome-based therapeutics in pre-clinical or clinical development are discussed. We end by putting this within the context of the current regulatory framework surrounding FMT and related therapies.

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Contents

1. Introduction

Our microbial inhabitants that form the human gut microbiome influence many aspects of our physiology that were previously unrecognized.

As the gut microbiome has recently become acknowledged as a virtual 'organ' in human biology, many studies have associated problems of this ecosystem with a growing number of both gastrointestinal (GI) and non-GI disease states. Because gut microbial composition tends to remain relatively temporally stable within a given individual, there is potential for this ecosystem to be exploited both as a predictor of health-status and a valuable target for therapeutic intervention (Faith et al., 2013). In this review, we will examine how our increased understanding of gut microbiota has led to a surge in microbiome-based therapeutics for human medicine.

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2. The Human Gut Microbiome

The human gut microbiome is the collection of microorganisms, their gene products and corresponding physiological functions found in the human GI tract (Gill et al., 2006). Much of our current understanding of the bacterial composition of the human gut microbiome has come from 'omics-based' survey initiatives such as the Human Microbiome Project (Human Microbiome Project Consortium, 2012). These studies have provided great insight into gut microbial diversity, however, understanding of the function of the microbiome has lagged behind. To appreciate how the gut microbiome influences health, we must consider not only the composition of the microbiome but also microbe-microbe and host-microbe interactions.

2.1. How Is a 'Healthy' Microbiome Defined?

Our gut microbial inhabitants collectively provide a myriad of beneficial physiological functions; for example, production of vitamins and nutrients, regulation of metabolism, exclusion of pathogens, and maintenance of the immune system (Lozupone et al., 2012). The average healthy adult colon contains a dense microbial community of approximately 160 bacterial species (Qin et al., 2010). Phylogenomic studies of stool samples, primarily from Western populations, have shown that although gut microbial composition varies greatly between individuals there are general trends among healthy adults. Bacteroidetes and Firmicutes are the most prevalent bacterial phyla, while Actinobacteria and Proteobacteria are also prominent members (Human Microbiome Project Consortium, 2012). The large inter-individual variation in the ratios of these representative phyla can be attributed to numerous influences; a recent study found 69 factors that significantly correlated with overall microbiota compositional variation including medication/drug use, health-status, age, sex, lifestyle, host genetics, diet, and animal exposure (Falony et al., 2016).

The functional profile of the gut microbiome is more conserved than the taxonomic composition (Qin et al., 2010). Multiple studies have shown that the overall metabonomic (complex system associated metabolomic profile) (Marchesi and Ravel, 2015) and genetic capabilities of gut microbial ecosystems appear to be somewhat consistent across healthy adults (Human Microbiome Project Consortium, 2012; Qin et al., 2010; Turnbaugh et al., 2009). For example, short-chain fatty acids (SCFAs), produced by many gut microbial species through fermentation, provide 10% of our daily energy requirements, regulate host energy demands, intestinal epithelial cell homeostasis, and immune function, and also support the growth of the gut microbiota (Marchesi et al., 2015). Overall, a diverse microbial consortium displaying functional redundancy will preserve and augment essential functions associated with gut homeostasis (Rampelli et al., 2015).

2.2. Gut Microbial 'Dysbiosis'?

Gut dysbiosis can be defined as an imbalance in a microbial ecosystem characterized by a shift in the composition or function of microbes, which can result in pathogenesis (Fig. 1). Antibiotics, toxic compounds, poor diet, medical interventions, and disease can cause disturbances to, and potentially loss of diversity within the gut microbiota ecosystem (Hell et al., 2013). Diet is a major modulator of the gut microbiota; in the absence of dietary complex carbohydrates (the main substrates for SCFA production), microbial metabolism will shift towards proteolytic fermentation, which results in the generation of potentially toxic, pro-inflammatory compounds such as amines, ammonia, phenols and sulfides (Vipperla and O'Keefe, 2016). Such compounds have been implicated in gut dysbiosis and the development of colorectal cancer and inflammatory bowel disease (IBD) (Vipperla and O'Keefe, 2016).

However, defining gut microbial dysbiosis is challenging because of the compositional variability of the microbial ecosystem across different individuals. Although currently there is no biomarker for dysbiosis, recent work suggests that certain correlations may become diagnostically useful. For example, low fecal chromogranin A (CgA) concentrations, alongside higher microbial diversity and functional richness has been found to associate with higher high-density lipoprotein (HDL) concentrations (Zhernakova et al., 2016).

3. Gut Dysbiosis in *Clostridium difficile* Infection (CDI)

C. difficile is an anaerobic, sporulating, bacterial pathogen that is the etiological agent of CDI (Abt et al., 2016). CDI is a leading cause of nosocomial, antibiotic-associated diarrhea and the disease pathology is mediated by bacterial secreted toxins (Abt et al., 2016). Under normal circumstances, the resident gut microbiota are thought to facilitate colonization resistance against *C. difficile* suppressing its pathogenic activity in the colon (Theriot et al., 2014). However, broad-spectrum antibiotic use disrupts host-microbiota homeostasis by decreasing gut microbiota abundance, diversity, community structure, and altering metabonomic functional profiles (Dethlefsen et al., 2008; Perez-Cobas et al., 2013a, 2013b; Theriot et al., 2016, 2014). Many studies have described microbial taxonomic alterations in CDI patients (Table 1). In general, the gut microbiota of CDI patients have reduced overall bacterial diversity, characterized by increases in Proteobacteria with decreases in Firmicutes and Bacteroidetes phyla in comparison to healthy subjects (Antharam et al., 2013; Chang et al., 2008; Fuentes et al., 2014; Rea et al., 2012; Schubert et al., 2014; Shahinas et al., 2012). However, taxonomic changes do not appear to be consistent between distinct patient cohorts likely because of the large inter-individual variation in human gut microbiota (Girotra et al., 2016; Schubert et al., 2014).

Metabonomic alterations as a result of antibiotic use are important in the context of CDI (Theriot et al., 2014). As an example, primary and secondary bile acids have markedly different effects on *C. difficile*; primary bile acids promote growth of this pathogen while secondary bile acids are inhibitory. Thus, an antibiotic-induced perturbation that reduces secondary bile acid production can promote CDI (Theriot et al., 2016). In addition, mucosal carbohydrate concentrations, which can increase when antibiotics are present, can promote *C. difficile* expansion (Ng et al., 2013).

The standard treatment for CDI is metronidazole or oral vancomycin, which kill *C. difficile*, providing symptomatic relief and allowing restoration of colonization resistance (Surawicz et al., 2013). However, in approximately 25–30% of cases, persistent *C. difficile* (as endospores) or introduction of a new strain can lead to disease recurrence, which is more likely when there is incomplete recovery of the gut microbiota (Cornely et al., 2012). Management of recurrent CDI (rCDI) is a major clinical challenge; patients that are non-responsive to antimicrobial therapy require alternative treatment options (Lapointe-Shaw et al., 2016).

4. Gut Dysbiosis in Ulcerative Colitis (UC)

UC, a form of IBD, is a chronic, relapsing, idiopathic, inflammatory disorder of the colon and rectum (Bouma and Strober, 2003). In the last decade, there have been increases in UC incidence and prevalence worldwide, making it an important emerging global disease (Molodecky et al., 2012). Although the pathogenesis of UC is complex, multifactorial, and not fully understood, aberrant host immune responses, a dysfunctional intestinal barrier, and gut microbiota dysbiosis have been associated with this condition (Lepage et al., 2011; Michail et al., 2012).

Various studies have demonstrated a reduction of gut microbial diversity and taxonomic compositional differences in UC patients
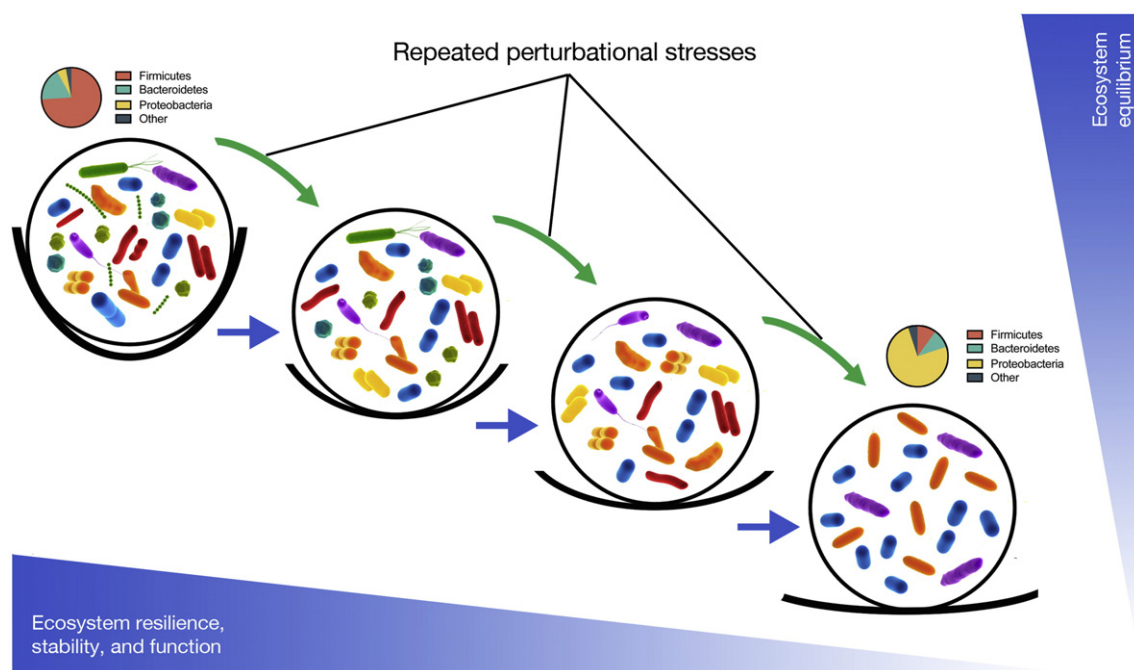
Fig. 1. Alternative stable states (ball and cup model) of human gut microbial ecosystems. Here we conceptualize the ecological stability of the human gut microbiota as inferred through its species richness. The gut microbial ecosystem is represented as a ball that exists within an equilibrium represented as a cup. The depth of each cup is symbolic of the resilience of a given ecosystem and is related to species richness, where lower diversity leads to lower resilience and the greater the likelihood that the "ball" will roll out of the "cup" into a new state of equilibrium that may be less stable that the first. Perturbational stresses caused by numerous factors such as poor diet, disease state, drug use (including antimicrobials), immunosenescence as examples can impact bacterial diversity and force the ecosystem into a less-stable equilibrium state. Repeated stresses can cause a situation where function may be reduced to the point that dysbiosis and the development of disease ensue.
Adapted from (Relman, 2012) and (Folke et al., 2004).

compared to healthy individuals, although less stereotypical trends are observed in taxonomic variation in comparison with CDI patients (Table 1). Notably, UC has been associated with decreases in species within the Lachnospiraceae family, and *Faecalibacterium prausnitzii* (Lepage et al., 2011; Rossen et al., 2015). As known butyrate producers, their reduction may account for the depletion of this SCFA observed in some UC patients (Lepage et al., 2011). Additionally, *Akkermansia muciniphila*, a commensal gut microbe that contributes to the homeostatic balance of the intestinal mucus layer was found to be reduced in UC patients (Png et al., 2010) potentially resulting in decreased mucosal thickness corroborating histologic findings in rectal biopsies from active UC patients (Pullan et al., 1994). Driven by observations of gut microbial dysbiosis in UC, there is interest in the development and application of microbiome-based therapeutics for this indication.

5. Gut Dysbiosis in Obesity

Obesity is a severe worldwide public health issue that has reached epidemic status in many industrialized countries (Villanueva-Millan et al., 2015), and is defined as the accumulation of excess adipose tissue to the detriment of health (Boulange et al., 2016) defined as a body mass index (BMI) > 30 in adults. Dietary intake is a principal contributor to the pathophysiology of obesity, however, gut microbes can modulate nutrient uptake and energy regulation providing an important, albeit underexplored environmental factor in metabolic syndrome and obesity-related disorders (Boulange et al., 2016). The connection between obesity and the gut microbiome is complicated by heterogeneity of patient diet, genetics, age, lifestyle, hormones, and disease as well as the complexity of clinical presentation of metabolic disorders (Boulange et al., 2016).

The gut microbiome has been implicated as an important player in obesity following the seminal discovery that the ratio of Firmicutes to Bacteroidetes (F:B), and energy harvest capacity differs in obese versus lean animal and human subjects (Ley et al., 2006; Turnbaugh et al., 2009). However, other studies of this link have provided conflicting results (Duncan et al., 2008; Fernandes et al., 2014; Schwiertz et al., 2010), thus, currently, there is no consensus of the importance of the F:B ratio as an indicator of obesity. In spite of this, many groups have found that both obese animal and human subjects have an altered gut microbiota compared to their lean counterparts characterized by reduced bacterial diversity, and altered colonic fermentation potential (Fernandes et al., 2014; Le Chatelier et al., 2013; Schwiertz et al., 2010; Teixeira et al., 2013; Vrieze et al., 2012) (Table 1). Metagenomically, the gut microbiome can be classified into two groups: high gene count (HGC) or low gene count (LGC). The HGC group, most often seen in lean individuals, was associated with increased *F. prausnitzii* and butyrate (SCFA) levels, while the LGC group, most often seen in obese individuals, was associated with lower butyrate production, but higher levels of *Bacteroides* spp. and *Ruminococcus gnavus* and more genes putatively associated with pro-carcinogenic metabolite production and oxidative stress (Le Chatelier et al., 2013). Diet-induced weight loss interventions in the LGC group could partially restore these metabolic changes, illustrating the plasticity of the gut microbiome and the significant impact of diet in obesity-related diseases (Cotillard et al., 2013).

6. Modulation of the Gut Microbiome Using Stool-based Therapeutics

In Table 2, we list representative examples of live microbial preparations and microbial-based products that are currently being developed with therapeutic manipulation of the gut microbiome or its associated host interactions in mind. Fecal-microbiota derived products, probiotic,

Table 1
Microbial taxa positively correlated to select gut conditions in human subjects.

Condition	Study size/details	Microbial taxonomic changes associated with condition[a]	Reference
CDI	Fecal samples from n = 14 recurrent CDI patients (4 within this group given FMT)	↑ Proteobacteria, ↓ Bacteroidetes, ↓ Firmicutes	Weingarden et al., 2015
	Fecal samples from n = 94 CDI, and n = 89 non-CDI inpatients	↑ Proteobacteria, ↓ Firmicutes (except Enterococcus and Erysipelotrichia and some Lachnospiraceae)	Schubert et al., 2014
	Fecal samples from n = 9 recurrent CDI patients given FMT in van Nood et al. (2013) study	↑ Proteobacteria, ↑ Bacilli, ↓ Bacteroidetes, ↓ Firmicutes, ↓ overall microbial diversity compared to post-FMT and healthy donor samples	Fuentes et al., 2014
	Fecal samples from n = 22 C. difficile culture-positive elderly subjects, and n = 252 healthy elderly subjects	↑ Proteobacteria, Firmicutes (↓ Enterococcaceae and ↑ Lactobacillaceae), ↓ overall microbial diversity	Rea et al., 2012
	Fecal samples from n = 39 CDI patients, n = 36 C. difficile-negative nosocomial diarrhea patients, and n = 40 healthy control subjects	↓ Firmicutes, ↑ Proteobacteria, ↓ Microbial diversity in both diarrheal groups compared to healthy controls	Antharam et al., 2013
	Stool samples from n = 6 CDI patients given FMT	↑ Proteobacteria, ↓ Bacteroidetes, ↓ Firmicutes (specifically Lachnospiraceae, ↓ overall microbial diversity	Shahinas et al., 2012
UC	Fecal samples from n = 15 UC patients and n = 15 control subjects	↓ Bacteroidetes, ↓ Firmicutes, ↓ overall microbial diversity	Rajilic-Stojanovic et al., 2013
	RCT on FMT for UC (58 fecal samples total from n = 34 UC patients, and n = 24 healthy donors)	↓ Firmicutes, ↑ Proteobacteria ↑ Bacteroidetes, ↑ Bacilli, no change in bacterial diversity	Rossen et al., 2015
	Sequencing completed on inflamed and non-inflamed mucosal biopsies from n = 6 UC patients, and n = 5 healthy controls	↑ Bacteroidetes, ↓ Firmicutes	Walker et al., 2011
	Fecal samples from n = 36 active UC patients treated with FMT, and n = 34 active UC patients given placebo	Reduced bacterial diversity, no information on phyla, but: ↓ Lachnospiraceae, ↓ Ruminococcus	Moayyedi et al., 2015
	Sigmoid colon biopsies from n = 8 monozygotic twin pairs discordant for UC, and n = 10 healthy individuals	↑ Actinobacteria, ↑ Proteobacteria (↓ Bacteroidetes), no change in Firmicutes, ↓ Overall bacterial diversity	Lepage et al., 2011
Obesity	Gut microbial communities profiled from n = 31 monozygotic twin pairs, and n = 23 dizygotic twin pairs generally concordant for obesity or leanness	↓ Bacteroidetes, ↑ Actinobacteria, no change in Firmicutes	Turnbaugh et al., 2009
	Stool samples from n = 2 lean, and n = 12 obese individuals	↓ Bacteroidetes, ↑ Firmicutes	Ley et al., 2006
	Stool samples from n = 1 normal-weight adolescent, and n = 1 obese adolescent	↓ Bacteroidetes, ↑ Firmicutes	Ferrer et al., 2013
	Collected fecal samples from n = 52 lean, and n = 42 overweight or obese individuals	No change in Firmicutes to Bacteroidetes (F:B) ratio between lean and obese groups	Fernandes et al., 2014
	Stool samples from n = 30 normal range, n = 35 overweight, n = 33 obese (defined by BMI)	↑ Bacteroidetes, no change in Firmicutes	Schwiertz et al., 2010
	Stool samples from n = 9 metabolic syndrome patients undergoing allogenic gut microbiota (n = 9) from healthy lean individuals or autologous FMT (n = 9)	↓ Overall bacterial diversity, and ↑ Bacteroidetes, ↓ Lachnospiraceae in obese subjects compared to lean prior to FMT	Vrieze et al., 2012

Abbreviations: Body mass index (BMI); fecal microbiota transplantation (FMT); *Clostridium difficile* infection (CDI); randomized controlled clinical trial (RCT); ulcerative colitis (UC).
[a] Phylum level taxonomic changes are listed unless otherwise described.

prebiotic and targeted molecule therapeutics have been developed for a variety of indications. In the following section, we specifically discuss the use of complex stool-based therapeutics as the most commonly used treatment modalities to date.

6.1. Fecal Microbiota Transplantation (FMT)

FMT aims to restore gut microbiota diversity by transferring feces from a healthy donor to a sick patient. The therapy has been extensively used in the treatment of rCDI with good success, likely because the donated gut microbial ecosystem can replace the microbiota lost through antibiotic use and thus suppress *C. difficile* overgrowth, promoting patient recovery (Seekatz et al., 2014). Recent studies have demonstrated effectiveness of FMT for clinical cure of rCDI around 90% (Agrawal et al., 2016; Kelly et al., 2016; van Nood et al., 2013; Youngster et al., 2016, 2014). Failure of a first FMT is strongly associated with previous history of CDI-related hospitalization events, preexisting IBD, being an inpatient while receiving FMT, or severe/complicated rCDI status (Fischer et al., 2016; Khoruts et al., 2016).

Donor strains introduced into the GI tract via FMT durably colonize and establish themselves alongside or in place of the pre-existing microbiota (Li et al., 2016; Seekatz et al., 2014). In general, decreases in Proteobacteria, and increases in Firmicutes and Bacteroidetes have been widely observed in CDI patients following FMT (Fuentes et al., 2014; Hamilton et al., 2013; Kelly et al., 2016; Khoruts et al., 2010; Seekatz et al., 2014; Shahinas et al., 2012; van Nood et al., 2013; Weingarden et al., 2015) (see Table 1). Butyrate-producing bacteria, mainly from Ruminococcaceae and Lachnospiraceae families, are depleted in CDI (Antharam et al., 2013), and the observation that FMT can restore SCFA production by the gut microbiota (Fuentes et al., 2014) has encouraged the investigation of metabonomic changes in the gut microbiome post-FMT. In one study it was shown that post-FMT patient metabolome profiles shifted from their pre-treatment state to closely resemble their donor metabolome counterparts (Weingarden et al., 2014). Increased amounts of secondary bile acids were found to be the main FMT-induced metabolic change, suggesting that restoring bile acid composition with FMT may play an important role in treating rCDI (Weingarden et al., 2014).

Given the success of treating rCDI with FMT, there has been great interest in extending this treatment modality to other gut diseases, notably UC (Table 2). However, while CDI clearly results from a simple breakdown in gut microbiota diversity, the underlying cause of UC is as yet unknown but likely to be more complex in etiology (Petrof and Khoruts, 2014). Data from individual case studies have suggested that FMT may be of benefit for UC, however there are mixed results from RCTs. To date, FMT has been assessed as a novel therapeutic for UC in two RCTs (Moayyedi et al., 2015; Rossen et al., 2015). The first study found that 24% of patients who received FMT

Table 2
Select gut microbiome-based therapies in clinical or pre-clinical testing.

Active agent	Presumed mechanism of action	Indication	Research status	Company/innovator	Reference/clinical trial identifier[a]
Stool	Restoration of colonization resistance through FMT	CDI	Non-profit stool bank, screening, and FMT product development	OpenBiome	Kazerouni et al., 2015
			Stool bank and screening	Advancing Bio	AdvancingBio, 2016
RBX2660	FMT-derived bacteria to restore gut microbiota	CDI, IBD	Phase II RCT complete for CDI	Rebiotix Inc.	NCT01925417
MET-1	33 strain defined bacterial ecosystem that restores colonization resistance	Recurrent CDI	Proof-of-principle clinical study	NuBiyota	Petrof et al., 2013
SER-109	Purified bacterial spore-based ecological formulation to restore gut microbiota after antibiotic treatment	Recurrent CDI	Missed primary endpoint in phase II RCT	Seres Therapeutics Inc.	Khanna et al., 2016; NCT02437487
SER-287		Mild-to-moderate UC	Phase I RCT		NCT02618187
VE-202	Oral formulation of live Clostridial bacteria that restore immune system homeostasis (increase regulatory T cells)	IBD	Pre-clinical	Vedanta	Atarashi et al., 2013; Furusawa et al., 2013
CBM588	*Clostridium butyricum* bacterial probiotic	Pediatric AAD	Phase II RCT	Osel Inc.	Seki et al., 2003
		UC with pouchitis	Phase II RCT		Yasueda et al., 2016
Lactin-V	*Lactobacillus crispatus* bacterial probiotic	Bacterial vaginosis	Phase II RCT complete		NCT00635622; Hemmerling et al., 2010
		Recurrent UTIs	Phase II RCT complete		NCT00305227; Stapleton et al., 2011
Lactobacillus plantarum	Probiotic to modulate the gut microbiome	CVD and obesity	Phase II RCT complete	OptiBiotix Health PLC	Patent #: WO2015067948A1
CNDO-201	*Trichuris suis* ova, nonpathogenic helminth probiotic to modulate immune system	Crohn's disease	Missed primary endpoints in two phase II RCTs	Fortress Biotech	NCT01576471; NCT01279577
Blautix	Proprietary live bacterial therapeutic	IBS	Phase I RCT complete	4D Pharma Research Ltd.	RNS Number: 4931E
Thetanix	Anti-inflammatory protein derived from *Bacteroides thetaiotaomicron*	Pediatrics Crohn's disease	Phase I RCT		NCT02704728; Eudract 2014-005666-29
SYN-004	Targeted molecule that degrades IV β-lactam antibiotics specifically in gut to protect microbiome	CDI, antibiotic-resistant infections and AAD	Phase II RCT	Synthetic Biologics Inc.	NCT02563106
Avidocins	Genetically modified R-type bacteriocins that have specific antibacterial activity	CDI, foodborne pathogens	Pre-clinical	AvidBiotics	Gebhart et al., 2015; Scholl et al., 2012, 2009
AG014	Genetically modified *Lactococcus lactis* probiotic strain that secretes anti-inflammatory factors	IBD	Phase I RCT complete	Intrexon	Vandenbroucke et al., 2010
SHP-01	Narrow-spectrum lysin (antimicrobial enzyme) specifically targeting *C. difficile*	Acute and recurrent CDI	Pre-clinical	Symbiotic Health	Hirsch et al., 2015; Wang et al., 2015
VT301	Genetically-engineered lactic acid bacterial probiotic that produce elafin to heal gut epithelial lining	IBD	Pre-clinical	ViThera Pharmaceuticals	Motta et al., 2011
SGM-1019	Small molecule inhibitor that modulates host-microbiota interactions	IBD	Phase I RCT complete	Second Genome	Ratner, 2015
NM504/505	Modulating the gut microbiome using prebiotics to improve glucose tolerance and other metabolic parameters	Metformin-intolerant type 2 diabetes	Phase 0 RCT	MicroBiome Therapeutics	NCT01866462; Burton et al., 2015

Abbreviations: Antibiotic-associated diarrhea (AAD); *Clostridium difficile* infection (CDI); cardiovascular disease (CVD); fecal microbiota transplant (FMT); inflammatory bowel disease (IBD); irritable bowel syndrome (IBS); randomized controlled clinical trial (RCT); ulcerative colitis (UC); urinary tract infection (UTI).

[a] Applicable clinical trial registry numbers or primary literature references are included where publicly available.

and 5% who received placebo entered remission after 7 weeks, and identified that FMT patients had increased microbial diversity in comparison to patients given placebo. An interesting trend was noted in that the majority of patients who went into remission after FMT received stool from a single healthy donor, suggesting particular benefits of this donor's microbiota above others (Moayyedi et al., 2015). Although the study did not reach its primary efficacy endpoint, it attained significance for the end point of remission. Interestingly, a greater success in inducing remission was seen in patients who had been recently diagnosed with UC (within the year previous to treatment) compared to patients with a longer history of disease (75% vs. 18%), suggesting a temporal window when UC patients may be more receptive to FMT (Moayyedi et al., 2015).

In contrast, Rossen et al. showed no statistically significant difference in clinical remission between UC patients who received FMT sourced from healthy donors or autologous FMT (their own fecal microbiota), and only 41% of patients who received donor FMT achieved clinical and endoscopic remission. The authors of this study carried out a detailed assessment of the fecal microbiota taxonomic composition pre- and post-FMT and demonstrated that the microbial ecosystems of patients who responded to FMT from a healthy donor increased in the numbers of bacterial species from *Clostridium* clusters IV, XIVa, XVIII while the amount of Bacteroidetes species was reduced (Rossen et al., 2015).

UC has a complex etiology and it is not yet understood whether gut microbial dysbiosis is a cause or effect of disease; this may underlie the apparent range of outcomes after FMT. When treatment is successful in inducing remission, the heterogeneity of patient, donor, and mode of delivery complicate the understanding of which factors contribute to optimal clinical resolution.

Overall, there have been a limited number of studies evaluating the effectiveness of FMT for the treatment of obesity. When microbiota contents were transferred from obese mice into lean germ-free mice, the recipient mice showed an increase in adiposity compared to control

animals (Turnbaugh et al., 2006). Similarly, the transfer of stool from monozygotic or dizygotic twins discordant for obesity into 'humanized' germ-free mice resulted in increased adiposity and the transmission of obesity-associated metabolic phenotypes (Ridaura et al., 2013). Because the gut microbiota imparts such clear phenotypic changes to distinct recipient mammalian hosts, this holds promise for the therapeutic manipulation of the microbiome to treat obesity-related disorders. There is currently only one published report investigating the effects FMT has on metabolic syndrome in human subjects. In this small RCT, FMT from healthy lean donors to patients with metabolic syndrome resulted in increased insulin sensitivity 6 weeks after transfer (Vrieze et al., 2012). This improvement was associated with increased gut microbial diversity (including butyrate-producing bacteria, particularly *Roseburia intestinalis*) (Vrieze et al., 2012). Butyrate is known to play an important role in promoting insulin sensitivity in mice (Lin et al., 2012), thus it is possible that an increase in butyrate production may be responsible for the observed therapeutic effect of FMT (Vrieze et al., 2012). Further studies investigating global compositional and metabolic changes resulting from FMT are ongoing and will be necessary to bring targeted-microbiome therapeutics into clinical practice.

6.2. Stool-substitute Therapies

Stool-substitute therapies aim to combine the high-effectiveness of FMT with the excellent safety-profile of probiotics to mitigate many of the concerns of each respective therapeutic option. To date, this approach has been used only for the treatment of rCDI. Stool-substitute therapies use defined, standardized preparations of stool-derived products, while retaining the compositional, metabolic and transcriptomic properties of fecal communities. Tvede and Rask-Madsen were among the first to use a stool-derived microbial consortium of 10 bacterial strains to cure six patients with CDI (Tvede and Rask-Madsen, 1989). However, at the time of the study, CDI was considered an uncommon complication of antibiotic use and no real progress was made in the field for almost three decades.

In a small proof-of-principle trial we demonstrated a successful cure of two rCDI patients, using a stool-substitute preparation of 33 purified bacterial strains (MET-1) which had been derived from a single, healthy donor (Petrof et al., 2013). As for FMT, the microbiota profiles of the treated patients indicated incorporation of the MET-1 composition strains after a single administration of the therapeutic. Signatures identifying with MET-1 strains were present in the patients 6 months post-treatment, suggesting that a subset was able to colonize the recipients; this sets MET-1 apart from conventional probiotics assessed to date, and highlights the therapeutic potential representative of a multi-species microbial ecosystem (Petrof et al., 2013). Seres Therapeutics Inc. has also developed stool-substitute therapies for rCDI with a varied approach by using a spore-based microbial ecosystem formulation. Results from their first RCT with product SER-109 were promising, however interim results from their phase II RCT have been largely disappointing (Seres Therapeutics Inc., 2016). In general, as with FMT, the utility of stool-substitute therapies for treatment of rCDI requires further research to understand, and capitalize upon, their mechanisms of action.

7. Regulation of Stool-based Therapeutics

There is persuasive evidence for the use of FMT in rCDI, and perhaps also for a number of different diseases where the gut microbiota may play a pivotal role. FMT is cost effective and now recommended for treating rCDI nonresponsive to conventional antibiotic therapy (HQO, 2016; Lapointe-Shaw et al., 2016; Merlo et al., 2016). As such, two registered stool banks have emerged in the USA that screen and provide FMT products for use in the treatment of rCDI (Kazerouni et al., 2015; Advancing Bio, 2016) (Table 2). These organizations aim to facilitate and expand FMT access, nonetheless, the FMT regulatory landscape poses one of the biggest obstacles to efficient delivery of FMT to rCDI

patients, as well as to the exploration of potential uses of FMT for other diseases.

In many countries that recommend FMT for rCDI (e.g. England, Austria, Australia), FMT is not considered a drug by regulatory bodies (GESA, 2015; Kump et al., 2014; NICE, 2014). In contrast, Health Canada, the Federal Drug Administration, (FDA, USA), and the Agence nationale de sécurité du médicament, (ANSM, France) all classify FMT as a drug (ANSM, 2014; FDA, 2013; Health Canada, 2015). Because fecal material is extremely complex containing living microbes, microbial metabolites, host cells, and food particles; it is not inert and thus cannot be standardized as a drug in terms of pharmacokinetics, pharmacodynamics, or consistency of chemical composition. Labeled as a drug, storage of FMT falls under the jurisdiction of the pharmacist, which has caused problems for implementing FMT in North America but perhaps more so in France, where the preparation and documentation of FMT material is the responsibility of the pharmacist (Kump et al., 2014). Pharmacies not equipped for preparation of FMT material must coordinate with a microbiology laboratory, adding an additional layer of complexity. In addition, for tracking purposes donor fecal samples must be stored for a minimum of two years. Across regulatory bodies, there is also no clear consensus on donor screening tests. The ANSM is the most specific, requiring a two-step screening process and indicates which types of culture media and PCR tests should be employed (ANSM, 2014). Health Canada provides less specific recommendations, suggesting a list of potential pathogens for testing at the discretion of the treating physician (Health Canada, 2015).

Currently the FDA will allow FMT for the treatment of rCDI without the need for an Investigational New Drug (IND) application, provided the donor and donor stool are adequately screened, and the health care provider obtains adequate consent from the patient after discussion of risks (FDA, 2013). An IND is required if the above conditions are not met, and for FMT for any indication other than rCDI. Health Canada has released similar restrictions (Health Canada, 2015). In March 2016, the FDA proposed an additional "enforcement discretion" clause that requires the donor be "known" to the treating physician, i.e. that the FMT product used is not obtained from a stool bank (IDSA, 2016). These restrictions, while aimed at improving safety, create delays, as healthy donors need to be sourced and screened. The FDA currently continues to exercise "enforcement discretion", however, the Infectious Disease Society of America (IDSA) has recently requested that IND regulations for using FMT from stool banks to treat rCDI be waived, as they may cause potentially fatal delays in providing treatment for rCDI (IDSA, 2016). IDSA also pointed out that stool banks very comprehensively screen their donors. Indeed, the development of centralized, registered stool banks, with rigorous donor screening and FMT processing protocols in place, would be more efficient overall.

Another issue raised by the IDSA and others is the urgent need to establish a national FMT registry in order to monitor safety and long-term adverse events of FMT (Hecht et al., 2014; Vyas et al., 2015). Earlier this year, the American Gastroenterological Association (AGA) received funding from the National Institutes of Health (NIH) for creation of the first national registry for patients receiving FMT (AGA, 2016). The registry, designed to assess efficacy, patient outcomes and safety, aims to collect data from over 4000 patients over a 10-year period and will begin prospectively collecting clinical data starting in 2017. Such registries have been established for other human tissue products (e.g. blood, semen, breastmilk and organs). The FDA regulates each of these products differently, and the development of a new category of "tissue" for FMT, as advocated by many (Vyas et al., 2015) would address most of the issues surrounding standardization of the practice of FMT. Though regulatory agencies have yet to adopt this stance for FMT, new knowledge obtained from such a registry may provide valuable information in this regard.

This leaves us with the emerging approach of stool-substitute therapies. Several groups are pursuing this avenue of research (Table 2) in the hope of expediting the commercial availability of an effective FMT-like treatment for rCDI that lacks many of its drawbacks.

Regulatory bodies acknowledge that their requirements are not perfect; their goal is to provide optimal safety conditions for the FMT recipient based on the current state of knowledge on FMT, and they recognize that more clinical trials and long-term outcome data are needed. However, regulation must be carefully balanced with the fact that stool is widely available, and overly restrictive policies will drive patients to seek DIY approaches, easily found on the web, which can be dangerous without appropriate medical oversight. As regulatory bodies continue to grapple with the most appropriate way to regulate FMT, either there will need to be a change in regulations to better reflect the complex nature of stool, or the field will be forced to move more towards developing defined mixtures and away from FMT.

8. Outstanding Questions

Given the potential of microbiome-based products for rCDI, there is interest in examining whether stool-derived therapeutics will prove beneficial for other indications including inflammatory intestinal diseases. As we continue to unravel the mechanisms behind the effectiveness of stool-derived therapies for rCDI, many findings emphasize the importance of a robust gut microbiota to host health. Investigating whether such mechanisms hold true for other GI and non-GI indications are important future research directions to pursue. As FMT has been adopted into clinical practice in many countries, the proper standardization of FMT across regulatory bodies, for example through the use of standardized preparations, guidelines surrounding indication, and a better definition of what is considered to be a healthy microbiota will be necessary for efficient, consistent and safe use of this treatment modality (Abt et al., 2016).

9. Search Strategies and Selection Criteria

Data for this review were identified by searches of PubMed, and references from relevant articles using the search terms "gut microbiome", "gut microbiota", "antibiotics", "probiotics", "fecal microbiota transplant", "clostridium difficile", "ulcerative colitis", "obesity". Unpublished reports were included in the FMT regulatory section. An exhaustive web search was conducted to compile the microbiome-based therapeutics in pre-clinical or clinical testing described in Table 2.

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