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Analyzing the Safety and Efficacy of Fecal Microbiota Transplantations for Inflammatory Bowel Disease using Clostridium difficile Infection as a Reference

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Analyzing the Safety and Efficacy of Fecal Microbiota Transplantations for Inflammatory Bowel Disease using *Clostridium difficile* Infection as a Reference

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Abstract

Fecal microbiota transplantation (FMT) is the process by which fecal suspension from a healthy individual is transferred into the gastrointestinal tract of another individual in an attempt to cure certain diseases. This transplantation process has been accredited as being a potential remedy for a growing number of diseases that have been associated with gut microbial imbalances. Interest in FMT has largely been driven by the science community's increasing interest in the gut microbiome and its role in potentially regulating a multitude of different functions and processes within the human body. One disease that has been found to respond exceptionally well to FMT treatments is *Clostridium difficile* infection (CDI). However, while FMT has demonstrated high cure rates for CDI, this transplantation process is no panacea. In fact, the results from FMT treatments on other diseases, such as Inflammatory Bowel Disease (IBD), have not been as impressive as CDI's. This review will examine the existing literature surrounding FMT usage on IBD and will propose a series of experiments and studies needed to truly test the safety and efficacy of FMT for IBD patients. This review will also reference current literature documenting FMT treatments for CDI as a comparative tool for investigating if this form of bacteriotherapy is indeed a viable therapeutic option for treating IBD.

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Introduction.

Intestinal dysbiosis has been theorized as being a causative agent of ill-being for a variety of different diseases. Fecal microbiota transplantation (FMT) offers promising therapeutic options for microbiota restoration; it has found great success in the treatment of *Clostridium difficile* infections (CDI), demonstrating high efficacy rates in the eradication of CDI and CDI-associated symptoms (Rossen et al., 2015a). In addition to intestinal disorders, recent studies have demonstrated relationships between alterations in the gut microbiome and disease causation beyond intestinal disorders, such as with metabolic diseases, neuropsychic diseases, and autoimmune diseases (Xu et al., 2015). Findings from such research have stirred an interest in evaluating the potential therapeutic role FMT may have on certain extraintestinal disorders, such as Inflammatory Bowel Disease (IBD). The etiology of IBD has long been speculated as being a combination of genetic and environmental factors (Foster et al., 2016 & Ek et al., 2014). Recently, intestinal dysbiosis and its association with IBD have fastly become accepted by much of the research community, as shifts in one's gut microbiome have been demonstrated to be an influencing factor in IBD's multifactorial pathophysiology in multiple studies (Colman et al., 2014). However, clinical trials that have attempted to test the efficacy of FMT for IBD patients have been met with varying degrees of success; there have been many severe side effects that have been associated with these FMT treatments as well (Rossen et al., 2015a). Because of these findings, this review will attempt to investigate the efficacy of FMT for IBD and propose a series of experiments aimed at testing the safety and effectiveness of FMT treatments for IBD. This review will also utilize current literature on FMT treatments for CDI as a guide to finding

existing FMT protocols and procedures as well as using it as a comparative tool for investigating FMT as a therapeutic option for IBD patients.

Clostridium difficile Infection.

Clostridium difficile infection (CDI) is a fastly growing problem in many industrialized countries, causing problems that range from diarrhea to pseudomembranous colitis (an inflammatory disease of the colon). CDI is caused by an opportunistic, gram positive bacterium, called *Clostridium difficile* (*C. difficile*), that has spore forming and toxin producing capabilities (Leffler et al., 2015). It has an oral-fecal route of transmission amongst humans and is spread when people come in contact with surfaces contaminated with feces containing *C. difficile* bacteria and subsequently touch their mouths (Leffler et al., 2015). CDI symptoms usually include: watery diarrhea (at least three bowel movements a day for two or more days), fever, loss of appetite, nausea, abdominal pain or cramping, and dehydration (Sunenshine et al., 2006). CDI is the leading causes of nosocomial (hospital acquired) diarrhea in Europe and North America, and it is estimated that there are 500,000 cases of CDI that are diagnosed in the USA annually (Rupnik et al., 2009). Recent outbreaks of CDI and the emergence of increasingly virulent strains have been associated with CDI's fastly growing morbidity and mortality rates, with there having been a 23% annual increase of cases from 2000-2006 (Zilberberg et al., 2008) and a 20 fold increase in mortality since its first diagnosis (Rupnik et al., 2009).

C. difficile's spore form allows it to survive even the toughest of circumstances as it permits the bacterium to stay dormant until it reaches better conditions that allow for it to thrive (Sunenshine et al., 2006). In humans, CDIs are transmitted when *C. difficile* spores are ingested. The spore form is resistant to the hostile gastric acid environment of the stomach and is then passed into the intestines, where conditions are considerably more favorable for the bacterium to grow (Sunenshine et al., 2006). The large intestine is where *C. difficile* colonizes and induces *C.*

difficile-associated diseases (i.e. CDI) as a result of changes or disruptions to a host's commensal microflora (Sunenshine et al., 2006). *C. difficile* germinates and produces toxins, which have both enterotoxin (targets intestines) and cytotoxin properties (Heinlen et al, 2010). These toxins can increase vascular permeability and hemorrhaging by opening up cellular tight junctions in the intestines (Karmali et al., 2013). The toxins can also cause severe inflammation and mucosal injury to the colon, potentially leading to pseudomembranous colitis, as a result of the toxins producing tumor necrosis factor-alpha and proinflammatory interleukins (Poxton et al., 2001). *C. difficile* toxins also cause damage to the GI tract, causing diarrhea in many CDI patients (Poxton et al., 2001). The primary pathophysiological hypothesis for CDI and the cause for *C. difficile* overgrowth is the bacteria's ability to colonize the GI tract following a shift to a person's normal microflora; the current leading cause for such disruptions to the flora is the use of broad spectrum antibiotics (Karmali et al., 2013).

As previously mentioned, *C. difficile* is pathogenic due to its production of toxins; the most predominant toxins produced are toxin a (TcdA) and toxin B (TcdB), both of whose mechanisms of action include: being endocytosed by the cell, degrading the actin cytoskeleton, and causing cell death (often in the colonic epithelium) (Heinlen et al,. 2010 & Poxton et al., 2001). TcdA and TcdB are located on a pathogenicity locus of the *C. difficile* bacterium and have similar primary structures. It is thought that the enzyme associated with cytotoxic activity for both Tcd A and TcdB is located on the N-terminus of this pathogenicity locus (Poxton et al., 2001). Binary toxin, *C. difficile* transferase, is another toxin associated with CDI; it has been attributed to causing hypervirulent strains of CDI by significantly increasing toxin a and toxin B production (Barbut et al., 2005). An example of a CDI that produces the binary toxin is variant

strain, Bi/naP1/027, which caused an epidemic of CDI between 2002-2005, accounts for 25%-50% of all CDIs, and is currently the predominant strain of CDI in hospital isolates (Ananthakrishnan, 2011). A distinguishing trait of Bi/naP1/027 is its resistance to fluoroquinolones (a broad spectrum antibiotic drug) and the current widespread usage of antibiotics may explain why there has been a selective pressure for the emergence of this particular strain in recent years (Pépin et al., 2005). Other hypervirulent, variant strains of CDI have also been on the rise since the early 2000s (i.e. ribotype 078); many of these strains have been associated with increased symptom severities and inflated mortality rates, making the eradication of CDIs an extremely important area of current research (Goorhuis et al., 2008).

In addition to escalating morbidity and mortality rates, many hypervirulent strains of CDIs have high recurrence rates. Patients with CDI are usually treated with rounds of the following antibiotics: metronidazole (for mild to moderate cases), vancomycin (for severe cases), and fidaxomicin (for severe cases) (Gerding et al., 2016). While these antibiotics eliminate a majority of CDI symptoms in patients, recurrences after treatments with antibiotics for an initial infection range from 15%-35%, within an 8 week timeframe, and recurrence rates have been found to increase to 77% after more than one CDI recurrence (Gerding et al., 2016). Because of the high failure rates of standard therapy regimens, alternative methods for treating CDIs have been highly sought after.

Inflammatory Bowel Disease.

Inflammatory Bowel Disease (IBD) is a class of autoimmune diseases that affect millions of people worldwide; it causes inflammation of the GI tract that leads to symptoms of diarrhea and abdominal pain in patients. IBD is primarily comprised of two major disorders: Crohn's Disease (CD) and Ulcerative Colitis (UC); chronic uncontrolled inflammation of the intestinal mucosa in the GI tract (esophagus, stomach, small intestine, large intestine, etc) is the hallmark of this class of diseases (Papadakis et al., 2000). In the United States, it is estimated that 1 million people currently suffer from IBD, and there are about 30,000 new cases that are diagnosed each year; the distribution of which is primarily divided amongst CD and UC (Hanauer, 2006). While the diagnosis of IBD can be made at any age, there seems to be a bimodal age distribution for these diseases with the first peak occurring around age 30 and the second peak occurring between ages 40-70 (Ali, 2015). Previous studies have also shown that an early onset of CD (<16 years old) may cause more severe forms of Crohn's when compared to adult onset CD (Ali, 2015).

While similar in many regards, CD and UC have many differences as well. For example, the location of CD inflammation is usually found in the distal ileum and the colon (although inflammation can occur in an part of the GI tract from the mouth to the anus), while UC inflammation occurs exclusively in the colon (large bowel) (Laass el al., 2014). The spread of inflammation in CD tends to be discontinuous and patchy with skip lesions; the spread of inflammation in UC is usually continuous from the rectum to the colon (Laass el al., 2014). In CD, all layers of the bowel wall are affected by inflammation (transmural inflammation), but in UC, only the mucosa and submucosa layers are affected (superficial inflammation) (Laass el al.,

2014). Other diagnostic features that typify CD are the presence of fissures (tears or cracks along the anus) and fistulas (sores or ulcers in the intestinal tract) (Laass el al., 2014). Furthermore, UC seems to affect males more than females; CD is marginally more frequently seen in females. However, both diseases seem to be more prevalent within urban populations and tend to be diseases associated with higher socioeconomic groups (Wiercinska-Drapalo et al., 2005).

The distribution of IBD amongst racial and ethnic groups is highly fluctuating. In the past, it was speculated that IBD occurred more frequently in white populations than in ethnic or racial minority groups (Hanauer, 2006). However, this gap has been fastly closing as studies have demonstrated marked increases in IBD diagnoses in African American populations and second generation south Asian populations that have immigrated to developed countries (such as the US and the UK), highlighting the importance of environmental factors in the development of these diseases (Foster et al., 2016). Adding on to the idea that environmental factors increase the prevalence of IBD, it is highly speculated that "Westernization" (i.e. consumption of processed foods and industrialization) is an influencing factor in the recent rise of IBD diagnoses as well, as populations that were previously considered low risk for IBD are now experiencing much higher rates of IBD incidence (Ponder et al., 2013). For example, IBD is most prevalent in developed countries, such as the US and Scandinavia (Hanauer, 2006). However, over the past few decades, IBD has been steadily increasing in Asian pacific regions, correlating to the industrialization and "Westernization" of these areas (Ng et al., 2013). As developing countries experience a rise in IBD diagnoses, a noticeable trend that appears is that UC tends to appear first followed by rising rates of CD (Bernstein et al., 2008). Other environmental factors that

seem to influence IBD prevalence include: stress, smoking, diet, breast feeding, and exposures to medication (Ponder et al., 2013).

In addition to environmental factors, the etiology of IBD is believed to be affected by genetic factors as well. Over the years, more than 160 susceptibility loci/genes have been associated with IBD; these genes tend to encode for various proteins that have a role in influencing and potentially downregulating: barrier functions, the immune system (both innate and adaptive), and microbial defense mechanisms (Cho et al., 2011). The focus of gene discovery for CD and UC have been largely distinctive, with there having been more attention paid to issues of autophagy, innate immunity, and abnormal bacterial processes for CD and barrier function for UC (Ponder et al., 2013). Through multiple twin studies and data compilation studies done on IBD, it has been demonstrated that there is usually a greater incidence of IBD within families (aka family aggregation/familial IBD); 5-23% of patients have first degree relatives who are also affected by IBD (Ek et al., 2014). In a twin study done on IBD, it was found that monozygotic twin pairs had higher concordances for CD than dizygotic twin pairs did, highlighting the effects of genetics in this disease pathology (Ek et al., 2014). Studies have also shown that UC patients are affected by genetic predispositions as well, although the results have not been as pronounced as those that have been found for CD (Orholm et al., 2000). It is hypothesized that the interplay between genetic predisposition and environmental exposures (which in turn can affect immune system functioning) are all influencing factors in IBD pathogenesis.

A theory on the etiology of IBD that demonstrates the intersection of: genetics, the environment, and host immune system functioning, is the hygiene hypothesis. The hygiene

hypothesis proposes that clean environments (i.e. germ free) and declining incidences of infectious diseases, as is the case in much of the Western world and in many developing countries, are the causes of the sudden rises in autoimmune diseases and allergy disorders seen in the past couple of decades (Azad et al., 2013). In support of the hygiene hypothesis, previous studies have shown that reduced microbial diversity (i.e. as a result of antibiotic usage or being raised with limited microbial exposure) in infancy has been linked to the development of allergy diseases later on in life (Azad et al., 2013). However, studies still show conflicting results in showing whether it is the diversity of the microbiota or the gut microbial composition itself (i.e. specific species) that has more weight in determining the evasion of disease (Azad et al., 2013).

A popular mechanism that seems to support the hygiene hypothesis is the Th1/Th2 model (Wills-Karp et al., 2001). Th1 cells of the immune system produce interferons and tumor-necrosis factors, which are important signalling cytokines for producing macrophage, phagocytosis, and immunity responses (Wills-Karp et al., 2001). Th2 cells, on the other hand, produce a different set of cytokines (i.e. interleukins), whose primary functions include inhibiting the activation of macrophages and producing antibodies (IgE) (Wills-Karp et al., 2001). Studies have shown that imbalances in Th1 and Th2 levels (i.e. compositions skewed toward Th2) tend to confer disease (i.e. allergies) (Prescott et al., 1998). In light of these findings, much research has gone into investigating if Th1 stimulation (i.e. through intentional microbial introductions) during early infancy has an effect on providing disease immunity later on in life, and has found varying degrees of success (Prescott et al., 1998). Therefore, more work still needs to be done on microbial exposures, its effects on regulatory T-cells, and its effects on maintaining a homeostatic balance between functions of: inflammation, anti-inflammation, and

immune tolerance, as disruptions in this balance tend to confer disease (i.e. autoimmune diseases).

Microbial exposure and its association with immunoregulatory defects seem to play a role in the pathogenesis of IBD as well (Hanauer, 2006). The IBD hygiene hypothesis states that children raised in an environment that is overly hygienic will experience negative impacts on their immune development, which causes them to be more susceptible to autoimmune diseases like IBD later in life (Weinstock et al., 2009). Because researchers have previously known that areas associated with higher socioeconomic status tend to have higher incidences of IBD, it was long speculated that something about the environment of developed countries conferred higher susceptibility to IBD and other autoimmune diseases. A theory stemming from the hygiene hypothesis, which may explain why IBD rates are so much higher in developed countries, is that there is a lack of helminth (large parasitic worms) exposure in developed countries due to sanitation efforts (Zhang et al., 2014). To live within their hosts, helminths have been shown to be able to activate cells of both the innate and adaptive immune system, suppressing inflammation in order to avoid being rejected by its host (Elliott et al., 2012). Helminth colonization has been shown to suppress intestinal inflammation as well, which is the primary pathology of IBD (Elliott et al., 2012).

In addition to increased efforts of becoming more hygienic in developed countries, which prevent the colonization of organisms that potentially provide protective advantages against autoimmune disorders such as IBD, many studies have hypothesized that a disruption in a person's own commensal microflora has a role in IBD pathogenesis as well (Colman et al., 2014). Microbiota colonization of the human gut starts from birth (in which the mother's own

microbiota provides the neonate with its first microbial inoculum); the composition of this microbial community changes as a baby is introduced to various changes in its life (i.e. diet, early microbial exposures, medications, etc) (Palmer et al., 2007). An infant's microflora increases in diversity and richness until around early childhood (typically 3-5 years old), when their microbiota starts to stabilize and resemble adult-like microbial compositions; shifts in this established microbial ecosystem (i.e. via use of antibiotics, surgery, etc) tends to confer disease (Rodríguez et al., 2015).

In recent years, many studies have shown that gut microbiota dysbiosis has an negative effect on the development of proper immune responses and that producing an appropriate immune response is highly dependent on the colonization of microbiota in the GI tract (Nagalingam et al., 2012). For example, a study done on germ free rabbits demonstrated that the sterilely derived rabbits acquired gut microfloras that were completely different than that of the conventionally raised rabbits; it was concluded that certain microbial species were needed for proper antibody repertoire diversification (although the exact species of influence remain to be elucidated) (Lanning et al., 2000). Another study that used gnotobiotic mice (germ free mice or mice whose microbial species are completely accounted for), found that these mice failed to produce normal immune responses (Rask et al., 2005). Further evidence that demonstrates that the gut microbiome has an effect on immune response regulation and modulation are experiments that have shown that germ free mice have fewer: Peyer's patches (which are crucial for the surveillance and eradication of pathogens in the GI tract), a thinner lamina propria (which is home to numerous immune cells), mature lymphoid follicles, T-cells, and Paneth cells (which secrete antimicrobial molecules) (Round et al., 2009). Other experiments have also shown that

certain bacterial species that inhabit the gut can produce defensins (i.e. *Bacteriodetes thetaiotaomicron*) and can activate the host immune system in order to improve their own fitness (i.e. *Salmonella enterica*), further demonstrating the role the microbiome has on immunoregulation (Stappenbeck et al., 2002 & Winter, et al., 2010). As shown from these studies, a healthy gut microbiome is tightly linked to proper regulation of host immune responses; a dysbiosis in this microflora can lead to defects in immunoregulatory pathways that lead to diseases.

In IBD, there has been increasing support made for the hypothesis that the gastrointestinal microbiome-host immune response axis has a role in IBD pathogenesis. In fact, there has been research indicating that a person's commensal gut microflora has a role in triggering, maintaining, and establishing IBD phenotype (Sartor, 2008). For example in CD patients with inflammation in the ileum, a study found that two genera of bacteria were decreased: *Faecalibacerium* and *Roseburia* (Sartor, 2010). Research has shown that these genera tend to produce short-chain fatty acids, which normally provide protection to the intestines, illustrating how microbiota dysbiosis can potentially be a factor in determining IBD phenotypes (Sartor, 2010). Research has also shown that CD patients, in a couple of studies, were found to have lower levels of *Faecalibacterium prausnitzii*, which has previously been linked to having anti-inflammatory capabilities, demonstrating how shifts in one's healthy gut microbiota might lead to the onset of intestinal inflammation associated with IBD (Sokol et al., 2008).

In a review article outlining the role of gut microbiota in IBD pathogenesis, it was stated that there are four broad mechanisms that help to elucidate the relationship between IBD (and its

pathology of intestinal inflammation) and one's commensal gut microflora: (1) there is a dysbiosis of the commensal enteric microflora (i.e. in several studies, IBD patients showed depletion of *Bacteroidetes* and *Firmicutes* groups and an abundance of *Actinobacteria* and *Proteobacteria* groups), (2) there are alterations in the gut microbiome, either pathogenically or functionally, that lead to inflammation of the intestines (i.e. alterations that lead to increased mucosal adherence of a foreign microbe, which causes an increased immune response leading to inflammation), (3) there are host genetic defects that lead to increased microbial antigenic exposure (i.e. mutations that lead to a more permeable epithelial layer in the intestines and a subsequent increased immune response), (4) there is impaired host immunoregulation (Sartor et al., 2012). All of these proposed mechanistic pathways cause an upregulation of T-cells and innate immune cells (i.e. natural killer cells, neutrophils, dendritic cells, etc) in the intestinal mucosa due to an increased exposure to bacterial antigens (Sartor et al., 2012). This increased stimulation of the immune system can ultimately cause chronic inflammation of the intestines, leading many to speculate that commensal microbial dysbiosis is indeed a contributing factor to IBD pathogenesis.

Currently, there is no cure for these autoimmune diseases and there are only treatment options to help control the disease symptoms such as: steroids (corticosteroids), anti-inflammatory drugs, antibiotics (for skin lesions and bacterial infections), and surgery (Cammarota et al., 2015). However, most of these conventional therapies have negative side effects as well. For example, while many IBD patients who choose to undergo surgical procedures, to cut out inflamed areas, often find themselves symptom free for the first couple of years post-surgery, their IBD symptoms almost always seem to return (Cammarota et al., 2015).

Potential dangers associated with using steroid treatments are issues of patients becoming steroid dependent, as well as the risk of developing a steroid-refractory disease (in which steroids no longer prove to be effective for disease treatment) (Cohen et al., 2016). Current studies have proposed a couple of new therapeutics, and they stem from the current hypothesis that gut microbial dysbiosis has a role in IBD pathogenesis. These therapy options include: probiotics (which are live microorganisms that are thought to confer health benefits when administered in small amounts) and prebiotics (which are fiber compounds believed to promote the growth of "good bacteria") (Cammarota et al., 2015). While a few studies have shown that some probiotics demonstrate higher rates of IBD symptom resolution when compared to their placebo drug counterparts (i.e. *VSL#3*, a probiotic cocktail), there are risks that come with this therapy as well (Cammarota et al., 2015). For example, the usage of probiotics as a treatment option for IBD patients has been recorded to cause sepsis in a handful of cases (Cohen et al., 2016). There is also very limited data showing the efficacy of probiotics on severely immunocompromised IBD patients, demonstrating the need for more clinical trials that test the effectiveness of probiotic usage on patients with IBD. In addition to probiotics, another emerging form of therapy for IBD treatment is fecal microbiota transplantation, which looks to be a promising therapeutic option for microbial restoration.

Fecal Microbiota Transplantation.

FMT is argued to have been implemented for some 1,700 years. The earliest practice of it is said to have taken place during 4th century China when Ge Hong, a traditional Chinese medicine doctor, administered human fecal matter orally to patients with food poisoning and severe diarrhea, and found great success with his new treatment regime (Zhang et al., 2012). So while FMT is not a novel concept in and of itself, the first medically documented case of FMT usage in modern times was not until 1958 when it was used to treat pseudomembranous colitis in a four person case study (Eiseman et al., 1958). The first usage of FMT as a means for treating CDI was in 1983 by means of enema (Schwan et al., 1983).

FMT is the process by which fecal suspension from a healthy individual is transferred into the GI tract of another individual in an attempt to cure certain diseases. The concept behind this treatment is that each living organism has a certain gut microbiota composition that makes up its microbiome (all microbial genes). Yet, even the microbiomes of healthy individuals vary greatly--the diversity of which still remains unexplained, although diets, early microbial exposure, and environmental factors are some of the many influences of microflora changes (Consortium, 2012). Although there is no specific microbiota composition across healthy individuals that confers well-being, studies have shown that certain taxa are usually more associated with a "healthy" microbiome while certain other bacterial species tend to be linked to disease; these revelations have been the basis for the fastly growing prebiotic and probiotic industry.

The gut microbiome is responsible for many different critical functions, including: immune functions, digestion, and colonization resistance (Schubert et al., 2014). Colonization resistance is the ability to prohibit and restrict the colonization and growth of pathogenic species. The idea of colonization resistance is one of the key concepts behind why FMT has found such great success with CDIs, as CDIs are thought to be caused by a significant decrease in an individual's healthy microbiota species, allowing for the colonization of pathogenic bacterial species that cause CDI (Schubert et al., 2014).

Because FMT has shown promising results as a treatment for CDIs, FMT has also been hypothesized as being effective for a variety of other diseases as well, such as: intestinal disorders (i.e. Inflammatory Bowel Disease, Irritable Bowel Disease, and metabolic diseases), neuropsychiatric disorders, autoimmune diseases, and allergy disorders (Malnick et al., 2015 & Xu et al., 2015). After CDIs, IBD has been one of the most highly studied class of diseases in the FMT treatment realm. However, FMT usage on IBD is in much more of a preliminary stage of testing than with CDIs, and scientists are still currently running many trial studies and experiments in an attempt to better elucidate how FMT can best be used to treat patients with IBD (Malnick et al., 2015). Results from many of these clinical trials, testing the efficacy of FMT on IBD, have been found to be substandard to those of CDI, both in terms of effectiveness and its safety profile, demonstrating a need for more research to reveal if FMT is truly a safe option for IBD patients.

Results.

Clostridium difficile Infection:

Methods of Administration

There is no singularly successful way of FMT administration. FMT is usually administered to either the upper GI tract (i.e. via Nasogastric tubes or NGT, duodenal tube, endoscopy) or the lower GI tract (i.e. via colonoscopy, retention enemas) (Dodin et al., 2014). In 2011, approximately 23% of FMT procedures were administered using a NGT or gastroscopy and demonstrated cure rates of 76% (Rohlke et al., 2012). In order to avoid aspiration, however, delivery of fecal suspension to the upper GI tract requires administration of smaller volumes of suspension (Dodin et al., 2014). A meta-analysis study (consisting of 182 participants from 12 published studies) compared and analyzed the success rates of using FMT via colonoscopy or nasogastric tube. Although it was found that there were higher cure rates with the colonoscopy group, ultimately there was no significant difference in treatment efficacy (between colonoscopic and NGT FMT) (Postigo et al., 2012). Recurrence of CDI after FMT treatment, between both methods of delivery, was also found to be statistically insignificant in this study (colonoscopy group: 5.4% and NGT group: 5.9%) (Postigo et al., 2012). Another review paper found that FMT administration through a gastroscope or NGT showed the lowest resolution rates (Gough et al., 2011). Therefore, in general, colonoscopy is considered to be the first line approach for FMT delivery in adults and upper GI tract FMT is used more for pediatric patients and patients with severe comorbidities, but methods of administration still largely remain up to a physician or researcher's discretion (Dodin et al., 2014).

Fecal Solution Preparation

The systematic analysis of Gough et al. demonstrated that using water as a diluent shows higher resolution rates than using saline solutions (Gough et al., 2011). However, the relapse rate is two times greater for solutions prepared with water than with saline (Gough et al., 2011). Other diluents such as: milk, saline with psyllium, and yogurt also appear to demonstrate great efficacy (Gough et al., 2011). Larger volume suspensions seem to be more effective than smaller volume suspensions, with a study finding a 97% resolution rate for suspensions greater than 500 mL and only a 80% resolution rate for 200 mL suspensions (Rohlke et al., 2012). Some studies have also found that relapse rates are four times higher in solutions that use less than 50 grams of stool (Rohlke et al., 2012). Other studies have shown that there is not a significant difference on resolution rates using either fresh or frozen stool samples in FMT treatments (Hamilton et al., 2012). Another clinical trial has also proposed the efficacy of using oral, capsulized, frozen fecal matter, asserting that their orally prepared FMT pills had resolution rates of 90% (Youngster et al., 2014).

Bowel Lavage Procedure

Polyethylene glycol electrolyte lavage was a standard protocol in many of the reviewed studies that used colonoscopic FMT procedures, as the lavage is presumed to flush out residual traces of feces, antibiotics, and CD (bacteria, spores, and toxins) before FMT administration (Rohlke et al., 2012). However, systematic analysis reveals that patients who receive both bowel lavage and an antibiotic before FMT tend to show the highest rates of relapse (Gough et al., 2011).

Patients

Inclusion of patients into clinical trials greatly varied from trial to trial. Studies often included patients who who had at least a couple (i.e. 2 or 3) documented recurrences of CDI despite standard antibiotic therapy (Hamilton et al., 2012). Multiple studies excluded patients who were under the age of 18 and/or had life expectancies of less than one year (Hamilton et al., 2012). Other patient participation exclusions included patients who were pregnant, severely immunocompromised (i.e. patients on chemotherapy), or taking other antibiotics aside from the standard treatments for CDI (Bowman et al., 2015). Patients were usually also maintained on antibiotics (i.e. a full dose of vancomycin) until a few (i.e. 2) days before the FMT procedure was to be done (Hamilton et al., 2012).

Donors

A variety of donors can be used in the FMT process (i.e. family, friends, partners, relatives, or unrelated healthy subjects) (Rossen et al., 2015a). In the past, descriptions of donors were not well documented as they were only described as being "healthy donors" (Malnick et al., 2015). Up until 2011, partners or family members were most frequently used as donors (Malnick et al., 2015). Nowadays, donors are screened intensively before they are allowed to make fecal donations. Donor screenings can include: questionnaires addressing risk factors for potentially transmissible diseases, a fecal test, tests for parasites (including *Blastocystis hominis* and *Dientamoeba fragilis*), tests for CD and enteropathogenic bacteria, serology tests, antibody tests (for HIV, human T-cell lymphotropic virus types 1 and 2, hepatitis A, B, and C, Cytomegalovirus, Epstein-Barr virus), and tests for certain microbes (i.e. *Treponema pallidum,*

Strongyloides stercoralis, and *Entamoeba histolytica*) (Rossen et al., 2015a). At times, donors were excluded from participation in certain studies if they: had gastrointestinal comorbidities, used antibiotics in the months prior to donation, and showed presence of features resembling metabolic syndrome, autoimmunity, or allergic diseases (Hamilton et al., 2012). It was previously believed that fecal donations from related donors demonstrated higher rates of resolution than with unrelated donors. (Gough et al., 2011). However, more recent clinical trials make the argument that there is no such effect on resolution rates (Youngster et al., 2014). Gough et al's meta-analysis also claimed that male donors showed 86% resolution rates with a 0% relapse rate, while female donors showed 100% resolution rates but had an 8% relapse rate (Gough et al., 2011).

Efficacy

In 2011, based on 27 case series, it was determined that FMT had a 92% efficacy rate, where 89% of patients experienced resolution after a single treatment; 4% of patients experienced relapses (Gough et al., 2011). Based on a systematic review on FMT published in 2015, which included 33 cases series published on CDI, the efficacy of FMT (defined as "resolution of diarrhea") ranged from 87.8% to 90.0% in repeated FMTs (Rossen et al., 2015a). A study showed that severe and complicated cases of CDI, hospitalized patients, immunocompromised patients, patients with more than three episodes of CDI, and patients with IBD comorbidities demonstrated efficacies of more than 80% (Rossen et al., 2015a).

Table 1. Studies on fecal microbiota transplantation in *C. difficile* infection with an emphasis on the potential side effects of these treatments.

Author	Year	Participants	Delivery Method	Results of FMT	**Side Effects**	Follow Up Period	Other Notes
Alang et al	2015	1	NM	CDI resolved	Obesity	NM	Daughter was stool donor; daughter was also gaining weight
Lee et al	2016	232	Enema	Clinical resolution: 83.5% for frozen and 85.1% for the fresh	Mild to moderate: transient diarrhea, abdominal cramping, nausea, constipation, etc	13 weeks	First randomized controlled trial to show that frozen FMT is non-inferior to fresh FMT in terms of clinical efficacy
Hourigan et al	2015	8	Colonoscopy	FMT provided clinical improvement in children with and without Inflammatory Bowel Disease (IBD)	Mild prolonged diarrhea for 6 months post-FMT, fecal urgency, intermittent fecal incontinence in 1 patient (without IBD)	6 months	Patients were all children; 5 patients had IBD comorbidities

**NM= not mentioned

Inflammatory Bowel Disease:

Methods of Administration and frequency:

Like with CDI, there is currently no standardized procedure of FMT administration for IBD patients. However, the majority of FMT for IBD patients seem to be administered via the lower GI tract (Anderson et al., 2012). Of the patients that had FMT delivery via the lower GI tract (which typically includes the anus, rectum, colon, and cecum), most the patients received enemas, followed by colonoscopic procedures (Colman et al., 2014). When FMT is administered via the upper GI tract (which typically includes the esophagus, stomach, and duodenum), the most popular route of administration seems to be through nasogastric/nasojejunal (NJ) procedures, followed by gastroscopic installation (Colman et al., 2014). A previous study has also been documented using a combination of upper and lower GI tract FMT administration (nasojejunal tube and enema) (Angelberger et al., 2012). However, this study found little efficacy in its FMT treatments after a follow up period of 12 weeks (Angelberger et al., 2012). Another study also found that one of its patients developed aspiration pneumonia as a result of NJ tube administration; the route of administration was changed to a rectal tube afterwards for safety reasons (Vermeire et al., 2015). A systematic review published in 2014 revealed that 41% of patients received FMT more than once; one study reported that a patient received as many as 70 FMTs (Colman et al., 2014). Another review found that only a small majority of IBD patients received only a single infusion, and all of those patients were also being treated for comorbid cases of CDI (Anderson et al., 2012).

Fecal Solution Preparation:

Fecal matter for IBD patients are delivered both as fresh stool samples and frozen stool samples; the majority of patients (data collected from five different studies) that seemed to achieve clinical remission received fresh stool samples (Colman et al., 2014). Many studies failed to report how their fecal solutions were prepared. When reported, fresh fecal sample collection took place from 10 mins to 6 hours before FMT administration (Anderson et al., 2012). The amount of stool used was generally between 200-300 grams, and the stool samples were usually suspended in 200-300 mL of normal saline, but there are fluctuations in these numbers across different studies (Anderson et al., 2012). Recently, in Nanjing, China, an automatic system has been developed to normalize the fecal purification process; the machine is called the GenFMTer (Cui et al., 2015a). The GenFMTer can cut the stool preparation time, from collecting the feces to delivery of the final product, from 60 minutes (when prepared by operators) to less than 15 minutes, and the GenFMTer is said to better preserve the microbiota isolated from stool samples (Cui et al., 2015a).

Bowel Lavage Procedure

It was not always noted whether or not bowel lavage procedures were performed on patients prior to FMT administration. When noted, polyethylene glycol was usually the solution delivered to patients in these procedures (i.e. via NJ tubes) (Grehan et al., 2010 & Angelberger et al., 2013). However, bowel lavage procedures have also been omitted from the FMT preparation steps as well (Kump et al., 2013). The efficacy of bowel lavage procedures on IBD patients still remains to be elucidated. Some studies required that their patients undergo “full bowel

preparations", but whether that refers to bowel lavage procedures or not remains unknown (Vaughn et al., 2014).

Patients

Each study had its only set of inclusion criteria that allowed for patients to partake in certain clinical trials. Studies tended to include both pediatric and adult patient demographics, with a few studies focusing exclusively on younger patients (Colman et al., 2014). Studies tended to include patients with active IBD flare-up symptoms (i.e. moderate or severely active UC), usually confirmed via endoscopy, colonoscopy, or histology procedures, and patients with chronic active therapy–refractory IBD (patients who no longer respond to standard medical therapies) (Kump et al., 2013 & Angelberger et al., 2013). However, some studies only allowed patients with refractory IBD to partake in their clinical trials (Vermeire et al., 2015 & Kump et al., 2013). Other studies purposefully excluded patients with refractory IBD (Vaughn et al., 2014). Additional exclusion criteria from studies included: smokers, patients on antibiotics, steroids, and/or probiotics, patients who had severe comorbidities (i.e. cardiac, renal, or hepatic comorbidities), patients with short bowels, patients who were pregnant, patients who were under 16, etc (Vaughn et al., 2014, Vermeire et al., 2015, Kump et al., 2013). One study required that its patients receive antibiotics (metronidazole, 500 mg twice a day) during the week prior to being admitted into the hospital for the FMT procedure (Angelberger et al., 2013). Some studies did not give their patients antibiotics prior to the FMT treatments (Vaughn et al., 2014).

Donors

Not all studies included descriptions of their donor screening processes and/or donor selection processes; others simply described their donors as being "healthy donors" (Colman et al., 2014). In many studies, donors were described as being either being anonymous donors or related donors (i.e. parents, siblings, children, partners) (Colman et al., 2014). However, several studies prohibited IBD patients from receiving stool samples from donors who were related to them and/or living in the same household as them (Kump et al., 2013). Some common donor criteria included a negative history: for intestinal diseases (and/or GI infections), autoimmune diseases, common viruses (i.e. hepatitis A, B, C, HIV, *Epstein-Barr, Herpes simplex*, etc), enteropathogens (i.e. *Salmonella spp., Shigella spp., Aeromonas spp.,* etc), etc (Kump et al., 2013 & Angelberger et al., 2013). Some recent studies have also been collecting donor stool samples for further microbiota analyses (i.e. via RNA pyrosequencing techniques) (Cui et al., 2015b). One study required that their donors undergo a lavage procedure prior to having their stool samples collected (Kump et al., 2013).

Efficacy

Overall, remission rates for IBD patients using FMT treatments vary greatly; while some studies found resolution rates of 68% other studies found resolution rates of 0% (Rossen et al., 2015a). In a systematic review written in 2014 (which included 119 patients), the clinical remission rate was said to be 45% after follow up (Colman et al., 2014). However, the length of the follow up periods varied greatly and the average length of the follow up periods was only 1.5 months (Colman et al., 2014). The systematic review also described that 6% of patients' health

conditions deteriorated post FMT treatment, but two of those patients seemed to have recovered by week 8 after their treatments (Colman et al., 2014). Another review reports that resolution rates fall to 36.2% when studies were excluded due to publication biases (Kelly et al., 2015). In a few pediatric studies, the remission rates were found to be around 60-65%, but were revealed to have short follow up periods post FMT (Colman et al., 2014). In a randomized control trial of FMT in IBD, remission was achieved in 24% of patients who received FMT and 5% of patients who received a placebo (Moayyedi et al., 2015). In general, studies that used methods of clinical scoring of disease activity (i.e. CD Activity Index, Mayo Index) and randomized control trials tended to show lower efficacy rates for FMT in IBD (Ianiro et al., 2014).

Table 2. Studies on fecal microbiota transplantation in Inflammatory Bowel Disease with an emphasis on the potential side effects of these treatments.

Author	Year	Participants	Delivery Method	Results of FMT	**Side Effects**	Follow Up Period	Other Notes
Quera et al	2014	1	Colonoscopy	Asymptomatic for CDI	High fever, Bacteremia (treated with aztreonam)	5 months	CDI comorbidity; patient had previous cases of bacteremia pre-FMT
De Leon et al	2013	1	Colonoscopy	Resolution of CDI symptoms; UC remission	Transient flare up of "quiescent" UC, abdominal cramping, tenesmus, etc	2 weeks	CDI comorbidity, Wife was stool donor
Angelberger et al	2013	5	NJ + Enema	Positive clinical response for 1 patient	Temporary increase of C-reactive protein, fever, vomiting, etc	12 weeks	Patients had severely active UC
Cui et al	2015(b)	30	Endoscopy	76.7% clinical improvement	Increased diarrhea, fever	15 months	Mesalazine (3.0 g) was given daily for 3 months post FMT for its anti-inflammatory effects
Kunde et al	2013	10	Enema	67% maintained clinical response at 1 month; 33 % maintained clinical remission after 1 week	Mild: cramping, fullness, flatulence, bloating, diarrhea, blood in stool Moderate: fever Adverse: self-limiting	1 month	Participants' ages: 7-21 years old

**NM=not mentioned

Discussion.

Fecal microbiota transplantation has been advertised as a promising microbial restoration panacea that has the capabilities to treat a broad spectrum of diseases and disorders ranging from gastrointestinal diseases (i.e. CDI and IBD) to a variety of non-GI diseases (i.e. obesity, Parkinson's, autism, multiple sclerosis, etc) (Aroniadis et al., 2013). While studies have hypothesized that these transplantation treatments have the capability to elicit positive results in curing and resolving the symptoms of many of the aforementioned ailments, there is a lack of literature and properly conducted clinical trials that support the efficacy of FMT usage on many of these diseases (Ianiro et al., 2014). The only disorder that seems to exhibit convincing success rates with FMT treatments is CDI, where cure rates tend to exceed 90% in many clinical trials that have tested the efficacy of FMT in CDI (Aroniadis et al., 2013). Because of its high cure rates and the relatively limited amount of adverse side effects it causes, FMT is now considered an effective therapy for treating recurrent *C. difficile* infections (Rossen et al., 2015a).

However, while FMT has generally been accepted as a relatively safe and effective therapy for treating CDI, clinical trials that have tried to duplicate the therapeutic designs of FMT for Inflammatory Bowel Disease have found inferior results to those found in CDI in terms of cure rates, symptom resolution rates, and safety profiles (Ianiro et al., 2014). Existing literature shows that the efficacy of FMT in IBD fluctuates greatly, and that the average clinical remission rate is said to be approximately 45% (Colman et al., 2014). These rates tend to be even lower when any sort of clinical measure of disease activity or objective score is used; the current cure rate may also potentially be further curtailed if studies had longer follow up periods (Colman et al., 2014 & Ianiro et al., 2014). In addition to the relatively low efficacy of FMT in

IBD, these transplantation treatments also tend to demonstrate a lower safety profile for IBD patients than for CDI patients; it has been noted that a worsening of disease is a potential adverse side effect associated with FMT in IBD (De Leon et al., 2013). Because of this, great consideration must be taken into account when determining whether these transplantations are truly safe options for treating Inflammatory Bowel Disease.

Because of these concerns, this review will propose a series of goals and studies aimed at testing the safety and efficacy of these transplantation treatments for Inflammatory Bowel Disease. While some of these proposals may go beyond standard approvable boundaries and may fail to pass the regulations stipulated by the Institutional Review Board, these suggested goals and experiments can offer invaluable knowledge and insight that might not otherwise be attained through proposals and experiments that pass review board guidelines.

This review proposes that both observational and experimental studies are necessary to further examine the safety profile and effectiveness of FMT in IBD. In terms of observational studies, this review proposes the establishment of two longitudinal studies that will follow two specific cohorts. These suggested longitudinal studies will attempt to establish a wider scope for genetic profiling the gut microbiomes of IBD populations that have previously received FMT treatments and populations that have an elevated prospect of developing IBD in the future.

The first proposal is a lifetime longitudinal study that will follow all IBD patients, worldwide, who have previously received FMT treatments. When possible, genomic sequencing of donor samples (documenting both the microbial community and microbiome of the stool samples) for each IBD patient should be kept on file as well. At regular time intervals (i.e. biannually), participants of this study will be required to fill out a self-reported questionnaire that

will assess their health conditions post-FMT in order to determine if time has an effect on disease symptom resolution (for IBD patients in which FMT was deemed unsuccessful in a prior study) and/or symptom flare ups (for IBD patients in which FMT was deemed successful in studies). This longitudinal study will also require that participants' stool samples be metagenomically analyzed (i.e. via Next Generation Sequencing techniques) at regular time intervals as well, in order to determine the gut microbial compositions, species interactions, and gene products that may influence disease symptom resolution and/or flare-ups (Koboldt et al., 2013). Genetically analyzing the stool samples on a regular basis will also potentially allow researchers to make important discoveries. For example, it might reveal that time is a necessary factor for a patient's gut microbiome to converge towards their donor's; it will also help to elucidate if this convergence over time allows for a higher rate of disease resolution. The proposal of this longitudinal study is based on information gathered from a couple of studies. A study previously suggested that the IBD patients who responded the most positively to FMT treatments tended to have microbiota profiles that were similar to that of their donors (after a 12 week follow up period), whereas the microbial diversity of nonresponders did not change over time (Rossen et al., 2015b). Another study demonstrated that FMT induced a transient flare-up of UC in a patient, which had previously been dormant for 20 years, when used to treat the patient's CDI symptoms, indicating the potential for FMT treatments to exacerbate or revive IBD symptoms post-FMT (De Leon et al., 2013). This longitudinal study can help to confirm whether the findings from these existing studies are valid, as well as contribute to elucidating the gut microbiome that is most susceptible to IBD genesis and what aspects of that microbiome must be restored in order for IBD eradication.

The second longitudinal study will follow a different cohort--pregnant women with IBD and the child/children they give birth to during that pregnancy. The participants of this study (mothers and children) would be required to give stool samples at regular intervals for metagenomic analyzation as well. The children would also be required to fill out self-reported questionnaires, either by themselves or by proxy, at regular time intervals that inquire about their main diets and when their IBD symptoms started (when applicable). The main aim of this study would be to examine the heritability of IBD and hopefully document the exact microbial shifts that lead to the onset of IBD. A secondary goal of this study would be to investigate the role diet has on changing one's commensal gut microbiome and if these changes eventually give rise to IBD. Existing literature that inspired the proposal of this study are those that document evidence of IBD's heritability and studies supporting the claim that diet has an effect on shaping one's gut microbiota (Cho et al., 2011 & Filippo et al., 2010). While it is understandable how these studies might not be approved by a review board, as they warrant meticulous protocols from participants and researchers, these proposed longitudinal studies have the potential to reveal valuable insight on IBD's pathogenesis that can lead to more streamlined and targeted approaches to microbial restoration therapies that have the possibility of replacing traditional FMT procedures.

In addition to longitudinal studies, this review will also propose a series of experimental studies that should be considered when examining the safety and efficacy of FMT in IBD. The two primary goals of these experimental studies are to: (1) establish a standardized FMT protocol specific to IBD, and (2) find IBD populations that are the most suited for FMT treatments. While many studies reference and use existing FMT algorithms for CDI in IBD, it is important to create a FMT protocol specific to IBD because IBD pathology differs greatly from the etiology of CDI.

While existing FMT protocols for CDI are a good place to start from, IBD's multifactorial pathogenesis makes it so that it is not practical to solely rely on the CDI's FMT methodologies for IBD. For example, despite the variability in FMT procedural protocols for CDI, FMT still demonstrates high resolution rates for CDI with little adverse side effects. However, procedural variability for FMT in IBD does not exhibit the same high cure rates and low side effects that CDI does, highlighting the importance of establishing a standard protocol tailored for IBD. A standardized protocol for IBD is also a crucial and necessary step to normalizing and minimizing the bias associated with FMT in IBD, which is a critical issue involving much of the existing literature documenting FMT usage in IBD. The lack of procedural standardization makes it not only extremely difficult to interpret and compare results across different studies, it is also a potential reason why FMT exhibits relatively lower resolution rates with IBD; FMT protocols regarding route of administration, fecal solution preparation, patient preparation, and donor selection processes for IBD are all ill defined. Furthermore, because existing literature has also shown that the efficacy of FMT treatments in IBD is highly variable, ranging from resolution rates of 0% to 68% in some studies, it would seem as though only certain IBD populations may benefit from FMT therapies (Rossen et al., 2015a). Overall, it is important to establish a standard FMT procedure for IBD as well as find the subsets of IBD patients that are most suited to receive FMT treatments.

In order to achieve the goal of protocol standardization, it is necessary to carry out experiments that are aimed at elucidating which route of administration is better for specific IBD populations in terms of efficacy. It is also important to determine which route of administration is better because a previous study has mentioned that certain delivery routes may be more

beneficial to particular groups of bacteria (Ianiro et al., 2014). A way to test which administration route is better suited for certain IBD populations is a series of experiments enrolling men and women, with IBD, of different age groups with varying states of health. For example, one clinical trial should only admit patients with mildly active disease states who are randomly assigned a method of delivery (i.e. oral, NJ/NG tube, enema, colonoscopy, etc). Some controls for this experiment should be: diet (i.e. patients should eat a diet low in fat and animal protein, but rich in fiber and plant polysaccharides as these foods have been associated with microbial species richness and diversity in a previous study), disease state (only mildly active IBD patients should be admitted to best demonstrate the effect of delivery route on disease resolution), immunocompromisation (participants should be relatively healthy besides mild IBD symptoms; BMI should be under 30), age (participants should be at least 16 years old to allow better retention of larger fluid volumes for enemas), fecal solution preparation (i.e. 50 grams of fresh fecal matter from anonymous donors suspended in 500 mL of saline, as these numbers demonstrated higher cure rates for CDI), and bowel lavage procedures (none to minimize the disruption of existing microflora) (Filippo et al., 2010 & Rohlke et al., 2012). This experiment will help to establish a baseline for what route of delivery is most effective at resolving symptoms for relatively healthy IBD patients. Further experiments can repeat this protocol with different populations of IBD patients (i.e. infants under the age of 3, severely immunocompromised patients, patients with refractory IBD, etc). Additional experiments can also take into account which delivery method has the highest patient "buy-in" and which route is the easiest to administer. It should also be noted that previous studies examining the effect of FMT in CDI have mentioned that a potential disadvantage of using colonoscopic procedures is

the risk of colon perforation, so future researchers should also take this into consideration when administering FMT via colonoscopy (Dodin et al., 2014). Previous clinical trials studying FMT in CDI have also shown that fecal matter suspended in at least 500 mL of diluent tended to exhibit higher resolution rates (Rohlke et al., 2012). However, this ideal volume might change depending on the route of FMT delivery; future researchers should take time to investigate the volume of diluent that is most effective for each method of administration.

Another method to achieving the goal of protocol standardization is figuring out what the donor requirements should be for FMT used in treating Inflammatory Bowel Disease. For example, in addition to the donor specifications used for FMT in CDI, stricter guidelines should be implemented for IBD patients' FMT donors as IBD patients are usually more immunocompromised than CDI patients (due to the intake of anti-inflammatory drugs). In light of this, donors for IBD patients should always have their stools metagenomically analyzed to detect for potential pathogens that may be likely to cause disease for IBD patients but not for CDI patients. Metagenomically analyzing donors' stool samples is also important because a previous study has shown that donor species richness has an effect on transplantation success for certain IBD patients (Vermeire et al., 2015). Additionally, genetic factors are an area of influence for IBD patients while it is not for CDI patients. Due to these genetic influences on IBD pathogenesis, an example of an experiment that should be executed should be one that tests this hypothesis: anonymous donors have higher rates of resolution than genetically related donors. If this first test proves to be true, a follow up test should be performed to test the hypothesis that: genetically related donors exhibit higher rates of relapse after initial resolutions. If this second

test also proves to be true then great caution should be taken when patients wish to use genetically related donors for fecal microbiota transplantations.

In addition to determining which route of administration is most effective and what the donor guidelines should be for FMT usage in IBD, it is also important to demonstrate which methods of fecal matter preparation and administration exhibit the highest resolution rates for IBD patients. An example of a first test that should be run is one that analyzes the hypothesis: fresh fecal matter has higher rates of resolution than frozen fecal matter (as a previous study indicated that using fresh fecal matter had higher cure rates than using frozen matter but the results were not significant, probably due to its small sample size) (Cui et al., 2015a). Important controls for this experiment would be: donor BMI, the amount of fecal matter used, the volume of diluent used, and what type of diluent is used (i.e. saline, water, milk, yogurt, etc). If it is demonstrated that using fresh fecal matter does indeed have a higher rate of resolution for IBD patients, then an additional experiment should done to test the following hypothesis: fecal matter purified by a machine (i.e. the GenFMTer) and then frozen demonstrates similar efficacy rates as using fresh matter. If this too is demonstrated to be true, then using frozen stool samples may be as effective as using fresh stool samples for IBD patients, which is important because using frozens stool samples allows for immediate administration while fresh stool samples must be prepared for a period of time before administration is possible. Other potential areas of procedural standardization that should be further explored in order to determine the safety and efficacy of FMT treatments for IBD patients are: establishing how many FMT treatments are typically needed for disease resolution and determining if combination therapies (i.e. FMT combined with steroid therapies) are effective (Cui et al., 2015c).

Along with designing experiments aimed at standardizing FMT procedures for IBD patients, another goal of the proposed experiments in this review is to find IBD populations that are the most suited for FMT treatments. A method of achieving this particular goal is conducting a series of experiments that test the efficacy of FMT treatments on a wide variety of IBD patients (i.e. pediatric populations, adult populations, immunocompromised patients, steroid dependent patients, refractory patients, newly diagnosed patients etc). An example of such an experiment is a randomized control trial that only admits pediatric IBD populations under the age of 3 (as ages 3-5 is when an infant's microbiota starts to converge to adult-like microbiota compositions) in order to see if FMT treatments can induce higher resolution rates in this population subset than a placebo counterpart can (i.e. a placebo using just saline) (Rodríguez et al., 2015). Some controls of this experiment should be: the amount of stool infused (i.e. 50 grams of fresh stool), volume of diluent (i.e. 250 mL of saline, as 500 mL of saline may be too much liquid for an infant to retain), the participant's state of health (should be relatively healthy with only mildly active disease symptoms), the method of administration (i.e. colonoscopy, based on a previous experiment that demonstrated high efficacy in treating CDI with FMT for two children under the age of 3), type of donor (i.e. anonymous donors only), and if a bowel lavage procedure is used (It is recommended that no bowel lavage is used due to its invasive nature and unproven efficacy in IBD symptom resolution) (Ritu et al., 2014). If this first experiment fails to demonstrate the efficacy of FMT treatments on this IBD population subgroup, it is recommended that the same control trial is repeated while changing one of the controls listed above (i.e. delivery via a NJ/NG tube instead of a colonoscopy or using a higher volume of saline if patients can tolerate it) to see if there are any changes in symptom resolution

rates. Similar randomized control trials can be conducted across the different IBD subgroups in order to better demonstrate which populations of IBD are the most suitable for FMT treatments.

Conclusion.

All in all, the proposals of goals and studies listed in this review are not exhaustive of all the concerns regarding safety and efficacy that must be addressed when determining if fecal microbiota transplantations should be considered a therapeutic option in the treatment of Inflammatory Bowel Disease. However, this review does demonstrate that there are still many areas of research that need to be further explored and studied before truly determining if FMT treatments are safe and effective therapies for IBD patients.

Appendix.

Notable Abbreviations Used

CDI	*Clostridium difficile* Infection
IBD	Inflammatory Bowel Disease
FMT	Fecal Microbiota Transplantation
UC	Ulcerative Colitis
CD	Crohn's Disease

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