The Effect of Probiotics and Fecal Transplantations on Crohn's Disease Symptoms

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Abstract

The effect of probiotics and fecal transplantations on Crohn's disease symptoms was investigated to determine which of these treatments is more effective in treating Crohn's disease. A systematic literature review was conducted to establish whether or not either probiotics or fecal transplantations can provide an alternative treatment to the accepted forms of treatment, including antibiotics and surgery. The relationship between the occurrence of Crohn's disease and the prevalence of major phyla in the gut microbiota of patients was investigated to demonstrate a connection between a bacterial imbalance in the gut microbiota and Crohn's disease. Data was analyzed from peer-reviewed papers and t-tests on that data indicated that probiotics do not have a statistically significant effect on inducing remission, while fecal transplantations do have a statistically significant effect on inducing remission. Based on the findings, it was concluded that fecal transplantations have a greater effect on Crohn's disease symptoms.

Key Terminology

Crohn's disease, gut microbiota, inflammatory bowel disease, probiotics, fecal microbiota transplantation, gastrointestinal tract, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*

Introduction

Crohn's disease is a chronic inflammatory bowel disease (IBD) that affects the gastrointestinal (GI) tract and can cause abdominal pain, diarrhea, weight loss, anemia, and fatigue. Symptoms occur due to inflammation of the GI tract in layers of affected bowel tissue,

where it can acutely spread. Furthermore, symptoms depend on the presence and severity of inflammation as Crohn's disease can affect any area of the GI tract, from mouth to rectum. When a person has Crohn's disease, there can be large healthy regions of bowel in between the diseased regions and all layers of the intestinal wall can be affected within those diseased regions.

There are about 70,000 new cases of IBD per year in the United States, while approximately 1.6 million people are currently living with Crohn's disease worldwide and those diagnosed are typically between the ages of 15 and 35 (National Institute of Health, 2018). Crohn's disease symptoms may occur aggressively in some patients, while others may experience infrequent flare-ups. The cause of Crohn's disease is unknown, but some experts theorize that a multitude of factors can cause it, including an autoimmune reaction in the immune system, genetics, smoking, taking nonsteroidal anti-inflammatory drugs, or consuming a high-fat diet (National Institute of Health, 2017).

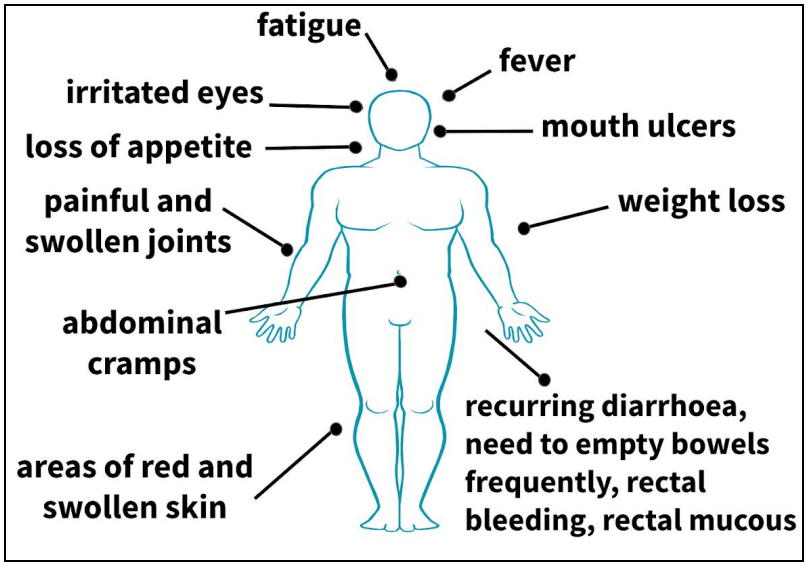

Figure 1. Diagram of the symptoms of Crohn's disease (Cheifetz, 2013).

Diagnosis of Crohn's disease includes lab tests, intestinal endoscopy, upper gastrointestinal series, and computed tomography scans. After the development of symptoms, diagnosis may take long periods of time, and once the disease has developed, patients are more at risk for non-IBD diseases such as gallstones and gastrointestinal infection (Cheifetz, 2013). Most patients take steroids or other immunosuppressants, but these forms of treatment can only slow the progression of the disease and lessen some of the symptoms. On occasion, patients may even require surgery when they do not respond to other forms of treatment, which can oftentimes be invasive and have high costs (Shadnoush et al., 2015). Current studies of potential therapeutic targets have important implications because there is no cure for Crohn's disease.

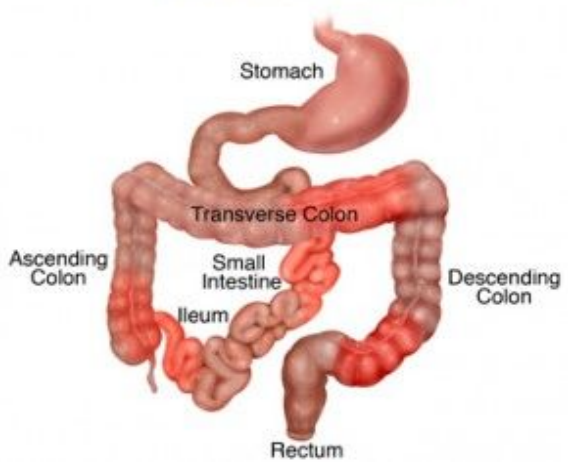

Figure 2. Areas of the GI tract that are most commonly affected by Crohn's disease (highlighted in red) and how Crohn's disease can evolve in any part of the GI tract (National Institute of Health, 2018).

Treatment for Crohn's disease depends on disease severity and symptom prevalence. Patients with mild symptoms will typically take antibiotics, while patients with moderate symptoms will take immunomodulators and patients with severe symptoms will require surgery

(Rogler, 2013). These treatments can be costly and put patients with socioeconomic hardships at a disadvantage as they may not have the means to afford the treatment they require. The cost of treatments for this disease has an average range of $25,282 to $26,192 annually per patient in the United States (Yu et al., 2008). Due to the substantial cost of treating Crohn's disease, it is imperative that new, efficient treatments that are more cost effective are devised. Despite the fact that antibiotics are typically used for treatment, the use of antibiotics has been shown to have negative effects on the health of the gut microbiota as they can sometimes kill helpful bacteria and can be a risk factor for the new onset of Crohn's disease (Lewis et al., 2015).

The human microbiome is the entire population of symbiotic microorganisms, including bacteria, archaea, viruses, and eukaryotic microbes, that lives in and on the human body. The gut microbiota is the community of microorganisms that is enclosed within the GI tract of the human body, accounting for nearly 70% of all microbes (Bamola et al., 2017). Within the gut microbiota, there are over one thousand species of bacteria that stay there either short-term or long-term and are acquired through diet (Shadnoush et al., 2015). The most abundant phyla that reside within the gut microbiota are *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria*, which account for 93.5% of the bacteria (Thursby & Juge, 2017). The gut microbiota is essential to maintaining the health of people as it contributes to life-sustaining functions, such as protecting against pathogens, helping the immune system, and contributing metabolic functions.

Dysbiosis, or a microbial imbalance, occurs when one of the bacterial colonies that make up the gut microbiota becomes unbalanced, throwing off the rest of the bacterial colonies. This can occur due to a number of scenarios, including a dietary change, accidental chemical

consumption, consuming more than two alcoholic beverages per day, and taking new medications. The occurrence of dysbiosis has been linked with a number of intestinal disorders like IBD, irritable bowel syndrome (IBS), asthma, and cardiovascular disease (Carding et al., 2015). The relationship between a functioning immune system and a well-balanced gut microbiota can prevent the development of certain diseases, including Crohn's disease (Carding et al., 2015). Furthermore, the function of the gut microbiota has shown to have different functions in Crohn's disease patients than in healthy patients (Scott et al., 2015).

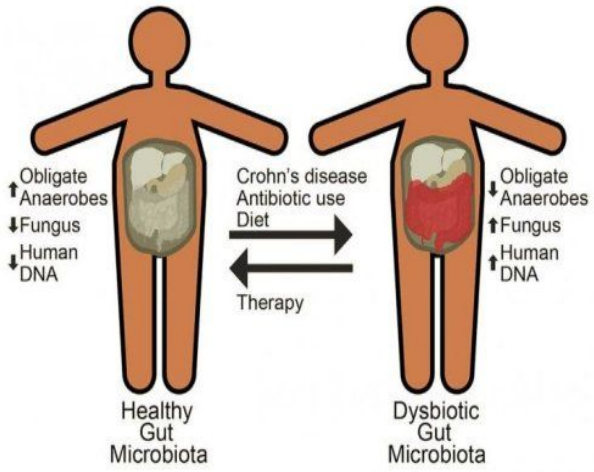

Figure 3. Diagram of the difference between a healthy gut microbiota and a dysbiotic gut microbiota. Crohn's disease and antibiotic use are associated with a dysbiotic gut microbiota, as seen on the right, while therapy with a diet change is associated with a healthy gut microbiota, as seen on the left (Lewis et al., 2015).

Patients with Crohn's disease have a lower fecal microbial diversity and an altered fecal bacterial composition compared to healthy people, but scientists have been researching methods of transplanting bacteria with potential beneficial properties into Crohn's disease patients to

counteract Crohn's disease-associated changes in the gut microbiota (Filteau et al., 2013). Certain foods containing probiotics, such as yogurt, cottage cheese, and sauerkraut increase microbial diversity and can in some cases reduce the symptoms of Crohn's disease (Shadnoush et al., 2015). Moreover, the composition of the microbiome can be altered due to a number of factors, including a change in diet (Bull & Plummer, 2014). The gut microbiota being thrown off balance or into dysbiosis has also been associated with the development of Crohn's disease (Ni et al., 2017). Finding an alternative treatment for Crohn's disease is imperative as current treatments, mainly antibiotics, can have negative effects on microbial diversity and can give patients a poorer quality of life compared to healthy people.

Previous studies have attempted to reduce symptoms in Crohn's disease patients by modifying their diet with specialized diets, such as an avoidance diet. Not enough substantial research has been conducted to fully determine whether or not a change in diet can cause increased rates of remission and an overall decrease in the symptoms of Crohn's disease patients. In not only Crohn's disease patients but also healthy patients, certain diets can increase microbial diversity and therefore boost immune system responses (Filteau et al., 2013). More research is being conducted that further links eating functional food with benefits beyond nutritional value. Doctors often recommend that their patients limit dairy products that contain added fiber, starches, highly sweetened drinks, and other problem foods to stop the aggregation of signs and symptoms of Crohn's disease.

Probiotics are live microorganisms intended to prevent and treat some illnesses and diseases. Certain foods, such as yogurt, miso, dark chocolate, cottage cheese, cheese, sourdough bread, and sauerkraut, contain probiotics. Beyond key foods, probiotics can come in the form of

supplements, suppositories, and powders. It has been suggested that probiotics improve intestinal microbial balance and the diversity of the gut microbiota as they can increase the amount of common anaerobic microorganisms that are needed for proper microbiome functions (Shadnoush et al., 2015). Additionally, several studies have proven that probiotics contain anti-inflammatory agents as well as nutritional benefits (Barajo, 2007). Some studies have shown that specific probiotics can have anti-inflammatory effects and help with the maintenance of remission, while others have shown that particular probiotics do not reduce inflammation or help patients remain in remission.

Bacteria in the *Firmicutes* phylum are typically gram-positive and have been shown to be involved with energy resorption and lessening the severity of inflammation in the GI tract (Kechagia et al., 2012). One of the most prevalent bacteria in *Firmicutes* is *Lactobacillus*, which is a well-known type bacteria that resides within the GI tract of humans and can be found in a number of probiotic foods (Kato-Kataoka et al., 2016). Probiotic *Lactobacillus* has been shown to improve intestinal health, reduce inflammation in the GI tract, normalize bacterial microflora balance, and lower the risk of developing various diseases (Kechagia et al., 2012). In patients with Crohn's disease, there has been shown to be an increased number of anaerobes and a decreased number of *Lactobacillus* (Goossens et al., 2003). Similarly, *Bifidobacterium* is another type of bacteria from the *Actinobacteria* phylum that is frequently found in many probiotics. When this strain of bacteria is transplanted into the gut microbiota, it can help maintain microbial balance by reducing the death and decline of healthy bacteria (Shadnoush et al., 2015). Although *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* have therapeutic benefits, *Proteobacteria* include

a wide range of pathogenic bacteria that can cause issues like diarrhea and duodenal ulcers (Kersters et al., 2006).

Fecal transplantations involve the transfer of stool from a healthy donor into the GI tract of a patient in order to treat their illness. Specifically, fecal microbiota transplantations involve transplanting fecal bacteria from a donor into a recipient via a stool sample. If conventional treatments fail, which happens to approximately one-third of patients, fecal microbiota transplantations may provide an optimal treatment for them (Bak, 2017). Altering the gut microbial flora through fecal transplantations demonstrates a promising new treatment for gastrointestinal diseases (Moayyedi et al., 2015). While fecal transplantations have been shown to have promising results when used to treat Crohn's disease, their standard treatment for Crohn's disease is still new and their long term effects have yet to be entirely explored.

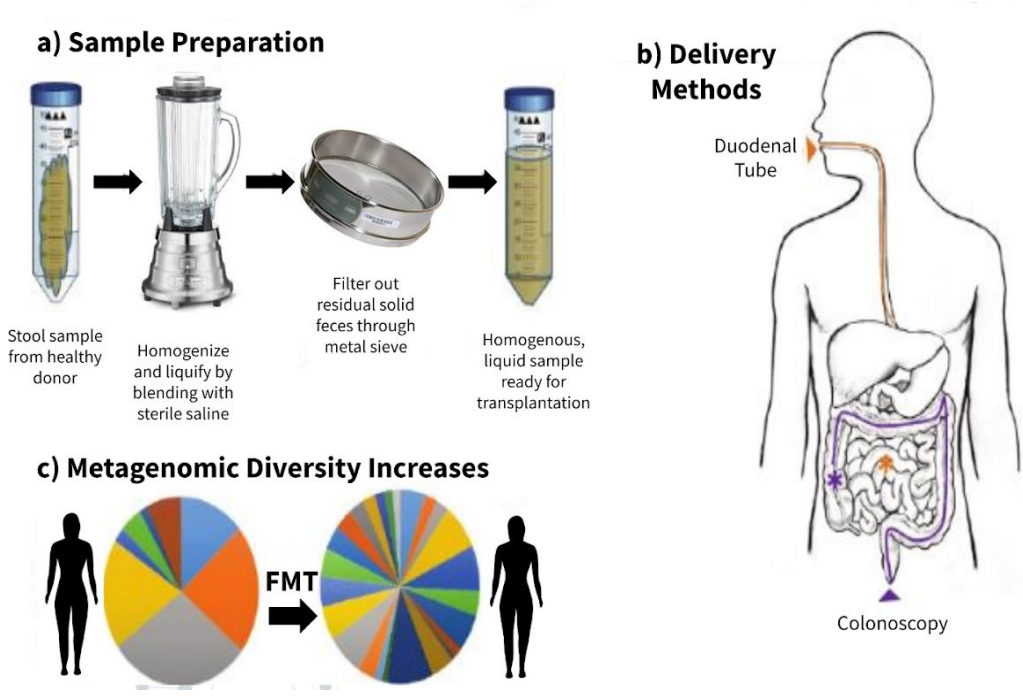

Figure 4. Diagram of a fecal microbiota transplantation in a Crohn's disease patient (Marotz & Zarrinpar, 2016).

During the fecal transplantation procedure, a stool sample is first taken from a healthy donor, homogenized and liquified in a blender with sterile saline, and filtered through a metal sieve to remove residual solid elements. Then, the homogenous, liquid stool sample is delivered into the patient's gut microbiota most frequently through a colonoscopy, but also on occasion with a duodenal tube. In the colonoscopy delivery process, a colonoscope is led through the patient's entire colon and as the colonoscope is withdrawn, the donor stool sample is delivered through the colonoscopy into the patient's colon. With the duodenal tube, the tube is inserted through the mouth or nose of a patient and advanced through the esophagus, into the stomach or duodenum, where the stool sample is placed. After the sample is delivered, the metagenomic diversity of the patient is expected to increase as a higher diversity of bacteria is introduced into the gut microbiota.

Purpose

The purpose of this study is to evaluate the efficiency of manipulating the gut microbiota by incorporating healthy bacteria into the gut microbiota with fecal transplantations and probiotic intake in the treatment of Crohn's disease. Currently, Crohn's disease has no cure, but finding ways to minimize some of the symptoms can help patients live a healthier, more functional life. Throughout the study, research was gathered to determine whether fecal transplantations and probiotic supplements can provide a minimally invasive way of changing the composition of the gut microbiota so that there are more bacteria residing there with therapeutic effects, in an attempt to induce remission.

Research Question

Do fecal transplantations have a greater effect on the symptoms of Crohn's disease than probiotics?

Alternative Hypothesis

Fecal transplantations have a greater effect on the symptoms of Crohn's disease than probiotics, as shown by greater remission rates in the patients who underwent a fecal transplantation, compared to those who underwent a probiotic treatment.

Null Hypothesis

Fecal transplantations do not have a greater effect on the symptoms of Crohn's disease than probiotics.

Methods

Peer-reviewed papers were retrieved and analyzed from various online databases, such as EBSCOhost, Medline, ScienceDirect, and JSTOR. Some relevant journals to my topic that were used were the Journal of Crohn's and Colitis, BMC Gastroenterology, Taylor & Francis Group, Cell Host Microbe, Clinical and Experimental Immunology, Institute of Nutrition and Functional Foods, Elsevier, and World Journal of Gastroenterology. When searching various online databases for peer-reviewed papers, keywords such as "gut microbiota in Crohn's disease," "probiotics effect on Crohn's disease," "fecal transplantations effect on Crohn's disease,"

"composition of gut microbiota in Crohn's disease patients," and "microbial diversity on human health" were used. The reference sections of peer-reviewed papers already used were also examined to find additional sources.

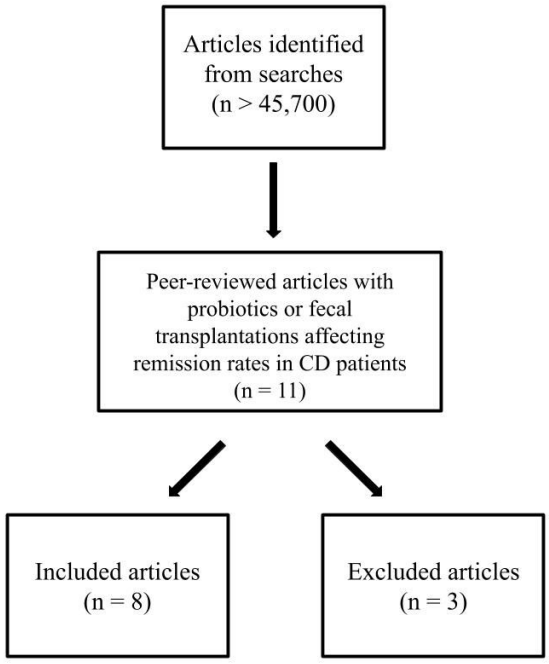

Figure 5. Flow chart for the article selection in this study.

Papers were excluded from this study if they were published before 2000 in order to make sure that the literature used contained the most relevant, up-to-date information. In addition, data from papers was only used if the papers were full-text and peer-reviewed and not systematic literature reviews or meta-data analyses. Papers that focused on maintaining remission rather than inducing remission were excluded because only the effect of probiotics and fecal transplantation on causing remission was looked at. Papers were not excluded if their studies focused on healthy participants rather than Crohn's disease or IBD patients because a association needed to found between the microbial differences of Crohn's disease patients and

healthy controls. The gut microbial populations of Crohn's disease or IBD patients and healthy controls were studied to draw a connection between Crohn's disease or IBD gut microbiota and a dysbiotic gut microbiota.

The population of bacteria in the gut microbiota was determined using 16s ribosomal RNA (rRNA) gene sequences, which are genetic markers that help study bacterial phylogeny and taxonomy. This method of bacterial classification is frequently used because the 16s rRNA gene sequence is present in most bacteria, has a slow rate of evolution, and is large enough for information processing (Janda & Abbott, 2007). The 16s rRNA gene codes for a part of the ribosome found in bacterial cells, so sections of all bacterial species have conserved parts of this gene. Complex samples from the gut can be studied simultaneously while identifying the many types of bacterial organisms living within the sample. The process of 16s rRNA gene sequencing includes sample collection, extraction of nucleic acids from the sample, PCR amplification to amplify DNA sequences, DNA sequencing, amplicon pooling, and sequence analysis.

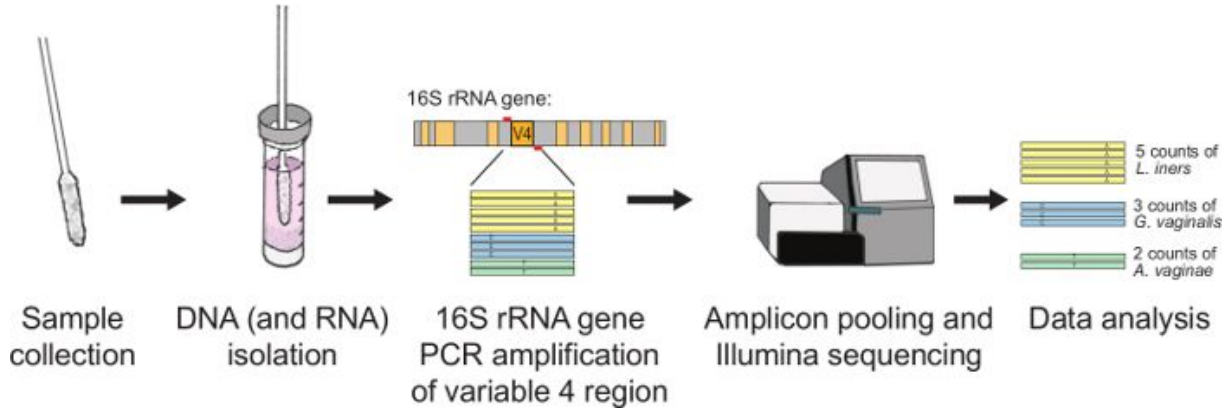

Figure 6. Process of 16s rRNA gene sequencing (Anahtar et al., 2016).

Clinical remission is defined by the absence of symptoms of a disease. Symptoms of patients were quantified using the Crohn's Disease Activity Index (CDAI). In many papers, this was used to determine whether patients had achieved clinical remission. The scale adds up points from 0 to 1100, based on answers to questions about symptoms and pain. Points are given based on the patient's various symptoms, including average abdominal pain over a seven day period, general well being each day over seven days, the number of complications, opiates for complications, the discovery of abdominal masses, anemia, and changes in weight. Between 0 to 149 points in considered complete remission, 150 to 220 points is considered mildly to moderately active Crohn's disease, 221 to 450 points are considered moderately to severely active Crohn's disease, and 451 to 1100 points are considered severely active Crohn's disease.

Variable	*Quantity*	*Multiple*	*Total*
Number of liquid or soft stools per day		2	
Abdominal pain (0 = none, 1 = mild, 2 = moderate, 3 = severe)		5	
General well being (0 = well, 1 = slightly under par, 2 = poor, 3 = very poor, 4 = terrible)		7	
Number of complications: arthralgias, iritis, erythema nodosum, pyoderma gangrenosa, aphthous ulcerations, anal fissure, anal fistula, anal abscess, fever > 37° past week, intestinal obstruction		20	
Opiates for diarrhea (no = 0, yes = 1,)		30	
Abdominal mass (no = 0, questionable = 2, yes = 5)		10	
Deviation from normal hematocrit (*N* = 42 for female, 47 for male)		6	
% deviation from standard weight		1	
Total CDAI			

Figure 7. Crohn's Disease Activity Index (Craig et al., 2003). CDAI<150=remission; >450=severely ill.

Periods of remission can vary between a few days to many years. For the purpose of this study, clinical remission was defined by the absence of Crohn's disease symptoms six weeks after the treatment ended. In the papers that looked at probiotics, papers were not included if the probiotic treatment was administered for less than three months. This ensured that the data found about the patients' altering Crohn's disease symptoms were a result of the probiotics or fecal transplantation approach and not other factors.

Most academic research uses the significance level of 0.05, so that was the significance level that was used when the t-tests were conducted. Two types of t-tests were run: a paired two-sample t-test and an unpaired two-sample t-test assuming unequal variance. Excel was used to perform these t-tests and construct the graphs to guarantee that no errors were made that could indicate a contradictory or incorrect result.

Results

Table 1. Comparison of altered colonies of *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* in the gut microbiota of Crohn's disease patients compared to healthy controls.

Phylum	Imhann et al.	Gevers et al.	Morgan et al.	Willing et al.
Actinobacteria	Down	Down	Not Reported	Up in colonic CD
Bacteroidetes	Up	Down	Not Reported	Not Reported
Firmicutes	Down	Down	Down	Up in colonic CD
Proteobacteria	Up	Up	Up	Up

Four studies evaluated the populations of four major phyla in the gut microbiota of Crohn's disease patients, compared to healthy controls. All four of the studies reported that *Proteobacteria* was up in Crohn's disease patients. Three of the studies recorded that *Firmicutes* was down in the patients, while one of the studies recorded that *Firmicutes* was up only in colonic Crohn's disease. The reports for *Bacteroidetes* were inconclusive as two of the studies did not report of this phylum and the other two studies did not disclose results that matched. *Actinobacteria* were found to be down in two studies, up in only colonic Crohn's disease in one study, and not reported in one study.

Table 2. Overview of the patient statistics and outcome of the probiotic trials interpreted and included in this systematic review.

Source	Type of study	Total number of patients	Patient treatment separation	Mean age	Effectiveness of treatment?
Marteau et al (2006)	randomized, double-blind, placebo-controlled GETAID trial	78	probiotic: 35 placebo: 43	probiotic: 32 placebo: 29	no
Van Gossum et al (2007)	multicenter, randomized, placebo-controlled clinical trial	49	probiotic: 27 placebo: 22	probiotic: 38.7 placebo: 35	no
Steed et al (2010)	randomized, double-blind, placebo-controlled study	24	probiotic: 13 placebo: 11	probiotic: 46.3 placebo: 49	yes

Abbreviations: GETAID: Groupe d'Etude Thérapeutique des Affections Inflammatoires Digestives

A total of 151 patients we included in the probiotic studies, 75 (49.67%) being the patients who received the actual probiotic treatment and 76 (50.33%) being the patients who received the placebo treatment and served as controls. The mean age throughout the studies was 38.33 years old, with a mean age of 39 for the probiotic group and a mean age of 37.67 for the placebo group. While one of the studies did find that probiotics were successful in inducing remission in Crohn's disease patients, two of the studies found that probiotics were not successful in doing this.

Table 3. Overview of the inclusion criteria and treatment protocol for the probiotic trials included in this study.

Source	Inclusion criteria	Probiotic treatment	Placebo treatment	Permitted medication	Not permitted medication
Marteau et al (2006)	-over 18 years old -cumulative small bowel resection(s) of <1 m -no other intestinal resection during the previous 5 years -undergone recent surgical resection for ileal, ileocolonic, or colonic CD -no macroscopic lesions	-two packets per day of lyophilized LA1 (26109 cfu per packet) for six months	-received two packets per day of placebo (maltodextrin) for six months	-corticosteroids (if after surgery) -loperamide -cholestyramine	-antibiotics for more than 15 day -aminosalicylates -glucocorticoids -nonsteroidal anti-inflammatory drugs -immunosuppressive drugs -anti-tumor necrosis factor agents -thalidomide -other probiotics
Van Gossum et al (2007)	-diagnosis of CD for at least 6 months -ability to start oral nutrition	-LA1 blended with maltodextrin at 1010 CFU administered	-maltodextrin only administered once a day for 12 weeks	-no other medication was permitted	-all other medication was prohibited

	within 7 days of operation -need for curative resection -resection margins free of inflammation	once a day for 12 weeks			
Steed et al (2010)	-CDAI score between 150 and 450 -over 18 and 79 years old -not pregnancy -no alterations to medication within the last 3 months -no antibiotic treatment within the last 3 months -no indeterminate colitis -no short gut syndrome - no use of commercially available prebiotic or probiotic preparations within the past 3 months	-2×10^{11} freeze-dried viable *B. longum* in a gelatin capsule, and a sachet containing 6 g of Synergy I twice a day for 6 months	-placebo in the same packaging and form as the probiotic twice a day for 6 months	-conventional CD medication	-not specified

Abbreviations: CD: Crohn's disease, LA1: *Lactobacillus johnsonii*, *B. Longum*: *Bifidobacterium longum*

Two of the studies used *Lactobacillus johnsonii*, which belongs to the *Firmicutes* phylum and the other study used *Bifidobacterium longum* (*B. Longum*), which belongs to the *Actinobacteria* phylum, when concocting their probiotic supplement. Although the two types of bacteria used in the probiotics are from different phyla, both are still types of therapeutic

bacteria. The permittance of other medication during the time in which the probiotics were being administered varied among the studies as each study had their own unique rules regarding this subject. Furthermore, all of the studies used a packet of probiotics in powder form mixed with another substance to give to the patients. Maltodextrin, a polysaccharide used as a food additive, was used as the placebo in two of the studies, while the other study did not list what their placebo was made out of.

Table 4. Overview of the included patients, donor criteria, and treatment protocol for the fecal transplantation studies investigated in this review.

Source	Total number of patients	Patient mean age	Donor Criteria	Treatment	Effectiveness of treatment?
Cui et al (2014)	30	38	-patient selected -preferable if related to the patient -no antibiotic use within the past year -no history of chemotherapy	-laboratory purified fresh fecal microbiota suspension was input into patients' mid-gut by a tube within gastroscope under anesthesia	yes
Suskind et al (2014)	10	16.2	-meets infectious and noninfectious exclusion criteria -no other specification	-received bowel cleanout and then transplant via nasogastric tube	yes
Vaughn et al (2014)	9	not given	-unknown to the patient -healthy individuals -no other specification	-a single infusion of 50 g of stool was suspended in 250 ml of solution and administered through colonoscopy	yes

Suskind et al (2015)	9	16.22	-no antibiotics 3 months prior to transplant -parent of patient -passed the American Association of Blood Banks Donor History questionnaire	-30 g of donor stool was mixed with 100–200 ml of normal saline and administered via a nasogastric tube	yes
He et al (2017)	25	not given (between 10-25 years old)	-no use of antibiotics, laxative or diet pills in the past 3 months -no recent gastrointestinal diseases -no history of illness -no diseases or conditions potentially associated with specific changes in the gut microbiota	-prepared microbiota liquid suspension was transplanted into the distal duodenum of patients through gastroscope under anesthesia	yes

Out of the five studies evaluated, 83 patients were administered a fecal transplantation. A gastroscope was used in two of the clinical trials, a nasogastric tube was used in two, and a colonoscopy was used in one. The trials differed in whether the donors were related to the patients or not. Most of the trials specified that the donors must be healthy, have not used antibiotics within at least the last three months, and have no diseases or illnesses that could impact their gut microbiota or fecal sample. After the transplantations were completed, remission rates were determined at the baseline of six weeks using the CDAI. All of the clinical trials that were tested, found fecal transplantations to be an effective way of treating Crohn's disease.

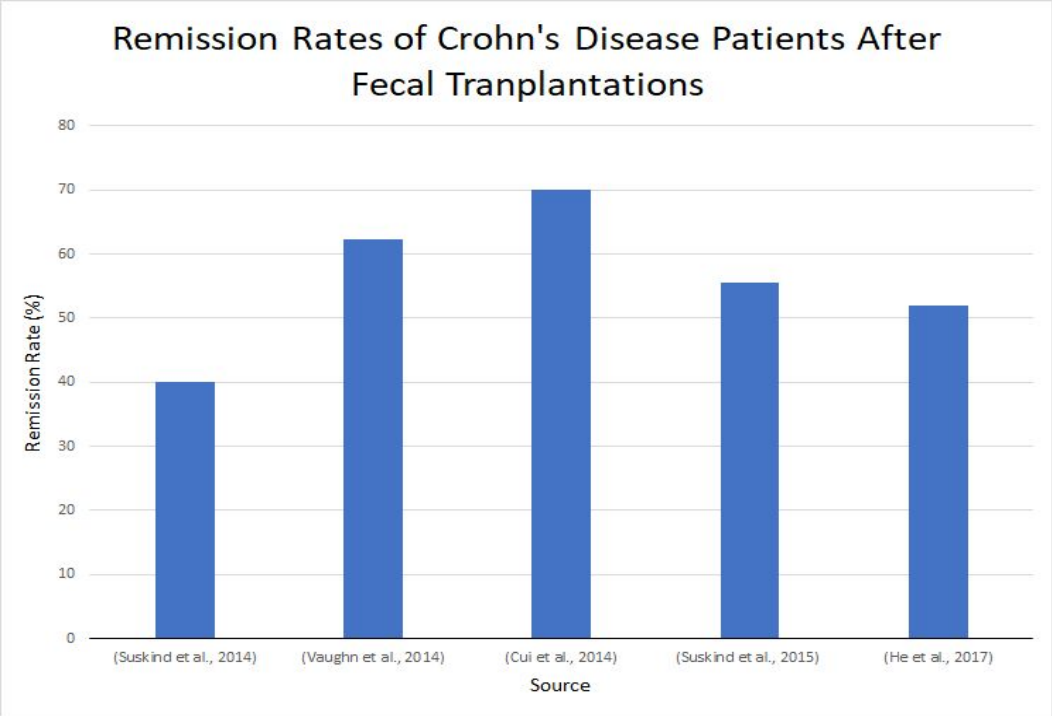

Figure 8. Graph depicting the remission rates of Crohn's disease patients after they underwent a fecal transplantation. Prior to the treatment, none of the patients were in remission.

Table 5. T-test results that compare the remission rates of Crohn's disease patients from various peer-reviewed papers before and after they underwent a fecal transplantation. Data was extracted from the sources used in Figure 8.

t-Test: Paired Two Sample for Means	***Before***	***After***
Mean	0	55.96111
Variance	0	126.8131
Observations	5	5
Hypothesized Mean Difference	0	
df	4	
t Stat	-11.1119	

P(T<=t) one-tail	0.000187
t Critical one-tail	2.131847
P(T<=t) two-tail	0.000373
t Critical two-tail	2.776445

A paired two-sample t-test was run to determine the statistical significance of fecal transplantations on inducing remission in Crohn's disease patients. The t-test was paired because the data compared the same patients before and after the treatment. The P one-tail value was the value that was used to determine the statistical significance because the remission rates at the beginning of the treatment were at 0%, so the data could only indicate a relationship between fecal transplantations and a positive change in remission rates. The P value of 0.00019 was compared with the significance value of 0.05.

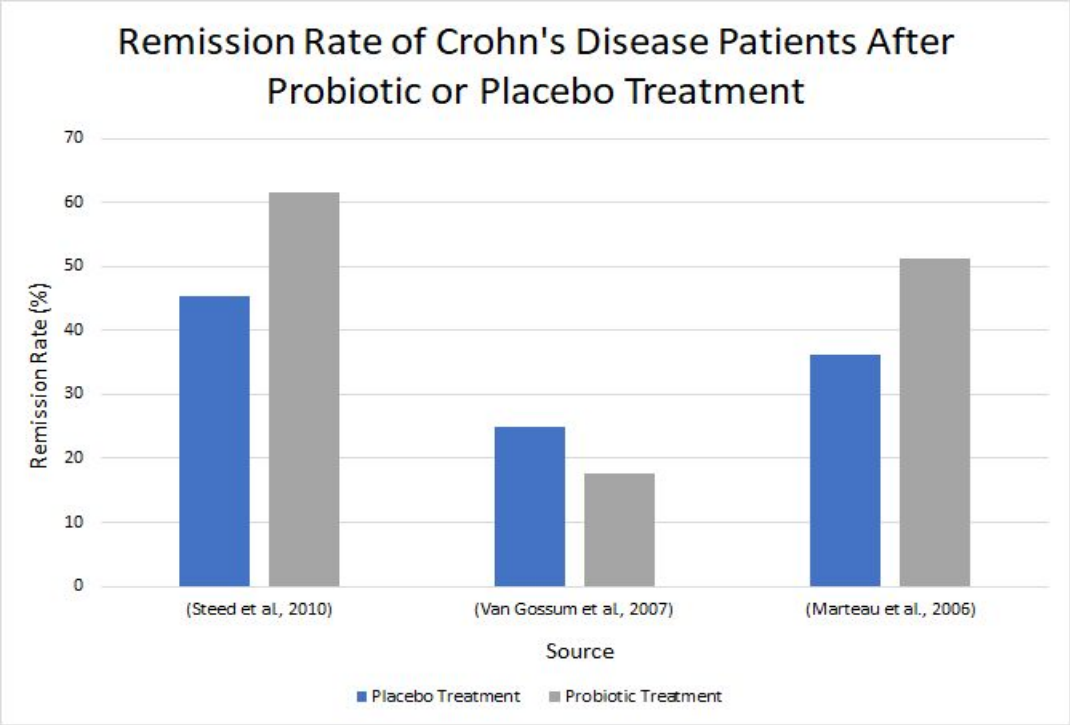

Figure 9. Graph of the remission rates of Crohn's disease patients from three controlled studies after they underwent a probiotic or placebo treatment.

Table 6. T-test results that show the remission rates of patients with Crohn's disease who were treated with probiotics or a placebo from various randomized controlled studies. Data is reflexive of the data portrayed in Figure 9.

t-Test: Two-Sample Assuming Unequal Variances	***Placebo Treatment***	***Probiotic Treatment***
Mean	35.54158167	43.44852067
Variance	104.8934735	526.2130997
Observations	3	3
df	3	
t Stat	-0.545152068	

P(T<=t) one-tail	0.311782711
t Critical one-tail	2.353363435
P(T<=t) two-tail	0.623565422
t Critical two-tail	3.182446305

An unpaired two-sample t-test assuming unequal variance was used to examine the discrepancies between the effects of a probiotic and placebo treatment on inducing remission in Crohn's disease patients. The t-test was unpaired assuming unequal variance because the probiotic group and the placebo group are two different groups of patients and the number of patients and the impact of the probiotics and placebo varied. The two-tail P value was used when drawing conclusions about the data because there was a possibility that the relationship between the two and groups an inducing remission could have gone in either direction. The P value used from the t-test was 0.62.

The studies used for Figure 8 and Table 5 were all randomized controlled trials that examined the effect of probiotics on remission rates in Crohn's disease patients. The number of patients included in the trials varied between 11 and 90. The probiotics were administered for a minimum of four months in each study and patients were only eligible for the studies if they had Crohn's disease. The age of the patients ranged varied between the studies as both children and adults with Crohn's disease were used. The CDAI was used to determine whether patients had achieved clinical remission after at least six weeks of being treated with either a probiotic or a placebo.

Discussion

When the gut microbiota is imbalanced, it loses its symbiotic relationship with its host and is thrown into dysbiosis. A dysbiotic gut microbiota has been connected with the development of Crohn's disease and the inability to reduce inflammation in the GI tract (Lewis et al., 2015). Table 1 displayed the changes experienced in the gut microbiota of Crohn's disease patients compared to healthy controls. In all of the examined studies, *Proteobacterium* was up and bacteria in this phylum and typically pathogenic, meaning they have the capability of producing or causing disease. Other studies have proven that *Proteobacterium* converts urea, a waste product that is excreted by the kidneys during urination, into ammonia that is then reabsorbed by bacteria to make amino acids associated with dysbiosis in the gut microbiota of Crohn's disease patients (Friedman et al., 2017).

Moreover, Table 1 showed that *Firmicutes* was found to be down in most of the studies. While the comprehensive therapeutic role of *Firmicutes* is still being discovered, it has been linked with aiding the host in maintaining homeostasis in their GI tract, which in turn assists with immune responses, immune system development, nutrient intake, and defending against pathogens (Distrutti et al., 2016). *Actinobacteria* and *Bacteroidetes* have likewise been shown to have similar therapeutic qualities, but based on the findings, it is inconclusive whether or not those phyla are increased or decreased in Crohn's disease patients (Distrutti et al., 2016). Since, *Firmicutes*, *Proteobacterium*, *Actinobacteria*, and *Bacteroidetes* are the four major phyla found in the gut microbiota, their interactions with the host and other phyla are important in conserving an accurately functioning gut microbiota.

For the t-test on fecal transplantations, the P value of 0.00019 was compared with the significance value of 0.05. The P value is the probability that the null hypothesis is correct, so a

low P value reveals that there is a low probability that the null hypothesis is correct. Since the P value is undoubtedly lower than the significance value, the null hypothesis was rejected. For probiotics, the P value of 0.62 was extracted from the t-test. Due to the high P value, it can be revealed that probiotics do not have a statistically significant capability of inducing remission in patients.

The method of delivery for the fecal transplantations did not seem to have an apparent impact on the effectiveness or the ability of the transplantation to induce remission rates in patients, which can be observed in Table 4. Similarly, whether or not the donors were related to the patients did not appear to influence the effectiveness of the transplantation. In addition, the age of the patients and the number of patients involved in the studies varied and did not seem to impact the outcome of the trials.

Conclusion

The data insinuates that fecal transplantations are a more effective treatment for Crohn's disease than probiotics. Although probiotics are adequate in some subjects, there are not adequate in all patients. However, some subjects may respond better to probiotics than fecal transplantations or traditional forms of treatment, such as surgery or antibiotics. Fecal transplantations provide a better option than probiotics but need to be further developed to be enhanced and reach their full potential. These two forms of treatment can be used as a minimally invasive form of treatment for Crohn's disease in some cases, but they can only help ease some of the symptoms, not fully cure the disease in all patients or even the patients that truly respond to these forms of treatment.

By fixing the imbalance of the gut microbiota in Crohn's disease patients, fecal transplantations and probiotics can help prevent a dysbiotic gut microbiota. This would assist patients in boosting their immune responses and protect against pathogens that could worsen their symptoms or cause further complications. Harmful bacteria were determined to be more prevalent in the gut microbiota of Crohn's disease patients, indicating that manipulating their gut microbiota would be beneficial in restoring the normal balance of bacteria.

Although probiotics are meant to increase the concentrations of helpful bacteria in the gut microbiota of Crohn's disease patients, they were not able to show compelling data to support that assertion. Notable differences in the remission rates between the probiotic and the placebo group could have been due to chance, symptom differences within patients, disease severity in patients, and participant dropouts. Due to this, the argument that the remission rates in the probiotic group were higher in the probiotic group is flawed.

Further Work

More research and clinical trials are required to optimize the effects of both probiotics and fecal transplantations. Using fecal transplantations and probiotic supplements is a relatively new form of treatment for Crohn's disease, so there is no formal standard treatment method for them and their long term side effects are relatively unknown. It is also unclear whether these forms of treatment can help patients remain in remission over the course of multiple years after they received the treatment. While current probiotics have not shown to cause statistically significant remission rates, it is plausible that a well-balanced probiotic cocktail, specially designed for the gut microbiota of each patient, could cause higher remission rates. Further,

additional research is required to determine if the donor should be related to the recipient or what should formally exclude a person from being a donor considering many papers differ in their exclusion criteria for donors.

Sources of Error

Since fecal microbiota transplants are such a new form of Crohn's disease treatment, the number of studies that have been conducted using them is limited. Research is currently being completed to find more evidence of their benefits, so their full advantages will not fully be understood until more studies have been conducted. In addition, the probiotics used in the studies varied in type and quantity. It is possible that some probiotics are more effective than others. Studies with a larger patient size had a more accurate representation of the remission rates of patients after a probiotic or fecal transplantation treatment because a smaller amount of participants leaves more up to chance.

The fecal transplantations varied when it came to their donor stool samples and their transplantation methods. It is feasible that one of the stool samples could have had more colonies of a certain type of bacteria that would have been more beneficial to a different patient than the patient it was administered to. Crohn's disease patients all experience different symptoms and severity of the disease, so one patient may have more difficulty achieving remission than a different patient. The studies focused on patients of all ages and genders and it was not analyzed whether or not age or gender has an effect on the effectiveness of probiotics or fecal transplantations.

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