Faecal Microbiota Transplantation: Alteration of the Gut Microbiome for the Effective Treatment of Crohn's Disease and Colitis

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By

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Declaration

"I hereby certify that the material, which I now submit for assessment on the programme of study leading to the award of Master's Degree, is entirely my own work and has not been taken from the work of others except to the extent that such work has been cited and acknowledged within the text of my own work. No portion of the work contained in this thesis has been submitted in support of an application for another degree or qualification to this or any other institution."

Signed:  Date: 23rd April 2023

Student's Name: Ciara Phillips 23rd April 2023

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Abstract

The Gut Microbiome is a community of microorganisms that colonise together in the gastrointestinal tract to play a crucial role in the body's immune defence, behaviour, and metabolism. The Gut Microbiome has been linked to the development of many gut dysbiosis related diseases including Crohn's disease and Colitis. As there is currently no cure for these diseases, treatment for the alleviation of associated symptoms is crucial for patient quality of life. Faecal Microbiota Transplantation is a restorative therapeutic technique involving the transplantation of healthy donor faecal bacteria to a receiving patient with the aim of altering their Gut Microbiome to resemble that of their donor's. The objective of this research was to investigate how alteration of the Gut Microbiome with Faecal Microbiota Transplantation can be used in modern Western medicine as an effective disease treatment method for Crohn's Disease and Colitis.

This thesis compared the effectiveness of Faecal Microbiota Transplantation to current, conventional disease treatment methods via secondary research through analysis of recently published scientific journals and other reliable government and non-government-based sources.

A major observation from this research was that patients undergoing Faecal Microbiota Transplantation showed higher success rates when higher levels of *Faecalibacterium prausnitzii* were present in their Gut Microbiome post treatment. Going forward, an extra step should be added to the donor screening process to help determine the presence of *Faecalibacterium prausnitzii* in the donor sample before its acceptance as suitable for donation. This will help increase both the receiver's treatment success rate and patience acceptance of the disease treatment method.

In conclusion, this thesis adds to the overall collective knowledge of the use of Faecal Microbiota Transplantation and gives evidence to its massive future potential as an effective main-stage, first-time disease treatment method in modern Western medicine for the alleviation of Crohn's Disease and Colitis symptoms.

List of Abbreviations

ANSA-Na	1-Amino-2-Naphthol-6-Sulphonate Sodium Salt
ARTG	Australian Register of Therapeutic Goods
BL	Baseline
CD	Crohn's Disease
CDED	Crohn's Disease Exclusion Diet
CI	Confidence Interval
CMC	Carboxymethyl Cellulose
COVID-19	Coronavirus Disease 2019
E. coli	*Escherichia coli*
EGD	Esophagogastroduodenoscopy
EPEC	Enteropathogenic *Escherichia coli*
ESPEN	European Society for Clinical Nutrition and Metabolism
FDA	The Food and Drug Administration
FMT	Faecal Microbiota Transplantation
GDPR	General Data Protection Regulation
GI	Gastrointestinal
GM	Gut Microbiome
HD-FS	Healthy Donor Faecal Suspension
HD-ST	Healthy Donor Stool
HPRA	Health Products Regulatory Authority
HTC/P	Human Cell and Tissue-Based Product
H_20_2	Hydrogen Peroxide

IBD	Inflammatory Bowel Disease
IBS	Irritable Bowel Syndrome
MHRA	Medicines and Healthcare Products Regulatory Agency
MTC	Microbiome Treatment Centre
NR	Non-Responders
OTUs	Operational Taxonomic Units
PEC	Percutaneous Endoscopic Cecostomy
PEN	Partial Enteral Nutrition
***p*-value**	Probability Value
P80	Polysorbate 80
rCDI	Recurrent *Clostridium difficile* Infection
RE	Responders
RR	Relative Risk
RSCI	Royal College of Surgeons in Ireland
R^2	R-squared
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SCD	Specific Carbohydrate Diet
SCFAs	Short Chain Fatty Acids
SOC	Standard of Care Therapy
spp.	Species
STEC	Shiga toxin-Producing *Escherichia Coli*
TET	Transendoscopic Enteral Tubing
TGA	Therapeutic Goods Administration
UC	Ulcerative Colitis

UK	United Kingdom
UPFs	Ultra-Processed Foods
USA	United States of America
W2	2 weeks post FMT treatment
W6	6 weeks post FMT treatment
W8	8 weeks post FMT treatment

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Chapter 1: Introduction

1.1 Chapter Overview

This thesis examines the role of Faecal Microbiota Transplantation (FMT) as a disease treatment method for Crohn's disease (CD) and Colitis when current treatment methods are no longer deemed as effective. The aim of this introductory chapter is to introduce the reader to the topic of FMT by sharing a general understanding of the importance of Gut Microbiome (GM) in the body, giving a brief overview of what FMT is, providing a brief synopsis of its historical usage, highlighting its most common uses in human health, outlining its associated health benefits, and discussing its current medical usage rates worldwide. In contrast, this chapter also highlights the controversies associated with the use of FMT as a disease treatment method.

1.2 The importance of the Gut Microbiome in the body

The GM is a community of microorganisms, colonising together in the gastrointestinal (GI) tract (Moszak, 2020). These microorganisms are present in the GM in vast quantities and are crucial to human health. The GM composition has been linked to bodily functions such as immune defence, behaviour, and metabolism. It has also been linked to the development of a vast number of gut dysbiosis related diseases including CD and Colitis. Due to the important role that the GM plays in the body, it is frequently referred to as a forgotten organ.

The composition of the GM majorly influences a person's mucosal immune system's functional and structural development. The composition of each person's GM is unique with a specific metabolic efficiency. As seen in Figure 1.1, there are several factors that alter its composition. These factors include environment, geographical location, genetics and ethnicity, delivery pattern at birth, age, diet, stress levels, socioeconomic status, the consumption of antibiotics, prebiotics or probiotics and the use of FMT. Altering any of these factors can lead to the alteration of the GM composition (Hasan and Yang, 2019). This alteration can be either positive or negative with negative alteration linked to many diseases.

Bacterial species (spp.) such as *Lactobacillus and Bifidobacterium* can be taken as probiotics as a method of altering the GM composition with the aim to help improve the patient's immunity to disease. Probiotics, prebiotics and FMT can all cause changes to

the structure or activity of the GM. Their ability to alter the GM composition indicates the influence they have on re-establishing human health.

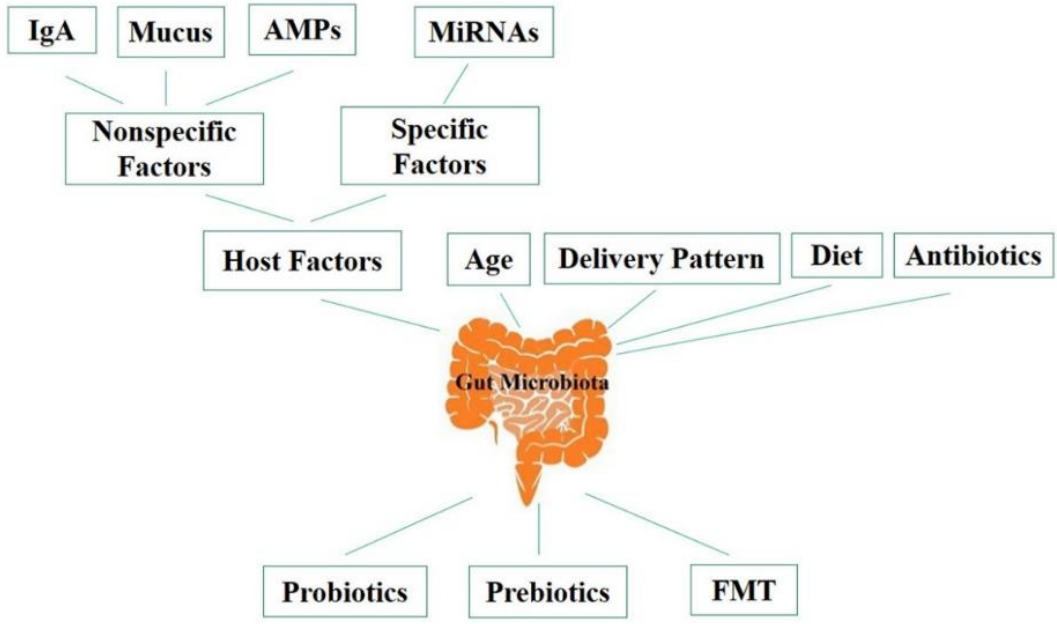


Figure 1.1: Factors affecting the Gut Microbiome and the ways to modulate it (Taken from (Hasan and Yang, 2019))

1.3 An overview of Faecal Microbiota Transplantation

While there is currently no accepted definition for FMT, it can be described as a restorative therapeutic technique that consists of the transplantation of healthy faecal bacteria from a healthy donor to a receiver who suffers from intestinal issues. This process allows for the long-term beneficial restoration of a naturally good GM in the receiving patient (Hasan and Yang, 2019). By having the ability to alter a patient's GM composition, FMT also allows for the analysis of the role that the GM plays in gut dysbiosis related diseases (Wortelboer and Herrema, 2021). The aim of this process is to restore natural diversity and function in the GM without the use of antibiotics or further medication (Ademe, 2020).

Figure 1.2 clearly outlines the modern process involved in FMT administration to patients. Faecal donors are first screened to ensure their health status. Stool samples are then collected and analysed for the presence of any infectious pathogens that may cause

harm to the receiver. Once the samples have been approved as safe for use, the delivery route is then determined. Once this process is complete, patients are then monitored to ensure the process was successful.

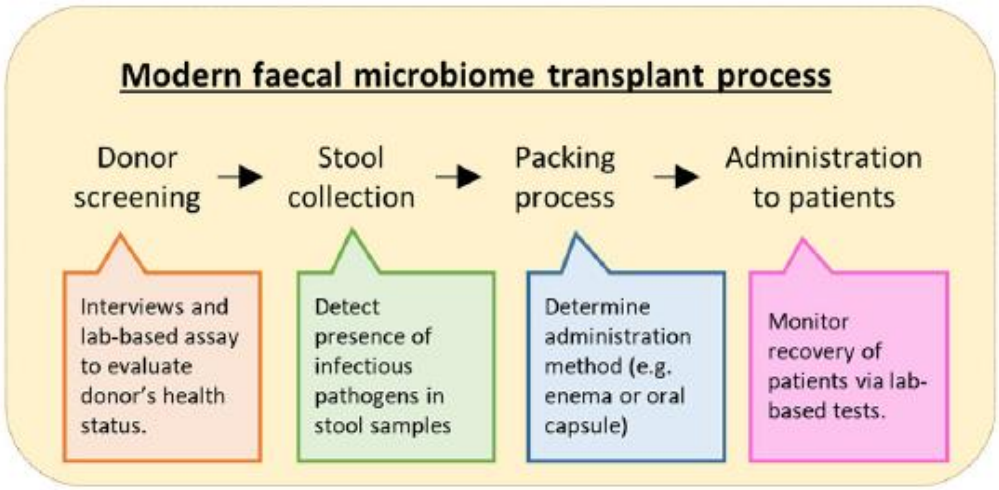
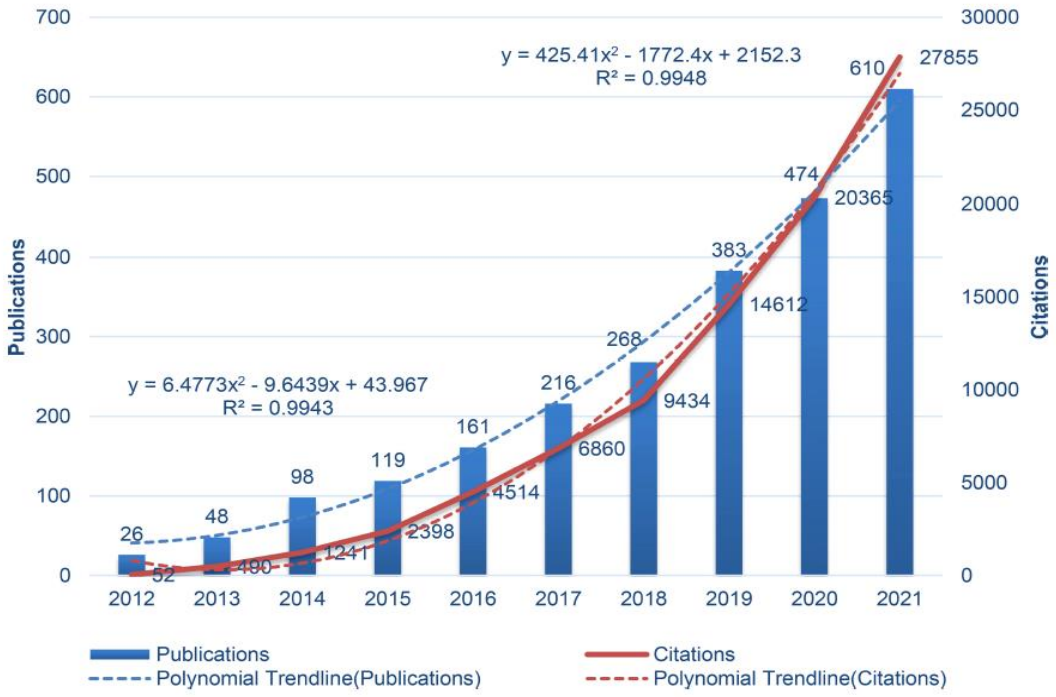


Figure 1.2: Modern FMT process (Taken from (Ser *et al.*, 2021))

Over the past two decades, the use of FMT to treat patients suffering from gut dysbiosis has gained rapid acceptance within Western medicine (Gulati et al., 2020). An advantage of using FMT as a disease treatment method is that it has shown to have a high success rate for causing the long-term beneficial alteration of the GM composition. Graph 1.1 gives a clear indication of how in the last decade, extensive research has been carried out on the important role that FMT can play in modern Western medicine. There has been a major increase in published studies from 2019 to 2021 with significant correlations between publications, citations, and year [R-squared (R^2) = 0.9943, R^2 = 0.9948]. There were 1,467 articles published on the topic of FMT from 2019 to 2021 (Fengwei Zhang *et al*., 2022).

As summarised by Merrick *et al.* in 2020, the use of FMT as a disease treatment method is generally considered as a safe method if extremely thorough and careful donor screening and testing is conducted. There are however short-term risks associated with the FMT process, but these are mainly associated with the delivery method used rather than the process itself. Reported long-term risks associated with FMT have been very rare but include the transmission of antibiotic resistant bacteria, and even death in extremely rare cases. In 2021, Marcella *et al.* analysed the health risks associated with the use of

this treatment method between the years 2000 to 2020. Based on the results from this systematic review, of the 129 studies (4241 patients) analysed, 19% of the patients reported side effects from the FMT treatment method with 1.39% of these patients suffering from serious health risks such as infection and even death. The most reported short-term risks post FMT treatment were diarrhoea (10%) and abdominal discomfort (7%).



Graph 1.1: Annual trends in FMT research publications and citations (Taken from (Fengwei Zhang *et al.*, 2022)).

1.3.1 Historical usage of Faecal Microbiota Transplantation

The FMT timeline is summarised in Figure 1.3. While considered a relatively novel treatment method for disease in Western medicine, first literary evidence of the FMT process as a therapeutic technique date back as far as over 1,700 years ago. This process first originated in ancient China in the 4th century as a treatment method for severe diarrhoea and food poisoning. This method was first completed by a man named Ge Hong who called it yellow soup (Wang *et al.*, 2019)**.** In the 16th century, Li Shizhen reported

the use of FMT for the treatment of various GI diseases in a book called “Ben Cao Gang Mu” (Compendium of Materia Medica) (Ser *et al*., 2021).

The first report of the use of FMT in Western medicine was in 1958 when Eiseman *et al.* reported its successful use for the treatment of Pseudomembranous Colitis in four different patients (Eiseman B *et al.*, 1958). In 1983, a report was published by Schwan *et al.* which documented the use of this medical process for the treatment of recurrent *Clostridium difficile* infection (rCDI) (Schwan *et al.*, 1983). Bennet and Brinkman reported the successful FMT treatment of the first patient with Ulcerative Colitis (UC) in 1989 (Hao *et al.*, 2023). In 2013, Els *et al*. carried out the first randomized controlled trial which showed that FMT treatment of rCDI resulted in an increased patient success rate when compared to the use of antibiotics alone (Wang *et al.*, 2019). In the last decade, FMT has re-emerged as a restorative therapeutic technique for the treatment of GM related diseases due to the extensive use of antibiotics resulting in the increased development of antibiotic-resistant pathogens. The overuse of antibiotics in Western medicine has also been linked with the undesirable alteration of the GM composition (Hao *et al*., 2023).

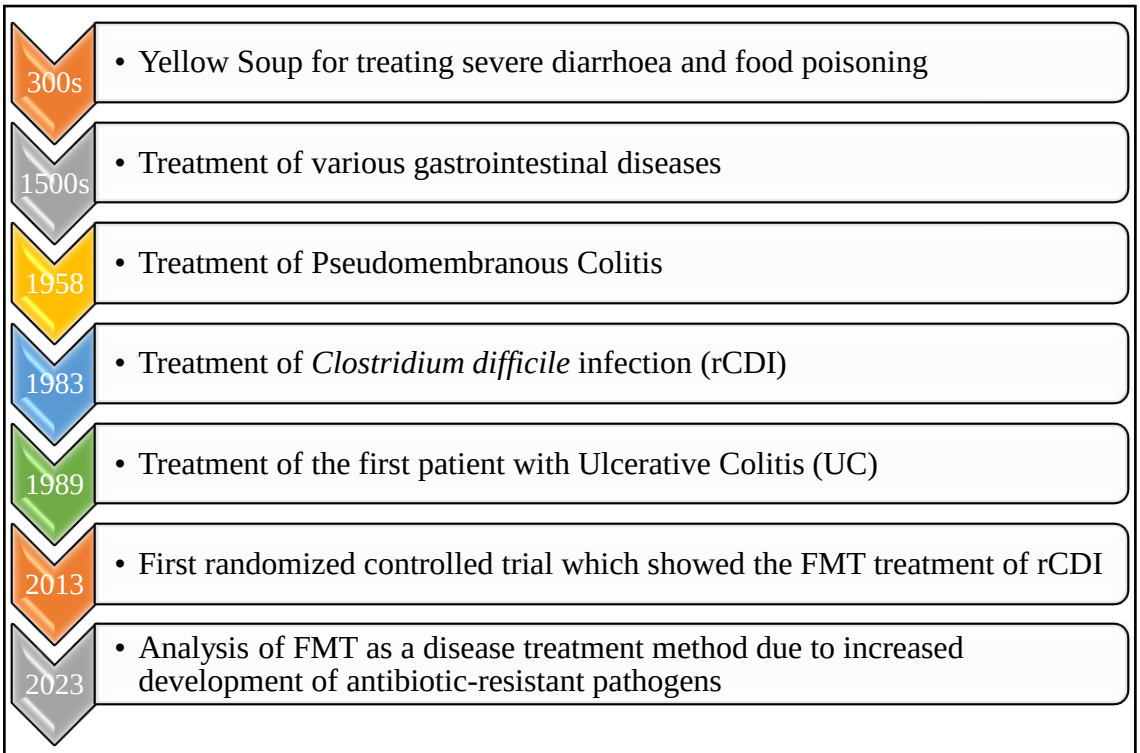


Figure 1.3 Historical usage of FMT

1.3.2 Health benefits linked to Faecal Microbiota Transplantation

The unsuccessful response of many medical therapeutic strategies in correcting gut dysbiosis related diseases and disorders has resulted in the increased analysis of FMT as a promising restorative therapeutic technique for the treatment of these diseases. Benefits associated with the use of this process include restoration of metabolites, immune function and colonisation resistance (Ademe, 2020). FMT can positively restore the GM structure and function which can be negatively altered due to the extensive use of antibiotics. This process has the advantage of being able to overcome antibiotic-resistant pathogens in the GM (Ademe, 2020). The process of FMT allows for the receiver's GM to replicate that of the donor's, thereby increasing the level of beneficial bacteria present (Shen *et al.*, 2018). By promoting leukocyte adhesion, T-cell activity, Th1 differentiation, and immune-stimulatory factors and by inhibiting proinflammatory cytokines secretion, FMT can restore the GM composition, reduce the risk of colonic inflammation and help treat gut dysbiosis related diseases (Shen *et al.,* 2018). Another health benefit associated with the use of this process is that it can result in the alteration of the receiver's intestinal pH value. This pH alteration can increase the adhesion of health benefitting bacteria and hydrogen peroxide (H_2O_2) which results in the inhibition of the adhesion and translocation of pathogens in the GM (Shen *et al.*, 2018).

FMT results in the increased production of intestinal short-chain fatty acids (SCFAs). SCFAs are biologically active. SCFAs have a strong immunomodulatory effect and therefore their production is a definite health benefitting property of this process. The three most common SCFAs found in the gut are butyrate, propionate, and acetate. Firmicutes produce butyrate and Bacteroidetes produce propionate and acetate (Ooijevaar *et al.,* 2019). Butyrate is a SCFA that is required as the main energy source for colonic epithelial cells. Increased levels of butyrate post FMT helps reduce intestinal permeability which helps maintain the integrity of the epithelial barrier (Shen *et al.,* 2018). In 2018, Shen *et al*. also highlighted how this process resulted in the increased level of *Lachnospiraceae* in the GM. *Lachnospiraceae* are bacteria that produce the SCFA butyrate. This indicated the significant role that butyrate plays in the FMT process.

1.3.3 Most common uses of Faecal Microbiota Transplantation

FMT is still in the experimental and research phase for many GM related diseases such as gastrointestinal, metabolic, cardiovascular, immunological, and neuropsychiatric diseases. However, since the completion of the first randomised clinical trial in 2013, it has become a routine treatment for rCDI as an alternative to antibiotics once they have been proven to be ineffective (Wortelboer and Herrema, 2021). In 2020, Ademe reported that the reason this process has become so popular for the treatment of rCDI is due to the fact that 20% to 60% of rCDI patients being treated via other methods have resulted in recurrences due to inadequate GM recovery, regardless of the antibiotic treatment methods used. In contrast, treating rCDI patients with FMT has reported cure rates of 90 to 92% with mortality rates of severe rCDI decreased from 42% to 12% (Ademe, 2020). In 2022, Fengwei Zhang *et al.* reported that rCDI treatment is the current main research topic for FMT worldwide for both basic and clinical research.

1.3.4 Delivery routes of Faecal Microbiota Transplantation

In 2020, Gulati *et al.* published a review on the available, anticipated, and aspired delivery routes for FMT as a disease treatment method. As Gulati *et al.* highlighted in Figure 1.4, there are many delivery routes for FMT as a disease treatment method in humans with the most efficient and successful route yet to be established. Examples of the main delivery routes include colonoscopy, retention enema, jejunostomy, sigmoidoscopy, nasoduodenal route, nasojejunal route, esophagogastroduodenoscopy (EGD), enterostomy, transendoscopic enteral tubing (TET), percutaneous endoscopic cecostomy (PEC) and oral capsules.

This paragraph outlines a brief description of the main delivery routes used for FMT. When suffering patients receive FMT via colonoscopy, the process consists of using a flexible tube which is inserted through the anus to the colon to deliver the donor's processed faecal matter. A camera is fitted into the colonoscope to allow for visualisation of the colon lining. A colonoscopy can be used to analyse the whole large intestine. Patients who undergo the procedure are usually sedated to help reduce discomfort during the procedure (Gulati *et al.,* 2020). During the retention enema procedure, the donor's processed faecal matter is softened to allow for more ease of passage through the body. The sample is then injected via the rectum to the colon (Gulati *et al.*, 2020; Rupawala *et*

al., 2021). Patients can also receive FMT via the jejunostomy surgical procedure which involves delivering the donor's faecal matter to the receiver by inserted a tube into the lumen of the proximal jejunum by making an incision in the patient's abdominal wall (Gulati *et al.*, 2020). A sigmoidoscopy procedure is similar to a colonoscopy but is less invasive and does not require the patient to be sedated. A sigmoidoscopy only reaches the lower third of the patient's colon. (Gulati *et al.*, 2020). The Nasoduodenal route consists of inserting a tube into the patient's duodenum via the nasal cavity. The donor's faecal matter is delivered to the receiver through this tube. When the tube is inserted further into the jejunum, this FMT delivery route is called the nasojejunal route (Gulati *et al.*, 2020). As can be seen in Figure 1.4, EGD is an oral route of administration of FMT. During this procedure, the donor's processed faecal slurry is delivered to the receiver through a flexible tube that is inserted into the mouth and then travels through the pharynx, oesophagus, stomach, and duodenum (Gulati *et al.*, 2020). An enterostomy is a procedure which involves using a long conventional endoscope, a capsule endoscope, or a double-balloon endoscope. During the enterostomy procedure, the endoscope is inserted into the patient via the mouth or the anus (Gulati *et al.*, 2020). TET is a FMT delivery route that involves a tube being inserted into the ileocecal junction via an endoscopy. The TET method uses titanium clips to fix this tube to the patient's cecum. The cecum is the first part of the large intestine and connects the colon to the small intestine. Patients who use this FMT delivery route are under anaesthesia during the procedure (Chuyan Long *et al.*, 2018; Gulati *et al.*, 2020). PEC is another FMT delivery route that allows for the delivery of the donor's faecal matter to the receiver by making an incision in the receiver's cecum for the placement of a cecostomy tube. This tube is also known as a catheter (Gulati *et al.*, 2020).

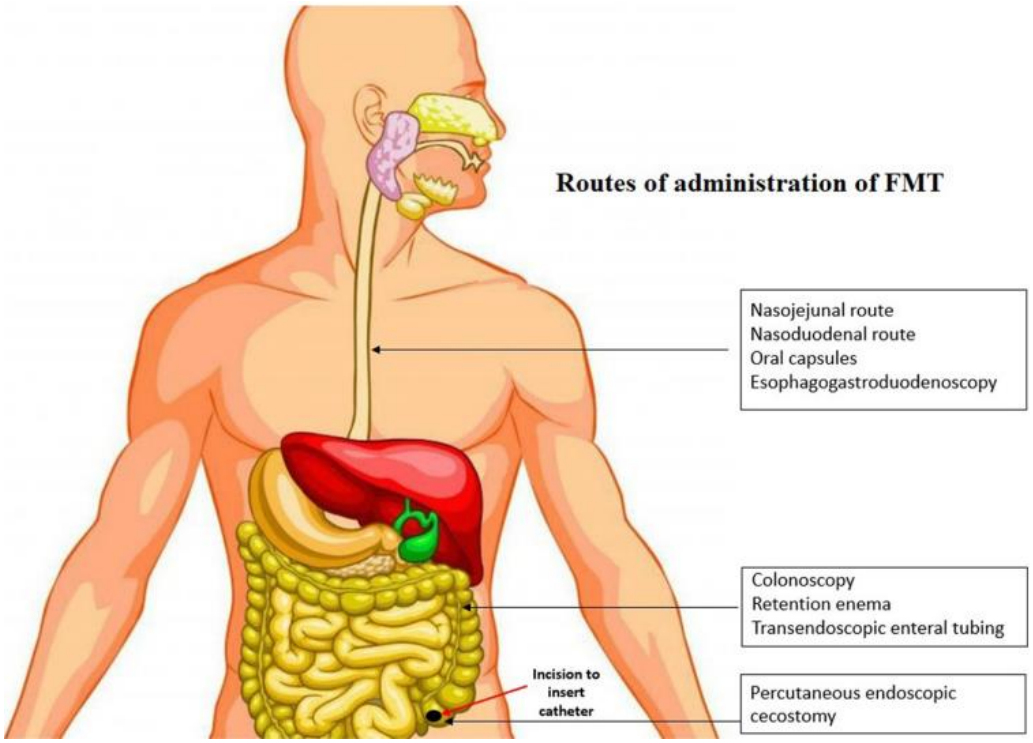


Figure 1.4: Routes of administration of FMT (Taken from (Gulati *et al.*, 2020))

As part of this review of FMT delivery routes, Gulati *et al.* (2020) composed a table similar to Table 1.1 to highlight how many factors must be considered before choosing the most suitable FMT delivery route. These factors include the degree of invasiveness, associated risks, convenience of administration, cost, the level of pre-treatment required, the delivery site and self-administration ability. As can be seen in Table 1.1, it is evident that there are multiple variables that may affect the effectiveness associated with its use as a disease treatment method. Also highlighted in Table 1.1, are the varying risk levels of different delivery routes of FMT administration. Risks associated with treatment via colonoscopy include perforation, bleeding, and bloating. The nasoduodenal route and nasojejunal route have both been linked with increased risk of aspiration. Administration via oral capsules is associated with less severe side effects such as nausea and vomiting. The risks of all delivery routes should be carefully analysed before a delivery route of FMT administration is chosen.

Based on the comparison of different FMT delivery routes outlined in Table 1.1, it is evident that oral capsules are the least invasive and the most cost-efficient delivery route with low-risk association. Oral capsules have high administration convenience by allowing for patient self-administration. To date, colonoscopy is the most common FMT

delivery route used. This is mainly due to the level of available observational data and completed placebo-controlled trials that supports its efficacy in the treatment of rCDI. The second most common delivery route is by rectal enema, followed by the nasoduodenal tube route. It must be noted that due to the many advantages and disadvantages associated with each of these delivery routes, there is no one-fits-all delivery route, and the chosen delivery route must be determined on a case-by-case basis.

Table 1.1: Comparison among different routes of administration of FMT (*Adapted from (Gulati *et al.*, 2020)).

Technique	Degree of Invasiveness	Convenience of Administration	Risks Involved	Delivery Site	Cost
Colonoscopy	+++	Low	Perforation bleeding, bloating	Colon	+++
Enema	+	Average	N/A	Colon	+
Nasoduodenal Route	++	Low	Aspiration	Colon and distal part of ileum	+++
Nasojejunal Route	++	Low	Aspiration	Colon and distal part of ileum	+++
Oral Capsules	-	High	Nausea, Vomiting	Colon and distal part of ileum	+

Esophageogastro-duodenoscopy (EGD)	++	Low	Perforation bleeding	Colon and distal part of ileum	+++
Transendoscopic Enteral Tubing (TET)	++	Low	N/A	Colon and distal part of ileum	+++
Percutaneous Endoscopic Cecostomy (PEC)	+++	Low	Catheter dislodgement, peritonitis	Colon and distal part of ileum	++

1.4 Current usage rates of Faecal Microbiota Transplantation worldwide

In 2020, Baunwall *et al.* conducted a Europe-wide, cross-sectional survey which reported that 1874 FMT procedures were performed in European hospitals in 2019 with the majority of these procedures being completed for the treatment of rCDI. Table 1.2 shows how this survey found that 1077 (58%) of these procedures were carried out to treat patients with rCDI, 791 (42%) were carried out for non-rCDI patients and 6% (0.03%) of the procedures were carried out for unknown reasons. The current European clinical usage rate of FMT as a treatment method for rCDI patients is only at approximately 10%. As clearly outlined in Table 1.2, The leading country for FMT clinical use in Europe is the United Kingdom (UK) with a total of 690 procedures. 411 of these procedures were for non-rCDI procedures and 279 were for CDI procedures. In contrast, the country with the second highest level of FMT procedures was Denmark at 305 procedures. Only 11 of

these procedures were for non-rCDI treatments while it was reported that the rest of the procedures were as a rCDI treatment method (Baunwall *et al.*, 2021).

Table 1.2: The clinical use of FMT in Europe in 2019 according to country, indication, and population size (Taken from (Baunwall *et al.*, 2021)).

Country	Centres no.	Total FMT procedures	Indication CDI no.	FMT for CDI per 100·000*	Indication Non-CDI no.	FMT for non-CDI per 100·000*
United Kingdom	3	690	279	0·417	411	0·615
Denmark	5	305	294	5·053	11	0·189
Italy	1	150	120	0·199	30	0·050
Sweden	2	96	66	0·642	30	0·292
Finland	1	90	60	1·087	30	0·543
France	4	88	68	0·101	14	0·021
Germany	3	86	39	0·047	47	0·057
Czech Republic	1	83	3	0·028	80	0·750
Netherlands	1	82	42	0·242	40	0·231
Norway	2	61	31	0·580	30	0·561
Austria	1	60	8	0·090	52	0·586
Belgium	2	27	15	0·131	12	0·104
Switzerland	1	20	16	0·187	4	0·047
Lithuania	1	18	18	0·646	0	0·000
Iceland	1	8	8	2·214	0	0·000
Bulgaria	1	5	5	0·072	0	0·000
Spain	1	5	5	0·011	0	0·000
Total	**31**	**1874**	**1077**	**0·257**	**791**	**0·189**

* Per 100·000 population
Abbreviations: CDI: *Clostridioides difficile* infection, no: Number.

In terms of worldwide usage rates of FMT for disease treatment, as summarised in Table 1.3, the leading country involved in this area of research worldwide is the United States of America (USA) with a count of 914, follow by China with a count of 682. China was followed by Canada with a majorly reduced count of 146. France was ranked as 4th highest FMT research country globally but 1st in Europe. In relation to centrality, the top leading countries were identified to be France (0.20), England (0.17), USA (0.16), Spain (0.15), and Germany (0.10). This level of centrality indicates that these are the top 5 most influential and cooperative countries involved in FMT research (Fengwei Zhang *et al.*, 2022).

Table 1.3: Leading top 10 countries involved in FMT research Worldwide (*Adapted from (Fengwei Zhang *et al.*, 2022)).

Rank	Count	Centrality	Country
1	914	0.16	USA
2	682	0	China
3	146	0.05	Canada
4	146	0.2	France
5	130	0.1	Germany
6	117	0.17	England
7	110	0.05	Italy
8	103	0.04	Netherlands
9	97	0.08	Australia
10	87	0.02	Japan

One of the main reasons that there is no agreed definition for FMT is because regulatory agencies across the world vary in opinion when it comes to its classification. Most regulatory agencies classify FMT into one of four categories. These four categories consist of a biological drug that is highly regulated with restricted use, a human cell and tissue-based product (HTC/P) which is a more process-focused regulation, a medicinal product which claims oversight and variable access and finally a practice of medicine which results in devolved oversight with unpredictable access (Scheeler *et al.*, 2019).

Table 1.4 summarises this worldwide variance in FMT regulatory classification. The Food and Drug Administration (FDA) in USA classifies it as an unapproved biological drug. In Europe, many countries vary in their classification of this process. In Belgium and Italy, it is classified as an HTC/P. In Denmark, UK, and Norway, it is classified as an unlicensed medicinal product (Scheeler *et al.*, 2019). In Oceania, countries such as Australia considered it an unapproved technique until July 2021 when Australia's

government authority for analysing and monitoring therapeutic goods, Therapeutic Goods Administration (TGA), released new regulatory requirements which outlined that most FMT products are now regulated as biologicals. These TGA regulations also highlighted how it may be regulated as a medicine rather than as a biological drug when a microbiological strain present in stool is grown from established isolates with standardised consistency. To use a FMT product in Australia, it must first be included in the Australian Register of Therapeutic Goods (ARTG) and all associated adverse effects must be reported to TGA (Therapeutic Goods Administration, 2022). Australia is known for having the world's highest-throughput private stool banks (Scheeler *et al.*, 2019). Based on this knowledge, it is evident that until a clear global regulation surrounding FMT has been determined, there will be continued major restrictions in its use as a treatment method, especially in European hospitals (Cammarota *et al.*, 2017).

In terms of the worldwide approach to stool banks, many countries including USA, Italy, the Netherlands, UK, Australia, and Israel have all confirmed their use of stool banks for FMT use (Scheeler *et al.*, 2019). There is however currently no international standardization for these stool banks. Increased global access to stool banks are necessary to help standardize the process and ensure its improved quality, safety, and ease of access (Chen *et al*., 2021).

In the UK, use of FMT is regulated by the Medicines and Healthcare Products Regulatory Agency (MHRA). As can be seen in Table 1.4, in the UK, it is categorised as an unlicensed medicinal product with clinical trials being required for its use. According to the MHRA, if choosing this route to treat patients suffering from rCDI, clinicians must obtain FMT on prescription for the patient as an unlicensed medicinal product. In this case, it can be prepared by a pharmacy. This is known as the magistral option. The officinal formula is used when the prepared sample is for the direct use of the pharmacy's patients or for use in an approved clinical trial. In the UK, where FMT is formulated to match the specifications of a physician for use by a patient under their direct care, this is classified as FMT under the ''Specials'' framework. In the UK, stool banks are available in both hospital and private settings (Scheeler *et al.*, 2019). Currently in the UK, FMT is only recommended for the treatment of rCDI. It is however under investigation as part of clinical trials to determine if it is suitable as a therapeutic treatment method for other non-rCDI gut dysbiosis related diseases such as complications of liver disease and

inflammatory bowel disease (IBD). The Microbiome Treatment Centre (MTC) was set up in 2017 and is the first MHRA licensed facility in the UK to provide this treatment method for clinical trials and for its use as a treatment method for rCDI (University of Birmingham, 2023).

Table 1.4: FMT regulatory classification summary (*Adapted from (Scheeler *et al.*, 2019)).

Country	FMT Regulatory Classification	Known Stool Banks
USA	Investigational biologic drug: clinical trial required for use	Yes, non-profit and hospital
Canada	Investigational biologic drug: clinical trial required for use	No
Austria	None; considered neither a drug, tissue, nor organ	No
Belgium	Human cell or tissue product; clinical trial required	No
Denmark	Unlicensed medicinal product	No
Estonia	None	No
Finland	Unlicensed drug product	No
France	Experimental drug: clinical trial required for use	No
Germany	No federal-level guidance; case-by-case decision by state	No
Ireland	None, considered practice of medicine	No
Italy	Human cell or tissue product; clinical trial required	Yes, under development with government support
Malta	None	No
The Netherlands	Unclassified treatment	Yes, non-profit
Norway	Unlicensed medicinal product	No

Portugal	None	No
Slovenia	None	No
Spain	None; considered neither a pharmaceutical product nor a tissue	No
Switzerland	Investigational medicinal product	No
United Kingdom	Unlicensed medicinal product: clinical trial required for use	Yes, hospital and private
Australia	Biologic drug	Yes, hospital, non-profit and private
Israel	Unapproved medical treatment: clinical trials required	Yes, hospital
Singapore	Under review	No

1.4.1 Current usage rates of Faecal Microbiota Transplantation in Ireland

In stark contrast to the increasing level of worldwide research and practise of FMT as a disease treatment method, there has been little acceptance of this process in Irish hospitals with a clear absence of any national protocol. As suggested by Scheeler *et al.* in 2019, This lack of protocol, along with a shortage of frozen pre-screened stool and challenges with donor selection, may be the reasoning behind this lack of Irish acceptance of this process. The Health Products Regulatory Authority (HPRA) is an Irish regulatory agency that advises the Irish government on public health. The HPRA does not regulate FMT use in Ireland. Irish clinicians have expressed a desire to use this process as a disease treatment method, especially for rCDI patients but until issues such as an adequate supply of suitable donor stool samples are available, this will be a huge challenge. FMT is currently considered as a practise of medicine in Ireland (Scheeler *et al.*, 2019).

To help tackle the lack of a coordinated approach to this treatment method in Irish hospitals, in 2019 a project called Stool Bank Ireland was founded. Stool Bank Ireland was funded by the Sláintecare Integration Fund. The Stool Bank Ireland team consisted of researchers and clinicians from the Royal College of Surgeons in Ireland (RSCI), Cork

University Hospital, Beaumont Hospital, and APC Microbiome Ireland. The aim of this project was to establish a national registry of all Irish FMT recipients and to investigate the feasibility of safely using it in Irish hospitals to treat rCDI. Unfortunately, the development of this project coincided with the onset of the coronavirus disease 2019 (COVID-19) pandemic. The COVID-19 pandemic resulted in a major set-back in the development of this project as FMT research and other non-COVID areas of the health service where no longer a top priority. The potential risk of transmitting the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from the FMT donor to the receiver also had a major impact on the development of the Stool Bank Ireland project. SARS-CoV-2 is a strain of the coronavirus that can result in serious health complications. Unfortunately, the project was closed out in 2021 due to a lack of substantial funding, donor samples importing issues, donor samples supply issues and EU data protection compliance issues. To date, there is currently no stool bank available in Ireland. (Department of Health, 2022).

1.5 Controversies associated with Faecal Microbiota Transplantation

The use of FMT as a disease treatment method is a very controversial topic, mainly due to the uncertainty of its efficacy. There is also a major taboo associated with it due to the fact that the bacteria used in the process originated from a stool sample of another person. This taboo has resulted in a lack of patient acceptance of the treatment method. Patients' disgust regarding the origin of the bacteria used in the process has had a knock-on effect on the speed of its research and development due to the lack of willing participants that are necessary for clinical trials. Therefore, it is clear that a key step in the progression of the FMT process is the acceptance of receiving patients. A positive social outlook of this treatment method will have a major impact on its future role in Western medicine (McLeod, Nerlich and Jaspal, 2019). As clearly highlighted by Zhang *et al*. in 2019, there are also many ethical issues associated with the use of FMT as a disease treatment technique. Ethical issues such as the informed consent and potential exploitation of vulnerable patients receiving the treatment method must be carefully considered. Another ethical issue that must be analysed is the associated public health implications.

The FDA is a federal agency of the Department of Health and Human Services in the USA. Due to the extensive FMT related research that has been conducted in the USA

over the past few years, the FDA have issued several warnings regarding its use as a disease treatment method. These warnings include both the potential risk of infections caused by Shiga toxin-producing *Escherichia coli* (STEC) and Enteropathogenic *Escherichia coli* (EPEC) and the potential risk of transmitting SARS-CoV-2 from the FMT donor to the receiver (FDA, 2020a; FDA, 2020b). As previously highlighted, the outbreak of COVID-19 resulted in a worldwide pandemic that has had a major effect on this area of research and development. The COVID-19 pandemic resulted in a major concern regarding the risk of transmitting SARS-CoV-2 during this process. As a result of this risk of the receiver's health, the FDA recommended that only stool samples donated before December 2019 could be used for any FMT procedures unless proper SARS-CoV-2 testing of both the donors and stool was completed. This concern even led to the suspension of new donations in some FMT centres (Ianiro *et al.*, 2020). This suspension of donations not only reduced the availability of donor stool samples, but it also reduced patient's trust in the process for the treatment of gut dysbiosis related diseases.

1.6 Chapter Conclusion

Chapter 1 provides a brief overview of the background of GM and FMT. This chapter highlights the important health benefits associated with FMT, its success rate as a treatment method for rCDI, and outlines the reasons why perusing its use as a treatment method for other gut dysbiosis related diseases is crucial for the future of Western medicine. This chapter also highlights how usage rates of this disease treatment method vary dramatically from country to country. Whilst there are still many controversies associated with FMT that must be first overcome before it is accepted as a disease treatment method globally, the backing and support via a clear global regulation on its usage and the development of globally accessible stool banks would help patients gain trust in the process and increase global usage rates of the disease treatment method.

Chapter 2: Crohn’s Disease and Colitis

2.1 Chapter Overview

The following chapter will outline how patients suffering from CD and Colitis may benefit from using FMT as a treatment method in the future. This chapter will discuss the health risks associated with both CD and Colitis along with the current treatment method used for these gut dysbiosis related diseases. This chapter aims to educate the reader on the links between the diet, the GM composition and the treatment of CD and Colitis. This chapter will also discuss the FMT donor selection process and the role it plays in the success of FMT. This chapter will conclude with a clarification of the concept behind this thesis and outline the research question.

2.2 Health risks associated with Crohn's Disease and Colitis

CD and Colitis are the two main types of Inflammatory Bowel Disease (IBD) with both diseases resulting in the chronic inflammation of the GI tract. As can be seen in Figure 2.1, Colitis is responsible for the inflammation of the large intestine while CD can be responsible for the inflammation of either the small intestine or the large intestine and can occur anywhere in the GI tract.

Many factors affect the pathogenesis of CD including diet, environmental factors, genetics, dysregulation of the immune system, and the GM composition (Núñez-Sánchez *et al.*, 2022). All these factors can cause intestinal inflammation in the GI tract. In 2022, Štofilová *et al.* highlighted that while all these factors are associated with the onset of Colitis**,** the exact pathogenesis of this gut dysbiosis related disease still remains unclear. The most common type of Colitis is Ulcerative Colitis (UC). Common symptoms of CD and Colitis include diarrhoea, constipation, fatigue, dehydration, abdominal pain, bloating and wind, bowel incontinence, rectal bleeding, weight loss, and joint and bone pain. Approximately 0.2% of the European population reportedly suffers from IBD (Štofilová *et al.*, 2022) with an estimated 0.003% of the European, North American, and Oceanic population being diagnosed with CD (Núñez-Sánchez *et al.*, 2022).

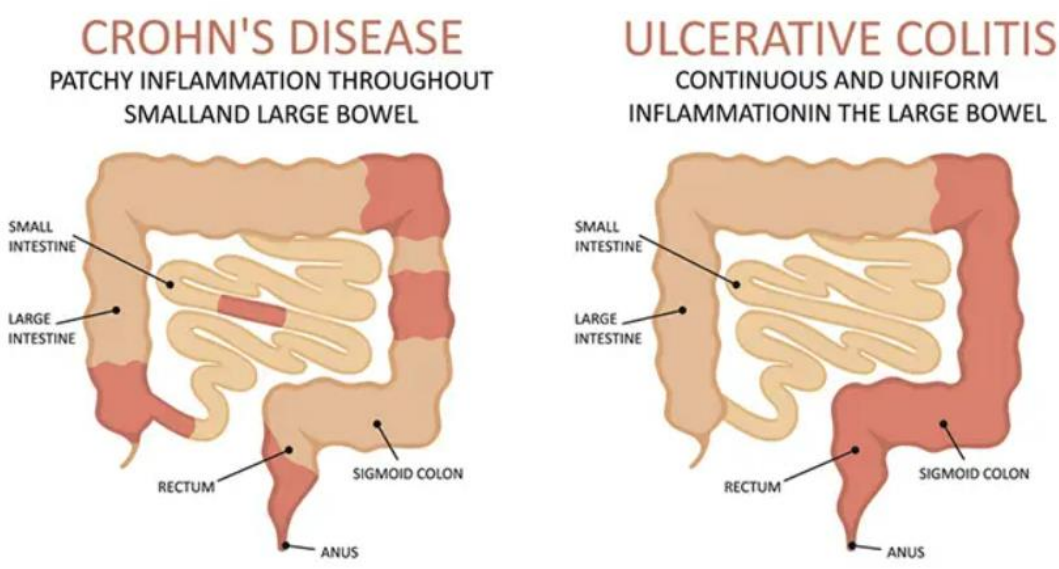


Figure 2.1: Distinctions between CD and UC (Taken from (Thourani, 2022))

2.3 Current treatment methods for Crohn's Disease and Colitis

To date, there is currently no cure for either CD or Colitis. There are however many different treatment methods available to help alleviate associated side-effects and symptoms of these relapsing diseases. Currently, the most common treatment methods used for these gut dysbiosis related diseases include corticosteroids, exclusive enteral nutrition (EEN), immunosuppressants, biological medicines and in severe cases, the patient may require surgery.

This paragraph outlines a brief description of the most common current treatment methods used for CD and Colitis. Suffering patients take an anti-inflammatory medicine called corticosteroids. Corticosteroids are commonly known as steroids. Prednisolone is an example of a corticosteroid which is taken to help reduce inflammation in the digestive system. EEN, also known as tube feeding, is another treatment method that can be used to reduce the symptoms of these diseases. EEN is a simple process that consists of replacing all the patient's oral intake of food and drinks with a specialised liquid nutrient filled formula and water (Bechtold *et al.*, 2022). Treatment via EEN is the recommended first-line induction therapy for paediatric patients suffering from either disease. EEN has shown to have the same remission benefits as corticosteroid treatments while also reducing the risk of complications with growth, bone and muscle development, mucosal

healing, and helping to reduce the risk of relapse in paediatric patients (Núñez-Sánchez *et al.*, 2022). Immunosuppressants are drugs that suppress the damage of healthy tissues and cells, caused by the immune system. Immunosuppressants such as mercaptopurine, methotrexate and azathioprine can be prescribed to patients to reduce the activity of the immune system. Biological medicines, also know and Biologics, are considered as a treatment method for Colitis and CD patients when other treatment methods have not worked. Biologics can be defined as medicines that are derived from a biological source such as living organisms or living cells. Biologics are usually composed of either solely one nutrient or a complex mixture of nucleic acids, carbohydrates, proteins, cells or tissues for transplantation (Favour Danladi Makurvet, 2021). Biologics used to help treat CD and Colitis patients include vedolizumab, infliximab, adalimumab, vedolizumab, and ustekinumab.

Traditionally, corticosteroids were the most frequently used treatment for CD and Colitis but based on research completed over the last two decades, there has been increased use of anti-inflammatory therapies, especially in patients that were unresponsive to other treatment methods (Núñez-Sánchez *et al.*, 2022). In 2021, Zhao *et al.* highlighted the European economic burden associated with the current treatment methods used for both diseases. Direct European health care costs were calculated to be on average €2,088 per UC patient per year and €3,542 per CD patient per year. Based on the high morbidity rates and expensive health care costs associated with the currently available treatment methods, the development of alternative safe and cost-effective treatment methods such as FMT have now become a priority (Núñez-Sánchez *et al.*, 2022).

2.4 Links between diet and the treatment of Crohn's Disease and Colitis

There has been much research carried out over the past few years with the aim to identify a link between the diet and the treatment of gut dysbiosis related diseases. Analysis of diet-based treatment methods can be dated as far back as the 1970s (Núñez-Sánchez *et al.*, 2022). In 2020, Khorshidi *et al.* completed a meta-analysis in order to analyse the possibility of a link between the diet and increased risk of developing CD and UC. This meta-analysis defined a healthy diet as a one high in fruit, vegetables, legumes, low-fat dairy products, fish, poultry, wholegrain foods, nuts, and dietary fibre. This meta-analysis

concluded that a healthy diet was linked with a reduced risk of CD and UC. It was also suggested as part of this meta-analysis that this beneficial link may be due to the antioxidant and anti-inflammatory properties associated with consuming a healthy diet. On the other hand, to date, the exact relationship between the increased risk of gut dysbiosis diseases and specific diets still remains unclear (Khorshidi *et al.,* 2020).

It is recommended that the use of EEN as a CD and Colitis treatment method, in paediatric patients especially, should coincide with the exclusion of sugars, fats, and food additives from the diet to reduce the patient's intake of pro-inflammatory foods. Other anti-inflammatory diets include the Mediterranean diet, the Specific Carbohydrate Diet (SCD), which involved the exclusion of complex carbohydrates from the diet, and the low-FODMAP diet involving the exclusion of fermentable oligosaccharides, disaccharides, monosaccharides, and polyols. However, it must be noted that most of these anti-inflammatory diets have reported contradictory results when used for the treatment of CD (Núñez-Sánchez et al., 2022). As a result of these contradictory results, the CD exclusion diet (CDED) was developed. This diet is low in animal protein and fat while high in dietary fibre and carbohydrates. This CDED diet combined with partial enteral nutrition (PEN) has reported positive results and could be considered in the future as an alternative treatment to EEN for paediatric patients. According to a study carried out by Levine *et al.* in 2019, CDED combined with PEN results in sustained remission, more tolerance, increased long-term modifications in microbiome profiles and reduced inflammation in children suffering from mild to moderate CD symptoms, compared to using the EEN treatment method. This diet method of treatment also resulted in positive changes in the faecal microbiome of treated patients.

In 2020, Khalili *et al*. reported that results obtained from two large Swedish prospective cohort studies indicated a positive link between following a Mediterranean diet and reduced risk of later-onset CD. These large cohorts consisted of 83,147 participants with an age range of 45 to 79 years old. Khalili *et al*. highlighted as part of this study that while these results indicated a strong link between following a Mediterranean diet and the reduced risk of developing CD, this was not the case for the development of UC.

In 2023, Gubatan *et al*. published a review of the dietary exposures and interventions that are used to treat IBD conditions such as CD and Colitis. The results from this review indicated that ultra-processed foods (UPFs), emulsifiers, and food additives are all linked

with the increased risk of developing gut dysbiosis related diseases. In contrast, Gubatan *et al*. reported that exclusion and elimination diets are linked with reducing associated symptoms in suffering patients. Specific diets such as the Mediterranean diet, high fibre diets, SCD and anti-inflammatory diets have been positively linked with reduced associated symptoms and improved inflammatory biomarkers (Gubatan *et al.*, 2023).

Many studies have been carried out which have analysed the link between the consumption of processed foods and the risk of developing CD and Colitis. In 2021, Narula *et al.* completed a prospective cohort study to analyse the link between the consumption of UPFs and the risk of developing IBD. This study involved 116,087 participants with an age range of 35 to 70 years old. These participants were followed prospectively at least every 3 years. Table 2.1 shows the results obtained from this study that highlight the link between increased consumption of UPFs and the increased risk of developing IBD conditions such as CD and UC. This study also reported that unprocessed red meat, white meat, dairy, fruit, vegetables, legumes, and starch showed no link with the increased risk of developing CD and UC. Due to no link being found between eating these foods and the development of these gut dysbiosis related diseases, Narula *et al*. concluded that the risk of developing these diseases may depend more on the way that the food is processed, especially if ultra-processed, rather than the actual food itself (Narula *et al.*, 2021).

In 2022, Meyer A *et al.* completed a European prospective cohort study involving 413,590 participants with the aim of determining if there is a link between the degree of food processing and the risk of developing CD or UC. This study involved a mean follow-up period of 13.2 years. The results from this study indicated that the risk of developing CD was lower in those with diets low in processed foods. This study reported no clear link between the consumption of processed foods and the risk of developing UC (Meyer A *et al.*, 2022). Also in 2022, a cross-sectional and prospective analysis of 187,154 participants in the UK Biobank was completed in order to analyse the effect that UPFs have on the risk of developing CD or UC. This study has a mean follow-up of 9.84 years and the results obtained from this study comparably indicated that there is a link between a high consumption of UPFs and increased risk of developing CD. However, this study did not find a link between consuming high levels of UPFs and the risk of developing UC (Chen J *et al.*, 2022). Similarly, another prospective cohort study was carried out in 2022

in the USA by Lo *et al*. to identify a link between UPFs and the risk of developing CD or UC. This study consisted of 245,112 participants with an age range of 29 to 85 years old. This study reported similar results to the previously mentioned studies with high consumption levels of UPFs being linked with the increased risk of developing CD but not UC (Lo *et al.*, 2022).

Table 2.1: Association between total ultra-processed food intake and risk of inflammatory bowel disease (*Adapted from ((Narula *et al.*, 2021)).

	Ultra-processed food intake		
	<1 serving/day	**1-4 servings/day**	**≥5 servings/day**
Inflammatory Bowel Disease			
Number of participants	76 415	25 453	11 742
Number (%) of events	199 (0.26)	134 (0.53)	95 (0.81)
Crohn's Disease			
Number of participants	76 415	25 453	11 742
Number (%) of events	34 (0.04)	23 (0.09)	30 (0.26)
Ulcerative Colitis			
Number of participants	76 415	25 453	11 742
Number (%) of events	165 (0.22)	111 (0.44)	65 (0.55)

As can be seen in Figure 2.2, additives high in salt and artificial sweeteners can cause harmful inflammation in the intestines, resulting in the onset of colitis. Excess salt levels can lead to increased intestinal permeability. Artificial sweeteners found in UPFs can also lead to intestinal inflammation and increased risk in the development of Colitis. Azo dyes such as yellow 6 and red 40 are synthetic food colourings which are often used in the food industry. These food dyes can be metabolized by commensal bacteria such as

Enterococcus faecalis and *Bacteroides ovatus* to produce a metabolite known as 1-amino-2-naphthol-6-sulphonate sodium salt (ANSA-Na). This metabolite has also been linked with the increased risk of developing Colitis, again indicating the harmful effects that food additives have on gut health. Also highlighted in Figure 2.2, are the harmful effects caused by emulsifiers in gut health. Consumption of common emulsifiers such as carboxymethyl cellulose (CMC) and polysorbate 80 (P80) can lead to reduced levels of beneficial *Bifidobacterium, Clostridium leptum* and important SCFA producers *Subdoligranulum* and *Faecalibacterium*. Consumption of these emulsifiers can also lead to intestinal inflammation (Gubatan *et al.*, 2023).

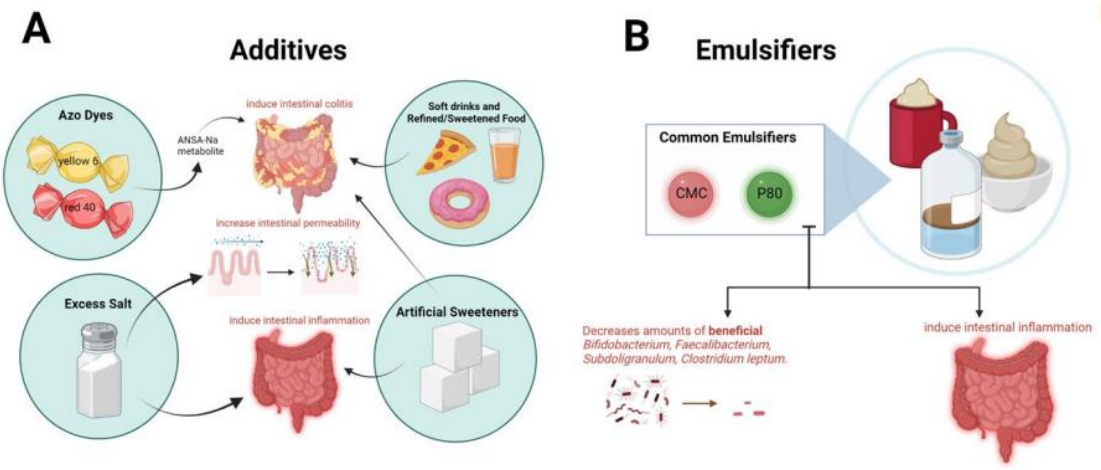


Figure 2.2: The harmful effects caused by additives (A) and emulsifiers (B) in gut health (*Taken from (Gubatan *et al.*, 2023))

While in recent years, specific diets such as the Mediterranean diet, the SCD, the low-FODMAP diet and CDED are starting to become more recognised as potential treatment methods for CD and Colitis, data on the long-term health benefits of these diets is still scarce. Furthering this knowledge is crucial for the development of nutritional intervention as a disease treatment method. More knowledge regarding the key molecular and cellular players involved in this process and their specific roles in the beneficial alteration of the GM composition is needed for the progression of this treatment method (Núñez-Sánchez et al., 2022). It must also be noted that while diet alone may not be

enough for disease prevention or treatment, it may play a crucial role in increasing the success rate of other treatment methods such as FMT (Gubatan *et al.*, 2023).

2.4.1 Links between diet and the Gut Microbiome composition

In 2022, Núñez-Sánchez *et al.* reported that following the Mediterranean diet or the SCD as a treatment method for gut dysbiosis related diseases has reportedly resulted in increased microbial diversity in the GM of patients with a reduced levels of *Bacillaceae* and Proteobacteria present, and slight increases in the presence of Bacteroidetes and *Clostridium* cluster IV and XIVa. Following the low-FODMAP diet reportedly results in reduced levels of GM Firmicutes such as *Clostridium* cluster XIVa and *Faecalibacterium prausnitzii*. Similar to the EEN method, post 6 weeks of following treatment, the use of the CDED diet combined with PEN has shown to reduce the level of Actinobacteria and Proteobacteria present in the GM while increasing the level of commensal Clostridia. However, unlike the EEN method which results in the GM composition reverting to its original microbial levels, post 12 weeks of treatment, the CDED diet combined with PEN indicated that the new healthy GM composition was still present in receiving patients with improved functionality being maintained (Núñez-Sánchez et al., 2022).

In 2021, Bolte *et al.* identified a correlation between dietary patterns and the presence of specific healthy bacteria in the GM. Bolte *et al.* linked consuming specific foods to the presence of certain bacterial spp. with pro-inflammatory or anti-inflammatory roles (Table 2.2). As per these results, Bolte *et al.* highlighted how the consumption of certain foods such as plant-based foods and fish can result in increased levels of certain bacterial spp. in the GM, thereby resulting in the positive production of SCFAs, reduced levels of pathobionts and increased microbial metabolism of polysaccharides. These bacterial spp. include *Lactococcus lactis, Bifidobacterium* spp., *Eubacterium* spp., *Lactobacillus delbrueckii, Roseburia* spp., and *Faecalibacterium prausnitzii*. In contrast, the increased consumption of foods such as animal protein, cheese, processed foods, and high-sugar foods have been linked with the increased presence of undesirable bacterial spp. in the GM. These bacterial spp. can result in increased levels of inflammation in suffering CD and Colitis patients. Examples of such bacterial spp. include phylum Firmicutes, clusters of *Lachnospiraceae* bacteria, *Ruminococcus gnavus, Coprobacillus, Bacteroides fragilis, Clostridium bolteae, Escherichia coli (E. coli), Erysipelotrichaceae, Blautia* spp., and

Streptococcus spp. These results clearly indicate the key role that the diet plays in the alteration of the GM composition. It also indicates the possibility of how nutritional interventions can be used as a treatment method of CD and Colitis.

Table 2.2: Overview of links between the diet and the GM composition (*Taken from (Bolte *et al.*, 2021)).

Taxa	Diet (↑)	Diet (↓)	Pro-inflammatory or anti-inflammatory role
Bifidobacterium spp	Plant protein, carbohydrates, bread, fruit	Protein, animal protein, fat, fish, savoury snacks, red wine, butter	SCFA synthesis (acetate); linked to dense mucosal barrier, reduced LPS levels and raised efficacy of cancer immunotherapy; depleted in IBD, IBS, obesity
Lactococcus lactis, Lactobacillus delbrueckii	Buttermilk, cluster of fermented dairy	No negative associations	SCFA and thiamine synthesis, anti-cancer activities
Eubacterium spp	Plant protein, cereals, fruit, red wine	Carbohydrates, non-alcoholic drinks, soft drinks	SCFA (butyrate) and phenolic acid synthesis; depleted in IBD
Roseburia spp	Fish, nuts, vegetables, plant protein, cereals, tea, legumes, vegetables, fruit	Total kcal, sugar, savoury snacks, meat, gravy, sweetened milk drinks	SCFA synthesis (butyrate) and anti-inflammatory effects; depleted in IBD
Faecalibacterium prausnitzii	Red wine, legumes, fruit, lean beef, fish, nuts, fat	Carbohydrates soft drinks, sweets, syrup	SCFA synthesis (butyrate) and anti-inflammatory effects; depleted in IBD
(phylum) Firmicutes and clusters of *Ruminococcus gnavus, Lachnospiraceae bacteria, Clostridium boltea, Coprobacillus*	Protein, animal protein, fat intake, cheese cluster of fast food and soft drinks	Plant protein, carbohydrates, bread	Enriched in obesity, increased energy harvesting capacity
Bacteroides fragilis	Cheese, custard	Cluster of breads and legumes	Opportunistic pathogen with increased abundance in IBD and colorectal cancer, raised LPS levels
Escherichia coli	No positive associations in the meta-analysis	Cluster of breads and legumes	Increased abundance in IBD and colorectal cancer, raised LPS levels
(family) Erysipelotrichaceae	Animal protein, soft drinks, syrup	Plant protein	Pro-inflammatory; associated with colorectal cancer, hypercholesterolaemia, and obesity.
Streptococcus spp	Protein, animal protein, fat, cheese, yoghurt drink, custard	Plant protein, nuts	Increased in IBD, alcoholism, liver cirrhosis, primary sclerosing cholangitis, colon cancer and IMIDs such as MS, ankylosing spondylitis and arthritis
Blautia spp	Animal protein, alcohol, meat, cheese, soft drinks, fast food pattern (*R. gnavus* cluster)	Plant protein, carbohydrates, fruit, bread	Increased in IBD, MS, ankylosing spondylitis and arthritis

* Diet (↑): positive relationship; Diet (↓): negative relationship; IMIDs: immune-mediated inflammatory diseases; kcal: caloric intake; LPS: lipopolysaccharides; MS: multiple sclerosis.

The GM is one of the main factors involved in the pathogenesis of gut dysbiosis related disease such as Colitis. Patients suffering from Colitis have been found to have a GM composition with high inter-individual variability and instability while also having decreased diversity. Colitis patients have an increased level of Proteobacteria and a reduced level of Actinobacteria and Firmicutes in their GM. This knowledge has led to the common use of prebiotics and probiotics intake as a treatment method for the

necessary alteration of a patient's GM compositions with the aim to improve its functionality. *E. coli* Nissle 1917, *Bifidobacterium* spp., *Saccharomyces boulardii*, and *Lactobacillus* spp. are all probiotic strains that can have beneficial health effects on the GM. In contrast, the use of prebiotics and probiotics as a treatment method for CD is not as successful as it has many contradicting results. Due to the uncertainty of results obtained from preclinical and clinical studies surrounding the benefits of probiotics for CD patients, the European Society for Clinical Nutrition and Metabolism (ESPEN) do not currently recommend the use of probiotics for the treatment of CD. While some studies indicate that prebiotics may be more beneficial than probiotics for the treatment of CD, to date, no study has yet verified their actual efficacy as a treatment method for CD patients (Núñez-Sánchez et al., 2022).

2.4.2 Links between the diet and the success rate of Faecal Microbiota Transplantation

In 2020, Sokol *et al.* completed a pilot randomized controlled study which analysed the role that FMT can play in maintaining CD remission in receiving patients. This small scale, randomized, single-blind, sham-controlled FMT pilot trial concluded that there is a link between prolonged remission in receiving FMT patients and a high level of colonisation by the donor microbiota (Sokol *et al.*, 2020). Similarly in 2021, Rinott *et al.* published the results of a 14-month human trial which analysed the diet of 90 patients who received autologous FMT for the treatment of weight regain after a weight-loss phase. The results from this trial concluded that patients who followed a green-Mediterranean diet, enriched with green-tea and Wolffia globosa (Mankai) green plant, combined with autologous FMT, reported a significant change in their GM composition during the weight loss phase and reduced weight regain afterwards. The green-Mediterranean diet consists of less red and processed meat and more plants and polyphenols compared to the standard Mediterranean diet. Regarding the alteration of the GM composition post treatment, Rinott *et al.* observed that patients who followed this specific diet combined with FMT treatment showed increased levels of spp. *Bacteroides uniformis, Bacteroides vulgatus,* and *Alistipes putredinis* which are all associated with the GM composition of a lean person. This result again further emphasised the link between the diet and the success rate of FMT *(*Rinott *et al.*, 2021).

In 2021, an international online survey was conducted to assess health professionals' and researchers' knowledge, current practice recommendations and overall attitudes regarding what diet patients undergoing FMT should follow. This survey covered topics such as current practice, demographics, beliefs and anticipated future directions regarding FMT and the diet. Results from this survey showed that participants did agree that diet was an important factor for both donors and receivers of FMT. As part of this survey, participants also confirmed that while they did believe following a healthy diet was linked with the success rate of the process, they were hesitant to recommend a specific diet to their patients based on the scarcity of data available on the associated long-term health benefits (Clancy, Gunaratne and Borody, 2021).

In 2021, a study was carried out in Australia to analyse the dietary intake of FMT recipients. This observational, pilot study was based on 18 participants with a median age of 35 years old. The results obtained from this study highlighted a high degree of variability in patients' fibre intake and noted that patients undertaking FMT do not always meet the recommended daily intake requirements of fruit, vegetables, fibre, grains, milk, or calcium. Due to fibre being a known GM modulator, this study highlighted how dietary fibre should be considered as a key variable that should be controlled for any patients undergoing the FMT treatment method (Clancy *et al.*, 2021).

2.5 Donor selection for successful Faecal Microbiota Transplantation

Careful donor selection is crucial for the success of FMT. As mentioned previously, the importance of using the right donor has led to the increased need for globally accessible universal donor stool banks to increase accessibility and to help ensure the safety and standardisation the FMT process (Chen et al., 2021). Historically, a family member or spouse were considered as ideal FMT donors due to having shared environmental factors but recent clinical evidence has indicated that using unrelated FMT donors may be more beneficial to the receiver, especially for the treatment of genetic related diseases (Wang *et al.*, 2019). Donors must be carefully screened and meet strict criteria to ensure their faecal donations do not contain any pathogens that could harm the receiver. This step in the FMT donation process became especially evident following the first report of a FMT-associated death that occurred when an asymptomatic carrier donor passed on a multidrug-resistant organism to the receiver (Murphy CL, Zulquernain SA, and Shanahan

F, 2019). Increased long-term success rates of FMT has been linked with specific donor selection (Odakura *et al.*, 2022).

As part of the donor selection process, donors must first meet strict criteria before being considered acceptable donors. Such criteria include ensuring that the donor has a good communicable disease-free health status as this could have a detrimental effect on the receiving patient's health. The donor must ensure that 6 months prior to their donation, they have not been involved in any high-risk sexual behaviours, received any tattoos or piercings, been incarcerated or have been associated with any intravenous drug abuse. Donors must also confirm that 6 months prior to their donation, they had not travelled to any endemic diarrhoea areas as this could also result in negative health effects in the receiver. To date, the donor's diet has not been reviewed as part of the donor selection process but based on the clear link between diet and the success rate of FMT, this may become a necessary part of the acceptance criteria in the future (Clancy, Gunaratne and Borody, 2021).

As highlighted by Zhang *et al.* in 2022, when using the FMT process in clinical trials, donor selection usually consists of using one single healthy donor for all receiving patients or by using the random selection of multiple pre-screened and approved donors. In future clinical trials, improved donor specific selection can help reduce the risk of false-negative trials and can also help with the development of using the FMT process as a novel treatment method for diseases such as CD and Colitis (Zhang *et al.*, 2022).

As outlined in Figure 2.3, the donor screening process for FMT can be quite extensive. Donors must first attend an on-site evaluation where they give informed consent for their donation to be used. They must also provide true and accurate information regarding their current and historical health status as part of a donor health questionnaire. Donors must then pass an in-person clinical interview and their stool sample must undergo laboratory screening. Once the stool sample has successfully passed all laboratory testing and analysis, it is then deemed acceptable and the donor is then fully approved for giving donations to the stool bank in the future (Chen *et al*., 2021).

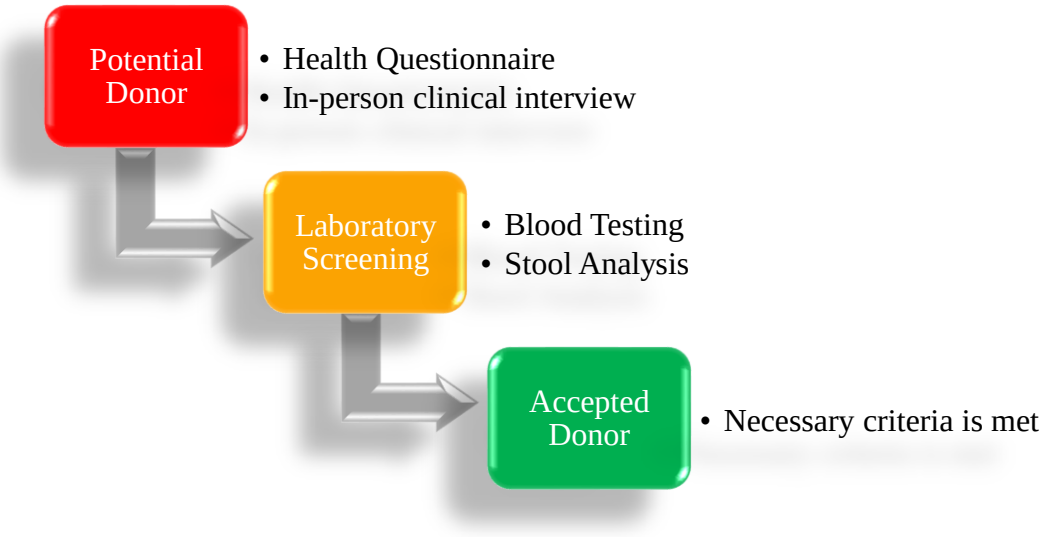


Figure 2.3: The donor selection process for stool banks

In 2020, Okahara *et al.* outlined how the genetic relationship and closeness of age between the donor and receiver can play a crucial role in the long-term maintenance after FMT in UC patients. Closeness in age between donor and receiver has been linked with an increased success rate of the FMT process for the treatment of UC due to the GM composition being affected by the aging process. Another important factor that must be considered when choosing a donor for the treatment of UC is the diversity of the donor's GM composition as this can help play a role in the stabilisation of the receiver's GM composition, again indicating the importance of choosing specific donors to matching receivers. Relatives have shown to have similar GM compositions, making healthy relatives ideal donor candidates for UC treatment cases. Siblings have been shown to reflect the same GM as the receiver's GM prior to the development of UC. It has also been noted that if non-relatives have a GM composition close to the receiver's GM prior to the development of UC, this donor selection may also lead to successful FMT (Okahara *et al.*, 2020). Based on all these facts, specific donor selection is clearly crucial for the success of long-term maintenance of FMT.

2.6 Thesis Outline

The aim of this thesis is to examine the role that FMT can play as a novel treatment method for CD and Colitis when current treatment methods are no longer deemed as effective. While there is currently no cure for either of these gut dysbiosis related diseases, treatment is still necessary for alleviating the patient's symptoms. This analysis of the use of FMT as a disease treatment method in modern Western medicine will be completed via secondary research. This process will involve reviewing recently published studies and clinical trials that have been carried out in this area of study and comparing the data from each to determine if there is in fact a clear benefit of using FMT in place of current treatment methods to treat these diseases. The concept behind this thesis is to gain a better understanding of the role that the GM plays in the development of gut dysbiosis related diseases such as CD and Colitis and how alteration of the GM can be used as either a prevention or treatment method for these diseases. The results gained from this thesis will also explore the impact that the use of FMT can have on the long-term treatment of these diseases. This thesis also aims to outline the current gaps in the available knowledge surrounding this area of study and to pinpoint the exact areas that require the most immediate focus and development.

2.6.1 Research Question

The focus of this study will be on researching the health benefits associated with the use of FMT as a disease treatment method for CD and Colitis. The previously mentioned aims that are clearly defined in the thesis outline led to the development of the following thesis research question: can the alteration of the Gut Microbiome with Faecal Microbiota Transplantation be used in modern Western medicine as an effective disease treatment method for patients suffering from Crohn's disease and Colitis?

2.7 Chapter Conclusion

Chapter 2 provides a brief overview of the currently available treatment methods used to alleviate the symptoms of suffering CD and Colitis patients. This chapter discusses how the high morbidity rates and expensive health care costs associated with these current treatment methods should be used as a valid reason for pursuing FMT as an alternative safe and cost-effective treatment method. This chapter also analyses the effect that certain

diets such as the green-Mediterranean diet have on the GM composition and how nutritional intervention can be used as a disease treatment method for gut dysbiosis related diseases. The correlation between the presence of specific healthy bacteria in the GM and the patient's dietary patterns indicates the pro-inflammatory or anti-inflammatory roles that the diet can play in the treatment of CD and Colitis patients.

This chapter highlights the important role that donor-receiver matching plays in the successful long-term maintenance of FMT and how there is no universally suitable donor. The donor chosen for each FMT treatment must be chosen case by case, based on a number of factors such as genetics, age difference to the receiver, and GM similarities between donor and receiver pre-diagnosis. This chapter also highlights the importance of using screened, healthy donors that have met all the necessary stool bank criteria to ensure the safety and increased success rate of the FMT process.

Chapter 3: Methodology

3.1 Study Design

This section outlines the methods used during this thesis to compare the effectiveness of FMT to current, conventional treatment methods used for treating CD and Colitis. This methodology section highlights the methods used during this research project to efficiently search reliable online databases for relevant information. This work was completed using dry research which involved analysing recently published, peer reviewed journals, clinical trials, and other reliable government and non-government-based sources. All work completed during this thesis was via secondary research and no ethical approval was required for the completion of this study.

The theoretical and academic background of this study has already been outlined in Chapter 1 and Chapter 2. Topics covered in Chapter 1 include an overview of GM and FMT, health benefits associated with FMT, most common uses, delivery routes, current usage rates and associated controversies. Topics covered in Chapter 2 include an overview of CD and Colitis, current treatment methods used, the role diet plays in disease treatment and the importance of specific donor selection in FMT. This chapter will outline the methodology used during this thesis to carefully analyse relevant data to help answer the research question. The next chapter will present all the results obtained from reviewing these relevant publications. Chapter 5 will consist of a thorough discussion and critical review of the findings from the results that were obtained in Chapter 4 in relation to the role that FMT can play in the future treatment of CD and Colitis. Chapter 6 will consist of conclusions obtained from this research, highlighting the limitations of this thesis, and recommending where future work should commence in relation to this area of research. Finally, Chapter 7 will list all necessary references used during the writing of this thesis.

3.2 Scope

The scope of this research question focuses on the potential of using FMT effectively as a more mainstream gut dysbiosis related disease treatment method in modern Western medicine. Specifically, the focus will be on the benefits that this treatment method could bring to patients suffering from CD or Colitis when current treatment methods are no longer deemed as a viable option. This research will also analyse the impact of current treatment methods compared to FMT with regard to the long-term successful treatment

of the patient. This research will use current literature to highlight the current gaps in our knowledge related to FMT and to outline which of these gaps require the most immediate focus and development.

3.3 Research Methods

As previously mentioned, the research methodology used during this work was completely secondary research with all necessary material obtained from online databases. The analysis of already published data allowed for the comparison of results obtained from different studies to determine if there is in fact a benefit to pursuing the increased use of FMT as a disease treatment method in modern Western medicine. These databases allowed for access to recently published, credible, peer reviewed research papers. The electronic databases used to obtain this material during this research were all access through the Technological University Dublin (Tallaght campus) library website. The databases used as part of this research include PubMed, Academic Search Complete, Science Direct, Scopus, Standards (Irish & ISO), FSTA (Food Science and Technology Abstracts), Web of Science and Wiley Online Library.

3.3.1 Search Strategy

On the initial search, the electronic databases were searched by using the words “Faecal Microbiota Transplantation for Crohn's Disease or Colitis’’. This resulted in 1,102,936 results. Due to the large volume of data that was returned based on this initial search, the search was then refined and filtered by date. The inclusion date filters were set from 2019 to 2023 to ensure all published data obtained was up to date and as accurate as possible. It was also filtered to only include peer-reviewed and full text documents. This reduced the number of searches to 212,907. The search was then further refined by removing the word “or’’ from the search list. This reduced the number of searches down to 4,824. The word search was again shortened to only include the key words “FMT Crohn’s Colitis’’. This reduced the number of searches to 2,371. As this was still too broad a range of published studies to review, the search was then further filtered by relevance to the following subject matter: Gut Microbiome, Faecal Microbiota Transplantation, Ulcerative Colitis, Crohn’s disease, Colitis, treatment effectiveness, FMT, disease remission, Colitis Ulcerative, Faecal Microbiota Transplant, disease relapse and

dysbiosis. This extra refined search reduced the number of relevant scientific papers down to a much more manageable number of 201 related sources. These 201 scientific papers were then reviewed and screened for relevance in relation to this research question. A further 77 journals were eliminated as being inappropriate for this research based on the lack of relevance relating to their title and abstract. Upon further reading of these related scientific papers, a further 64 were also eliminated as being irrelevant to the research question. Due to the suitability of content on the remaining recently published journals, these 60 journals were identified as being relevant to the research question and were included in the thesis. An additional 6 sources from reliable government and non-government- based websites were also included as being relevant to the research question. Of these 66 reliable sources that were used to research this topic, the results obtained from 14 of these scientific papers were analysed in detail to help answer the research question. Figure 3.1 highlights how each additional search criteria reduced the number of acceptable scientific papers that could be analysed as part of this research work. This gives a visual representation of the number of scientific papers and reliable government and non-government-based websites used during this research to help answer the research question.

Figure 3.1: Number of scientific papers and reliable government and non-government-based websites selected during research to help answer the research question.

3.3.2 Data Inclusions

The data included in this thesis has been obtained from recent studies, with the majority of this data being collected from 2019 onwards. Any data that was used from older publications was only taken into consideration when the data used was still applicable and relevant, with no more recently published data available that would contradict these results. The oldest issued publication used in this research was from 1958 but was only referenced in relation to the historical usage of FMT. All data included in this research, met the following criteria: was ideally published within the last 7 years (if possible), had access to the full text, was relevant to the research question and the thesis aims, and was from a reliable and credible source. The data analysed during this research was all obtained from human studies.

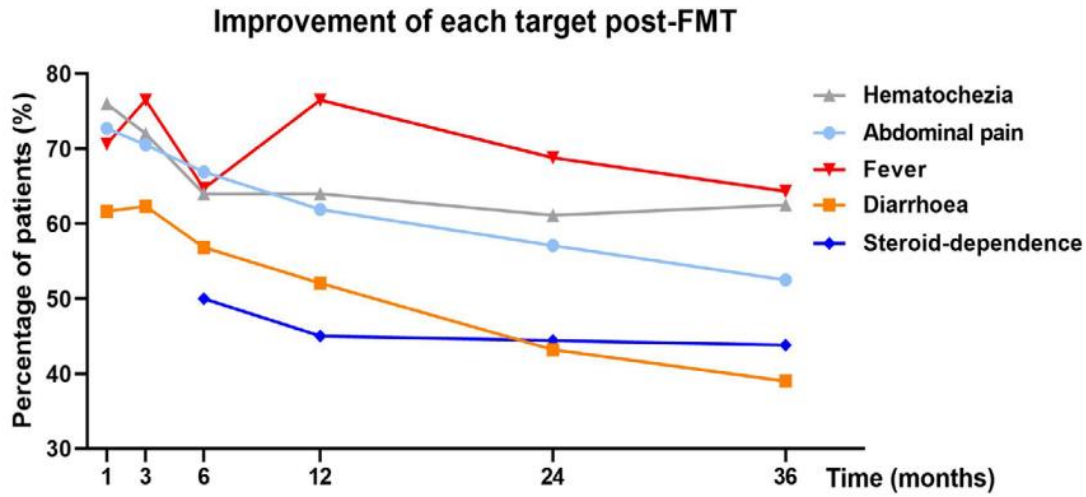
3.3.3 Date Exclusions

Data excluded from this research includes non-peer reviewed papers, papers that did not supply open access to the full text and those that had been published over 7 years ago where there was a risk of the data being irrelevant, misleading, and outdated. Scientific papers with unsubstantiated data were excluded from this research. Scientific papers that had not been written in the English language were also excluded from this research.

Chapter 4: Results

4.1 Success rate of treating Crohn's Disease with Faecal Microbiota Transplantation

In 2020, a study was completed to determine the efficacy of FMT in patients with CD. In the study, prior to being treated with FMT, 79.9% of patients complained of abdominal pain. 83.9% of patients reported suffering from diarrhoea. 14.4% of patients reported suffering from Hematochezia, also known as rectal bleeding. 9.8% of patients complained of fever and 11.5% of patients reported steroid-dependence. As can be seen from Graph 4.1, and Table 4.1, post 36 months of FMT treatment, the percentage of patients suffering from each of these symptoms were reduced. 62.5% of patients reported improvements in Hematochezia symptoms, 52.5% of patients had reduced abdominal pain, 64.3% of CD patients reported reduced fever and 39% and 43.8% of patients reported reduced diarrhoea and steroid-dependence respectfully (Xiang *et al.*, 2020).

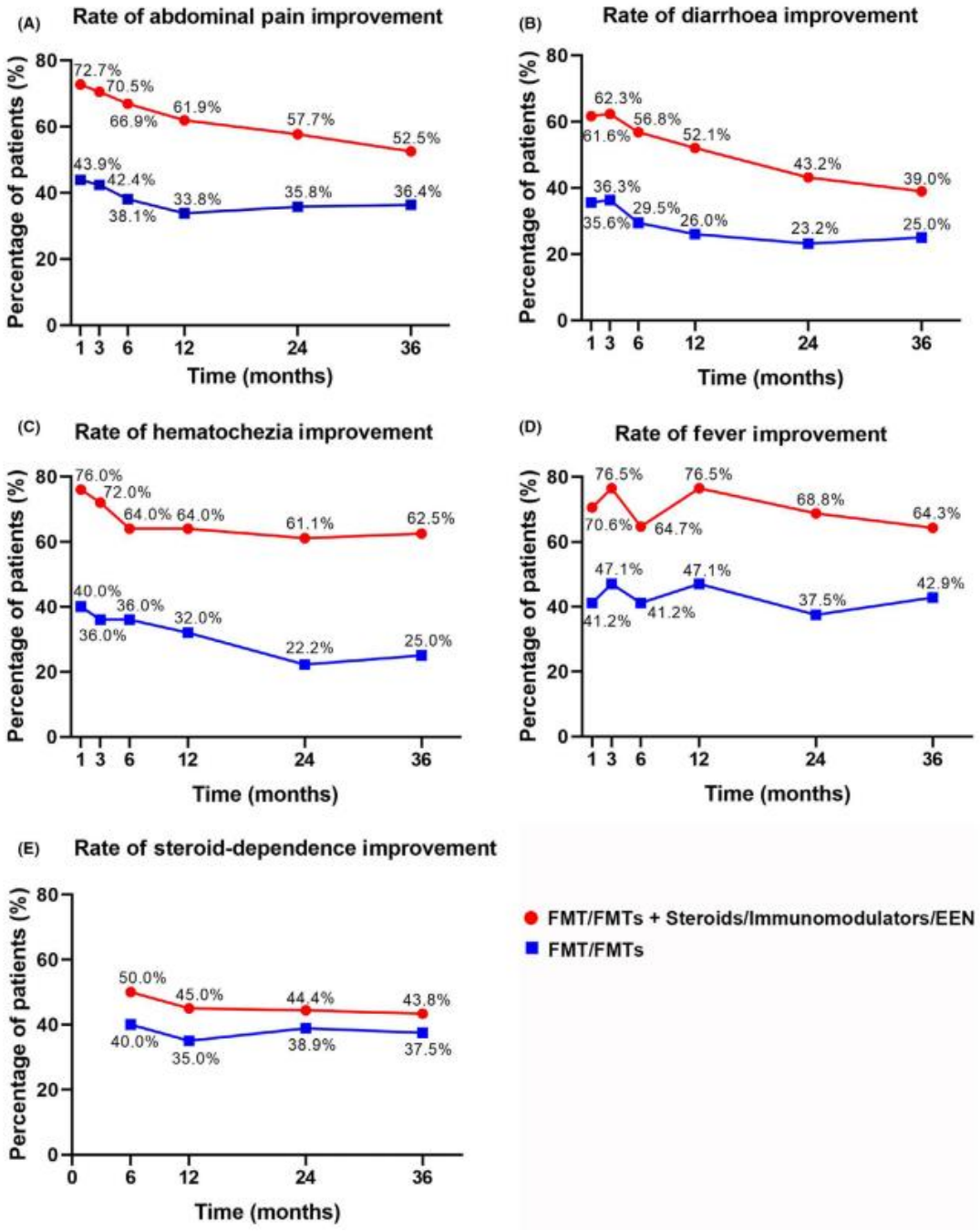


Graph 4.1: Improvement of each target post-FMT (*Taken from ((Xiang *et al.*, 2020)).

Table 4.1: Improvement of each target post-FMT (*Taken from ((Xiang *et al.*, 2020)).

Month (s) / Targets	1	3	6	12	24	36
Hematochezia, % (*n*)	76 (19/25)	72 (18/25)	64 (16/25)	64 (16/25)	61.1 (11/18)	62.5 (10/16)
Abdominal pain, % (*n*)	72.7 (101/139)	70.5 (98/139)	66.9 (93/139)	61.9 (86/139)	57.7 (71/123)	52.5 (52/99)
Fever, % (*n*)	70.6 (12/17)	76.5 (13/17)	64.7 (11/17)	76.5 (13/17)	68.8 (11/16)	64.3 (9/14)
Diarrhoea, % (*n*)	61.6 (90/146)	62.3 (91/146)	56.8 (83/146)	52.1 (76/146)	43.2 (54/125)	39 (39/100)
Steroid-dependence, % (*n*)			50 (10/20)	45 (9/20)	44.4 (8/18)	43.8 (7/16)

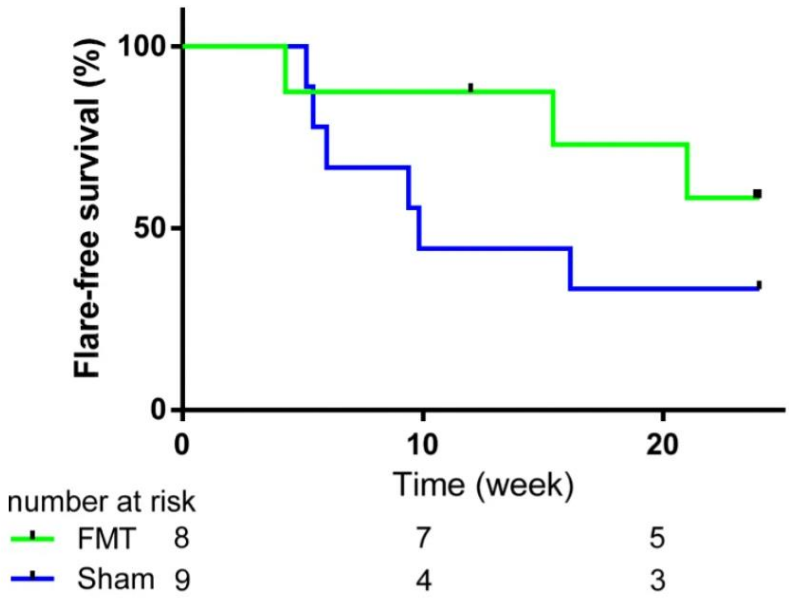
Graph 4.2 to Graph 4.6 show in more detail how each CD patient reported an improvement in each of the symptoms. In these graphs, patients have also been separated into two groups depending on if they received FMT alone or FMT with steroids, immunomodulators or EEN, based on single or multiple FMTs. As can be seen in Graph 4.2 to Graph 4.6, based on these five symptoms, over a 36-month period, there was an overall range of 22.2% – 47.1% of CD patients that reported reduced symptoms based on single and multiple FMTs. It is also evident from these graphs that when using FMT combined with steroids, immunomodulators or EEN, patients reported an even higher rate of reduced symptoms (Xiang *et al.*, 2020).



Graphs 4.2 – Graph 4.6: Percentage of patients who achieved improvement in abdominal pain (A), diarrhoea (B), Hematochezia (C), fever (D) and steroid-dependence (E) in two groups divided by whether they received steroids, immunomodulators or EEN, based on single or multiple FMTs (*Taken from ((Xiang *et al.*, 2020)).

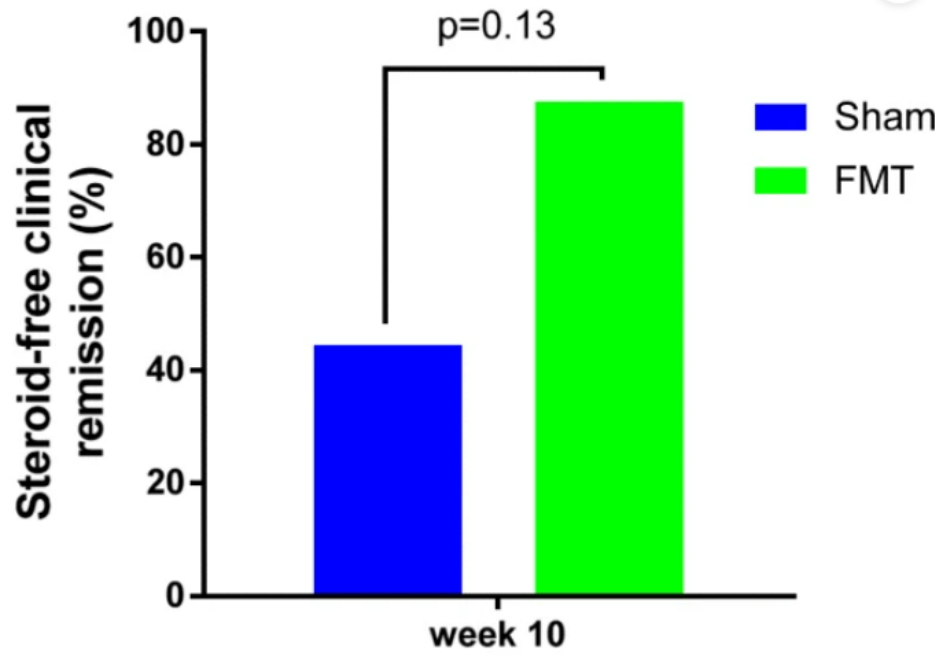
In 2020, a randomized, single-blind, multicentre, placebo-controlled 24-week trial was carried out to analyse how FMT can be used to maintain remission in CD patients. This trial consisted of 2 cohorts of patients. One cohort received FMT (8 patients) while the other cohort received a sham transplantation during a colonoscopy (9 patients). Starting at 6 weeks post FMT treatment (W6), administration of corticosteroids was reduced each week. At W6, patients from both cohorts underwent another colonoscopy. Results from this trial regarding the colonisation of the donor microbiota indicated that at W6, no patient had achieved the primary endpoint of >60% similarity with the donor GM composition. It was reported that at W6, the similarity between the donor and receiver was ≥0.6 (Sokol *et al.*, 2020).

As can be seen in Graph 4.7, the flare-free survival % of patients was higher in the FMT cohort than the sham cohort. At week 10, 7 out of 8 FMT patients were considered to be at risk of a flare-up. By week 20, this had reduced to 5 patients. For the sham cohort, 4 out of 9 patients were considered at risk of a flare-up with this number only reducing to 3 patients by week 20 (Sokol *et al.*, 2020).



Graph 4.7: Flare-free survival % of patients in the FMT cohort and the sham cohort over the course of a 24 week trial (*Taken from (Sokol *et al.*, 2020)).

Similarly positive results were also reported when the steroid-free clinical remission % was analysed. As can be seen in Graph 4.8, at week 10 the steroid-free clinical remission % of sham patients were only at 44.4% (4 out of 9 patients) while the cohort who received FMT had a much more positive result at 87.5% (7 out of 8 patients). This resulted in a probability value (*p*-value) of 0.13. These results indicate the positive link between FMT and the maintenance of clinical remission in CD patients (Sokol *et al.*, 2020).



Graph 4.8: Steroid-free clinical remission % of patients in the FMT cohort and the sham cohort at week 10 (*Taken from (Sokol *et al.*, 2020)).

4.1.1 Success rate of treating Colitis with Faecal Microbiota Transplantation

In 2022, changes in the GM composition of 15 UC patients post-FMT were assessed as part of an 8-week-follow-up study. Both donors and receivers from this study had their stool samples analysed by 16S rRNA gene-based microbiota analysis. All patients had similar baseline (BL) characteristics at day 0. As can be seen in Figure 4.1, results from this study indicated that 66.7% of the patients (10 patients) were classified as responders

(RE) to the treatment while 33.3% (5 patients) were classified as non-responders (NR) (Huang *et al.*, 2022).

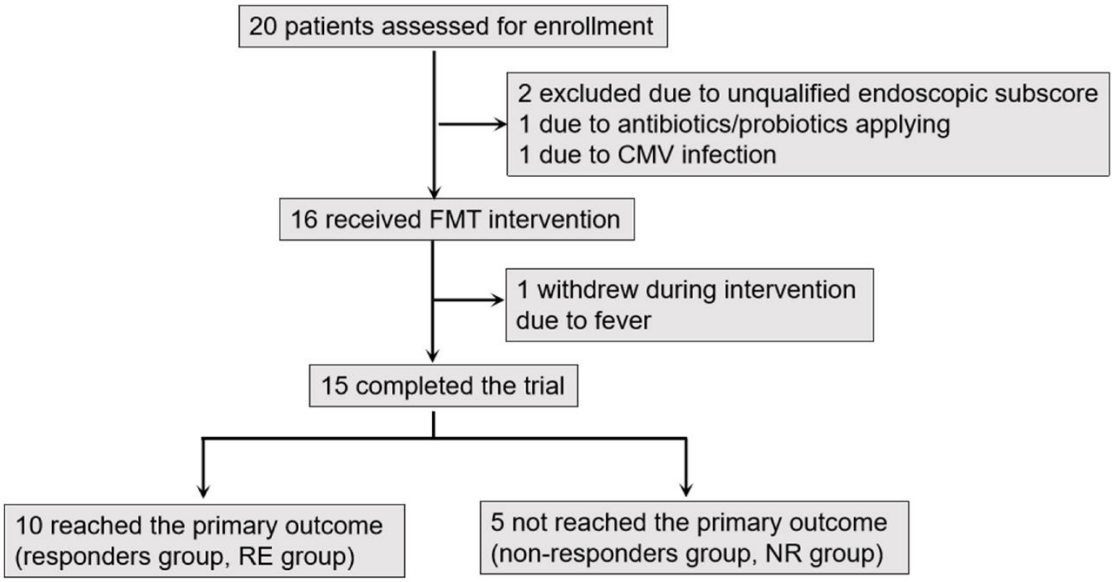
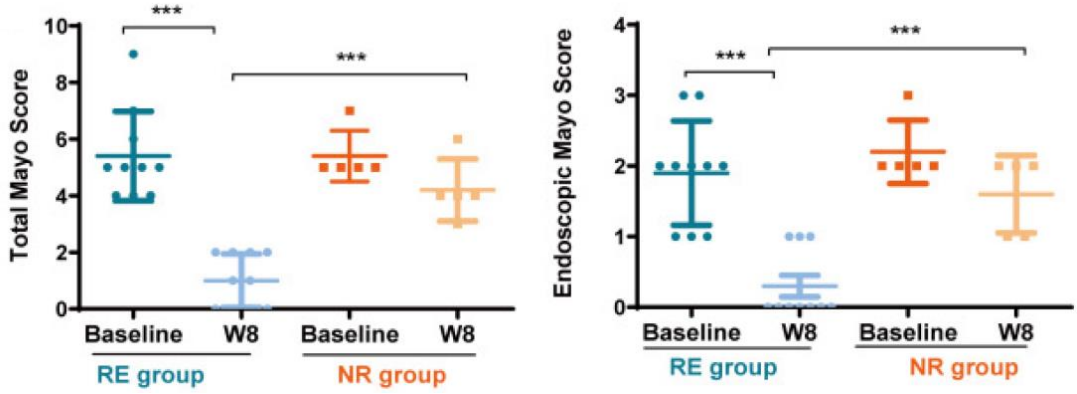


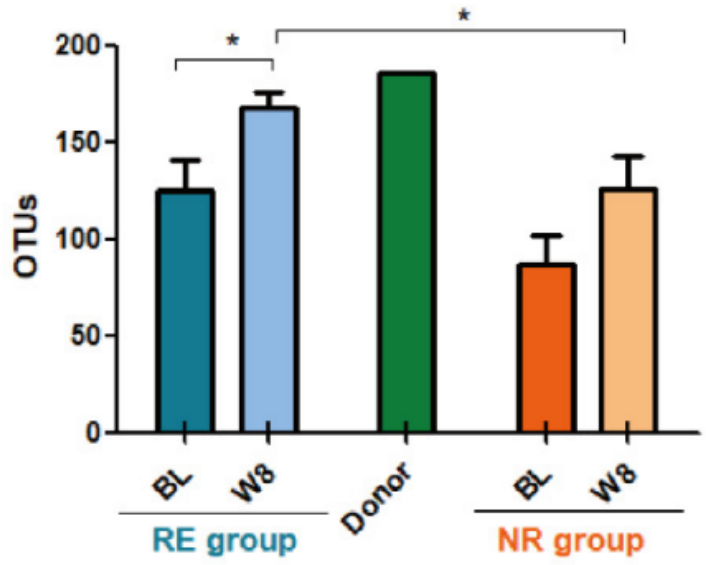
Figure 4.1: The flow of UC patients in a FMT trial (*Taken from (Huang *et al.*, 2022)).

The UC patients were classified as belonging to the RE or NR group depending on their total Mayo score and endoscopic Mayo score. The Mayo score ranges from 0 to 12 and was used to monitor patients' disease severity during the study. As can be seen from Graph 4.9 and Graph 4.10, 8 weeks post FMT treatment (W8), there was a significant decrease in the total Mayo score and endoscopic Mayo score of patients in the RE group. This result indicated improved clinical and endoscopic remission in the patients belonging to the RE group (Huang *et al.*, 2022).



Graphs 4.9 – Graph 4.10: The total and endoscopic Mayo scores of patients who received FMT for the treatment of UC (*Taken from (Huang *et al.*, 2022)).

There was a significant difference between the microbial diversity of the NR and RE patient groups after FMT. Stool samples from both donors and receivers were analysed for microbial community profiling at BL and at 2 weeks post FMT treatment (W2), 4 weeks post FMT treatment (W4) and W8. Results show that the UC patients at BL had lower operational taxonomic units (OTUs) than the donors. These OTUs are grouped based on similarity. There was no significant difference in the α-diversity of OTUs between the NR and RE patient groups at BL (124.556 ± 16.119 versus 86.2 ± 15.021, *p*-value = 0.142). As seen in Graph 4.11, at W8, there was a significant difference in the microbial diversity of patients in the RE group while there was no reported difference for the patients in the NR group. Graph 4.11 visually indicates through the use of a bar chart that by W8, the GM of the RE group had been altered to resemble that of the donor's (Huang *et al.*, 2022).



Graph 4.11: Bar chart showing changes in intestinal microbiota diversity in NR and RE groups at BL and W8 with α-diversity reflected by observed community OTUs (*Taken from (Huang *et al.*, 2022)).

In 2019, Sood *et al.* published a study that analysed the positive role that FMT can play in the maintenance of UC patients' clinical remission. As part of this study, 61 UC patients received FMT with standard of care therapy (SOC) over multiple sessions. 31 of these patients then received FMT every 8 weeks, for 48 consecutive weeks while the remaining 30 patients received placebo colonoscopic infusion instead. Table 4.2 shows the clinical outcome of the patients post 48 weeks. Of those that received FMT and SOC, 87.1% (27 patients out of 31) achieved maintenance of clinical remission at 48 weeks while 66.7% (20 patients out of 30) of the placebo group maintained clinical remission. For secondary endpoints of endoscopic remission, 58.1% (18 patients out of 31) of those in the FMT group achieved endoscopic remission while only 26.7% (8 patients out of 30) of the placebo group achieved remission. Table 4.2 also shows that for histological remission, 45.2% (14 patients out of 31) in the FMT group achieved remission while only 16.7% (5 patients out of 30) of the placebo group achieved this result. This indicated the positive results achieved in UC patients in clinical remission who underwent maintenance FMT treatment versus the control placebo group (Sood *et al.*, 2019).

Table 4.2: Clinical outcomes of patients (*Adapted from (Sood *et al.*, 2019)).

Outcome Measure	**FMT**	**Placebo**	***p*-value**
Clinical Remission	27/31 (87.1%)	20/30 (66.7%)	0.111
Endoscopic Remission	18/31 (58.1%)	8/30 (26.7%)	0.026
Histological Remission	14/31 (45.2%)	5/30 (16.7%)	0.033

In 2021, Crothers *et al.* completed a randomized, prospective, single-centre pilot study to analyse the use of daily, oral FMT for long-term maintenance therapy in UC patients. This study consisted of a small cohort of 12 UC patients. 50% of the cohort received a 12-week FMT treatment and daily encapsulated oral FMT. The rest of the cohort (6 UC patients) received a colonic installation and longitudinal oral placebo capsules. This control group was given the subject code: placebo. Patients who received the FMT treatment were allocated the following subject codes: E, W, F, A, P and N. As can be seen from Figure 4.2, 2 out of 6 subjects who received FMT (subjects E and W) achieved clinical remission during this study [relative risk (RR) = infinity, 95% confidence interval (CI) = 0.38-infnity, *p*-value = 0.45]. This was not the case for any of the patients in the placebo group. Overall, results from this study showed that 3 of the 6 subjects who received FMT (subjects E, W, and P) met the study definition of clinical response. However subject P required steroid therapy at week 6 of this study, making their results of improvement due to FMT invalid. For the placebo group, only 1 subject met the study definition of clinical response (RR = 3.00; CI: 0.42– 21.20, *p*-value = 0.55). There was a strong correlation between the donors' and the receivers' GM compositions for up to 20 weeks. These results indicate the positive health benefits associated with UC patients consuming daily encapsulated oral FMT (Crothers *et al.*, 2021).

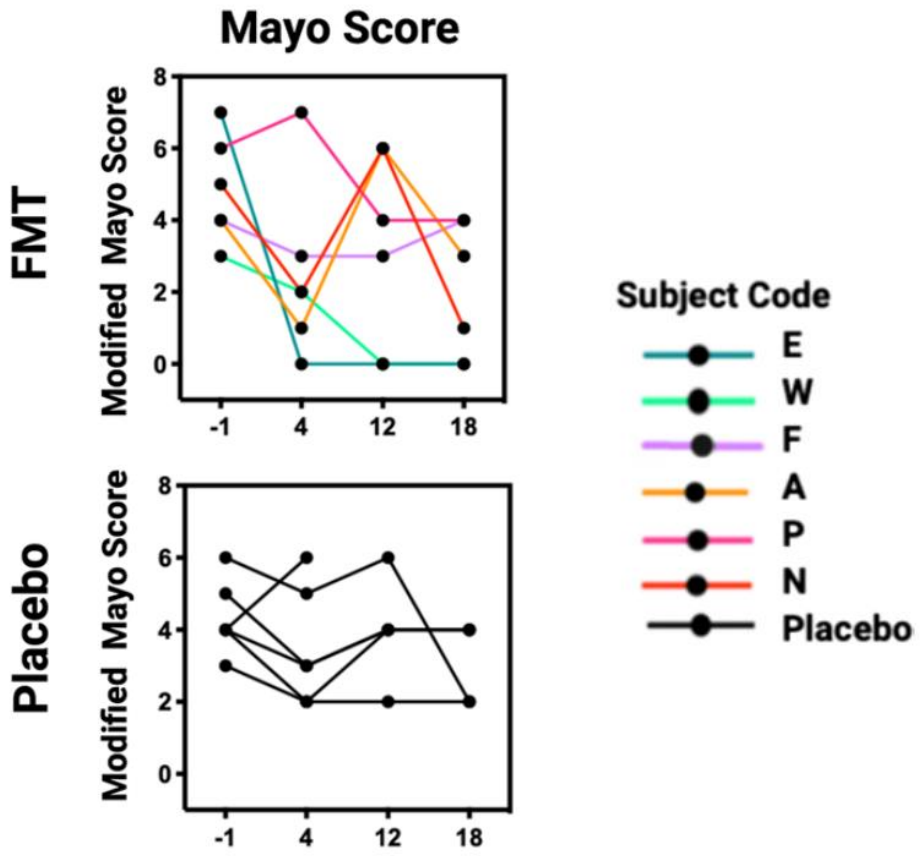


Figure 4.2: Modified Mayo Score for both the FMT patients and the placebo group with each line represents a single subject over time (*Taken from (Crothers *et al.*, 2021)).

A placebo-controlled, randomised, double-blind trial was carried out in Australia in 2022 with the aim to determine the efficacy of oral lyophilised FMT as a UC treatment method. As part of this trial, patients in both the placebo cohort (20 patients) and the cohort receiving oral lyophilised FMT (15 patients), first received a 2-week course of antibiotics such as doxycycline, metronidazole, or amoxicillin before FMT treatment or consumption of the placebo capsule commenced. Post an 8 week follow up, 53% of the patients who received oral lyophilised FMT were reported to be in corticosteroid-free clinical remission while this was only reported for 15% of the placebo cohort (difference 38.3%, 95% CI 8.6–68.0; *p*-value = 0.027; odds ratio 5.0, 95% CI 1.8–14.1). In regard to patients experiencing mild and self-limiting gastrointestinal complaint, 67% of UC patients in the

FMT group reported negative symptoms and 85% of the placebo-control group experienced these symptoms. 2 patients in the FMT group reported severe symptoms including worsening UC while 1 patient in the placebo group reported rectal bleeding. 8 weeks after FMT, the 15 UC patients who received treatment were further divided with 4 patients in the cohort continuing FMT treatment for another 48 weeks and the rest of the cohort withdrawing from the FMT treatment. 100% of UC patients who continued the FMT treatment were in clinical, endoscopic, and histologic remission at week 56. In contrast, none (0%) of the patients who withdrew from the FMT treatment were still in clinical remission at week 56. These results indicated that the consumption of antibiotics for a 2-week period followed by oral lyophilised FMT resulted in clinical remission in UC patients with a longer treatment period resulting in clinical remission for a longer period of time (Haifer *et al.*, 2022).

The first pilot, randomized, controlled trial of treating UC paediatric patients with FMT started in 2015 and was completed in 2018. This 36-month trial consisted of 25 screened UC patients with ages ranging from 4 to 17. Figure 4.3 shows how of these 25 patients, 13 received FMT while 12 patients were allocated to the placebo group. Figure 4.3 also highlights how 50% of the placebo group (6 patients) continued with treatment until the end of the trial while over 69% (9 patients) in the group who received FMT continued with treatment until the end of the trial. In 2021, Pai *et al.* discussed the results from this trial and reported that there were improved UC symptoms in 91.7% of the paediatric patients who received FMT while only 50% of the placebo group reported improvements. At week 30, 50% of the FMT group reported a clinical response which was defined as an improvement from the BL Paediatric UC Activity Index. In comparison, only 25% of patients in the placebo group reported clinical response at week 30. This indicates the success associated with using FMT to treat UC in paediatric patients (Pai *et al.*, 2021).

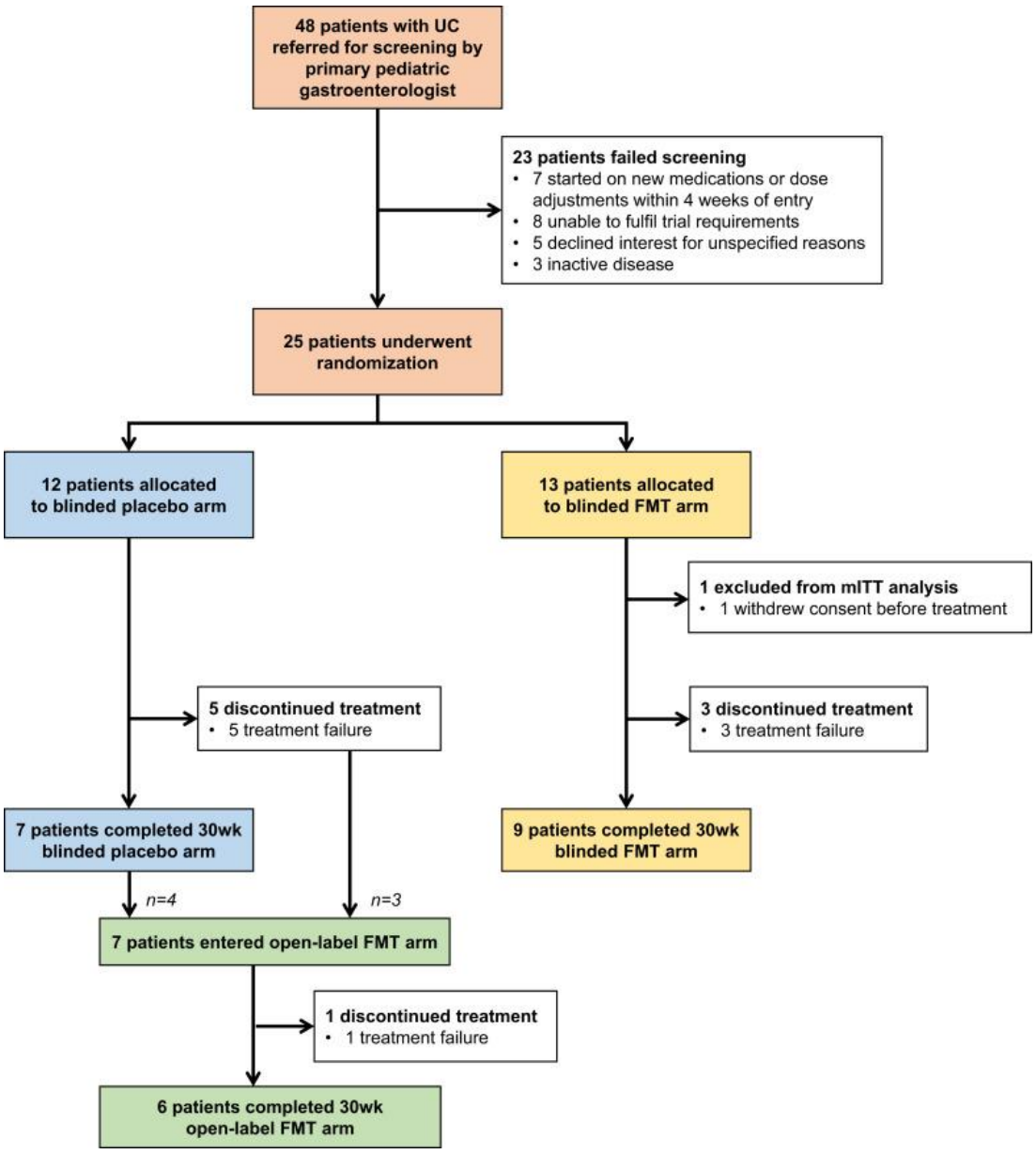


Figure 4.3: Flow of screened, and randomized UC paediatric patients in a FMT trial (*Taken from (Pai *et al.*, 2021)).

4.2 Limitations associated with treatment of Crohn's Disease and Colitis with Faecal Microbiota Transplantation

While generally considered as a safe method for treating gut dysbiosis related diseases, there are some limitations associated with FMT in terms of undesirable side effects. As can be seen in Table 4.3, common side effects associated with this treatment method

include short-term symptoms such as diarrhoea, fever, abdominal pain and bloating while associated long-term effects including obesity and the development of IBD in receivers. It must be noted however that most of these short-term side effects are often associated with the delivery route used for FMT rather than treatment method itself (Seon-Young Park and Geom Seog Seo, 2021).

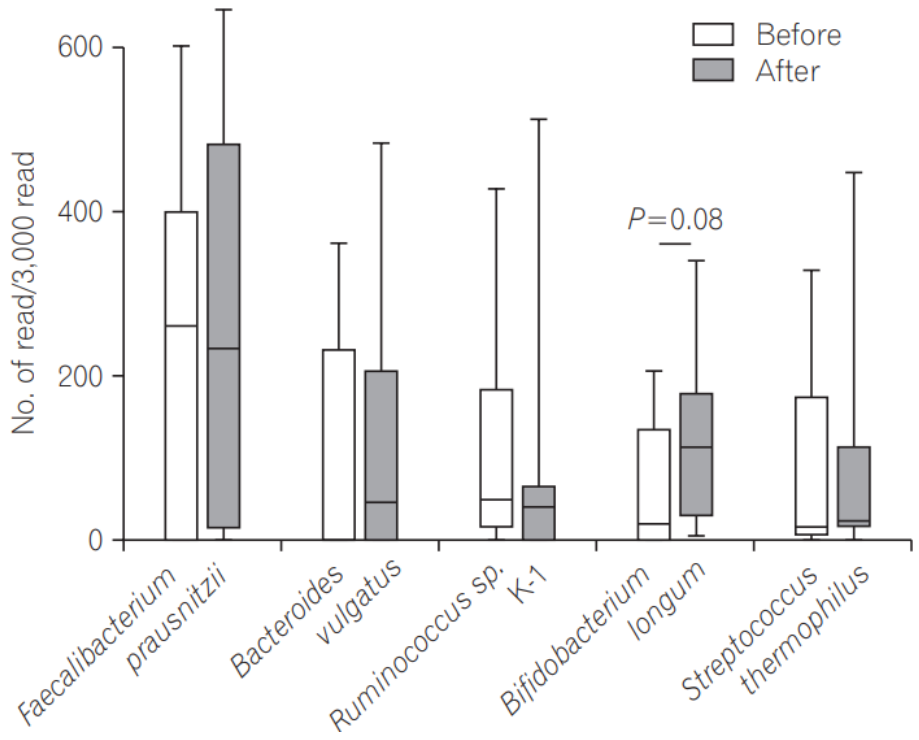
Table 4.3: Negative side effects reported from patients who underwent FMT (*Adapted from (Seon-Young Park and Geom Seog Seo, 2021)).

Short-Term Side Effects	**Long-Term Side Effects**
Bloating	Obesity
Gaseousness	Immune Thrombocytopenia
Diarrhoea	Rheumatoid Arthritis
Irregular Bowl Habit	IBD
Irritable Bowel Syndrome (IBS)	IBS
Constipation	
Abdominal pain, tenderness	
Fever	
Nausea	
Hematochezia	
Aggravation of IBD	
Gram negative bacteraemia (gram negative bacteria in the blood stream)	
Bowel perforation	
Belching	
Death	

4.2.1 Failure rate of treating Crohn's Disease and Colitis with Faecal Microbiota Transplantation

A trial was carried out in 2020 to determine if FMT could be used to maintain remission in CD patients. Results from this trial reported that increased FMT failure rates in CD patients post treatment were linked significantly with higher BL levels of several taxa belonging to the Gammaproteobacteria class of the Proteobacteria phylum being present in the receiver's GM. Examples of such gram-negative bacteria include *Haemophilus*, *Actinobacillus*, and *Klebsiella*. This indicates the receiver's BL microbiota may play a role in the success rate of donor microbiota colonisation in FMT treatment. Increased failure rates were also associated with the lack of donor microbiota engraftment and resulted in the patients no longer being classified as being in clinical remission. This trial reported that FMT failure occurred in 2 patients as their GM were not colonised by the donor's microbiota post treatment (Sokol *et al.*, 2020).

In 2017, a Japanese clinical trial was published. The purpose of this trial was to assess the efficiency and safety of FMT in UC patients. The results from this trial indicated that the use of single FMT failed to alter the GM of these patients, indicating the limited effectiveness associated with the use of single FMT as a treatment method for UC. As can be seen in Graph 4.12, there was no reported significant difference (p-value = 0.08) between the levels of faecal bacterial spp. present in the patient's pre-FMT stool samples compared to their post-FMT stool samples (Shinta Mizuno *et al.*, 2017).



Graph 4.12: Top 5 faecal bacterial species present in patients' pre-FMT stool samples compared to their post-FMT stool samples (*Taken from (Shinta Mizuno *et al.*, 2017)).

4.3 Analysis of microbial species crucial for the success of Faecal Microbiota Transplantation

Alteration of the GM composition is linked with better outcomes for patients who receive FMT. Huang *et al.* reported from their study completed in 2022, that after treating UC patients with FMT, there was a reduction in the levels of Proteobacteria present in the patients' GM with the RE group having significantly lower levels of Proteobacteria present when compared to the NR patient group (0.030 ± 0.009 versus 0.117 ± 0.044, *p*-value = 0.040). As can be seen in Figure 4.4, the NR group patients had higher levels of *Enterococcus* present in their GM while patients from the RE group had higher levels of *Faecalibacterium* present. The NR_BL group also showed increased GM presence of *Escherichia−Shigella* while the RE_BL group showed the presence of higher levels of *Enterobacter*. These results highlight how the presence of some bacterial groups such as *Escherichia−Shigella* in the GM may be linked with increased risk of FMT failure.

Similarly, it also highlights how the presence of some bacterial groups such as *Faecalibacterium* in the GM may be necessary for the alleviation of symptoms for suffering UC patients (Huang *et al.*, 2022).

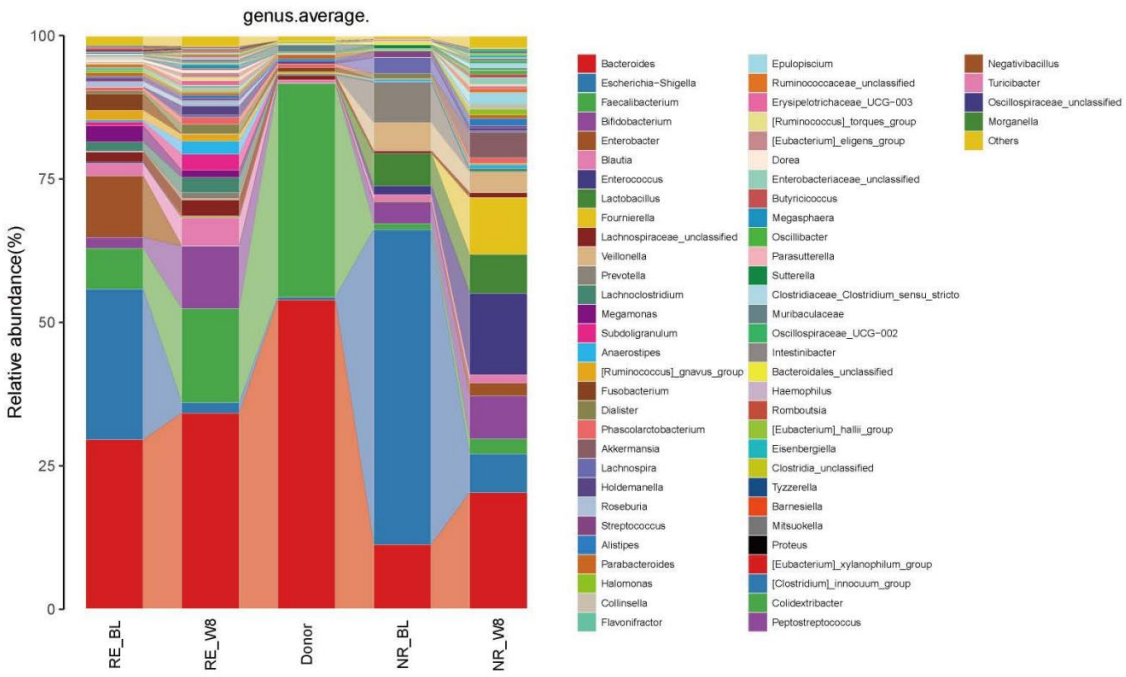


Figure 4.4: Relative abundance of genera in the intestinal microbiota - indicating the positive microbiome community shift in the RE group of UC patients after FMT (*Taken from (Huang *et al.*, 2022)).

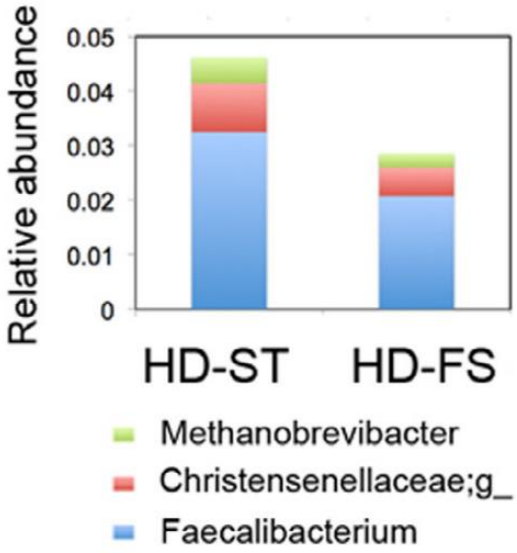
Crothers *et al.* reported that based on the results obtained from their analysis on the use of daily, oral FMT for long-term maintenance therapy in UC patients, the dominating phyla present in the GM of these patients were Firmicutes and Bacteroidetes (88.90%). Proteobacteria and Actinobacteria were also present but at much lower levels. Results from this study indicated that at the genus level, the analysed stool samples showed the presence of high levels of Clostridiales and Bacteroidales. Burkholderiales, Bifdobacteriales, Enterobacteriales and Lactobacillales were also present but at much lower levels. Following a 7-day course of antibiotics including Ciprofoxacin and Metronidazole, the results from this study showed a change in the GM composition of the patients with an increase in the level of gram positive Actinobacteria, including the orders Lactobaccilales and Bifdobacteriales. In contrast, there was a reduction in the level of

gram negative and anaerobic bacteria of the Bacteroidetes and Firmicutes phyla (Crothers *et al.*, 2021).

Results from a study published in 2020, based on determining the safety and efficacy of different methods of FMT for the treatment of CD, found that of the 27 patients who received FMT, either by colonoscopy or gastroscopy, 66.7% achieved clinical remission with neither method being highlighted as being superior. This study also reported that compared to donors, the receiving CD patients had higher levels of *Streptococcus, Clostridium, Cronobacter,* and *Fusobacterium* present in their GM and lower levels of *Faecalibacterium, Roseburia, Eubacterium,* and *Bacteroides* present (Yang *et al.*, 2020).

As previously highlighted, results from the trial carried out by Sokol *et al.* in 2020 which analysed how FMT can help maintain remission in CD patients, indicated that patients who's FMT treatment was unsuccessful, had a significant association with higher BL levels of numerous taxa belonging to the Gammaproteobacteria class of the Proteobacteria phylum. When the overall microbial population was examined for microbial based predicators for flare-ups, before the end of W6, several taxa were identified as being associated with a flare-up. These taxa belonged to the Gammaproteobacteria class and the Clostridiales order comprising of *Ruminococcus gnavus*, *Desulfovibrio, Coprococcus*, and *Ruminococcaceae*. These results show how certain taxa can be linked with remission maintenance in CD patients (Sokol *et al.*, 2020)).

A study completed by Sarrabayrouse *et al*. analysed the mucosal microbial load present in the GM of CD patients. As part of this study, it was reported that the success rate of FMT was determined by the inflammatory state of the intestinal mucosa. A low bacterial load indicated better colonisation by the donor microbiota, resulting in a stronger anti-inflammatory response when compared to intestinal mucosa of those with a higher bacterial load. This highlights the dependency that the success of FMT has on the bacterial load of the receiver's intestinal mucosal microbial load. As can been seen in Graph 4.13, Using 16S sequence analysis, the presence of 3 genera required to correct gut dysbiosis in CD patients, were detected in this study. Both healthy donor stool (HD-ST) and the healthy donor faecal suspension (HD-FS) displayed the presence of *Methanobrevibacter*, a genus from the *Christensenellaceae* family, and *Faecalibacterium.* The HD-FS was reported to have a GM composition similar to that of the HD-ST (Sarrabayrouse *et al.*, 2020).



Graph 4.13: Detection of 3 of the genera required to correct gut dysbiosis in CD patients, in both the HD-ST and HD-FS (*Taken from (Sarrabayrouse *et al.*, 2020)).

4.4 Donor-patient matching for Faecal Microbiota Transplantation

Pre-FMT, donors' stool samples should be screened and only those with low levels of *Parabacteroides, Enterococcus faecalis,* and *Bacteroides* present should be chosen for use in FMT. Presence of these bacteria at low levels in the GM have been linked with pro-inflammatory cytokine release (Sarrabayrouse *et al.*, 2020). In 2021, Olesen and Gerardin re-evaluated the results from 12 previously published studies that analysed the use of super donors in FMT for the treatment of IBD conditions such as CD and Colitis. Out of the 12 studies that underwent statistical analysis, 10 studies (83.33%) indicated that there was no statistically significant donor effect found. One of the studies analysed, was carried out by Jacob *et al.* in 2017. Upon analysis of this study, Olesen and Gerardin reported that the results obtain from this study indicated a statistically significant efficacy difference by pool of donor stool but not in relation to the donor's GM composition or α-diversity. The last study analysed was published by Vermeire *et al.* in 2016. This study reported that the bacterial α-diversity of donor samples varied with increased bacterial diversity resulting in more successful outcomes for the receivers (p-value = 0.012). During this study, it was reported that there were large CI associated with quantifying the

uncertainty regarding the power of the donor effect. These CIs ranged from no donor effects to very sizeable donor effects. The results obtained from this review indicate the current gap in available knowledge surrounding the clinical importance that may be associated with the donor effect. (Olesen and Gerardin, 2021).

A study was carried out in 2022 which analysed the role of optimal timing and donor-patient matching when treating UC patients with FMT. This study consisted of 62 donors and 93 UC patients who underwent autologous FMT. Results from this study reported that 4 weeks after patients underwent treatment, there was a reported 36.1% remission induction rate and 63.9% efficacy rate. An age gap between donors and receivers affected the cumulative non-relapse rate (p-value = 0.01). There was also a reported significantly higher cumulative non-relapse rate in patients whose donor was their sibling when compared to those in the parent-child donor group (p-value = 0.001). This study reported that the optimal timing for receiving FMT treatment was before any corticosteroid or tacrolimus treatments. This study concluded that the short-term efficacy of the treatment was affected by the receiver's own characteristics while long-term efficacy was affected more by what donor was used for the treatment. This highlights the importance of donor-patient matching in the FMT process (Odakura *et al.*, 2022).

4.5 Level of patients willing to accept the Faecal Microbiota Transplantation Treatment Method

As previously mentioned, the taboo associated with the FMT process has been well documented over the past few years. In 2020, Zhong *et al.* completed a survey to analyse the awareness and attitude of Chinese IBD patients in relation to receiving FMT via the TET method. This study consisted of 620 questionnaires. The results obtained from this survey indicate that 44.6% of patients did not even know that FMT was a treatment option available to them, highlighting the importance of patient education on available treatment methods. 80.6% of patients were unaware of or did not understand the concept of TET. 63.2% of the surveyed participants confirmed that they would agree to receive FMT treatment via TET if needed. 62.4% of the surveyed patients actually underwent FMT treatment via TET with 95.6% of these patients reporting being satisfied with the results of the procedure. The patients who underwent FMT via TET reported having a much more positive attitude toward FMT than those who did not receive this treatment method

(98.5% versus 87.8% respectfully, *p*-value = 0.017). Results from this survey indicate that the taboo associated with FMT is linked with a lack of understanding of the safety and efficiency of the procedure (Zhong *et al.*, 2020).

4.6 Synopsis of major findings

Based on these results, it is evident that CD patients can have improved symptoms up to 36 months post-FMT treatment (Xiang *et al.*, 2020). FMT combined with steroids, immunomodulators or EEN has been reported to result in an even higher rate of reduced CD related symptoms (Xiang *et al.*, 2020). FMT has also been linked with both an increased flare-free survival % and an increased steroid-free clinical remission % in receiving patients. These results highlight a positive link between the maintenance of clinical remission in CD patients and the use of FMT as a treatment method (Sokol *et al.*, 2020). Results from a 2022 study show that at 8 weeks after successful FMT, the GM composition of receivers have been significantly altered to resemble that of their donor's while there was no reported alteration of microbial diversity for those who reported the treatment as being unsuccessful. This indicates the important role that FMT can play in treating CD and Colitis by altering the GM composition (Huang *et al.*, 2022). Results from a study carried out in 2019 highlight the positive role in the maintenance of UC patients' clinical remission that FMT can play. 87.1% of patients that received FMT achieved maintenance of clinical remission at 48 weeks post treatment while a placebo group only maintained clinical remission in 66.7% of the patients. Similarly positive results were reported for histological remission with 45.2% in the FMT group achieving remission compared to only 16.7% of the placebo group (Sood *et al.*, 2019). Results from an Australian trial carried out in 2022 reported clinical remission in UC patients can be achieved by the consumption of antibiotics for 2 weeks followed by oral lyophilised FMT. This study also reported that a longer treatment period can result in a longer period of clinical remission (Haifer *et al.*, 2022). In 2021, another study reported an improvement in the symptoms of 91.7% of UC paediatric patients who received FMT compared to a placebo group which reported an improvement rate of 50% (Pai *et al.*, 2021).

In contrast, FMT failure rates have been linked significantly with the presence of higher BL levels of several taxa belonging to the Gammaproteobacteria class of the Proteobacteria phylum in the receiver's GM. FMT failure has also been linked with the

lack of donor microbiota engraftment (Sokol *et al.*, 2020). The use of single FMT has also been reported to have high increased failure rates compared to multiple rounds of FMT (Shinta Mizuno *et al.*, 2017).

Successful FMT has been linked with low levels of Proteobacteria, *Enterococcus faecalis, Escherichia−Shigella, Parabacteroides, Bacteroides,* Burkholderiales, Bifdobacteriales, Enterobacteriales, and Lactobacillales present in the GM and high levels of *Faecalibacterium, Enterobacter,* Clostridiales and Bacteroidales (Sarrabayrouse *et al.*, 2020; Crothers *et al.*, 2021; Huang *et al.*, 2022). Increased levels of gram positive Actinobacteria and a reduction in gram negative and anaerobic bacteria of the Bacteroidetes and Firmicutes phyla are also associated with a healthy GM composition (Crothers *et al.*, 2021). When the GM composition of receiving CD patients was compared to their donors', the FMT receiver had higher levels of *Streptococcus, Clostridium, Cronobacter,* and *Fusobacterium* and lower levels of *Faecalibacterium, Roseburia, Eubacterium,* and *Bacteroides* (Yang *et al.*, 2020). Taxa belonging to the Gammaproteobacteria class and the Clostridiales order comprising of *Ruminococcus gnavus* have been associated with undesirable flare-ups in receiving patients. In contrast, taxa including *Desulfovibrio, Coprococcus*, and *Ruminococcaceae* have been linked with remission maintenance in FMT receiving patients (Sokol *et al.*, 2020). The presence of *Faecalibacterium,* a genus from the *Christensenellaceae* family and *Methanobrevibacter* in the GM have been linked with increased FMT success (Sarrabayrouse *et al.*, 2020).

Donor-patient matching is a crucial part of the FMT process. Optimal timing for receiving FMT should be before any corticosteroid or tacrolimus treatments. Short-term efficacy of FMT can be affected by the receiver's own characteristics while donor selection has more of an effect on the long-term efficacy of the treatment method (Odakura *et al.*, 2022). While patients' acceptance of FMT is on the rise, there is still a major lack of patient education available surrounding the process. A survey carried out in 2020 reported that 44.6% of patients surveyed did not even know what FMT was (Zhong *et al.*, 2020).

Chapter 5: Discussion

5.1 Chapter Overview

The purpose and objective of this research was to examine the role of FMT as a novel treatment method for alleviating the symptoms of CD and Colitis when current treatment methods are no longer deemed as effective. This chapter will provide a synopsis of the major findings obtained from the 66 sources reviewed as part of this research, consisting of scientific papers and sources from reliable government and non-government-based websites. This chapter will also discuss the results obtained from the 14 scientific journals that were analysed in depth during this research to help answer the research question: can the alteration of the Gut Microbiome with Faecal Microbiota Transplantation be used in modern Western medicine as an effective disease treatment method for patients suffering from Crohn's disease and Colitis? The aim of this chapter is to compare the results obtained in this dry research and to determine if any similarities or differences were noted between these analysed studies in relation to the effectiveness of FMT as a novel treatment method for CD and Colitis patients.

5.2 The clinical efficacy of Faecal Microbiome Transplantation as a disease treatment method

As can be seen in Graph 4.1, it was observed that FMT can result in the alleviation of CD associated symptoms such as Hematochezia, abdominal pain, fever, diarrhoea, and steroid dependency. As mentioned previously in Section 2.3, there is currently no cure available for gut dysbiosis related diseases such as CD and Colitis. This highlights the importance of the availability of treatment methods to patients to help alleviate their associated symptoms and help improve their quality of life. As outlined from the results summarised in Table 4.1 and visualized in Graph 4.4, a study on the efficacy of FMT reported that only 1 month post FMT, 76% of patients who suffered from Hematochezia (14.4% of the total cohort) reported reduced incidents of rectal bleeding. At 36 months post treatment, there was a high percentage of patients still reporting improved results (62.5%). This reduction in patients suffering from rectal bleeding was the first ever report of FMT's efficacy against reducing Hematochezia in CD patients (Xiang *et al.*, 2020).

Table 4.1, Graph 4.2, Graph 4.3, Graph 4.4, Graph 4.5, and Graph 4.6 all clearly outline the major improvement in overall CD and Colitis patients' symptoms post FMT treatment that was observed in the study carried out by Xiang *et al*. As can be seen in Graph 4.2, it

was observed that of the patients suffering from abdominal pain, 72.7% of these patients (79.9% of the total cohort) reported having improved symptoms 1 month post FMT. At 36 months post treatment, 52.5% of these patients still reported improved symptoms. Graph 4.5 and Graph 4.3 show how patients suffering from a fever and diarrhoea respectfully reported similarly promising results with 70.6% of those suffering from fever (9.8% of the total cohort) and 61.6% of those suffering from diarrhoea (83.9% of the cohort) reporting improved symptoms at just 1 month post FMT. 64.3% of these patients suffering from fever and 39% of these patients suffering from diarrhoea still reported a positive reduction in symptoms at 36 months post receiving treatment. Graph 4.6 gives a clear visual of how at 6 months after receiving FMT, 50% of steroid-dependent patients (11.5% of the total cohort) reported that they were steroid-free. At 36 months post treatment, this percentage was still very high at 43.8%. The importance of this result is that it outlines the potential success rates of using FMT as an alternative to steroids for the treatment of CD. This study also reported that 22.2% – 47.1% of the overall cohort of patients reported improved symptoms as a result of undergoing FMT treatment. This result further emphasises the clinical efficacy associated with the use of this process as a disease treatment method. (Xiang *et al.*, 2020).

The first randomized controlled study to evaluate FMT in CD was published in 2020 by Sokol *et al.* This study analysed the effect of single FMT via colonoscopy in CD patients who had achieved clinical remission through the consumption of systemic corticosteroids. As can be seen in Graph 4.8, the cohort who underwent FMT had a much higher rate of steroid-free clinical remission when compared to the control (sham) group. While the difference between the group who underwent FMT and the sham group was not statistically significant (p-value = 0.13), this result is still an important part of this research as it shows its similarities to the results obtained by Xiang *et al.*, with both highlighting the positive link associated with undergoing FMT and an increase in rates of clinical remission in CD patients (Sokol *et al.*, 2020). The overall results obtained from this research indicate that there is a clear beneficial link between undergoing FMT treatment and the reduction of reported CD and Colitis related symptoms in suffering patients. Graph 4.7 visually highlights how FMT can also benefit CD patients by reducing their risk of flare-ups. This risk was reportedly lower, but not statistical significance (p-value = 0.23) in the FMT group compared to the sham group. During this study it was reported that flare-ups occurred in 66.67% of the sham group (6 out of 9 patients) while

only 37.50% of the FMT group (3 out of 8 patients) reported flare-ups. This study indicated that these reported flare-ups in patients were associated with the receiver's GM consisting of several taxa belonging to the Gammaproteobacteria class of the Proteobacteria phylum and *Ruminococcus gnavus* from the Clostridiales order (Sokol *et al.*, 2020).

5.3 The relative abundance of genera existing in the Gut Microbiome post Faecal Microbiome Transplantation treatment

The importance of the level of *Ruminococcus* genus present in the receiver's GM post FMT was highlighted in a 2022 clinical trial that evaluated the response rate of FMT in Colitis patients (Huang *et al.*, 2022). It was previously highlighted in Section 2.4.1, how low levels of Actinobacteria, Proteobacteria, *Bacillaceae*, *Ruminococcus gnavus*, *Clostridium bolteae*, *Bacteroides fragilis*, *Erysipelotrichaceae*, *Streptococcus* spp., *E. coli*, *Coprobacillus*, *Blautia* spp., *Lachnospiraceae* bacteria, *Fusobacterium* spp., phylum Firmicutes and increased levels of *Clostridium* cluster IV, *Clostridium* cluster XIVa, Bacteroidetes, *Lactococcus lactis*, *Bifidobacterium* spp., *Eubacterium spp.*, *Lactobacillus delbrueckii*, *Roseburia* spp., and *Faecalibacterium prausnitzii* are associated with a healthy GM composition (Bolte *et al.*, 2021; Núñez-Sánchez et al., 2022). Graph 4.13 gives a visual indication that the presence of genera including *Faecalibacterium*, *Christensenellaceae* and *Methanobrevibacter* are required in the GM to correct gut dysbiosis in CD patients (Sarrabayrouse *et al.*, 2020). These results expand the boundaries of our understanding of the vital role that the presence of certain bacteria in the donor's sample can play in the clinical remission of the receiver. This important role is again highlighted in Figure 4.4 which shows the relative abundance of genera existing in the GM post FMT treatment. This positive microbiome community shift in the GM of UC patients after FMT indicates how this treatment method can be used in modern Western medicine as an effective treatment method to successfully alter the GM of patients suffering from CD and Colitis (Huang et al., 2022). This knowledge of both the beneficial and detrimental effects of certain bacterial spp. present in the GM could alter the use of future FMT treatments. Focusing only on the presence of certain bacterial spp. in the GM instead of completing a whole microbiome transplantation could help increase the success rate of future FMT processes. This could also lead to increased patient

acceptance of this disease treatment method. Due to the promising progression of FMT as a disease treatment method, it is evident that further future knowledge on the ideal bacterial spp. needed for successful treatment is required urgently. It was pinpointed during this research as one of the main areas in FMT that requires the most immediate focus and development.

Figure 4.4 also highlights that the patients who responded positively to FMT had higher levels of *Faecalibacterium* present in their GM (Huang et al., 2022). This correlates with results obtained from a review completed in 2023 which analysed the dietary exposures and interventions in IBD. This review outlined that an important role of *Faecalibacterium prausnitzii,* the sole spp. of the *Faecalibacterium* genus, in the GM is the production of biologically active SCFAs (Gubatan *et al.*, 2023). The main SCFA produced by *Faecalibacterium prausnitzii* is butyrate. Butyrate plays a key role in the success of the FMT process. As previously discussed in Section 1.3.2, the role that FMT can play in the increased production of GM derived SCFAs has been linked with a strong immunomodulatory effect. Increased levels of butyrate post FMT helps reduce intestinal permeability which is necessary for maintenance of the epithelial barrier's integrity (Shen *et al.*, 2018). The role that SCFAs play in the diet was discussed in detail in Section 2.4. Similarly, Section 2.4.1 discussed results obtained by Bolte *et al.* which reported that the consumption of fish and plant-based foods can result in increased levels of *Faecalibacterium prausnitzii* in the GM. The presence of this bacterial spp. in the GM has also been positively linked with increased microbial metabolism of polysaccharides and reduced levels of pathobionts (Bolte *et al.,* 2021). The correlation between the presence of this gram-positive, mesophilic, anaerobic bacterial spp. in patients' GM post FMT and the alleviation of symptoms for these suffering patients would indicate that the presence of certain bacterial groups such as *Faecalibacterium in* the receiver's GM may be necessary for the success of the FMT treatment method. During this research, it was observed that high levels of *Faecalibacterium prausnitzii* present in the GM composition showed best results for a successful FMT treatment. Going forward, the presence of high levels of this bacterial spp. in the GM should be used as an indication of a healthy GM composition. When the FMT receiver's GM levels of *Faecalibacterium prausnitzii* increase to match that of the donor, it is a strong indication of the success of the treatment. The strong anti-inflammatory response and protection of the epithelial barrier associated with the presence of this bacterial spp. ensures host homeostasis in the receiver. In the

future, high levels of *Faecalibacterium prausnitzii* present in the receiver's GM post FMT should be used as a clear indicator of successful FMT and increased levels of remission in receiving patients. By alleviating intestinal inflammation, the increased gut microbial presence of this bacterial spp. through use of FMT can play a major role in the treatment of CD and Colitis. It was noted during this research that while the analysed studies did mention the importance of high levels of *Faecalibacterium prausnitzii* being present in the receiver's GM post FMT, no study analysed the presence this bacterial spp. in the donor sample during the donor screening process. Future studies in this area should analyse in great depth, this link between the presence of *Faecalibacterium prausnitzii* in the donor sample and the reduction of CD or Colitis symptoms in patients who underwent FMT. Future clinical studies providing more scientific data on this positive link are necessary before any associated claims can be clinically proven. If this novel angle of analysing the FMT success rate is clinically proven as a result of these future studies, it would ensure the success rates of FMT in suffering patients being increased dramatically, based alone on careful donor selection and increased steps in the donor screening process.

5.4 The importance of patient acceptance of Faecal Microbiome Transplantation

It is evident from the research completed during this study that while usage of this treatment method is on the rise, and the associated health benefits have been clearly outlined, there are still a lot of challenges that FMT must overcome before being fully accepted as a CD or Colitis treatment option in modern Western medicine. Such challenges include lack of patients' education on what the treatment process actually entails, lack of physicians making this treatment method available to their patients, the major level of taboo and lack of patient acceptance associated with this treatment method due to the origin of the bacterial spp. used, the slow speed of its research and development due to the lack of willing participants that are necessary for clinical trials, the lack of a clear global regulation regarding its usage and the need for globally accessible universal stool banks. As outlined in Section 2.5, future access to universal stool banks is necessary to ensure the standardisation, superior quality, and safety of using this process for the treatment of CD and Colitis (Chen et al., 2021). In order to become accepted as a commonly used treatment method in modern Western medicine, FMT must also

overcome many ethical issues. As highlighted in Section 1.5, the ethicalness of FMT is one of the main controversies associated with it. Examples of such ethical issues include the associated public health implications, ensuring patients are fully educated on the process before treatment and issues regarding the informed consent and potential exploitation of receiving patients (Zhang *et al.*, 2019).

As outlined in Section 4.5, Zhong *et al.* published the results of a survey in 2020 which analysed the awareness and attitudes of IBD patients in China regarding the use of FMT as a treatment method. Results from analysis of this survey showed that 44.6% of patients who completed this survey did not know that FMT was a treatment option available to them. This highlights the gap of knowledge and lack of patient education surrounding the process involved in this treatment method. It also highlights the lack of patient knowledge on the availability of this treatment method to them as an alternative novel approach to alleviating the symptoms of diseases such as CD or Colitis. These results indicate that there is a direct link between FMT taboo and a lack of knowledge on the safety and efficiency of the procedure. Another interesting result from this survey was that 63.2% of participants stated that if needed, they would agree to receiving the FMT as a disease treatment method. This high percentage of participants that were open to the treatment method is very impressive when considering that up to 44.6% of these patients did not even know that this treatment method was an option available to them at the start of the study. This result again emphasizes the fact that the taboo associated with FMT is strongly linked with patients' lack of understanding of its efficiency and safety rates. During this research, it was evident that CD and Colitis patients have an overall poor recognition of what the FMT process consists of. In future studies, the reasoning behind this lack of education should be explored in detail with the hope of increasing awareness of the process and educating CD and Colitis patients on the uncommon alternative disease treatment methods that are available to them. Physicians' knowledge on the FMT process must also be assessed in future studies to ensure they are supplying their patients with up-to-date and accurate information on this alternative disease treatment method. This study also reported that 95.6% of surveyed patients who underwent FMT reported that they were satisfied with the results. This high percentage of patient satisfaction shows that FMT can be used efficiently and safely to treat gut dysbiosis related diseases such as CD and Colitis but common use of this disease treatment method is still limited due to both the lack of education and the lack of knowledge related to the treatment method (Zhong

et al., 2020). The findings from this research indicate that there is an overall poor recognition and understanding of the process of FMT amongst CD and Colitis patients when compared to other treatment methods used in modern Western medicine. Future studies need to focus on the reasoning behind the low awareness of this procedure in order overcome it. Patient education on the FMT procedure through physician's recommendation will help increase the acceptance of this procedure in the future (Zhong *et al.*, 2020).

Based on all the research analysed on the taboo associated with this alternative treatment method for gut dysbiosis related diseases, it was determined that an obvious way to increase future patient acceptance of FMT would be the development of a new delivery route which would originate from a synthetic group of specific microbial spp. This would allow for the treatment method to become patient specific as it would allow for the alteration of the receivers GM with specific beneficial bacteria only. If this new delivery route were specific to the patient's GM needs, it would also reduce FMT failure rates, making the treatment method even more desirable to receiving patients. This delivery route could also be encapsulated and consumed orally to help increase convenience and ease of administration while also reducing associated risk, the degree of invasiveness and treatment costs. In the future, in order to increase patient's acceptance of this disease treatment method, the actual name of the treatment method itself should also be analysed to determine if this is in fact one of the major reasons behind the taboo and lack of patient acceptance associated with FMT. Having the word faecal in the name of this disease treatment method may be very off-putting for some patients, making the delivery method undesirable in their opinion. Future studies in this area should analyse if renaming this process alone would result in increased patient acceptance. Going forward, increased education on the topic of FMT will be crucial to its progression and will lead to increased acceptance for its use in modern Western medicine as an effective disease treatment method for patients suffering from CD and Colitis.

5.5 The health benefits associated with the use of Faecal Microbiome Transplantation

During this research, it was established that many health benefits have been associated with the use of FMT as a restorative therapeutic technique for the treatment of gut dysbiosis related diseases. As outlined in Section 1.3.2, these health benefits include reduced colonic inflammation, increased production of SCFAs, increased immune function, maintenance of the epithelial barrier integrity, colonisation resistance and the restoration of metabolites in the GM. FMT can also overcome antibiotic-resistant pathogens in the GM, making it the ideal treatment option for patients when antibiotics are no longer and effective treatment method (Shen et al., 2018; Ademe, 2020).

5.5.1 The role that the Gut Microbiome plays in the maintenance of a healthy body

During this research, the role that the GM plays in the maintenance of a healthy body has clearly been defined. Section 1.2 discusses in detail the major influence that the composition of the GM has on a person's mucosal immune system's functional and structural development. The GM is also necessary for healthy bodily functions such as immune defence, metabolism, and behaviour. During this research, many factors were identified as been responsible for this microbial GM composition. Such factors include antibiotic, prebiotic or probiotic consumption, stress, diet, age, genetics and ethnicity, geographical location, environment, socioeconomic status, and birthing delivery route (Hasan and Yang, 2019). Due to the large number of factors associated with this composition, it is evident that future studies are required to the determine the exact factors that have the most influence on the GM composition. Gaining this knowledge in the future will help to determine what exact GM altering factors are responsible for the presence of certain bacterial spp. in the GM. In terms of the link between the alteration of the GM composition and the development of many gut dysbiosis related diseases, it is crucial that this gap in knowledge regarding the most influencing GM altering factors must be further analysed. Gaining this knowledge is crucial to the progression of FMT as a readily available disease treatment method as it will ensure the future maintenance of long-term patient clinical remission post FMT. Since altering the GM composition to match that of a healthy donor through the use of FMT has shown to reduce the symptoms associated

with many gut dysbiosis related diseases such as CD and Colitis, it can be hypothesised that improving the long-term success rate of this treatment by ensuring controllable limiting factors are reduced, will help improve patient quality of life and increase patient acceptance of the treatment method.

5.5.2 The scarcity of data available on the long-term health benefits for Faecal Microbiome Transplantation for patients who maintain a specific diet

Section 2.4.2 outlines the important link between the success rate of FMT and the patient's diet. This section discusses in detail the results from a 2021 study which analysed the diet of patients who received autologous FMT as a treatment method to help prevent weight regain after a weight-loss phase. These results reported successful FMT in patients on a green-Mediterranean diet, enriched with green-tea and Wolffia globosa (Mankai) green plant. These results reported that the success rate of FMT was linked to increased levels of bacterial spp. that are associated with the GM of a healthy lean person. These bacterial spp. included *Alistipes putredinis, Bacteroides vulgatus,* and *Bacteroides uniformis*. These results are relevant to this research as they highlight the important role that diet plays in ensuring successful FMT in patients (Rinott et al., 2021). Another study in 2021 outlined the importance of fibre in the diet of FMT patients due to fibre's role as a GM modulator (Clancy et al., 2021). During this research, the scarcity of data currently available on the long-term associated health benefits of patients maintaining a specific diet was clearly evident. Future studies are urgently needed to determine if there is in fact an ideal diet needed for increased success rates of FMT in patients suffering from gut dysbiosis related diseases or as with the delivery route if there is no one-fits-all solution.

5.6 The importance of choosing patient specific delivery routes for Faecal Microbiome Transplantation

Table 4.2 shows results from a pilot study carried out in 2019 which analysed the role of FMT in the maintenance of remission in UC patients. The results summarised in this table highlight that the patients who received FMT with SOC reported a higher rate of maintenance of long-term steroid-free clinical remission compared to those in the placebo group. The success rate of the FMT procedures obtained in this pilot study were

accredited to the use of a colonoscopy as the delivery route. This hypothesis that undergoing a colonoscopy is the best FMT delivery route was based on the findings that gastric acid has the ability to destroy Bacteroides and Firmicutes, making administration via the upper GI tract less effective (Sood *et al.*, 2019).

In a review carried out in 2021 which analysed the negative side effects and safety issues associated with FMT, it was reported that the delivery route chosen had a direct link with the development of negative, short-term side effects. Table 4.3 lists the negative side effects reported from patients who underwent FMT via different delivery routes. In agreement to the results obtained by Sood *et al*., it was reported in this review that FMT via the upper GI tract resulted in more negative effects (43.9%) than if the treatment was delivered via the lower GI tract (20.6%) (Seon-Young Park and Geom Seog Seo, 2021). However, based on the research of many scientific papers that was completed as part of this thesis, it is evident that there are many advantages and disadvantages associated with using each of the available FMT delivery routes, with no specific delivery route been deemed as being the best overall administration route. In some cases, it has even been reported that in order to achieve best results, patients receiving multiple FMTs have undergone one FMT delivery route and followed it with a different delivery route (Gulati *et al* 2020). As outlined in Section 1.3.4, the suitable and most effective delivery route must be determined on a case-by-case basis as there is no one-fits-all delivery route. During this research on the FMT delivery routes, it was noted that the accessibility of these delivery routes needs to be improved going forward. More convenient, less expensive delivery routes will help improve usage rates of this treatment method.

The previously discussed study published by Zhong *et al.* in 2020 reported that results obtained from a survey on awareness and attitudes of patients regarding FMT showed that their choice of delivery route was influenced by their disease. UC patients reportedly preferred colonic TET as their FMT delivery route while CD patients had a preference of mid-gut TET. Zhong *et al*. hypothesised that the reasoning for this preference may be due to the fact that colonic TET via whole-colon medication administrations may benefit UC patients while giving EEN to CD patients through mid-gut TET may result in more success rates. (Zhong *et al.,* 2020). This again highlights the importance of having numerous FMT delivery routes available to receiving patients. The incorrect delivery route can increase chances of FMT failure and result in a lack of patient willingness to

retry the treatment method again, hindering the growth of FMT usage rates as a novel disease treatment method for CD and Colitis. Another important factor that must be considered regarding FMT delivery routes is that while use of a colonoscopy may ensure the survival of Bacteroides and Firmicutes in the donor's sample, if multiple FMTs are required, there is a low chance of patient acceptance of a colonoscopy when oral capsules are available as an alternative delivery route. The limitations of this statement by Sood *et al.* regarding a colonoscopy being the best FMT delivery route are also highlighted when compared to the results obtained from a randomized, prospective, single-centre pilot study completed in 2021. This study reported a strong correlation between the composition of the donors' and the receivers' GM for up to 20 weeks post treatment when FMT was delivered via daily, oral FMT. Figure 4.2 gives a clear visual of the Modified Mayo Score for the patients who underwent FMT and also for the placebo group. As can be seen from Figure 4.2, only 1 patient in the placebo group met the study definition of having a clinical response compared to 3 out of 6 of patients in the FMT group (50%). However, it must be noted that subject P in the FMT group underwent steroid-therapy at W8 which means their results could not be considered as valid. No patient in the placebo group achieved clinical remission while 33.33% of the patients who underwent FMT achieved clinical remission post treatment. These results show the positive health benefits for UC patients that can be achieved when undergoing treatment via consumption of daily encapsulated oral FMT (Crothers *et al.*, 2021).

During this research, it was discovered that the results obtained from this study completed by Crothers *et al.* were very similar to the results obtained from an Australian randomised, placebo-controlled, double-blind trial that was carried out in 2022. As outlined in Section 4.1.1, this Australian trial was completed in order to establish the efficacy of oral lyophilised FMT as a UC treatment method. At W8, 53% of the patients who underwent treatment were reported to be in corticosteroid-free clinical remission compared to only a 15% success rate in the placebo cohort. Negative symptoms were also lower in the group who underwent treatment at 67% versus 85% in the placebo group. For patients who underwent multiple FMTs, there was a 100% success rate in clinical, endoscopic, and histologic remission at week 56. For those who underwent single FMT, there was no patient still in remission at this stage. The importance of this study is that it highlighted the success rate of delivering FMT through the consumption of oral lyophilised FMT

while also indicating the importance of multiple FMT procedures compared to single FMT for ensuring a longer clinical remission in patients (Haifer *et al.*, 2022).

It must also be noted that while oral lyophilised FMT does show very promising results for use as a treatment method for CD and Colitis, there are still some obstacles that this delivery route must overcome. One of these obstacles is the storage conditions required for these capsules. As highlighted by Crothers *et al.*, there are still concerns regarding the freeze–thaw cycles that these capsules undergo as a result of poor storage conditions during patient transport and home freezer conditions. Regarding real-world clinical use of frozen FMT capsules, this obstacle must first be overcome before this FMT delivery route can become a frequently used treatment method for gut dysbiosis related diseases in modern Western medicine. It is worth noting that future research and development in this area should be focused on a more temperature stable formulation in order to eliminate this concern (Crothers *et al.*, 2021).

5.7 The importance of the patient's baseline characteristics in the success rate of Faecal Microbiome Transplantation

57% of the selected studies, (8 out of 14), highlighted the importance of the BL (day 0) characteristics of the patient. FMT failure occurs when the receiver's GM is not altered to match that of their donor's. One study reported FMT failure to be a result of the receiver's BL being high in multiple taxa from the Gammaproteobacteria class of the Proteobacteria phylum. This result would suggest that the receiver's BL can influence the clinical outcome of the FMT treatment by having an effect on the success rate of the alteration of the receiver's GM composition post treatment to match that of the donor's GM (Sokol *et al.*, 2020). This hypothesis was further backed up by another study completed in 2021 which outlined that the categorisation of UC patients into RE and NR phenotypes, based on their GM composition at BL, would determine their suitability for the FMT treatment method. Those belonging to the NR phenotype tend to have increased histologic and endoscopic scores and more severe mucosal damage at BL (Crothers *et al.*, 2021). Contradictory results were reported in a 2022 study that investigated UC patients' response to FMT. This study reported that BL characteristic were similar for patients in both the RE group and NR group (0.386 ± 0.153 vs. 0.561 ± 0.195, p-value = 0.438) but as evident from Graph 4.9 and Graph 4.10, at W8, there was a significant difference in

the microbial diversity of these two groups (0.030 ± 0.009 vs. 0.117 ± 0.044, *p*-value = 0.040). Graph 4.11 highlights via bar chart that at W8, the GM of the RE group had been altered to resemble that of the donors' GM. The results from this study therefore indicate that the GM composition at BL did not play a role in the FMT success rate (Huang *et al.*, 2022). Regarding future use of FMT as a gut dysbiosis related treatment method, analysis of patients' BL characteristics may become a crucial step in the process to help determine the success rate of the procedure, however due to the contradicting results obtained during this research, it is evident that further research on this topic is urgently required before any claims can be made. FMT failure has also been linked with mis-communication between the receiver's intestinal mucosa and the donor's GM (Sarrabayrouse *et al.*, 2020).

5.8 The importance of donor-patient matching in the success of the Faecal Microbiome Transplantation treatment method

As part of this dry research, it was discovered that a study was carried out on how the mucosal microbial load could be a potential predictor of a CD patient's response to FMT. The results obtained from this study indicate that tissue damage and cytokine release were significantly lower in non-inflamed CD tissues compared to inflamed CD tissues. During this study it was also reported that the GM composition of receivers whose mucosal samples had an initial low microbial load, was successfully altered to resemble that of the donor's post FMT with increased levels of *Faecalibacterium prausnitzii* present in their GM. These patients also reported having higher secretion levels of anti-inflammatory cytokines compared to the patients who had a high microbial load. These results indicate that undergoing FMT treatment during active inflammatory disease reduces the risk of a successful outcome. Based on these results, it is reasonable to hypothesis that in the future, in order to ensure successful colonisation, a patient's ability to undergo FMT may be determined by their tissue microbial load, with lower levels resulting in increased chances of the treatment being successful (Sarrabayrouse *et al.*, 2020). This study also put major focus on the importance of donor-patient matching. This study chose donors based on their stool sample's microbial stability, richness, composition, and diversity in order to reduce FMT failure. Results from this study found that the receiver's bacterial load was dependant on the bacterial load of the recipient tissue but independent of the donor origin (Sarrabayrouse *et al.*, 2020). Based on these results, it could be suggested

that the donor GM composition does have an effect on the tissue response in receivers. The results obtained in this study are crucial to the answering of the thesis research question regarding the effectiveness of FMT as a treatment method for patients suffering for CD and Colitis as they show that during the FMT process, the donor stool sample does not negatively affect the recipient mucosal barrier integrity. This knowledge proves the safety of using donor samples to help treat gut dysbiosis related diseases. This study also outlined how donor samples should contain low levels of *Enterococcus faecalis, Parabacteroides and Bacteroides*, as they are all linked to pro-inflammatory cytokine release. Results from this study regarding the donor selection process are relevant to this thesis as they highlight the importance of donor-patient matching in the success of the FMT treatment method. The importance of the donor screening process and donor-patient matching was also discussed in detail both in Section 2.5 and Section 4.4. A study carried out in 2022 found that the short-term efficacy of the FMT was affected by the receiver's own characteristics while long-term efficacy was affected by the donor selection. This again highlights the importance of donor-patient matching in the FMT process. Results from this study also reported a significantly higher cumulative non-relapse rate in patients who received their stool donation via their sibling compared to those who received their donation from a parent (p-value = 0.001)(Odakura *et al.*, 2022). This result highlights how age and genetics should play a combined major role in the donor selection process in the future. While these studies focused on the importance of donor-patient matching, a review carried out in 2021, assessed the results from 12 different previously published studies to analyse the use of super donors in FMT. This review reported that 83.33% of these studies found no statistically significant donor effect, indicating the uncertainty surrounding its actual clinical importance. The importance of these results is that they reported that the receiver factors were the main factors responsible for FMT success rather than donor factors (Olesen and Gerardin, 2021). This result contradicts the results obtained by Odakura *et al.* and Sarrabayrouse *et al.* regarding the importance of the donor effect. This contradiction highlights the immediate need to eliminate the current gap in available knowledge surrounding the donor effect to ensure the future of FMT as a disease treatment method for CD and Colitis.

5.9 The overall worldwide acceptance of the Faecal Microbiome Transplantation process and the absence of any Irish national protocol on this treatment method

The data obtained as part of this research highlighted how the current worldwide usage levels of FMT as a disease treatment method, is still mostly used to treat rCDI, but only as an alternative treatment method when other methods such as antibiotics are deemed to be ineffective. Section 1.4 outlines how the current European clinical usage rate of FMT as a treatment method for rCDI patients is still relatively low at approximately 10% (Baunwall *et al.*, 2021). It is evident from the data obtained during this research that there is a major worldwide variance in FMT regulatory classification. The leading countries involved in FMT research worldwide are reported to be the USA, followed by China, Canada, and France respectfully. In relation to centrality of FMT research, the top 5 most influential countries were reported to be France, England, USA, Spain and Germany (Fengwei Zhang *et al.*, 2022). It must also be noted that one major limitation of the FMT process is the lack of a clear global regulation surrounding FMT. Until this global regulation has been determined, there will be continued major restrictions in its use as a disease treatment method in modern Western medicine. There should be a global focus on the completion of future studies to help determine a global regulation surrounding FMT, in order to reduce this limitation in the progression of this process as a novel disease treatment method for CD and Colitis. Section 1.4.1 of this thesis outlines the clear contrast between the overall worldwide acceptance of the FMT process and the absence of any Irish national protocol on this treatment method. To date, there is an incredibly low acceptance of this treatment method in Irish hospitals regardless of a clear desire by Irish clinicians to use this process as a disease treatment method. It was observed during this research that the reasoning behind this lack of Irish acceptance of this process may be due to the lack of access to frozen pre-screened stool and challenges with donor selection (Scheeler *et al.*, 2019).

5.10 Limitations of Faecal Microbiome Transplantation

While the research completed during this study did observe a clear beneficial link between the use of FMT and the alleviation of symptoms in CD and Colitis patients, it was also noted that there are still some major gaps in the available knowledge surrounding this

area of study which have limited its progression as a commonly used disease treatment method for CD and Colitis. Examples of these limiting gaps of knowledge include determining the most efficient way to ensure accurate donor-patient matching, the effectiveness of using super donors, the level of uncertainty surrounding the power of the donor effect, the lack of certainty surrounding the ideal dosage range needed for successful treatment and the lack of focus on the presence of certain bacterial spp. in the GM instead of completing a whole microbiome transplantation. These are some of the main issues related to the FMT process that require immediate future analysis. When these gaps in our knowledge no longer exist and there is reduced uncertainty in the safety and efficiency in the process, the use of FMT as a gut dysbiosis related disease treatment method will then be able to progress at rapid speed for use in modern Western medicine. In future studies, these knowledge gaps should be analysed in the form of long-term, large-scale, placebo-controlled clinical trials. It must also be noted that during this research, it was evident that none of the analysed scientific papers had considered the role of fungi and viruses in the GM composition. Future studies must determine the part that fungi and viruses play in the maintenance of a healthy GM and outline the effects that FMT has on the alteration of their levels in the GM post treatment.

5.10.1 The limited effectiveness of using only single Faecal Microbiome Transplantation

Interestingly, during this research, it was found that single FMT has a much lower success rate than multiple FMT procedures. As seen in Graph 4.12, a Japanese clinical trial that analysed the safety and efficiency of FMT in UC patients, reported that there was no significant alteration of the receivers' GM compositions (p-value = 0.08) post FMT. The lack of a significant change in the GM levels of microbial spp. such as *Faecalibacterium prausnitzii, Ruminococcus* spp. K-1, *Bacteroides vulgatus, Streptococcus thermophilus, and Bifidobacterium longum*, shown in this graph, gives a clear indication that the single FMT procedure failed. This failure indicates the limited effectiveness of using only single FMT as a way to treat gut dysbiosis related diseases (Shinta Mizuno *et al.*, 2017). In contrast, multiple FMT treatments report much higher levels of success. It is clear from the research completed in this study that by undergoing multiple FMT treatments, patients can increase their chances of being in clinical remission for a longer period of time.

5.11 The major findings from this research

The analysis of the results obtained in the selected 14 scientific papers, allowed for a much wider evaluation of the effects of FMT as a disease treatment method. Comparing and contrasting the results obtained from different studies allowed for the results to be viewed from an unbiased novel angle. This investigation resulted in the observation of a clear link between high levels of *Faecalibacterium prausnitzii* present in the GM of the receiver and the success rate of FMT. It was also determined from this research that while the donor selection process is very extensive, there is currently more of a focus on the donor's health status rather than on the exact bacterial spp. present in their GM. Going forward, analysis of the exact bacterial spp. present in the donor's sample must be analysed during the selection process. Only specific GM compositions such as those with high levels of *Faecalibacterium prausnitzii* and low levels of *Streptococcus* spp. *and E. coli* should be accepted for the stool donation process. The anti-inflammatory response associated with the presence of high levels of *Faecalibacterium prausnitzii* in the GM should be studied in the future in greater detail to determine the exact levels required to ensure the success of FMT as a disease treatment method for CD and Colitis.

In terms of the future of FMT, its safety levels in high-risk patients must be further studied in detail and acceptability of its use for high-risk patients must be fully established. To date, the majority of FMT trials have been carried out on only low-risk patients. As highlighted by Seon-Young Park and Geom Seog Seo in 2021, future trials must also ensure that for any patients with a greater risk potential, addition donor screening and donor-patient matching must be completed to help eliminate the risk of transmitting undesirable microorganisms to the immunocompromised receiver. Failure to detect pathogens in donor samples during the donor screening process could have fatal consequences for high-risk patients. The lack of studies being completed in CD and Colitis patients with more severe symptoms has hindered the acceptance of this disease treatment method due to the fact that the exact potential and limits of this process are still not fully analysed or understood. In order to overcome this issue, more large-scale, long-term, clinical trials that focus on high-risk patients are required for the progression of the acceptance of this disease treatment method.

As outlined by Allegretti *et al*. in 2019, there are many potential directions that can be taken in the future to eliminate issues associated with the use of FMT. For example, due

to the relative novelty of using this disease treatment method for CD and Colitis patients, there is currently very limited data available on its effect on the long-term clinical remission of these patients. Future trials on the long-term remission rates of FMT will allow suffering patients to make educated decisions on its efficiency (Allegretti *et al.*, 2019). Future progression in the knowledge of the long-term effects of this process will help determine the exact benefits associated with using FMT as a long-term treatment for CD and Colitis. Increased knowledge in this area may also lead to a wider patient acceptance.

5.12 Chapter Conclusion

It was observed during this research that all analysed studies discussed in detail the potentially positive use of FMT as an alternative treatment method when other treatment methods were no longer a viable option, yet no study outlined the actual potential of this treatment method as a first-time approach to tackling these gut dysbiosis related diseases. Based on the positive results observed during this research, it can be concluded that FMT has the potential to be successfully used as a novel first-time main-stage treatment method for the treatment of symptoms associated with CD and Colitis. Future studies should analyse the success rate of the alteration of the GM via FMT as a first-time treatment method for treating diseases such as CD and Colitis.

The research completed during this thesis adds to the overall knowledge base of the use of FMT as a disease treatment method. The knowledge gained during the research gives evidence to the massive future potential of the FMT process in modern Western medicine. From analysis of all the results obtained during this research, it is evident that the alteration of the composition of the GM with FMT can be used effectively in the future as a disease treatment method in modern Western medicine for the alleviation of associated symptoms in patients suffering from of CD and Colitis. These symptoms include bloating and wind, bowel incontinence, diarrhoea or constipation, abdominal pain, rectal bleeding, fatigue, dehydration, weight loss, joint pain, and bone pain. As there is currently no cure for CD or Colitis, future use of FMT as a main-stage treatment method for these gut dysbiosis related disease has a huge potential role in increasing patient quality of life by alleviating these associated symptoms. By focusing on what specific bacterial spp. are necessary for the successful alteration of the receiver's GM instead of

completing a whole microbiome transplantation, FMT can be used effectively as a first-time approach treatment method in the future to help alleviate patients' symptoms. Going forward, by focusing solely on ensuring the presence of high levels of *Faecalibacterium prausnitzii* in the chosen donor sample through an additional step in the donor screening process, future FMT patients may have increased success rates of higher levels of this anti-inflammatory and epithelial barrier protecting bacterial spp. being present in their GM composition post treatment. This additional step in the donor screening process may also lead to more long-term clinical remission in receiving patients.

Chapter 6: Conclusion, Limitations and Future Work

6.1 Conclusions

In conclusion, this dry research has outlined how FMT can be used as an effective disease treatment method in modern Western medicine for alleviating the symptoms of CD and Colitis through the alteration of the GM. The results obtained during this research indicate the clinical efficacy associated with the use of FMT for the alleviation of CD & Colitis symptoms such as Hematochezia, abdominal pain, fever, diarrhoea, flare-ups, and steroid dependency. As there is currently no cure available for these gut dysbiosis related diseases, patient quality of life and the alleviation of these associated symptoms is the main focus for their treatment.

From the analysis of all the relevant and available data that was gathered on this topic during this research, it was evident that the alteration of the receiver's GM with specific bacterial spp. results in a positive microbiome community shift in the GM and is crucial for ensuring the success of FMT as a disease treatment method. The relative abundance of genera present in the GM post FMT has been strongly linked with the receiver's treatment outcome and highlights the importance of both careful, specific donor-patient matching and the need for increased steps in the donor screening process to determine the bacterial spp. present in the donor sample. The safety of using donor samples in FMT for the treatment of gut dysbiosis related diseases has been outlined during this research through the analysis of studies completed on the receiver's bacterial load being dependent on the bacterial load of the recipient tissue but independent of the donor origin (Sarrabayrouse *et al.*, 2020). This research indicates that the donor GM composition positively effects the tissue response in receivers while still maintaining the recipient's mucosal barrier integrity.

A major observation from this research is that patients responded more positively to FMT when higher levels of *Faecalibacterium prausnitzii* were present in their GM post treatment. The presence of this anaerobic gram-positive, mesophilic, SCFA producing bacterial spp. is very desirable in the GM as it has been positively linked with a healthy GM composition due to its protective, and anti-inflammatory properties. Going forward, in order to increase the success rate of FMT in patients, it was concluded from this research that an extra step should be added to the donor screening process to help determine the presence of this bacterial spp. in the donor sample before it is accepted as suitable for donation. The presence of high levels of *Faecalibacterium prausnitzii* in the

donor sample would increase the receiver's treatment success rate and would also increase patience acceptance of the disease treatment method.

Overall, FMT has a very promising future as a disease treatment method for CD and Colitis. It is however evident from this research that there are still many gaps in knowledge and limitations associated with this treatment method that must be first analysed and understood before it can be fully accepted as a mainstream disease treatment method for CD and Colitis in modern Western medicine. One major limitation of FMT is the taboo associated with it. Future acceptance of this disease treatment method in modern Western medicine is largely dependent on patient acceptance. It is evident from this research that future studies must put a major focus on limitations such as the reasoning behind this taboo, the lack of associated patient education and the scarcity of medical awareness of this procedure, in order improve its future usage levels. Future studies should also focus on ways to limit the taboo associated with FMT. An area of concern that should be further analysed is the number of patients whose FMT acceptance is based alone on the fact that the word faecal is in the name of this treatment method. Another current limitation of FMT is that there is currently no available data on the role that fungi and viruses can play in the GM. Future studies are required to analyse these roles and determine if they play any part, positive or negative, in the alteration of the GM post FMT treatment.

The evaluation of available data that was completed during this research allowed for a broad unbiased observation of the FMT process and its role as a disease treatment method for CD and Colitis from a novel angle. This unbiased observation allowed for the conclusion that there is a future potential for the use of FMT as a first-time, main-stage disease treatment method for CD and Colitis in modern Western medicine with the aim of improving patient quality of life.

6.2 Limitations of this research

It must be noted that this research was subject to some limitations. One major limitation of this research was the lack of access to current, on-going medical trials that are closed off from public access due to General Data Protection Regulation (GDPR) and ethical reasons. Restricted access to such trials has limited this research by preventing the

analysis of the newest developments in this area of medicine. The inaccessibility of this new data may have limited and affected the overall conclusion of this study.

Another limitation that must be noted in this research is the methodology used to obtain the results. The methodology chosen was dry research. This means that no trials or surveys were conducted as part of this research. The completion of a trial which assessed the long-term effects of FMT on the clinical remission of high-risk CD and Colitis patients could have further emphasised the results obtained in this research but due to time constraints and available resources, this route was not a viable option.

Data excluded from this research includes scientific papers that have not been written in the English language. Going forward, these papers should be carefully examined as the results obtained may lead to increased knowledge on the topic of FMT as a treatment method for gut dysbiosis related diseases. The exclusion of these scientific papers from this research may have limited the findings of this study.

All of these limitations should be considered when interpreting this research and be addressed in future studies to reduce any gaps of knowledge on the topic of using FMT as a disease treatment method for CD and Colitis.

6.3 Recommendations and Future Work

As previously mentioned, future FMT studies must focus solely on the presence of certain bacterial spp. in the GM instead of completing a whole microbiome transplantation. Gaining knowledge on how the presence or absence of certain bacterial spp. in the GM alters the patient's health could help determine the exact GM composition required in a donor sample to ensure increased success rates in future FMT procedures. Determining the presence of high levels of *Faecalibacterium prausnitzii* in the receiver's GM post FMT could be used in the future as an indication of clinical remission in these patients. However, it must be noted that future clinical studies providing clear scientific data on this link must first be completed before any claims can be clinically proven. If clinically proven, this novel approach to ensuring increased success rates of FMT, by careful donor selection and increased steps in the donor screening process, would result in the progression of its approval as a disease treatment method for CD and Colitis in modern Western medicine.

Based on this research, it has been concluded that future work on the development of a new synthetic, patient specific, FMT delivery route is urgently required to help reduce FMT failure rates and increase patient acceptability. It was also noted during this research that there is very limited available data on the GM altering effects of FMT on high-risk CD and Colitis patients. Future studies on the safe use of FMT in high-risk immunocompromised patients are urgently required. The scientifically proven safety of this disease treatment method through the completion of multiple long-term clinical trials is necessary in the future before this treatment method can be accepted as suitable for these patients (Seon-Young Park and Geom Seog Seo, 2021). The lack of studies on high-risk patients has slowed the overall progression of the use of this disease treatment method in modern Western medicine for the treatment of CD and Colitis. The completion of future trials on the long-term remission rates of FMT are also necessary to determine the long-term efficiency of this disease treatment process (Allegretti *et al.*, 2019).

Future studies are required to examine the role that specific diets can play in increasing the success rate of clinical remission in CD and Colitis patients post FMT treatment (Gubatan et al., 2023). These future studies should also examine the role that the donor's diet plays on the success of the FMT process and determine if there is a need for the analysis of the donor's diet to become part of the donor screening process in the future (Clancy, Gunaratne and Borody, 2021).

Chapter 7: References

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