Microbiome and Inflammatory Bowel Diseases
A Review of literature of therapeutic strategies in treating gut Microbiota in Inflammatory bowel diseases.

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Summary

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Research Title: Microbiome and Inflammatory Bowel Diseases-A review of literature of therapeutic strategies in treating gut Microbiota in Inflammatory bowel diseases.

Aims: To do a review of literature about therapeutic strategies in treating gut Microbiota in inflammatory bowel disease.

Objectives:

1. To identify the changes of diversity of Microbiota during Inflammatory bowel disease.
2. To analyse the relationship between gut Microbiota and intestinal immunity in inflammatory bowel disease.
3. To review the effectiveness of Faecal Microbiota transplantation and probiotics in treating inflammatory bowel disease.

Abstract of thesis

This report is a summative review of research literature pertaining to the therapeutic strategies in treating gut Microbiota in Inflammatory Bowel Disease (IBD). Intestinal Microbiota has demonstrated some role in treating IBD and in pathogenesis. There are two debatable theories on this subject. The first one advocates a causal relationship with dysbiosis as a cause for IBD. The second theory states that a decrease in Microbiota diversity (dysbiosis) is a secondary phenomenon caused due to intestinal inflammation. This report has reviewed multiple reliable and credible research papers and publications and has concluded that the cause-effect relation is still controversial as it is could not be empirically established how dysbiosis leads to mucosal inflammation. Moreover, therapeutic treatments in correcting deficient microbial diversity through the use of antibiotics, probiotics and Faecal Microbiota transplantation (FMT) is studied and evaluated in detail for its merits and demerits. This review concludes that antibiotics and probiotics have their minor role in specific IBD
situations, and FMT can be beneficial and promising in treating IBD, despite its limitations which call for further controls and standardisation.

**Methodology**

For the purpose of this literature review, electronic searching of published articles were extracted and reviewed from sites such as PubMed, Science direct, and European Crohn’s and Colitis organisation (ECCO). 70% of the selected articles are dated within the last ten years.

**Conclusion**

Radical shifts have occurred in the knowledge surrounding the etiopathogenesis of IBD in the past 10 years with the introduction of modern techniques that can distinguish the gut microbiome more precisely and analyse host genome so that the vast genetic world of IBD can be studied. Dysbiosis of the gut microbiome can be found at the centre of the inflammatory process of IBD, which could be influenced by host genetics and environmental factors such as diet. However, the exact role of dysboisis is not clear as studies are still attempting to determine whether if dysboisis is truly a causative factor or a consequence of the intestinal inflammation. Many studies have suffered to formulate final conclusions due to limitation in their studies.

The coming 10 years will assist to dismantle the details of the host immune defences which regulates the host Microbiome ecology. Clarity is required on the relationship of the host genotype and the degree of influence it has on the composition of the Microbiome. This could possibly introduce more personalised therapeutic interventions, incorporating gene expression profiles of immune cells, Microbiome composition, host genotype as well as serotype and the disease phenotype to deduce the most appropriate treatment strategy. Patient management will be greatly altered as tailored procedures would be implemented according to patient need rather than general standard therapy.
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Conflicts of interest

The author reports no interest of conflicts

Ethics committee approval

Approval from the bioethics committee was not required
Abbreviation

1) IBD- Inflammatory Bowel Disease
2) UC- Ulcerative Colitis
3) CD- Crohn's Disease
4) GI- Gastrointestinal
5) FMT-Faecal microbiota transplantation
6) CDI- Clostridum difficile infection
7) RCDI- Recurrent Clostridum infection
8) GMP- Good manufacturing practice
9) OTU-operational taxonomic units
10) FDA- food and drug administration
11) MDR- multidrug resistance
1. Introduction

Inflammatory bowel disease (IBD) is a chronic, relapsing-remitting inflammatory disorder of the gastrointestinal (GI) tract, which is comprised of two primary disorders: ulcerative colitis (UC) and Crohn’s disease (CD) [1]. UC is a chronic inflammatory condition characterised by relapsing and remitting episodes of inflammation limited to the mucosal layer of the colon. In contrast, Crohn disease can affect any part of the gastrointestinal (GI) system from the mouth to the pre-anus and is characterized by transmural inflammation and by skip lesions [2]. These disorders have both distinct and overlapping pathologic and clinical characteristics; however there is a knowledge gap in the pathogenesis of this disease. Contributing factors are considered to be genetics, alteration gut microbiota and environmental factors. IBD commonly presents in early adulthood; 50% of diagnoses occur prior the age of 35[3] and studies indicate that IBD in children is rising worldwide [4][5].

There are limited known methods of treatment such antibiotics, probiotics and Fecal Microbiota Transplantation (FMT). The clinical usage of FMT is on the rise for the remission of IBD through manipulating the Microbiota of the gut, since the success of using antibiotics to treat IBD is varied and minimal in extreme cases. Due to the adverse effects of antibiotics and the temporary nature of probiotics as a therapy for IBD, FMT is presented as a solid treatment to influence the intestinal Microbiota composition in treating IBD. Studies indicate that FMT is a successful method to treat C. Difficile infections. FMT offers a better solution compared to antibiotics by enhancing the diversity levels of the bacterial composition in the IBD patients. Unlike, the use of probiotics, FMT offers a long term engraftment of the bacterial base in the receiving individual (Hamilton et al, 2013) [6]. Bojanova and Bordenstein (2016)[7] noted that the scale of FMT transmission is wider and broader in composition as compared to probiotic therapy since a gram of stool contains more than 1011 bacterial cells in addition to other taxa. Given that IBD results from an alteration in the gut microflora, increasing diversity within the microflora provides FMT merits and makes it more viable compared to use of antibiotics or probiotics in treating IBD.
2. Aims and Objectives of thesis

Aim: To do a review of literature about therapeutic strategies in treating gut microbiota in inflammatory bowel disease.

Objectives:

1. To identify the changes of diversity of Microbiota during Inflammatory bowel disease.
2. To analyse the relationship between gut Microbiota and intestinal immunity in inflammatory bowel disease.
3. To review the effectiveness of faecal Microbiota transplantation and probiotics in treating inflammatory bowel disease.
3. Review of Literature

3.1. Epidemiology

Highest incidents of paediatric IBD is prevalent in North America and Europe, however it is increasing in developing countries due to lifestyle changes. Kugathasan et al [8] explored the occurrence of IBD in a city of the United States and discovered that 5 per 100,000 to 11 per 100,000 were in children (4.56 per 100,000 for CD and 2.14 per 100,000 for UC), highlighting that CD is more prominent in children than UC [9]. A Canadian study published incidence of paediatric IBD increased from 9.5/100,000 in 1994 to 11.4/100,000 in 2005 [10]. Another study conducted in Scotland by Herderson et al [9] revealed the incidence of IBD was 7.82 per 100,000 person per years in subjects younger than 16 years. Hildebrand et al[10] observed a significant increase in the incident of paediatric IBD (7.4 per 100,000 person per year) in northern Stockholm between 1990-2001[10]. Sydney Children’s Hospital, Randwick (SCHR) studied the incidence of IBD in children of Middle Eastern descent, which highlighted that incident of IBD in 2006 was higher (33.1 per 100,000 children per year) than the control group (4.3 per 100,000 children per year) [11].

Studies from a range of countries portray results of an increase in IBD, particularly in adults [12][5]. Around 25% of patient diagnosis of IBD commonly occurs in the first 2 decades of their life [13][14]. Most patients are commonly diagnosed in childhood (13-18 years) and it’s incidence is rising in the early second decade of life [4]. The highest prevalence for UC and CD is in Europe, 505 per 100,000 persons and 322 per 100,000 persons respectively.

Systematic review led by Molodescky et al [12] [15] to analyse the incidence and prevalence of UC and CD across different regions and over time revealed the incidence and prevalence of IBD were the highest in western countries, specifically in Northern Europe and Canada. It was deemed that the rise in incidences of IBD in developing countries could possibly be attributed by limited treatment availability, particularly in severely ill patients. Even though current research has not been able to elucidate detailed pathogenetic mechanisms of IBD, a strong correlation between IBD and environmental factors have been consistently detected worldwide.
3.2. Diversity of Microbiota

The Microbiota in the human gut is almost equivalent to ‘an organ within an organ’ which developed and evolved along with humans in a balanced symbiotic manner leading to intestinal homeostasis [16]. The recent ten years of research in the medical field related to IBD has developed the concept of changes or alteration in the gut microbiome which is otherwise terms as dysbiosis.

A healthy human gut contains diverse microbiomes in trillions which includes bacteria, comprising of more than 1100 species with a minimum of 160 species in every individual. An in-depth analysis of the human gut worldwide has shown the existence of bacterial ecosystem and communities that reveals the possibility of distinct and unique enterotypes including Bacteroides, Prevotella or Ruminococcus which are mostly dependant on the dietary habits, age of the individual and their Body Mass Index (BMI). Research has shown the existence of Bacteroides enterotype which is mostly associated with a western diet that is protein rich as against the Prevotella enterotype that is mainly a carbohydrate based diet. It is not yet established if western enterotype may influence or cause the development of IBD.

There is a change in diversity of Microbiota in patients with IBD. The most unique and significant change in IBD patient’s gut, is the reduction in the levels of phyla Firmicutes. Within these particular phyla, it is documented that there is a massive decrease in the abundance of F. prausnitzii in the case of patients with Crohn’s Disease. Yet in the case of paediatric patients it is noted that there is a massive increase in the levels of F.prausnitzii, which indicates that this particular bacterium has a dynamic function of being protective and hence multiplies at the beginning of the IBD.

Additionally, there is a specific reduction in the diversity of Firmicutes, with a reduction in the constituent elements in case of patients with IBD. On the other hand, there is an increase in the bacteria namely Bacteroidetes, which belong to a different phylum. However, certain studies have revealed that there is a decrease in the number of bacteria related to these species in certain instances. This could be possible due to the reorganisation of the bacteriodes in a different spatial order, in IBD patients, with Bacteroides fragilis contributing to the growth
and adherence of biofilm mass in the IBD patients. In a normal healthy microbiome, 90 percent of the bacteria belong to these two phylogenetic species. The alteration of these microbiome in IBD patients is observed in varied and disparate ways.

Pathogens from the phylum *Proteobacteria*, play a major role in IBD. An increase in this particular bacterial species is found while analysing the diversity of the microbiome which indicates that it plays an aggressive role in creating chronic inflammation in IBD patients. In case of ileal Crohn’s disease, there is an increase in the concentration of E.coli bacteria along with its pathogenic variants.

Metagenomic analysis has shown that there is a massive alteration in the microbiome in IBD patients showing 25 percent lesser genes and reduction in proteins and functional paths. Moreover, in CD patients there were changes in the bacterial metabolites as well interaction between the hosts and the bacteria leading to changes in the enzymes secreted by the host.

Diversity in the Microbiota can be measured through microbiome diversity index that helps in analysing the specific taxa in the gut and its deviations from the normal gut microbiome. Though studies have found that there are alterations in the microbiome in IBD patients, there exists a question whether dysbiosis is an epiphenomenon of IBD or whether is the cause for IBD still remains to be answered in detail [16].

It is essential that further research needs to be undertaken to establish the correlation between the Microbiota and their role in IBD. The composition of the Microbiota alone cannot help comprehend their functions since the bacteria is non-cultivable. Although diversity indices emphasise on the variety in gut microbiomes in the host, it remains unexplained on how the microbes actually acts upon the individual. Hence the need for metagenomic studies which is aimed at understanding microbial functions and the potential of these non-cultivated bacteria to cause any impact on the gut Microbiota [17].

The microbial populations of Lachnospiraceae and Bacteroidetes are diminished in IBD patients while the Proteobacteria families increase in volume. The changes or variations in the Microbiota are different in case of UC and CD. In case of UC patients, observations show there is a decrease in the volume of butyrate-producing bacteria *Roseburia hominis* and *Faecalibacterium prausnitzii* relative to a healthy person. Nevertheless, it is exactly opposite
in the case of CD patients who show an increased level of *F.prausnitzii* along with reduced diversity on the whole.

In CD patients, another research exhibited a decrease in *Dialisterinvisus*, which is mainly a different and uncharacterized species of Clostridium spp., in *F.prausnitzii*, and in Bifidobacterium adolescentis and an increase in *Ruminococcus gnavus*. The CD patients also exhibit a decrease in *F. prausnitzii* [18]. Additionally, another study by Sokol et al 2008, [19] proved that oral administration of *F.prausnitzii* as a probiotics reduced the severity of colitis in patients with CD and restored the dysbiosis. Although, the application of these bacteria sounds promising in the treatment of CD. However, this result does not show the relationship between Microbial dysbiosis and IBD but rather indicates that gut microbial imbalances are the most likely reason of disease severity[19].

Furthermore, In a research study that was conducted among six healthy participants and six CD patients, the metagenomic library drawn from their fecal samples found a existence of a broad diversity of microbiomes in the healthy individuals whereas, the diversity was inverse in case of the CD patient’s faecal library. This latter exhibited a reduction in the diversity of species that belonged to Firmicutes, mostly from the Clostridium leptum group.

In line with the findings of the earlier phylogenetic research studies, the metabolome of the IBD patients displayed lowered levels of butyrate,acetate, methylamine, trimethylamine,and increase in levels of amino acids. It was found that this showed the variation in the overall microbiome composition in the IBD patients which affected the short-chain fatty acid production and nutrient absorption. The metabolome of the CD patients showed more inflammation as compared to the UC patients. This is mainly caused due to the breakdown of amino acids and fats leading to production of metabolites. Another research study in both UC and IBD displayed an increased quantity in both taurine and cadaverine with IBD patients showing greater bile acid levels [16].

The viral elements in the gut microbiome, as seen from existing studies state that ‘phages’ comprise of the major viruses in the healthy as well as in individuals with dysbiosis. Certain studies have mentioned that healthy viromes coexists in a healthy gut, but others have stated that there is an increase in phage diversity in IBD patients [20].
3.3. Immunogenicity of Microbiota

Despite the changes detected in the intestinal microbiome, there remains a question whether the alterations in the gut microbiome is a response from the aberrant immune system or whether the abnormality in the intestinal Microbiota has led to aberrations in the immune response? This is a similar concept to what comes first ‘the chicken or the egg? Studies have found it difficult to conclude whether the genetic defects have led to dysbiosis or whether it is the other way round [17]

It is believed that the imbalance in the symbiotic relation between the host and the Microbiota, results in immune imbalance of the intestines which leads to IBD. This is based on the evidence that the Microbiota in the intestines is determined by the genotype of the host and their susceptibility and history of infections, usage of medicines and antibiotics and their dietary habits. On the contrary, it is worth noting that IBD cannot be necessarily caused only due to dysbiosis alone.[21].

Due to the significant role played by the gut microbiota in developing inflammation in the intestines, it is more likely to make it easy to speculate that it could be specific strains of bacteria that causes IBD in patients[22].

3.3.1. The relationship between dysbiosis and inflammation in the intestines

A research study conducted on IL-10 deficient mice, which was inoculated with a specific bacterial strain, showed inflammation in the cecum was caused due to *E.coli* induction, Distal colitis was caused due to *Enterococcus faecalis* induction, and there was no evidence for colitis in *Pseudomonas fluorescens* induction. Observations indicated that there was an increase in colitis in IL-10 (interleukin 10) deficient mice when induced with *Helicobacter hepaticus*. These evidences have presented that any alteration in the intestinal microbiome can lead to immune responses that are unique and distinct in nature, leading to a possible conclusion that dysbiosis has the potential to modulate intestinal immune responses.
Research studies have shown that the Microbiota in intestines has an important role in developing the immunity in the host gut. This is evident from the example where *Bacteroides thetaiotaomicron* has an impact on immune abilities of the host since the anti-microbe peptides like angiogenin are regulated in the epithelium tissue by activating the toll receptors. Furthermore, certain bacteria have shown the ability to enhance the immune functions in the host. The immune system in the healthy individual is able to tolerate the bacteria in the intestines, but for individuals having IBD, this mucosal immune system is less tolerant. This has caused abnormalities in the immune responses leading to susceptibility towards IBD in vulnerable persons.

Moreover, abnormality in the composition of Microbiota in the gut, namely dysbiosis is related with the IBD pathogenesis. This is evident from the massive reduction in the diversity of the gut Microbiota. Variations in the gut balance and the resultant intestinal dysbiosis have a massive relation in the pathogenesis of IBD. Yet, it is not clear on how the host develops inflammatory responses and ensures balance within the mucosal immune system and the intestinal Microbiota [23].

In conclusion, it is yet to be empirically established if dysbiosis consists of a primary or secondary phenomenon in IBD or is it causal in immune deficiency. As alteration in gut Microbiota is seen to be related to developing IBD, it is worthwhile to explore the results on administering probiotics and antibiotics in treating IBD. Hence the need to consider varied and customised treatment methods for individuals with IBD, for which we consider the use of probiotics, antibiotics and FMT as therapeutic intervention to induce clinical remission.

### 3.4. Antibiotics

Only a small number of studies point towards the benefits of using antibiotics specifically in the case of post-surgical or fistulising Crohn’s disease. This is due to the fact that antibiotic therapy reduces the symptoms of inflammation in the sinus tract caused due to bacterial overgrowth [24]. However certain studies by Wu et al (2015) [25] have pointed out that the severity of the disease cannot be always generalised or reproduced as the inflammation is not
measured on identifiable parameters but rather justified using symptomatic parameters. The overall bacterial growth is reduced through antibiotics thus decreasing substantial symptoms like diarrhoea or bloating, thereby reducing the disease severity. However, this does not always reduce the mucosal inflammation. Use of antibiotics holds the adverse effect of reducing the bacterial diversity thereby causing more harm to the underlying inflammation.

3.5. Probiotics

Probiotics are live organisms that are beneficial to the host. Probiotics may influence the gut microbiota composition, metabolic activity and immunomodulation when ingested in sufficient quantities[26]. Microbial diversity is altered as probiotics competitively inhibit other microbes, by increasing mucosal barrier function and through production of short chain fatty acids (SCFA) that stimulate the intestinal dendritic cells and triggers an anti-inflammatory response [27][28][29]. In order to cause the maximum effect, probiotics are obtained from humans which enables the gut bacteria to survive. Probiotics strains such as *Lactobacillus sp*, *Bifidobacterium sp*, *Sacchromyces boulardii*, *E. coli Nissle 1917* and VSL#3 are often used in the treatment of IBD [30][31][32][33][34][35][36][37]. The use of probiotics in IBD has been highly recommended a therapeutic strategy in treating patients with pouchitis for UC patients post-operation [37]. However, the information in regards to the role of probiotics maintaining remission in UC is not very reliable and the Cochrane Database System’s Review has advised against its use[38]. In terms of treating patients with CD probiotics does not report a significant body of evidence and the European consensus document have determined such treatments to be futile [39].

Use of probiotics is advocated as an additional method to improve the Microbiota in the gut through introduction of specific bacteria, in order to shift the gut composition towards a healthy state. This has the potential to curb the growth of pathogens in the intestines. Probiotics like *E. coli Nissle 1917* is proven to be effective as a mesalazine to promote remission of UC [40]. IBD is also controlled through the use of probiotics like *Lactobacillus GG*, *bifidobacteria* strains, and the yeast, *Saccharomyces Boulardii* (Zocco et al, 2006)[41]. Another proven supplement in probiotics is VSL#3 that has a significant impact on reducing severity of disease and causing remission in mild to moderately active UC patients [42].
Nonetheless, probiotics treatments act as a temporary measure since it is not seen to be engrafted in the host after 2 weeks of ceasing the probiotics intake [43].

There is a possibility that probiotics may cause an effect by modifying the intestinal microbiota [44] and the intestinal inflammatory response [45]. *F. prausnitzii* is a probiotic bacterium that protects humans from IBD, by suppressing the chronic inflammation in patients [46]. This bacteria triggers IL-10 in the murine dendritic cells which helps in preventing the build-up of chronic inflammation [47]. Moreover, probiotics are known to reinstate the barrier functionality of the stopping the apoptosis of the intestinal cells. It also assists in increasing the mucus layer and protecting the same by promoting the synthesis of proteins that are vital for the intestinal health [48]. Probiotics facilitate the healthy composition of Microbiota in the gut through inhibiting the growth of pathogens by producing bacteriocins that kills the pathogens and can create acidic milieu that stops the growth of pro-inflammatory strains of bacteria. It also supports the growth of good gut bacteria like lactobacilli and bifidobacteria [49]. Bincy P et al (2017) [50] states that probiotics is beneficial for enhancing the bacterial diversity in the gut and reducing the fungal diversity. Additionally, it also produces anti-inflammatory and anti-carcinogenic fatty acids that are beneficial to the body [51].

A double blind study (Rembacken et al 1999) [52] which stated that remission in patients with UC can be maintained by using probiotic with *E coli Nissle 1917*, which is proven to be just as effective as mesalazine in treating UC. The study analysed 327 patients who took part in a double blind trial and were given either the probiotic drug (n=162) once daily or mesalazine (n=165) at 500mg three times daily. Following a 12-month period, the patients were assessed clinically, endoscopically and histologically. The results showed that relapse occurred in 40 out of 110 (36.4%) patient who were treated with E coli Nissle 1917 and in the mesalazine group, 38 out of 112 (33.9%). The study concluded that adding probiotics may have some benefits but, the supporting data is limited. Similarly, there is a lack of sufficient data to draw conclusions about effectiveness of probiotics in treating CD [38][53].

In the case of Crohn’s Disease, it was observed that the activity index scores substantially improved in case of two open label studies conducted on a total of 14 patients. The study made use of certain probiotics namely *Lactobacillus rhamnosus GG* and a combination of *Lactobacillus* and *Bifidobacterium* species (Gupta et al, 2000)[54]. Nevertheless, another study involving 11 patients, who were initially subjected to antibiotic and steroid therapy for
one week and was later introduced to random placebo or *Lactobacillus* GG, did not show any significant improvement in the remission of CD. It was noted that only 5 patients out of the total 11 completely participated till the end of the study [55]. The latter studies cannot be considered as a credible outcome since there was a possibility that the outcome could have been different, in case all the 11 participants completed the study. The former research supports the successful use of probiotics in treating CD.

Furthermore, a review conducted by Cochrane in 2007[38] to analyse the usage of probiotics specifically *S. boulardii* and VSL#3 as a form of treatment for mild to moderate UC, found that probiotics along with conventional remedies helped reduce disease activity modestly, though there was very little impact on improving remission rates. This study involved a total of 244 patients [38]. Subsequently, two other similar research found a positive impact for VSL#3 [56]. This study noted that the remission rates was improved drastically at 12 weeks’ time where there was a massive reduction in the UC activity index score by more than 50 percent along with improvement in mucosal health. The shorter duration of the research and a massive dropout of participants from the placebo group, it is questionable whether the outcomes can be generalized to a wider population.

Furthermore, a randomized controlled trial (RCT) conducted on 100 patients in a Japanese research, which tested the usage of probiotic bifidobacteria-fermented milk, containing *Bifidobacterium* and *Lactobacillus acidophilus* strains, found that there was a massive reduction in endoscopic and histological scores in patients having UC as compared to patients that were administered placebo [31].

The above evidences demonstrate that probiotics are beneficial as in improving gut Microbiota thereby having a positive impact as a remedial solution to treating IBD. The results are mostly in favourable for UC, However, the data shows inconsistency in efficacy of Crohn’s Disease. It is important to treat these findings with professional scepticism since the following factors are not considered in trials. Firstly, UC and CD are actually heterogeneous in nature among patients. Yet in a single analysis, diverse species and strains are pooled together proposing that they are homogeneous. Overall from the study it can be determined that there is an irregular impact on the efficacy in Crohn disease, and moderate to inconsistent evidence for remission of UC.
It is also worth noting that in order for probiotics to be effective at their site of action, probiotics need to survive the acidic condition of the stomach and bypass bile and the digestive enzymes so that they can be viable past their shelf lives[50]. Marketed probiotics in supermarkets have not obtained the basic requirement or standards. Many probiotics have not even been subjected to clinical trials or evaluation for any given diseases and base their claims on extrapolation from other strains, which minsters and ineffective strategy.

SaifUl Islam (2015) [57] stresses the need for safety when using probiotics and addresses that care should be taken when administrating probiotics to immune compromised patients or severely ill patients as there has been reports of severe and rare side effects such a sepsis, endocarditis, or liver abscess during use of probiotics with Lactobacillus. Additionally, Fungemia has been noted in patients with severe co-morbidities [58] [59] as the side effects such as constipation, flatulence, hiccups, nausea, infection and rash have been noted to be the most common among patient.

3.51. Conclusion

Generally, probiotics are considered to be safe; however, probiotics are not safe for critically ill or immune compromised patients or during pregnancy. Probiotics appear to have some beneficial properties in treating GI-related diseases. However, the risks and benefits must be taken into account before it’s use. Moreover, the symptoms of side effects in patients need to be considered before recommending this as a therapy.

Ailsa et al (2003)[60], published a clinical review in the Journal of Gastroenterology analysing the efficacy of probiotics for the treatment of IBD. However, they concluded that there needs to be a larger controlled trial in order to understand the definitive criteria in regards to the use of probiotics when treating IBD. In addition to this another study demonstrated the efficacy of probiotics in pouchitis and UC but there was no significant body of evidences for the use of probiotics in CD [61][55].
Despite the above evidences and the assuring record of safety in probiotics usage for treating IBD, there exists areas of concern and care in certain cases namely, patients who are immune suppressed, acute pancreatitis, or those having central vein line in situ. Besides, the case of systemic sepsis reported in a new born due to usage of a certain probiotic strain i.e. *E coli* strain *Nissle 1917*, is an area of caution. Additionally, probiotics strains do not inhabit the intestine permanently and hence the need for repeated intake for continued impacts. This suggests that the effects are temporary and calls for a permanent solution that ensures efficacy and safety in IBD treatment.

Though the evidences are compelling in favour of probiotics usage in clinical purposes, the range of clinical probiotics available for treating IBD requires more clarity that is convincing. The study can be critically concluded by stating that there is a lack of consistent results that proves the clinical impact of probiotics in IBD. This can be further explored through wider longitudinal research that establishes the relation between Microbiota and IBD. This will help replenish individual strains of deficient gut bacteria (e.g., *F prausnitzii*), leading to customised medicines in future.

Another issue related to the promotion of probiotics as a therapeutic intervention is the lack of quality controls and regulations in the market which has tainted the entire market and cast a doubt on its credibility as a clinical product. Also, there is a lack of adequate clinical testing for the viability of the bacteria, their appropriate shelf life, accurate composition etc. in the probiotics introduced and sold in supermarkets. This casts a problem for the medical personnel in being able to authoritatively prescribe or recommend certain probiotics to the patients for their IBD issues, leaving the patients to experiment the products at their own risks.

This leaves the need for additional trials and research studies with a wider patient population and over an appropriate span of time in order to analyse and evaluate the optimal strain, dosing, formulation, and route of administration for treating in each subtype of IBD. Thus, there has been a demand of the evaluating alternative modern methods namely FMT as a therapeutic strategy in the recent decade which has been subject to clinical trials.
3.6. Faecal Microbiota Transplantation

In recent years one of the key areas of research in biomedicine and clinical medicine has been Faecal Microbiota Transplantation (FMT). The clinical response from research in FMT has facilitated knowledge surrounding Microbiota-host interactions related to an array of disorders, such as Clostridium difficile infection (CDI), IBD, diabetes mellitus, cancer, liver cirrhosis, gut-brain disease and others. The concept of using healthy human stool to treat diseases has been utilised as early as the fourth century in China [62]. As a result of this long standing tradition, physicians in China are accepting of using fecal therapy according to a survey in 2014 [63]. The first successful case of FMT to treat IBD was published in 1989 (Bennet and Brinkman) [64].

Recognition of the extensive benefits of FMT has only been understood in recent years through Modern structured clinical trials, even though the practice of FMT has been traced back to 1700 years. Fecal Microbiota and fecal Microbiota transplantation has 197 clinical trials registered on www.clinicaltrials.gov as of 1st September 2016 for a range of diseases including sclerosing cholangitis, non-alcoholic steatohepatitis (NASH), type II diabetes mellitus, irritable bowel syndrome, IBD, hepatic encephalopathy and eradication of colonization by multi-drug resistant organisms. FMT is a natural form of bacteriotherapy that employs the use of diverse microbial gut community from a healthy donor. FMT provides patients with an effective, inexpensive and fairly safe method to enrich the human gut microbiota. The popularity of FMT has increased exponentially in the past 10 years and some studies have reported that it is a highly effective treatment. The expanded interest of FMT has now developed into other diseases associated to the microbiota for example antibiotic resistant infections, IBD, hepatic encephalopathy, neuropsychiatric disorders, and metabolic disease.

Michael Laffina et al (2016)[65], advocates a positive view of FMT as a therapeutic strategy in treating Inflammatory Bowel Disease such as Ulcerative Colitis and Crohn's disease. The efficacy of this treatment in both Clostridium difficile infection (CDI) and its recurrent form as well as in treating diseases related to the change in Microbiota such as antibiotic resistant infections, hepatic encephalopathy, neuropsychiatric disorders etc. FMT is promoted as an alternative method against the resistance to antibiotics in humans caused due to its continued usage and unwarranted overuse in both humans and in the food chain.
Kamada et al (2013)\cite{66} refers to the term ‘colonisation resistance’ as a mechanism by the healthy gut Microbiota in preventing pathogen colonisation by competing for the nutrients, producing antimicrobial enzymes, changing the PH levels and preventing access to certain areas related to the mucosa to inhibit other organisms. Colonisation resistance is found to be improved through use of FMT in order to restore the healthy intestinal environment and thus treat the recurring pathogen colonisation. This therapy has demonstrated effectiveness rates of up to 80 to 95 percent based on a single FMT therapy. FMT is also advocated as a method of treatment to decolonise the pathogens from the gut by identifying, isolating and treating the recipients with the target of completely eradicating the pathogens from the individual\cite{67}.

Conversely, Cho et al (2016)\cite{68} has contended that FMT holds the effective possibilities in decolonising and preventing the surge of disease outbreaks related to intestinal Multi Drug Resistant (MDR) Organisms, though at present individuals affected by MDR organisms are managed through isolation precautions without any predefined protocols to minimise its spread. Cho et al\cite{68} also stated that effectiveness of FMT is evident only controlling the MDR organism in the intestines and hence the need for other strategies.

Brandt et al (2012)\cite{69} has shown through his study that FMT has delivered mixed results in treating UC in adults. This was seen through short term benefits in the form of improvements, though none of them achieved complete remission. A randomised trial by a group from McMaster University to evaluate the efficacy of FMT for UC found that almost 24 percent of the recipients were in clinical remission while the other exhibited symptomatic improvements through FMT. The results on the whole suggested that the success of FMT is mostly dependant on donor faecal composition, the use of multiple FMTs, and early treatment of UC, since the composition and the microbial diversity in the recipients resembled that of the donor.

Anderson et al (2012)\cite{70} did a random study of a large section of previous research data and found that in one particular review, a majority of the individuals who had FMT for IBD, displayed a massive decrease in symptoms, and a cease in IBD medication as well as displayed achievement of clinical remissions. Similarly, Sha et al (2014)\cite{71} found positive
results with almost 70 percent of the patients which included children and adults achieving remission from their disease.

On the other hand, a recent review showed lower positivity in remission with only a mere 45 percent success overall (22% of patients with UC, 60.5% of patients with CD) [72]. This variability in the remission results questions the fundamental heterogeneity in the studies, which could be dependent on variable determining factors like selection of the donor, the process of preparing the FMT, delivery of infusion, and length of diagnosis or the course of the disease.

Wang et al (2016) [73] conducted a review of the negative impact of FMT, in which he reviewed 50 articles, covering a population of 1089 patients who underwent FMT treatment. 310 out of 1089 patients notably reported experiences of side effects and negative effects after FMT in the form of abdominal pain and discomfort, diarrhoea, transient fever, nausea, vomiting and constipation. It has been noted the adverse effects were more prominent in patients who had FMT administered through upper route. It is seen that there exists a consensus across the medical fraternity, on advocating the significance of screening the donor for transmissible conditions.

Though rarely reported, there are incidences of UC flare following FMT, which shows the possibility of adverse effect on the patient [74]. Yet there are reports mentioned which notes that the side effects of flare up are mild and limited, though the symptoms include fever, abdominal tenderness, and CRP elevation which was successfully resolved by administering acetaminophen [72].

Faming Zhang et al (2018) [75], analysed the past use of single species of microbes to treat diseases through the isolation of bacteria in order to use as probiotics. Historically this is a common practice [76]. This article states the limitations of using single species of bacteria and the effectiveness of using the whole Microbiota when carrying out FMT[77]. Research is currently focused on new microbes and multiple species in order to remodel gut Microbiota. From such studies it has been reported that a mixture of 18 strains of probiotics could reduce chronic constipation and IBS [78].
Brooke C. Wilson et al. (2019) [79] identified that the success of FMT is highly dependent on the microbial diversity and composition of the donor, this leads to the concept of FMT super-donors. This publication focuses on identifying the required criteria of super-donors and the ways in which this facilitates present knowledge of microbial component of chronic diseases, thus in the future enabling more targeted bacteriotherapy approaches. Both genetic and environmental influences continually shape the composition of gut Microbiota [80] therefore, fluctuations throughout an individual’s lifespan are to be expected [81]. The gut in healthy adults is primarily populated by bacteria that are members from the strictly anaerobic Firmicutes and Bacteroidetes phyla, with small representations from members of the Proteobacteria and Actinobacteria phyla [82]. It is complicated to determine what comprises a healthy gut microbiome from an inventory viewpoint given that no two gut microbiomes are identical (Human Microbiome Project Consortium, 2012) [83]. Regardless, a healthy intestinal state is associated with having a stable and diverse gut community [84] and variations to the Microbiota is linked to negative functional outcomes on the gut physiology, for example localized inflammation or interrupted metabolic processing (gut dysbiosis) [85].

Paramsothy et al. (2017) [86], noted observations under intensive doses of FMT and also the use of multiple donors. The treatment was linked with distinct microbial alteration and results illustrated clinical remission and endoscopic improvement in active UC. He et al [87] reported that three months after the first FMT, following up with a second fresh FMT is a safe and effective course of action confirming that the current clinical response to CD is sufficient.

It is still undetermined if the primarily focal point of FMT success should be based on a positive clinical response in the recipient, or by the gut microbiome profile of an individual transitioning to reflect the profile of a donor. A combination of both elements may help with determining FMT success, first identifying if the transplantation has been inserted and enhanced the local Microbiota community and secondly if the observations after are indicating improvements. Some studies have better results as compared to others, which could be possibly dependent on the higher percentage of engraftment in the recipient [88].

Success of FMT for treatment of IBD was first reported in 1989. A male with refractory UC underwent FMT and experienced clinical emission for 6 months[64]. Since then a vast
amount of FMT studies have been carried out on IBD patients producing varied clinical outcomes, remission rates, and other effects [62] [75]. Through systematic review [86] produced a meta-analysis of 53 studies of FMT in IBD patients comprising of four randomized control trials, 30 cohorts, and 19 case studies[86]. Analysis of cohort studies indicated that FMT was more effective in CD (52%) than UC (33%) in achieving induced remission. Another observation was that rates of remission were enhanced by a larger number of FMT infusions and administration from the lower GI tract. Contrasting to CDI studies, FMT performed on IBD patients have proven to exhibit different responses, which may very well be linked to the variations in the donor stool [89].

Moayyedi et al[88] was the first study to record the super-donor effect through a randomized control trial to study the effectiveness of FMT in patients with UC. 75 patients with active disease participated in weekly enemas containing either faecal material or brown water as a controlled placebo. The trial took place for a period of 6 weeks and the results indicated that FMT revealed to have much higher rates of clinical remission than the placebo after 7 weeks (24% and 5% respectively). The same donor stool was used on 7 out of 9 patients who entered remission, hence supporting that FMT success was donor-dependent. Nonetheless, clinical efficacy of a donor cannot presently be predicted prior to FMT in IBD patients. Studies have suggested increasing remission rates by pooling donor’s stool together, to diminish the chances of a patient being given ineffective stool [90] In order to homogenize donor-dependent effects, tests used multiple donors’ stool to administer FMT in diseased patients. The mixtures contained stool from up to 7 different donors, which greatly increased microbial diversity compared to using stool from a single donor based on operational taxonomic units (OTUs) count and phylogenetic diversity measures. This study also revealed a super-donor effect. The FMT batches which included contribution from the super donor displayed a greater remission rate (37%) than batches that did not (18%) [86].The results from this study are very reliable given that publication bias was avoided by Egger’s regression asymmetry test as well as funnel plots.

In a double-blind study[91] 72 patients with CDI randomly received fresh, frozen or lyophilized FMT product via colonoscopy. The results of the test showed the group that received the fresh product has the highest cure rate (25/25 100%), while the lyophilized group exhibited the lowest cure rate (16/23 78%) and intermediate (20/24 83%) in the frozen
Woodworth et al (2017) [20], explores the guidance and recommendations from professional societies and Food and Drug Administration (FDA) authority in selecting and screening the fecal donors for FMT. Certain exclusion criteria for the donors with diabetes mellitus, prior cardiovascular events, and clinical healthcare exposure while selecting the fecal donors must be set in place for the safety of patients. Further, exclusion criteria include condition of the intestinal microbiome of the donor. FDA has published numerous guidelines, though in the draft form, about exercising discretion in the selection of the donors through appropriate screening and enforcement. This is evident from the fact that FMT results are dependent on diversity of the donor stool Microbiota and hence sensitive to the donor selection[93].

It’s also important to test the donor in order to evaluate the diversity index of the Microbiota and its impact on the metabolic activity of the donor, cardiovascular issues, colorectal health and the multidrug resistance (MDR) levels in the donor. These factors need to be carefully considered during the screening process while selecting the donor. There exists a speculative preference for stool donors known to the recipient, though clinical evidences have proved that stools sourced or banked from screened donors unfamiliar to the recipient display a usual trend in usage.

The use of donor’s faecal screening as a robust method and suggest that as analogous to blood donor screening which is administered through testing in a laboratory and donor self-declaration questionnaires [94]. Guidance also mentions the use of questionnaire to detect risks where there are not sufficient laboratory methods available or where the diseases are usually undetectable or are at an early stage and to use proper diagnostics methods for testable medical issues [39].

Gathe et al (2016) [95] states that there exists the need for further screening and in depth study on the area whether donors known to the patients are relatively holding more merit in improving FMT remission rates. There has been a continuous speculative concern about the transmission of infections to the gut of the recipients and this has formed the basis of driving and formulating the framework for excluding the donors by setting stringent criteria. However, there has been very little notable evidence to suggest the transmission of harmful
effects which can be directly attributed to FMT and this is a positive signal that vouches for the effectiveness of the current screening process. Negative effects mostly noticed during clinical trials are mostly caused due to the procedural deficiencies in the FMT process instead of the actual faecal material as such.

The detrimental effects of using inappropriate techniques and procedures of infusing Microbiota into the small bowel resulting in nausea, vomiting and aspiration [96] [97]. The article advises to use x-ray fluoroscopy or other non-invasive techniques such as inserting a nasojejunal tube into the patient’s intestine when the patient is at high risk of aspiration under anaesthesia.

Other controls including thorough screening of donors should be conducted. Examples given in the publication includes a case where a woman developed new-onset obesity after receiving stool from a healthy but overweight donor [98]. Additionally, a study published by Wong et al. (2017) [99] demonstrated that the faecal Microbiota from patients with colon cancer promoted tumorigenesis in germfree and carcinogenic mice. Thus, reaffirms the importance of strict donor screening, to avoid transmission of diseases through FMT.

Moreover, this paper also evaluates the implications of contaminated microbes in the donor stool. It stresses the imperative need for the laboratory preparations of FMT materials to meet Good Manufacturing Practice (GMP) requirements. GMP is typically set for pharmaceutical companies to manufacture oral medications. Unqualified materials must be excluded and stools from familiar donors should still be screened repeatedly to prohibit infectious pathogens.

Currently there are gaps in the screening process. Critical information such as the receiver’s genetic background and dietary habits are not regularly considered. In some cases of IBD genetics may be a contributing factor [100], however due to a lack of genetic information, researchers focused on donor-dependent effects instead. Moreover, super-donors could assist with explanations surrounding varied recipient responses.

Patient perspectives were investigated through studies [101] that conducted surveys on patients’ attitudes. The data gathered illustrated that 56.19% of 105 patients with CD presented satisfactory clinical efficacy and 74.29% were willing to receive the second FMT.
Furthermore, 89.52% (94/105) showed their willingness to recommend FMT to other patients, which was largely due to effectiveness and the low-costs associated. Reluctance in patients arises when presented with options of Microbiota transplantation into organs beyond the gastrointestinal tract. Although studies provide evidence of potential role of Microbiota in extra-intestinal sites, such as vagina [102], sinus [103], urinary tract [104] and skin [105]. Therefore, further research must be carried out, which focuses on specific use of Microbiota in different organs[106], a strategy called selective Microbiota transplantation (SMT). SMT should be promising in precision medicine.

3.61. Conclusion

In the case of treating IBD, through the therapy of decolonising the gut, FMT is found to be more effective and holding considerable benefits over the usage of antibiotics. Additionally, the adverse effects of FMT is stated to be minimal and less serious [69]. FMT as holding restorative potential in addressing antibiotic resistance but requires further investigations. Nonetheless, (Alexa R et al (2017) [107] suggests that FMT cannot be fully relied upon consistently for all trials because there exists’ variations in the delivery methods which plays a significant role in ensuring clinical remission. The variation involves dose of bacterial infusion, the method of filtration of stool, the number of times it is administered and elements in the donor diet. These diverse factors play an important role in truly enhancing its success levels and it remains to be explored further to interpret clinical success in terms of these determining elements.

The failure of the previous trials can also be attributed to the fact that it was a single FMT infusion and there is a possibility of bias in selecting and recruiting the donors as well as patients. This could have caused a compromise in the research protocols. It was hard to draw a conclusion on the efficacy of FMT due to the limited number of case studies and lack of control in the selection of the group.

A collaboration between the varied health departments, Pharmaceuticals, stool banks, laboratories and researchers is required to closely observe the changing dynamic in the sector and understand the determining interventions that can enhance, decrease or amend the microbial composition of the gut.
Additional research study on the human gut microbiome will further enhance the understanding of which elements is likely causal and need to be considered during the screening process for donor selection, in order to improve efficacy and ensure safety of the patient. This will help select specific donors for specific microbial conditions instead of relying on finding an ideal donor. It is essential that a clearer understanding of microbial index needs be achieved in order to help narrow down and streamline the screening of stools, thus reducing the costs and increasing access to FMT treatment.

Moreover, FMT is classed as a cost effective, relatively safe and effective method to enrich the human gut in treating IBD through enhancing the healthy Microbiota. It is noted that numerous medical and academic research centres are established to study and research this subject of FMT and this shows its increasing popularity and demand in the medical field as a viable alternative treatment for IBD.

Regarding donor screening this paper further emphasises the need for a convenient, precise and fast faecal pathogen detection method. This field requires further research in order to find the most appropriate process to ensure patient safety is at the forefront of medical advancements.

This article states that FMT carried out on patients with CDI showed good efficacy, nonetheless IBD patient results are much more controversial.

It is to be noted that FMT also faces limitations, in the form of variable methods of administration, aesthetics consideration, concerns related to patient attitudes and safety concerns. Moreover, the issues related to patient resistance for extra intestinal applications can be countered through appropriate educational awareness and increased credibility of FMT as a clinical therapy as against promoting FMT as a clinical trial.

Also, there remains a large scope of areas of improvement in preventing contamination by raising the standards of GMP and promoting good practices in the industry through adequate screening and laboratory preparation practices. As against the popular notion that FMT will face patient unwillingness, evidences have proven otherwise by showing a significant positive attitude towards FMT as a therapeutic intervention.
FMT research is still in its early stages especially in comprehending its mechanism of effect even though earliest reports of FMT in literature date back to the fourth century. The existence of super-donors and other observations of FMT are not yet strongly supported by empirical data given that there is an immense deficiency of large randomized and controlled clinical trials. As a result of this, many smaller studies are emerging but still its efficacy is not fully conclusive. The results from such research are difficult to compare with each other yet they do provide support for the notion that the donor has a key influential role in FMT outcomes.

While, the best method to predict a patient’s response to FMT is not fully clear, it is generally accepted that high diversity of the gut Microbiota in the donor appears to provide improved results. FMT outcomes depend on the restoration of metabolic deficits which contribute to the disease in recipients; therefore, the efficacy of FMT heavily relies on the donor’s ability to supply the necessary taxa in order to restore the imbalance. Therefore, it is vital to expand the current knowledge about super donors as it has the potential to standardize the therapy through refined FMT infusion. FMT success can solely be predicted accurately by comparing the faecal profiles of various donors and studying its effect on IBD patients. Moreover, this throws open the scope of future developments in this subject through the introduction of capsule form administration methods which can help counter the longevity concerns related to FMT.

Also, using a larger sample size may assist in collating more reliable results. On the other hand, finding patients who fit the criteria to be part of the study and patients who are willing to participate in the investigation is understandably a difficult task. The survey of patient attitudes that this study highlighted is an important aspect of FMT that is not often given enough importance as well as emphasising the strong need for development of standardized FMT into a mainstream therapeutic option to allow benefits to reach more patients.

Although cohort based studies are lacking, it is evident that donor’s microbial diversity is a contribution factor in the therapeutic success of FMT [93] The correlation between FMT and increase of gut Microbiota diversity has been demonstrated consistently, usually the composition profile alters towards that of the donor stool [93][86] Some studies conclude that microbial diversity of the donor stool is the most critical factor in FMT results Observations identified by [93].In an IBD cohort showed that donors that produced a clinical response to
FMT have far superior bacterial richness than those who did not [93]. There is a fast increasing interest in FMT as a therapy as there is an existence of significant scope of fully determining and realising its full therapeutic potential in treating patients with IBD.

4. Final conclusion

1. To sum up, significant progress has been made in the last five years, identifying the different genetic variations in IBD. There are over 160 different genetic variations of IBD published to date. Although, diversity is significantly reduced in patients with IBD and further reduced in a flare it is evident to state that microbiota does have some roles in IBD ranging from causative to protective elements. However, the exact role of dysbiosis is not clear as studies are still attempting to determine whether if dysbiosis is truly a causative factor or a consequence of the intestinal inflammation. Many studies have suffered to formulate final conclusions due to limitation in their studies.

2. Rapid advances through DNA sequences and metagenomics have given fundamental insights into the enteric Microbiota communities and allowed us to understand how these bacterial species and their metabolic products interact with the host genes to initiate intestinal inflammation. This allows a promising prospect of personalised therapeutic interventions such as Probiotics and FMT. The effectiveness of probiotics in treating IBD to date is uncertain as basic questions regarding optimal compositions, administration strategies and its durability remains in question

3. In regards to treatment success and safety, Faecal Microbiota Transplantation (FMT) has shown to be promising despite reporting limitations. Although numerous questions still await to be answered, for example the long-term effects of FMT. However, this therapy could be beneficial treatment for IBD, especially those with concurrent CDI or with pouchitis. While questions are being answered, there is a constant need for additional data from randomised controlled trials since more questions arise through the study of gut Microbiome. Substitutes such as synthetic and multi-microbial stool are foreseeable advancements that we will probably see in the close future.
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