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Faecal microbiota transplantation - stool, a tool for wellness

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Abstract
The process of stool transfer from healthy donors to the sick known as faecal microbiota transplantation (FMT) has an ancient history but, only recently researchers have started investigating its application in an evidence-based manner. Donor selection, stool preparation and delivery mode are the underlying prerequisites to achieve maximum efficacy from the application of FMT. Presently, patient-directed donors, and super donors via stool banks, are available options for FMT resources. The review proceeds with collating and discussing the updated information on protocols involved in the collection, preparation, processing, storage and administration of stool samples for its effective translation into recipients. The prospect of FMT in treating gastrointestinal and extra intestinal diseases like neuro-psychiatric diseases via gut-microbiome homeostasis has been elaborated while concomitantly pondering upon the current regulatory bottle necks associated with its commercialisation. Determining the characteristics of a healthy microbiome, its causality with different diseased states, and ensuring cost-effectiveness treatment are the major challenges for addressing. Ongoing advances in integrated multi-omics technologies will provide more insights into defined microbial consortia targeted to treat specific disease conditions, wherein FMT is poised to emerge as a new therapeutic duly supported by a regulatory framework in place and validated findings of clinical studies.

Keywords: *Clostridium difficile* infection, faecal microbiota transplantation, microbiome, regulations, safety

1. Introduction
The human body is host to trillions of microorganisms ranging from different types of bacteria, viruses, and eukaryotes and they survive and grow by exhibiting either ammensalism, commensalism, mutualism, antagonism or parasitism. The ratio of microbes to the human body popularly estimated to be 10:1 (Luckey, 1972) [37] has been revised to 1:1 in the recent past (Sender *et al.*, 2016) [52]. Much research is going on to establish the microbial interactions within the human body's ecosystem and their importance to health and diseases. The human gut microbiota, which evolves with humans from birth, consists of 1000-1500 phylotypes, constitutes the biggest population of microbes in our body and acts as an interface between food and epithelium. They remain in continuous contact with immune cells and neural cells of the body. A number of disease conditions are associated with the disturbance in the normal balance of microbes.
Many countries are actively involved in research to understand the importance of gut microbiota and associated health benefits. The richness and diversity of human gut microflora act as an indicator of health and many metagenomic studies demonstrate this very precisely (Claesson *et al.*, 2012) [10]. Besides popularly known antagonism to pathogens, there are a number of physiological factors in the human body attributed to gut microbes such as modulation of the immune system, epithelial cell proliferation and differentiation, insulin resistance and having a bearing on behavioural and neurological functions. The host encourages and felicitates the selective growth of the benign microbes by releasing microRNAs, antimicrobial peptides, mucus and immunoglobulin. Further, there is a list of factors which are known to affect the composition of gut microbiota *viz*. host genetics, diet, age, mode of birth, administration of antibiotics, (Hasan and Yang, 2019) [22] travel, lifestyles, stress, and anxiety (Hollister *et al.*, 2014) [24]. There has been increasing interest

and investment in the field of medical science to transform faecal microbiota transplantation (FMT) as an effective tool to combat various gastrointestinal disorders and other disease conditions including neurological and psychiatric issues. Approved modes and modalities make this treatment a remedial measure, but need much refining seeing the associated safety and regulations. Many ground-breaking kinds of research that yielded positive outcomes in tackling bowel health problems and won regulatory approval in the world for a microbiome-based therapy using donors will definitely cure the sick (URL-https://indaily.com.au/news) [75]. Recently Food and Drug Administration (FDA) approved the first microbiota product for life-threatening *Clostridium difficile* infection (CDI) in patients of 18 years and above (URL- https://www.fda.gov/news) [74]. Considering the huge potential of FMT as a microbiome-based therapeutic for treatment of local and systemic diseases, this review provides a one-step access to FMT by collating and discussing the updated information beginning from the several protocols to achieve the maximum efficacy followed by the prospects of its application in treatment of gastrointestinal and extra-intestinal diseases, and finally the addressal of regulatory impediments for its successful translation. The coming years will surely emerge with many such products with all approvals for branding FMT as the therapeutic of the 21st century for the wellness of the population.

2. Faecal microbiota transplantation – definition, history and beyond

Faecal microbiota transplantation is the administration of a solution of faecal matter from a healthy screened donor into the intestinal tract of a recipient in order to directly change the recipient's microbial composition and confer health benefits (Smits *et al.*, 2013) [56]. This procedure is also termed bacteriotherapy. Ancient records pertaining to Ge Hong in fourth-century China, detail the use of human faeces in suspension form for treating food-borne diseases administered via the mouth. Cow faeces were recommended for gastric allied discomforts in India, some 3000 years ago. 'Handy therapy for emergencies in the Chinese handbook of emergency medicine was considered to be the first written document narrating various modes of application. Later in the 16th century, Le Shizen described using fermented faecal solution, fresh faecal suspension, dry form and infant faeces for severe diarrhoea, pain, vomiting and constipation. Alternatively, this practice was labelled as yellow soup therapy. Records also suggest that in the 17th century, this method was used for treating animals and was termed transfaunation. In 1958, faecal enema was used as a methodology to treat pseudomembranous colitis (Eisman *et al.*, 1958) [15]. This was the incident that marked the introduction of FMT into mainstream medicine. Successful use of FMT for ulcerative colitis (UC) was first reported by Bennet in 1984 when he documented the reversal of his own colitis following retention enema from a healthy donor (Bennet and Brinkman, 1989) [5]. In India, the FMT was successfully carried out for ulcerative colitis in the year 2014 without any adverse effects (Seth *et al.*, 2016) [54]. Several changes had happened in route of administration of FMT in patients and nowadays washed microbiota transplantation, a new trend in treatment since 2020 for numerous disease conditions (Zhong *et al.*, 2021) [72]. Several microbiota-based products are getting approved after years-long studies enlightening the possibilities of effective treatment of gastric-associated diseases.

2.1 Gut/Faecal Microbiota – The Clinical Importance

As the gut microbiota is closely associated with human wellness and disease, the organisms are otherwise been acclaimed with the status of 'Invisible organ'. The gut microbiota acts like an organ in the human body and positively influences the metabolism, nutrition, and immune system of the host. There is a balanced homeostasis that exists between the host and gut microbiota. Dysbiosis is always been a reason for different diseases including *Clostridium difficile* infection (CDI), inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), autoimmune disease, allergy, cardiovascular disease and metabolic disorders. The gastrointestinal system is known as the second brain as it is in contact with the first pool of immune cells and the second pool of neural cells in the body. It can act autonomously and even influence behaviour by sending messages up to the vagus nerve to the brain. The intestinal tract is supported by glial cells with 500 million neurons. The GI tract is engaged in the production of 50% of all dopamines and 95% of all serotonin (Yano *et al.*, 2015) [68] Available literature also points out, disturbed microflora can lead to or aggravate several neurological and psychiatric disorders like Parkinson's disease, Alzheimer's disease, autism spectrum disorders (ASD) and epilepsy, because the most applauded 'Gut-Brain Axis' is connected much to all these ailments (Hao-Ming *et al.*, 2021) [21]. Some researchers reason that bipolar disorders, depression, anxiety and other system-related neurological diseases such as hepatic encephalopathy, neuropathic pain, and sepsis-associated encephalopathy are also associated with neurological dysfunction, where dysbiosis plays a potential causative factor. The imbalance of gut microbiota especially in children can lead to various non-gastrointestinal diseases like asthma, type I diabetes, Tourette's syndrome etc. (Zhong *et al.*, 2021) [72] Similar to the gut-brain axis, another term called the gut-skin axis is quite new, where the involvement of gut microflora is under investigation in skin disorders like atopic dermatitis (Lee *et al.*, 2018) [35]. The skin and intestine both have an important role as immunological barriers and immune regulators. The intestinal microbiota has a positive effect on the skin through the modulatory effect of the gut on systemic immunity. Changes in the SCORAD (Scoring Atopic Dermatitis) scores offer clinical evidence for the importance of gut microbiota in Atopic Dermatitis patients and demonstrate that FMT may be an effective therapeutic intervention without any side effects (Mashiah *et al.*, 2022) [38] Faecal microbiota transplantation is an emerging treatment intended to rebalance disturbed symbiosis by introducing faeces from healthy donors to unhealthy individuals. There is growing interest in FMT as a treatment for various gastrointestinal diseases as well as metabolic disorders and cardiovascular diseases. Recent studies also suggest that FMT can be effectively used for refurbishing disturbed balance owing to alcoholic hepatitis and the treatment supports in alcohol addiction therapy (Philips *et al.*, 2022) [46]. It is also evident from research findings that alcohol abuse can lead to dysbiosis even after FMT therapy. This will lead to the predominance of *Gammaproteobacteria* including *Klebsiella* species that was observed in post-FMT patients. This can catalyse the chance

of being infected with CDI (Kellingray *et al.,* 2018) [29]. Moreover, several authors are of opinion that obesity in humans and animals can be effectively managed with the help of FMT. (Wang *et al.,* 2020, Mocanu *et al.,* 2021) [64, 41].

2.2 FMT – Methods and Methodologies

FMT is an emerging technique necessitating high-standard donor selection, screening, collection, preparation and storage of faecal microbiota, mode of delivery, preparation of the recipient, safety control and related activities. A standard FMT technique is still underway due to differences in existing methodologies. Stool banks are generally following a standardised protocol, but most of the institutions that are offering this procedure still require a uniform method for material preparation, because the process is dependent on many factors.

As of today, safety concerns and acceptability are the main constraints of FMT as a medical procedure. The faecal microbiota can be obtained from related or unrelated donors. No significant difference between related and unrelated donors has been identified yet (Kassam *et al.,* 2013) [27]. In the case of selective, maternal line first-degree relatives may have a theoretical advantage due to more adaptive antigen-specific antibodies. Similarly, it is speculated that men might be preferred donors over women because the latter may harbour microbiota that is more likely associated with autoimmune disease and IBS. But scientific evidence is lacking to prove these hypotheses (Kelly *et al.,* 2014) [31]

2.2.1 Donor Selection and Screening

The major step in FMT is donor selection. Many adverse conditions related to the infusion of donor material can be averted by proper scrutiny itself. An interview or answering a questionnaire will be highly useful in eliminating undesirable donors having a history of transmittable diseases. A three-step rigorous method is implemented on the selected candidates to be categorised as active donors. Reports from US stool banks confirm that less than 3% of the total respondents get qualified for emerging as active donors. Out of these, 65% of individuals are excluded based on their responses in a pre-screen survey, owing to abnormal body mass index, logistical constraints and recent antimicrobial use. Again 80% of the selected participants are rejected based on the clinical evaluation. Then only half of the qualified prospective donors will become successful and the rest get ejected due to unacceptable laboratory findings such as the presence of *Dientamoeba fragilis*, *Blastocystis hominis*, *Clostridium difficile* and *rotavirus* in their stool samples (Van Nood *et al.,* 2013) [61]. Due to the outbreaks of COVID-19, the European Society for Blood and Marrow Transplantation and US Food and Drug Administration (USFDA) introduced guidelines on testing for the COVID-19 virus checking in donors and collecting faecal samples. SARS-Cov-2 virus may be transmitted in faecal samples during the FMT process (Nicco *et al.,* 2020) [8].

The donors of faecal microbiota should be vigorously screened based on screening criteria available at respective institutions or national guidelines. The eligible donor should match an eight-dimensional criterion including age, physiology, pathology, psychology, integrity, time, living environment and recipient status, where some of the points were already discussed above (Zhang *et al.,* 2018) [71]. Individuals aged 18-60 years are preferred. Although the donor age has not been limited, adolescence is most preferred. Because they develop a stable and diverse microbiota benefitting from their energetic exercise and regular diet. The donor should meet the following criteria such as no history of surgery or exposure to epidemic areas, no infectious diseases, obesity, diabetes, IBD, IBS, chronic diarrhoea, chronic fatigue syndrome, colon polyps, immune disease, metabolic syndrome, allergic diseases, malignant tumours and other diseases that may be associated with intestinal microbiota disorders, no intestinal disorders, no infection signs, no use of antibiotics, laxatives, weight loss pills, immunosuppressants, chemotherapy drugs and other drugs in the past three months that may change the composition of the intestinal microbiota (Schmidt *et al.,* 2018) [51]. In addition to this, blood routine, faecal routine, occult blood, virology (hepatitis A, hepatitis B, hepatitis C, AIDS, syphilis, etc.), bacteriology (*Treponema, Salmonella, Shigella, Campylobacter, Helicobacter pylori, Vancomycin-resistant enterococci, etc*.) are to be closely verified (Ding *et al.,* 2020) [14]. Testing for additional pathogens can be considered in certain clinical situations, such as when the recipient is immunocompromised or in cases of potential donor exposures. Donor stool can be collected from 2 sources – directed donors and universal donors through stool banks (Kelly *et al.,* 2015) [30].

The concept of "super donors" is getting momentum these days due to the therapeutic success of donor materials. It is a term suggested to describe donors, whose stool is therapeutically significant and more effective when compared to other donors' treatment outcomes (Wilson *et al.,* 2019) [65]

2.2.2 Collection, Preparation and Storage

2.2.2.1 Collection

In the last 1700 years, when the first recorded use of FMT was practised, there is not much change that has happened in the mode of collection and preparation of faecal microbiota. To overcome the visual and olfactory discomfort of researchers and technicians, several changes, and innovative methods are in place, especially in the case of washing.

Once the rigorous screening process is over, samples can be collected within a month from the successful donors. A consecutive screening has to be performed after a particular period of donation to ensure the safety of the collected and preserved material. Cleaning and sanitisation of equipment are very crucial. Donors can use a clean opaque plastic bag with a proper opening and closing facility, for collecting the sample. Either on-site donation or collecting samples at home can be performed. If samples are collected at home, they shall be delivered in chilled conditions using ice bags within an hour of defecation. The stool can be stored for a period of 8 h while kept chilled at 4 °C. Bacterial cell activity and viability diminish at room temperature or keeping samples at 4 °C for more than 8 h (Ott *et al.,* 2004) [44].

2.2.2.2 Cryo-conservation

Significant differences have been reported in the case of treating *Clostridium difficile* infection using frozen versus fresh FMT. As mentioned above, the period of time and holding temperature are very crucial. Cryopreservation methods employing electrical freezers for maintaining -80°C

and or using liquid N_2 for keeping at -196°C are widely practised (Prakash *et al.,* 2013) [47]. Refreezing of defrosted stool samples is not permitted. Long-term preservation of transplanted faeces at -20 °C will lead to instability, especially *Actinobacteria and Bacteroidetes* which will affect the clinical outcome of FMT therapy (Takahashi *et al.,* 2019) [59]. Practically the stored material is kept for a period of a maximum of six months. Thereafter it is discarded as clinical waste. Many studies are happening to explore the most suitable storage temperature and time for storing the product without any compromise on stability.

2.2.2.3 Washing

Washing is a key step in FMT material preparation or washed microbiota preparation. The washing step is mainly of three types. Methods are added in the washing process to obtain the maximum viable cells possible. One of the methods is Rough filtration (RF), then filtration plus centrifugation (FPC) and the final type is microfiltration plus centrifugation (MPC). The automatic purification system (GenFMTer) is widely used in clinical practice, using MPC as the technique. With this equipment "from defecation to infusion" or "from defecation to freezing" can be reduced to one hour (He Z *et al.,* 2017) [23]. The FMT based on the automatic purification system and the related delivery routes was named washed microbiota transplantation (WMT) (Zhang *et al.,* 2018) [72]. WMT can reduce FMT-related adverse effects without discounting the efficacy of FMT. The WMT process assures the delivery of a precise dose of enriched microbiota. Both fresh and frozen microbiota can be effectively used for treatment, but recent studies prove that fresh FMT is more effective than frozen FMT, especially in CDI treatment (Agarwal *et al.,* 2021) [1]

For successful FMT, the quantity of donor material employed is around 30 g. Practically there is no correlation exists between the stool quantity and the number and type of microbial flora. It can change with the donors and even with the donations. Hence a sample size of 60 g is preferred for each treatment (Gough *et al.,* 2011) [17]

2.2.2.4 Procedure

Once the sample is collected and found fit for processing, approximately 60g of material (25%) is made into a homogeneous solution with around 150 ml of saline (65%) using a sterile mortar and pestle or suitable blender. The resulting slurry is sequentially passed through a stainless-steel laboratory sieve of varying pore sizes 2.0, 1.0, 0.5 and 0.25 mm respectively, to remove all possible suspending matter. The final filtrate is centrifuged at 6000g for a period of 15 min and the resulting pellet is diluted to one-half of the original volume using non-bacteriostatic normal saline. This suspension is then amended with glycerol (as cryoprotectant) to a final concentration of 10%. Later the suspended stool sample is filled into individual cryotolerant tubes and quickly frozen to -80^{0}C. An interim blood and stool analysis is to be performed to affirm the purity of the sample. The suspension is to be thawed in warm water at 37^{0}C before administering. It shall be applied within 4 h without any temperature abuse on the thawed material (Satokari *et al.,* 2014 and Gough *et al.,* 2011) [50, 17]

Faecal microbiota capsules could be prepared by concentration of the diluted, blended slurry as per the protocols detailed above. The concentrated faecal solution is filled in capsules that it contains 1.6 to 2.0 g of prepared material. Commercially available acid-resistant hypromellose capsules are usually used. Capsules are also stored at -80 °C for a maximum period of 6 months (Youngster *et al.,* 2014) [70].

2.2.2.5 Administration of Fresh and Frozen FMT

With the expertise of the clinician and the patient's condition, the mode of administration of FMT varies. Many routes of administration are possible and do not stick to a particular or optimal way of the protocol. It can be performed through the upper gut, mid gut or lower gut. Each mode of administration has its own pros and cons. As in the case of other procedures, here also immediate medical attention of the recipient followed by periodical follow-up of the patient and donor to be conducted. In the case of upper GI tract mode, the various procedures used are upper endoscopy, nasogastric/naso- intestinal tubes (NGT), ingestion of pills/capsule etc. In the case of the mid-GI tract method, esophagogastroduodenoscopy (EGD), percutaneous endoscopic transgastric jejunostomy (PEG-J tube) etc are in practice. Colonoscopy or rectal endoscopy, transendoscopic enteral tubing (TET), Sigmoidoscopy, and retention enema are the common methods associated with the Lower GI tract mode of administration (Ding *et al.,*2020) [14].

In case of immediate administration, cryoprotectants are generally not used. The filtered, diluted and homogenised sample in saline is applied within 6 h of collection. For frozen FMT, thawing can be done for 2-4 h in an ice bath before the FMT procedure (Hamilton *et al.,* 2012) [20] For patients suffering from swollen colon, severe colitis and discomfort with colonoscopy, the upper GI route is mostly performed. Nasogastric or naso intestinal routes may be uncomfortable and less appealing to the patient. It also requires radiology assistance to confirm tube placement. The efficacy rates were reported to be between 81% and 86% (Kim and Gluck, 2019) [33]. All forms of upper tract delivery increase the risk of vomiting or aspiration. Capsule delivery is an alternative mode for patients, where colonoscopy is risky. Although the standard dose per capsule is not yet defined and studies reveal that a mean of 1.6 g of stool/capsule yields a 70% cure rate without many adverse events (Kao *et al.,* 2017) [26].

Oral capsule for FMT is another mode of therapy. As it doesn't require much invasiveness, the patient's acceptability is high, but the expenditure and the burden of taking large capsules pull them back to normal modes. Many researchers, however, believe that colonoscopic administration has a 5–10% higher cure rate in recurrent CDI (rCDI), with the added benefit of ensuring that the FMT product reaches the colon because a water jet can be used to spray the material directly on the mucosa (Vindigni and Surawicz, 2017) [63].

Lower GI tract mode of administration, in colonoscopy, bowel preparation is recommended to improve the visualisation of the colon. Endoscopic delivery carries the same procedural risk and increases health care utilisation and costs. It has got an efficacy of 84 to 93%. About 50-100 g of donor faecal material that has been diluted to 250-500 ml of saline is most commonly used (Cammarota *et al.,* 2014) [6]. The main modalities are colonic TET, sigmoidoscopy and inexpensive methods like retention enema. Enema is an easy way of delivering faecal microbiota, but the access only arrives at the rectum and the sigmoid colon, making it difficult for patients, especially

children to hold the delivered microbiota for enough time. TET is considered to be an excellent alternative to overcome these issues. TET as a procedure has been reported as a safe and convenient procedure for multiple WMTs and colonic medication administration with a high degree of satisfaction among patients and has been approved as a mode of treatment by many countries (Peng *et al*, 2016) [45]. Here, the infused bowel lavage can control the existing pathogenic flora and support colonization of transferred useful microbiota (Ding *et al.,* 2019) [13].

2.2.2.6 Preparation of Recipient

For better adsorption of microbiota, bowel preparation of the recipient is a must when colonoscopy is used as the administration mode. Patients may be given loperamides to retain the faecal solution in the gut. During NGT, a proton pump inhibitor may be given in advance to increase the survival of the transplanted bacteria. Several observations advised against taking antibiotics 12-48 h before FMT, and antibiotic treatment before FMT was also discouraged (Cammarota *et al.,* 2017) [7]. But in some protocols, antibiotics are administered several days prior to transplantation to clear the recipient's gut microbiota. Immunocompromised recipients at risk of CMV/EBV-related disease (Cytomegalovirus and Epstein-Barr virus) should undergo viral testing prior to FMT. Recipients should perform blood testing (HIV, HBV, HCV and syphilis) for transmissible infections before transplantation. Nowadays screening for SARS-CoV-2 is mandatory for both the donors and recipients even though they are vaccinated.

3 FMT in Gastrointestinal and Neurological Diseases

FMT, the therapeutic method has been proven to be incredibly successful in the treatment of many gastrointestinal diseases such as CDI, IBD, IBS and several neurological and many other disease conditions.

3.1 *Clostridium difficile* Infection (CDI)

Clostridium difficile is a gram-positive, anaerobic, spore-forming and toxin-producing bacillus and is responsible for antibiotic-associated diarrhoea. CDI is one of the important nosocomial infections and is highly contagious and affects more of the population (Miller *et al.,* 2011) [39]. CDI causes symptoms ranging from mild watery diarrhoea to lethal pseudomembranous colitis. Vancomycin or metronidazole or fidoxomicin is widely used to treat CDI, but 15-30% of patients experience symptomatic recurrence after discontinuation of antibiotics (Kelly *et al.,* 2015) [30]. *Clostridium difficile* overgrowth occurs in the colon when the diversity of the gut flora is reduced by the administration of antibiotics. To an extent, FMT can restore the diversity of the gut flora similar to that of healthy donors. It has shown a rapid response and cure rate of around 90% with a negligible significant adverse event rate, regardless of the route of administration (Kassam *et al.,* 2013) [27]. The treatment can lead to the restoration of key *Firmicutes* and *Bacteroidetes* species with a decrease in *Proteobacteria* (Shahinas *et al*., 2012) [55]. The mechanism underlying decelerated growth of *C. difficile* after FMT though remains elusive, but likely includes niche exclusion, competition for nutrients and creation of a nutrient condition, unfavourable for growth like production of bacteriocins, increase in secondary bile acid production etc. (Kitahara *et al.,* 2000) [34]. *Bacillus thuringensis* secretes a bacteriocin, thuricin CD with narrow-spectrum activity against *Cl. Difficile* (Rea *et al.,* 2010) [48]. The *Lachnospiraceae* group supports the conversion of primary bile salts to secondary bile salts. Secondary bile salts such as lithocholates favour sporulation of vegetative cells (Sorg and Sonenshein, 2008) [57]. FMT also exert positive benefit by reducing sialic acid content in the gut with the help of commensal bacteria, thereby depriving *Clostridium* of a vital carbohydrate energy source (Ng *et al.,* 2013) [43].

3.2 Inflammatory Bowel Diseases

Ulcerative colitis (UC) and Crohn's disease (CD) are two major forms of Intestinal disorder popularly known as IBD. Chronic inflammation of the gastrointestinal tract and the cyclic nature of progression and remission are the peculiar characteristics of this disorder (Tan *et al.,* 2020) [60]. The onset of this disease condition is the outcome of ongoing unpleasant antigenic stimulation of intestinal epithelium caused by undesirable gut microflora (Loftus, 2004) [36] Although no specific infectious bacteria have been proven to cause IBD, accumulating evidence has demonstrated various alterations of the gut microbiota in IBD like, altered composition, reduced diversity, and a decreased number of bacteria in the gut microbiota. The dysbiosis is initiated due to the lack of species diversity. It is mainly associated with the reduction of *Bacteroidetes* phylum and the *Lachnospiraceae* group of phylum *Firmicutes.* The overshoot of *Proteobacteria* and *Actinobacteria* in the large intestine can also induce the disease condition (Morgan *et al.,* 2012) [42]. Studies have also shown lower levels of *Clostridium* cluster IV species, *Facelibacterium prausnitzi*, which has been associated with anti-inflammatory properties in patients with Crohn's disease (Varela *et al.,* 2012) [62]. Conventional whole stool FMT offers an untargeted approach to modify the underlying dysbiosis in IBD. FMT enemas in patients with UC and CD were found to be promising reporting that patients achieved clinical remission and maintained remission over long-term follow-up in many cases (Moayyedi *et al.,* 2015) [40]. Presently, many people are preferring FMT as an alternative treatment option over drug therapy. However, FMT is not as effective in IBD as it may be due to the multifunctional pathophysiology of IBD (Gupta *et al.,* 2016) [19].

3.3 Functional Gastrointestinal Disorders

Irritable Bowel Syndrome is very common among the population of North American countries. While medical treatment for IBS is still limited, the burden of overall illness is high and patients report a low quality of life, low work efficiency and absenteeism in the workplace (Gralnek *et al.,* 2000) [18]. The pathophysiology of IBS is not well defined, but studies point toward altered gut microbe inpatients. IBS can be classified into four types based on pronounced symptoms. The first one is IBS-D, where diarrhoea is most common. In the case of IBS-C, constipation is in most patients. The combined burden of diarrhoea and constipation is obvious in IBS-M. But in the case of IBS-U, one cannot be characterised by a pertinent symptom (Yao *et al.,* 2012) [69]. The reasons for dysbiosis such as visceral hypersensitivity, poor barrier function, and affected gut-brain axis are associated with IBS. The cure rate with FMT is higher than conventional treatment with antibiotics. FMT by restoring intestinal microbial balance

has appeared to be effective at improving the symptoms by increasing the IBS-stool scale score (Francis *et al.*, 1997) [16]. The treatment effect declines over time hence periodic and repetitive treatment is necessary because many studies on alpha diversity and beta diversity measurements had indicated variations in the microbiota as diversity did not reach up to the level identified in the healthy donors (Claesson *et al.*, 2011) [9].

3.4 Neurological and Psychiatric Diseasesc

GI symptoms are often associated with various neurological and psychiatric conditions. This targets the prime role of the gut microbiota and their imbalance and malfunctioning towards neuropathological progression (Hao-Ming *et al.*, 2021) [21]. The destruction of the intestinal epithelial barrier, loss of intestinal neurons and overproduction of pro-inflammatory cytokines are evidenced to be the major causes of the altered gut-brain axis. FMT can significantly improve the richness and diversity of gut microflora and regain the proportion of anti-inflammatory mechanisms. This will increase the amount of anti-inflammatory SCFA producing bacteria and the level of dopamine and tryptamines (Sun *et al.*, 2018) [58]. FMT will also lead to a reduction in α-synclein and trimethyl-N-oxide, which act as major reasons of Parkinson's disease and stroke (Kim *et al.*, 2019) [32]. Animal studies show that FMT is effective in treating psychiatric disorders like autism spectrum diseases, bipolar disorder, depression, anxiety etc. and is a mechanism for controlling obesity also (Yang *et al.*, 2020) [67]. The occurrence of GI diseases is more in ASD children compared to those with no ASD and is linked to problem behaviour mainly irritability in them (Restrepo *et al*., 2020) [49]. FMT is extensively used as a tool to correct ASD in children (Kang *et al.*, 2019) [25]. A recent study on mice models reported that FMT from aged donor mice to young germ-free mice led to a decline in strategic thinking and memory in young adult recipients (D'Amato *et al.*, 2020) [12]. Proteins supporting neurotransmission in hippocampus were affected by this process and directed to a condition of synaptic plasticity. It was evident from the presence of an ageing like phenotype in mice that underwent FMT from aged donors. Recent landmark studies in which patients with metabolic syndrome received FMT from lean, healthy donors have demonstrated metabolic improvements, especially in male patients. Another such study has demonstrated that low fermentable fibre supplementation following oral FMT, improved insulin sensitivity from baseline to six weeks in patients with severe obesity and metabolic syndrome (Mocanu *et al.*, 2021) [41]. For example, cellulose can act as a bulking and binding agent and that can influence gastrointestinal transit and modulate the donor microbe – host mucus layer interface. FMT can improve the adverse effects of hepatic encephalopathy by reducing urease-positive pathogens and increasing the expression of anti-inflammatory cytokines (Kawaguchi *et al.*, 2019) [28].

4 Step-up FMT Strategy

The step-up FMT consists of three steps. Step 1 refers to a single FMT, mostly used for CDI or refractory intestinal infections. Step 2 refers to multiple FMT, and is mostly used for IBD and partially refractory CDI. Step 3 denotes either single FMT or multiple FMTs together with typical medications (steroids, anti-TNF-α- antibody, enteral nutrition etc) for treating refractory complicated diseases and cancer (Wu *et al.*, 2019; Cui *et al.*, 2016) [66, 11]. The FMT restores or alters the immune status of the host, the function of the intestinal mucosal barrier and the sensitivity to regular medications.

5 Safety of FMT

So far FMT appears to be a safe process with no serious adverse events (SAE) reported irrespective of the abrupt announcement made by the FDA on the possibilities of infection, which had happened in the mid of 2019 following a reported mortality after the FMT procedure. The presence of Shigatoxin-producing *Escherichia coli* (STEC) and Enteropathogenic *Escherichia coli* (EPEC) have also been reported in the product supplied by a stool bank company based in the US. (URL-www.fda.gov) [73]. Here the donor may appear healthy even though acting as a career of some pathogenic microorganism. Hence use of next-generation sequencing (NGS) can act as a check measure to predict the risk of pathogenesis of the sample used in the FMT procedure (Ser H-L *et al.*, 2021) [53]. Adverse events can be divided into short-term and long-term, and short-term events can further be divided into those related to the method of FMT delivery and those related to the FMT itself. Many a times the patients who undergo FMT for various disease conditions are already suffering from other comorbidities. Some minor adverse events such as abdominal discomfort, bloating, flatulence, diarrhoea, constipation, vomiting and transient fever occur immediately after FMT. Transmission of enteric pathogens via FMT is also an important concern but appears to be rare with current screening. A greater theoretical risk may be the induction of chronic disease based on alterations in the gut microbiota. Clinical follow-up of patients over many years ideally combined with analyses of banked donor and recipient specimens will be crucial in assessing the possibility that FMT may increase or decrease the risk of several common chronic conditions.

6. Regulatory Issues with FMT

Worldwide, many countries are yet to accept FMT as a listed procedure for treating various diseases. To date, FMT is not fully considered as an integral service in modern health care even though being recommended for rCDI with a resolution rate of more than 90% and low hospital expenses (Allegretti *et al.*, 2019) [3]. But countries like Canada consider FMT as a 'New Biologic Drug' and require more refinement through the process of clinical trial application. The USFDA is also not questioning the efficacy of FMT as a treatment protocol, but strict regulations must be developed first. Major concern over the administrators in considering FMT as an integral service is the occurrence of unethical commercialisation of human material when the stool is being considered a drug. Moreover, severe pressure is happening from the pharma industries to classify FMT as a drug for huge business opportunities. To move one step ahead, the USFDA has taken an initiative to classify FMT as government-controlled drug therapy with no intervention by pharma companies in the stool business. Many countries are in the process of reclassifying FMT rather than a doctor's choice to a listed health or clinical practice. In the last November 2022, an Australian stool bank company got therapeutic goods administration approval for the first time in the globe for a microbiome-based therapy using donors to treat recurrent CDI. In Europe, the number of procedures between 2019-2020 is astounding and indicates FMT is a

widely accepted treatment. But with respect to other clinical practices, the numbers are still insignificant and a huge gap exists that needs to be filled by raising the clinical awareness and safety of the procedure in order to increase the FMT activity by 10-fold (Baunwall *et al.*, 2021) [4]. Swiss-based Ferring pharmaceuticals had received FDA approval for their product 'Rebyota', a novel first-in-class rectally administered microbiota-based live therapeutic product for treating CDI patients of eighteen and above (URL-www.fda.gov) [74]. The product is applied to patients who have completed antibiotic treatment for rCDI. Very recently in last month FDA has also approved first orally administered faecal microbiota product, 'Vowst' after several clinical studies (URL-www.fda.gov) [76]. Thus, gradually the FMT in its different forms are getting approved and certified as a treatment procedure for an array of diseases as explained. Due to some religious and ethical taboos, the use of FMT as a treatment is not practised in middle east countries like Jordan, but it might be considered on the failure of other treatments (Al-Bakri *et al.*, 2021) [2]. Research and development of FMT in clinical settings are crucial to assure the continued scrutiny of FMT procedure, effects and mechanism of action.

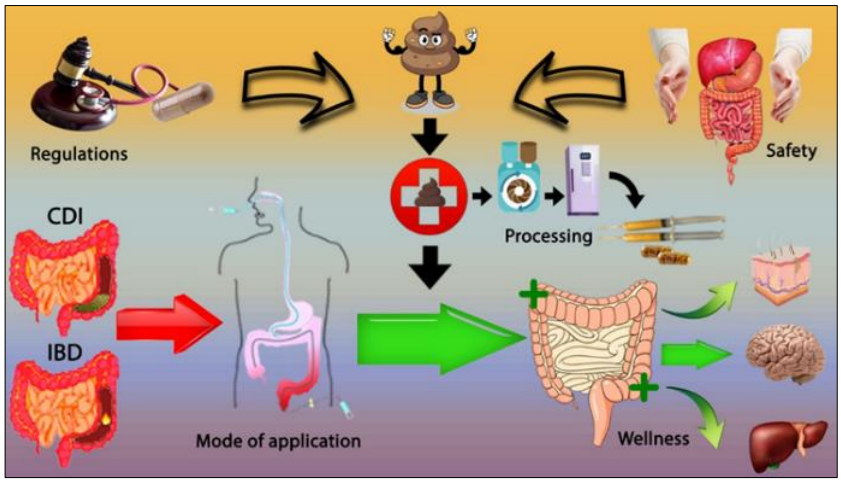

Fig 1: FMT- Stool a tool for wellness

7 Conclusion

The high therapeutic efficacy of FMT for various diseases like CDI, rCDI, and IBD is gaining momentum. People are becoming more aware of the capabilities of faecal microbiota and a number of diseases, both within and outside the GI tract may soon be treated with faecal microbes. There are many research findings that link dysbiosis with ill health and FMT will be an economical way to address them. Faecal therapy will continue to be refined beyond whole-stool transplants. But to say, FMT is not a 'free size' for all ailments, because it involves many parameters for the successful treatment of various conditions mentioned. Ongoing advances in the multi-omics technologies with integrated advanced bio-computational tools to analyse large and complex high-dimensional data sets will facilitate the development of defined microbial consortia targeted to treat specific diseases. In the current 21st century itself, FMT is poised to emerge as a new therapeutic duly supported by a regulatory framework in place and validated findings of clinical studies.

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Conflict of interests

The authors declare that there are no conflicts pertaining to this manuscript.

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