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Chapter

The Therapeutic Potential of the "Yin-Yang" Garden in Our Gut

Shabarinath Srikumar and Séamus Fanning

Abstract

The gut microbiota is made up of trillion microorganisms comprising bacteria, archaea, and eukaryota living in an intimate relationship with the host. This is a highly diverse microbial community and is essentially an open ecosystem despite being deeply embedded in the human body. The gut microbiome is continually exposed to allochthonous bacteria that primarily originates from food intake. Comprising more than 1000 bacterial species, the gut microbiota endows so many different functions—so many that can be considered as an endocrine organ of its own. In this book chapter, we summarize the importance of gut microbiota in the development and maintenance of a healthy human body. We first describe how the gut microbiota is formed during the birth of a human baby and how a healthy microflora is established overtime. We also discuss how important it is to maintain the microbiota in its homeostatic condition. A discussion is also given on how alterations in the microbiota are characteristic of many diseased conditions. Recent investigations report that reestablishing a healthy microbiota in a diseased individual using fecal microbial transplant can be used as a therapeutic approach in curing many diseases. We conclude this chapter with a detailed discussion on fecal microbial transplants.

Keywords: microbiome, microbiota, gut, antibiotic, IBD, FMT

1. Introduction

We, animals, live in a microbe-dominated planet. We are all covered, filled, and fueled by bacteria. All body surfaces like the skin, gastrointestinal tract, urogenital, and respiratory tract are in constant contact with the environment and are, therefore, colonized by bacteria. The realities of life associated with a microbe-dominated planet have led to the coevolution of animals with bacteria. This coevolution has led to close inhabitation of bacteria on different surfaces of the human body, especially the gut. Here, many bacteria and their phages, viruses, fungi, archaea, protists, and nematodes intermingle to form a microbial consortia collectively called "microbiome" or "microbiota". The presence and abundance of each taxonomic group may vary within population based on their access to adequate health care and local sanitation condition or within individuals based on their metabolic, medical, diet, and various other factors.

Despite being embedded deeply in the human body, the gut is essentially an open ecosystem—with constant exposure to environmental factors. The closure of the NIH-funded human microbiome project has given advanced understanding

about the composition and functional characteristics of the gut microbiota composition. The composition of the human microbiome varies significantly depending on the habitat [1]. For example, the gut microbiota is mainly populated by bacteria [2], while the skin harbors mainly fungi [3]. It was always considered that human microbiome outnumbered human nucleated cells by at least a factor of 10. However, reports suggest that the ratio is closer to 1 [4]. Recently metagenomic analysis concluded that the gene set of the human microbiome is 150 times larger than the human gene complement raising the possibility that the large bacterial genetic repertoire aids the human component in performing the essential functions that are not encoded by the human genome. Since more than 99% of these genes are bacterial in origin, the number of bacterial species were calculated to be around 1000–1150 bacterial species. The figures collectively emphasize the biological importance of the microbiome, and the genetic complement can be rightly considered as the second genome [2]. However, the taxonomic diversity of the gut microbiome notwithstanding, this chapter will deal only with the bacterial populations and all further references to gut microbiota means gut bacterial microbiota.

A typical human gut microbiota contains about 10^{14} bacteria and is made up of more than 1000 bacterial species [5, 6]. The human gut microbiota is composed of six bacterial phyla—*Firmicutes*, *Proteobacteria*, *Bacteroides*, *Fusobacteria*, *Actinobacteria*, and *Verrucomicrobia*. Of this, *Bacteroides* and *Firmicutes* occupy 70–90% of the total bacteria present in a healthy gut, while others are present in lower abundances [7]. Under healthy circumstances *Proteobacteria* and *Verrucomicrobia* members are also present but in lesser abundance [8]. The huge complexity and the variability of the microbiome make the determination of precise metabolic functions and the host-microbe cross talk very difficult. However, recent advances in deep sequencing and computational biology have contributed to large advances into understanding the unique biology of the gut microbiota and the subject is still in its infancy.

The functions bestowed by the bacterial gut flora are so enormous that it can be considered as an endocrine organ on its own [9]. It is well understood that the gut microbiota and their metabolites play important roles in host homeostasis, such as providing important nutrients like secondary bile salts and B/K group vitamins [10, 11], help in fermenting the otherwise indigestible complex plant carbohydrates such as dietary fibers into short-chain fatty acids (SCFA) [12], contributing to an effective intestinal epithelial barrier and activation of both innate/adaptive immune responses of the host [13]. In addition, the healthy gut microbiome drives intestinal development by promoting vascularization, villus thickening, mucosal surface widening, mucus production, cellular proliferation, and maintaining epithelial junctions [14–16]. The influence of the gut microbiota, either directly or indirectly, affects the physiology of most host organs even the brain [14, 17–20].

The taxonomic composition of the microbiota is very subtle—subject to change with variations in the diet [21], feeding time changes [22], sleep wake cycles, and even jet lag [23]. The unique taxonomic signature of the gut microbiota has to be strictly maintained for a healthy gut. Any disruption of the taxonomic composition of the gut could lead to conditions such as inflammatory bowel syndrome (IBS), asthma, obesity, metabolic syndrome, and cancer [24]. In some cases, resuscitation of the gut microbiota using probiotic bacteria like *Bifidobacteria* and *Lactobacillus* leads to the mitigation of gut inflammation consequently regaining health.

In this chapter, we will sequentially detail how the microbiota establishes itself in a human body, how minor or major variations in the gut microbiome

introduces diseased conditions, and how the recent therapeutic approaches aimed at resuscitating the healthy microbiota can cure many dysbiotic microbiota-associated conditions.

2. Our internal garden—on how gut microbiota is planted and nurtured

How do we become colonized with gut microbiota? Where do we get our initial inoculum from and how does this initial inoculum proceed in to a well-flourished, well-established microbiota? The first step to understanding this is to identify the initial inoculum and how this inoculum develops into a the full-fledged adult ecosystem following a series of ecological succession steps.

2.1 The initial colonization

The most prevalent concept, and a very incorrect one, is that babies are borne sterile and after birth, the body becomes immediately colonized with microbes from the surrounding environment. Placental mammals such as humans are borne through a birth canal, which is colonized by microbes. A baby acquires its first inoculum from the birth canal. A healthy vaginal microbiota is composed of few bacterial species [25, 26] and is predominated by *Lactobacilli* [27]. Consequently, naturally borne babies acquire vaginal microbes like *Lactobacillus, Prevotella*, and *Sneathia* spp. [28]. The bacteria are present in the mouth, skin, and even in the meconium. Therefore, all neonates are colonized by essentially the same vaginally derived bacteria obtained vertically from the mother. Once this microbiota is established, the microbiota becomes highly differentiated depending on their ability to colonize different body sites. For example, in the gut, facultative anaerobes establish and reduce the environment [29]. This highly reduced environment facilitates the colonization of obligate anaerobes [30–33]. In addition, breastfeeding will also enrich vaginally acquired lactic-acid-producing bacteria in the baby's intestine [34]. From then on, it is the physiology of the host habitat that selects the community that becomes well adapted to colonize that particular habitat. For example, the physiochemical, immunological, and the diet play an important role in determining the microbial community that will colonize the small and large intestines. The host genotype also appeared to influence the composition of the gut microbiota [35].

Cesarean section babies, in contrast to vaginally borne babies, are dominated by skin-associated bacteria like *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* spp. [28]. The *Staphylococcal*-rich microbiota could be obtained from the skin of the humans the baby is in contact with. The lack of a natural first inoculum in C-section babies affects the bacterial community in the GI tract [36, 37]. This variation from the naturally borne baby's microbiome will increase the susceptibility of the C-section babies to certain pathogens. For example, about 70% of the MRSA-caused skin infection happens to C-section babies. In addition, there is an additional risk to atopic diseases [38], allergies, and asthma [28, 39].

2.2 Development of the microbiota

The establishment, composition, and the density of the microbiome in the gastrointestinal (GI) tract depends on the biochemical factors like pH, oxygen gradient, antimicrobial peptides (AMPs), bile salts, etc. There is a pH gradient across the GI tract—lowest in the stomach and gradually increase to the terminal ileum, and a drop in caecum and increases toward the distal colon. Oxygen also exhibits a gradient across the length of the tract. The levels are highest in the upper GI tract, which

decrease to anaerobic conditions in the distal colon. Radially across the tract too, there exists a oxygen gradient. Anoxic conditions exist in the lumen, while there is an increase in the oxygen tension near the mucosa, and this oxygen is rapidly consumed by the facultative anaerobes [40]. Each area in the gut produces its own AMPs. Saliva contains lysozyme, which is antibacterial. Small intestine produces α-defensins, C-type lectins, lysozyme, and phospholipase A2. The large intestine produces β-defensins, C-type lectins, cathelicidins, galectins, and lipocalin. Mucus also plays an important role in the distribution of the gut microbiota. The mucus in the stomach and colon can be discriminated into two layers—outer loose layer, which is densely populated by bacteria, and the inner "solid" layer, where bacteria is sparce [41, 42]. Only mucin-degrading bacteria like *Akkermansia muciniphila* reach to the inner solid layer. Some pathogens like *Helicobacter pylori*, *Salmonella*, *Yersinia*, *Campylobacter*, etc. can also reach the inner mucus layer [43, 44]. All these factors play an important role in the establishment and the distribution of the gut microbiota.

The initially colonized microbiome has relatively few bacterial species. But during the initial phase of life, bacterial diversity increases in the microbiota. This may be because of the constant exposure of the baby to the environment. The gradual increase in the length of the GI tract can also provide a new niche for the bacteria to colonize. The bacterial diversity also depends on the diet as the introduction of a more plant-based diet increases the proportion of the Firmicutes [45]. Though lifestyle, illness, puberty, and other variable factors affect the microbiome, family members tend to have similar microbiomes with shared bacterial strains [35]. Thus, during the first year of life, the microbiome proceeds through a very variable phase. A distinct composition resembling the "adult microbiome composition" is established once an adult diet is established after weaning [35]. Once established, the gut microbiome composition seems to remain stable for a long time, possibly lifelong [46].

3. Maintain the flora!—on how any alterations could be disastrous

Biologically, the gut microbiome is very essential for the normal functioning of the human body. This complex ecosystem is responsible for many critical functions like (1) metabolism and energy regulation [47]—up to 10% of our daily consumed calories are provided by the microbes who break down complex plant-derived carbohydrates into short-chain fatty acids (SCFA), the main energy source of the enterocytes. From this perspective, alterations in the gut microbiome can contribute to obesity [48, 49] and consequently type II diabetes [50]; (2) immune system activation [51–53]—the colonic mucosal immune system plays a dual role and in that it must tolerate the gut microbiome and at the same time react against pathogenic organisms. This homeostasis is achieved by the intricate interplay between the microbiome and the host; (3) colonization resistance—physiologically colonized body surfaces are intrinsically protected from pathogen colonization. It is the intricate interplay between the above mentioned three major functions of the gut microbiota that brings about the physiological healthy state of the host.

Colonization resistance is the native ability of the host to suppress the invasion by exogenous microorganisms [54]. The concept of colonization resistance originated from the studies of Dubos in 1965 who demonstrated that indigenous gut microbiota neutralize colonization by a potential pathogen [55]. Slightly earlier, it was noted that loss of obligate anaerobic bacterial population in the lower intestinal tract correlated with infection, suggesting that the commensal anaerobic organisms were providing colonization resistance [56–59]. At the time, colonization

resistance was thought to result from microbe-mediated inhibition. We now know that the multiple mechanisms like microbiota-mediated activation of host immune responses are also involved. Colonization resistance provides broad protection against bacteria, virus, and other categories of pathogens [60]. On the other hand pathogenic microorganisms can out compete commensal microorganisms, subvert the immune response and invade the epithelia. For example, some pathogens can cause inflammation in the gut and utilize the consequent nutrient-rich inflammatory environment to outgrow other Proteobacteria. However, in a healthy intestine, the gut microbiota maintains the stiff colonization resistance by three mechanisms (1) directly inhibiting or killing the invading organism, (2) maintaining a protective musical barrier, and (3) stimulating a strong immune response that can neutralize a pathogen.

3.1 Direct inhibition

Bacteria produce many bioactive molecules, such as antimicrobial peptides, bacteriocins, etc., to selectively kill or inhibit the growth of competing bacteria [61]. These bioactive molecules are the primary source of antibiotics in the pharmaceutical industry [62].

3.2 Barrier maintenance

Gut microbiota regulates the strength of the intestinal barrier and sequesters themselves within the intestine. For example, the mucus layer is an important deterrent for many pathogenic microorganisms to reach the underlying epithelial cells. Mucus production is enhanced when a germ-free mice epithelium is exposed to some bacterial products [63], which means that an intact microbiota is essential to maintain the required thickness of the mucosal layer to keep pathogenic microorganism at bay. Diet can also influence the thickness of the mucosal layer. An assessment of intestinal microbiota localization with immunofluorescence shows that the absence of microbiota-accessible carbohydrates in the diet resulted in a thinner mucosal layer, thus exposing the underlying epithelial cells to pathogenic organisms [64]. The thinning of the mucosal layer made mouse susceptible to colitis.

3.3 Immune maturation and inflammation

A healthy microbiota is essential for a healthy immune system. Almost one and half decades ago, it was observed that microbial products secreted from the microbiota induced the colonic immune system by activating anti-inflammatory cells and cytokines. In 2005, a polysaccharide from *Bacteroides fragilis*, an important member of the gut microbiota, was shown to be important in the cellular and physical maturation of a developing immune system [51]. Perhaps, the most immunologically characterized bacterial metabolite synthesized by the microbiota is the Short Chain Fatty Acid (SCFA). In 2009, it was shown that SCFA directly bind G-protein-coupled receptor (GPR43) and activated immune responses [65]. Butyrate is the most characterized SCFA. Butyrate was shown to induce the differentiation of colonic regulatory T cells (Treg) cells in mice. A comparative NMR-based metabolome analysis showed that the concentration luminal butyrate correlated with the number of Treg cells in the colon [52]. Treg cells expressing transcription factor Foxp3 are also important in regulating intestinal inflammation. It was also found that SCFA plays an important role in regulating the function and the size of the colonic Treg pool [53].

Microbes can also directly activate the colonic immune system. Segmented filamentous bacterium (SFB) from the microbiota was shown to adhere tightly

to epithelial cells of the terminal ileum with Th17 cells, and this adherence correlated with the induction of inflammatory/antimicrobial defense genes [66]. More recently, bacteria in human feces were subjected to selection based on their potential to induce anti-inflammatory T regulatory cells. It was found that bacteria belonging to cluster XIVa clostridial group induced anti-inflammatory T regulatory cells along with bacteroides species [67, 68]. Gut microbiota can also activate the expression of bacterial C-type lectins in intestinal epithelial cells. The lectin, RegIIIγ, is essential to create a 50-μm clearance zone between the gut microbiota and small intestinal epithelial cells. Abrogation of the RegIIIγ synthesis increased the proximity of gut microbiota to the epithelial cells [69]. This shows that microbiota-activated lectin synthesis can directly act to suppress bacterial activity. In addition, gut microbiota can also enhance systemic antiviral activity [70]. Therefore, it is very important to maintain a healthy microbiota to drive efficient pro-inflammatory and anti-inflammatory immune responses in the host.

Simple alterations to the gut microbiota can often lead to very unhealthy consequences. For example, in the esophagus, the composition of the microbiome is heavily dependent on the microbes originating from the oral cavity and is dominated by *Streptococcus*, *Prevotella*, *Veillonella*, and *Fusobacterium* [71–73]. Any alteration to the microbiota composition could lead to inflammation and tumorigenesis. Such altered microbiota compositions were consistent with conditions like gastro-esophageal reflux disease (GERD), Barrett's esophagus (BE), and adenocarcinoma of the gastro-esophageal (GE) junction. Here, *Streptococcus* were found to be depleted while *Veillonella*, *Prevotella*, *Campylobacter*, *Fusobacterium*, *Haemophilus*, and *Neisseria* were enriched [74, 75]. Certain taxa present in the oral cavity like *Campylobacter concisus* and *Campylobacter rectus* were found to be enriched in the diseased mucosa-associated with GERD and BE [76, 77]. Similarly, for eosinophilic esophagitis (EoE), increased levels of *Neisseria*, *Corynebacterium*, and *Haemophilus* are reported [78].

The most important stomach associated bacterium is *H. pylori*. *H. pylori* has symbiotically co-evolved with humans and therefore are highly adapted to humans [79]. Early life time infection of *H. pylori* is beneficial for humans because it significantly lowers the risk of asthma in later years [80]. This beneficial association is brought about by the immune system modulation by the bacterium due to the high induction of regulatory T cells. The bacterium therefore qualifies for the position of a pathobiont -host determines whether the bacteria remains as a harmless symbiont or becomes pathogenic in nature. In a diseased condition of the host, the bacterium outcompetes the normal microbiota in numbers and becomes the most dominant pathogen.

Sampling the fecal material represents the colonic microbial population. However, sampling the small intestine is difficult because it is accessible only by invasive sampling. The small intestine is populated by distinct microbial communities that are less diverse and are dominated by *Veillonella*, *Streptococcus*, *Lactobacillus*, and *Clostridium* [81–83]. In the small intestine, alterations in the microbiome are associated with celiac disease (CeD). Gut microbiota is able to differentially degrade gluten. In CeD patients, there is an over growth of an opportunistic pathogen *Pseudomonas aeruginosa* producing a elastase called LasB. This enzyme degrades gluten and releases peptides that translocate the intestinal barrier, triggering a T-cell response [84]. A small-intestine-associated autoimmune disease where microbiome plays an important role is graft versus host disease (GvHD)—caused by the activation of T cells where host cells are recognized as antigens cause autoimmune attacks in the GI tract, liver, lung, and skin [85]. Germ-free mice had less propensity to develop GvHD—this led to the thought that microbiome could play an important role [86–88]. Loss of microbiome diversity and consequent

butyrate deprivation pushed cells to apoptosis bearing hallmark histological signs associated with GvHD. An overabundance of *Enterococcus* (*E. faecium* and *E. faecalis*) was observed in patients with GvHD associated with hematopoietic stem cell transfer confirming the association of GvHD with alteration in the microbiome diversity [89].

3.4 Antibiotic-associated colitis

Antibiotics considered as "wonder drugs" were implemented into therapy years before and have saved millions of lives. Even though antibiotics can reduce morbidity and mortality associated with bacterial illness, no antibiotic is pathogen selective. The application of antibiotics lead to collateral damage of accompanying microorganisms in a population, for example in a microbiota. Studies investigating the impact of antibiotics on microbiota confirm that antibiotic treatment increases the susceptibility of an individual to bacterial pathogens by compromising colonization resistance [90–93].

The observation that antibiotic therapy reduced colonization resistance making the host susceptible to bacterial infections was observed very early in the literature [56–59]. Gut microbial compositional analysis of an antibiotic-treated mice showed the expansion of γ-proteobacteria and enterococci, suggesting that gut microbiota somehow suppressed the expansion of oxygen-tolerant species [94, 95]. A study on healthy volunteers treated for a week or less with antibiotics reported persistent effects on their bacterial flora that included a loss of biodiversity on the gut flora, insurgence of antibiotic resistance strains, and upregulation of antibiotic resistance genes [96]. Antibiotic treatment can also induce long-term defects in the microbiota. For example, a single dose of clindamycin induced long-term susceptibility to *Clostridium difficile* infection [92]. A prior treatment with antibiotics not only disturbed the gut microbiota enabling the expansion of pathogenic commensals but also helped exogenic bacterial pathogens to establish inside the gut. When antibiotic-treated mice was infected with vancomycin-resistant *Enterococci* (VRE), the bacteria displaced the whole normal microbiota of the small and large intestine. In the clinical setting, this initial domination by the VRE preceded the bloodstream infections in patients undergoing hematopoietic stem cell transplant [91].

Microbiota establishes itself very early in the life cycle of every human, and this development is very crucial for a healthy lifestyle [97]. So, administration of antibiotics in the early stages of life predisposes the individual to diseases in late infancy or adulthood, particularly allergic or metabolic syndromes [28]. Antibiotic exposed prenatal mice resulted in exacerbated asthma following intranasal challenge with ovalbumin [98]. This case is true in children who are administered with antibiotics in the first year of life and may develop asthma during sixth or the seventh year [99]. Early use of marcolids in Finnish children led to the development of asthma and increased BMI associated with a dysbiotic gut microbiota [100]. Effects of antibiotic administration in early life are not limited to development of asthma alone but also to obesity. A low dose of penicillin delivered at birth transiently shifted the microbiota, and this transient shift induced sustained effects in body composition, leading to obesity [101]. All these reports emphasize the detrimental microbiota-associated effects of antibiotics and their implications in health.

Clostridium difficile is perhaps the most characterized pathogen associated with antibiotic associated colitis [102]. With >25,000 annual cases worldwide, *C. difficile* colitis is almost always associated with prior antibiotic use. The suspicion that microbiota-mediated colonization resistance resisted *C. difficile* in the gut was finally proven in 2013 [103]. 16S rDNA sequencing alone could distinguish between *C. difficile*-associated diarrhea and *C. difficile*-negative diarrhea [104]. Antibiotic

treatment reduces secondary bile salt production making the host susceptible to *C. difficile* infection [105, 106]. Apart from *C. difficile*, *Klebsiella oxytoca* also caused antibiotic-associated hemorrhagic colitis (AAHC)—a patchy hemorrhagic colitis usually observed after penicillin therapy typically dominating the right colon. Here, the pathogen is intrinsically resistant to β-lactams and the production of enterotoxin tilivalline can lead to intestinal epithelial apoptosis and colitis [107, 108]. Antibiotic-treated mice had impaired innate and adaptive antiviral immune response, and when the mucosa was exposed to influenza virus, the clearance was substantially delayed. On the other hand, these mice had severe bronchiole epithelial degeneration and increased host mortality when exposed to influenza. This is due to the macrophages from an antibiotic-treated mice had decreased expression of genes associated with antiviral immunity [70].

3.5 Inflammatory bowel disease (IBD)

IBD perhaps is the first diseased condition where alterations in microbiota are studied most extensively. IBD is a term mainly used to describe two disease conditions—Crohn's disease and ulcerative colitis. Here, intestinal cells play an important role in integrating the interactions among intestinal microbiota, mucosal immune system, and environmental factors [109]. It was observed very early that IBD conditions had a genetic component—there was a 10-fold increase in risk if related closely to the patient [110]. Genome-wide association studies (GWAS) reported many genetic factors that are associated with IBD [111]. As more GWAS based studies began identifying genetic factors associated with IBD, it was soon noted that some IBD genetic factors were also associated with other disease conditions like diabetes [112]. Curiously, GWAS and meta-analysis identified considerable overlap between susceptibility loci for IBD and mycobacterial infections [113]. The genetic associations notwithstanding, alterations in the gut microbiome of IBD patients have always been an interesting topic for microbiome researchers. The most significant alteration in the composition of the gut microbiome associated with IBD is the reduction in the abundance of the protective bacterium *Faecalibacterium prausnitzii* [114]. However, patterns of gut microbiota dysbiosis was not consistent across different studies. In a large cohort study involving more than 400 pediatric cases, multiple samples obtained from multiple locations of the GI tract before and after the onset of Crohn's disease were analyzed. Increased abundance of *Enterobacteriaceae*, *Pasteurellaceae*, *Veillonellaceae*, and *Fusobacteriaceae* and decreased abundance of *Erysipelotrichales*, *Bacteroidales*, and *Clostridiales* were found to be strongly consistent with the diseased condition. Oddly enough, there seems to be prevalence of oral bacteria in IBD and Crohn's disease patients. For example, the prevalence of oral and stomach-associated *C. concisus* was very high in both diseased conditions [115]. Furthermore, another Gram-negative oral bacteria *Fusobacterium nucleatum* was found to be abundant in Crohn's disease [116]. *F. nucleatum* was shown to be highly proinflammatory and protumorigenic [117–119]. The bacterium can activate the epithelial cell proliferation and induce a protomeric microenvironment, while inactivating the immunological tumor surveillance.

3.6 Colorectal cancers (CRC)

CRC is the fourth leading causes of death causing cancer and is the third important cause of malignancy. The CRC incidence is growing fast worldwide in low and middle east countries and is expected to increase by 60% by 2030 worldwide [120]. The transformation from a healthy epithelial cell to a malignant cell requires three steps: (1) induction of oncogenic mutations within Lgr5+ intestinal stem

cells, (2) altered β-catenin/Wnt signaling, and (3) proinflammatory cascades such as TNFα-NFκB and IL16-STAT3 catalyzing CRC development (Garret 2015 EN). Initially, there was increasing evidence about the role of bacteria in CRC. Bacteria such as *Fusobacterium nucleatum, E. coli*, and *Bacteroides fragilis* were shown to be associated with CRC [117, 121]. *F. nucleatum* was first shown to be highly enriched in tumors [117, 122]. The bacterium produces the FadA antigen, a ligand of E-cadherin in the intestinal epithelial cells that activate the β-catenin pathway leading to uncontrolled cell growth [119]. Furthermore, *F. nucleatum* is shown to be overrepresented in the colonic mucosa in the cases where the CRC relapses postchemotherapy. This was shown to be an interplay of intricate mechanisms including TLRs, miRNAs, and autophagy induction [123]. Some strains of *E. coli* harbor the polyketide synthase (pks) island encoding colibactin, capable of inducing DNA damage and mutation in epithelial cells [121]. A metagenome-wide association study on stools collected from patients with advanced adenomas, CRC, and healthy controls identified that certain *Bacteroides* species (*B. dorei*, *B. vulgaris*, *B. massiliensis*) and *E. coli* were overrepresented in the microbiome. Similarly, *Parvimonas*, *Bilophila wadsworthia*, *Fusobacterium nucleatum*, and *Alistipes* spp. were also overrepresented, suggesting that the gut microbiome signature can be used for early diagnosis and treatment [124].

4. The therapeutic potential of the gut microbiota

Humans have used live bacteria, particularly probiotic bacteria, for therapeutic purposes from time immemorial. We have seen some examples in earlier sections of this chapter. Perhaps, the best example of using live bacteria to cure infectious disease comes from antibiotic-associated CDI illness. *Clostridium scindens*, an obligate anaerobic bacterial species that inhabits the colon, has the rare ability to convert primary bile salts to secondary bile salts and is highly associated with resistance to *C. difficile* colitis [125]. Administration of *C. scindens* to susceptible mice resuscitated the secondary bile salt deficiency and rendered the animal more resistant to CDI. *C. scindens* and *C. difficile* have a negative correlation-could be the reason for *C. difficile* resistance in a healthy human gut microbiota [105]. However, the clinical benefit of using a single bacteria is limited. This is because, as we have seen earlier, many of the disorders are caused by a dysbiotic microbiota. Since a microbiota is very diverse in nature, resuscitation of a healthy gut microbiota cannot be achieved by the administration of a single bacterium. The concept of "putting back the bugs" was demonstrated in 1993 by using a combination of probiotic strains to cure chronic constipations and IBS [126]. Even with CDI, a cocktail of 10 gut commensal bacteria including obligate anaerobes could effect a cure [127]. Since then, many experiments have shown that by replenishing the healthy composition of a normal microbiota, many disease conditions can be controlled. Therefore targeting gut microbiota has gathered much attention and many options are currently being evaluated to achieve this goal of re-establishing the healthy gut microbiota to regain health—leading to the concept of fecal microbiota transplant (FMT).

FMT is the procedure where fecal matter is collected from a tested donor, diluted in an isotonic solution, strained, and transplanted into the patient using colonoscopy, endoscopy, sigmoidoscopy, or enema. The history of using stool of healthy donors to treat human diseases dates back to the fourth century in China, during the Dong Jin dynasty (AD 300–400 years) [128]. Fecal suspensions or "yellow soup" was used to treat serious disorders such as food poisoning, febrile disease, typhoid fever, etc., becoming the first record of the utilization of human feces to

treat human diseases. There are striking similarities between the earlier yellow soup and modern day FMT technology—(1) the inoculum originated from human fecal matter, (2) administration route is digestive tract, and (3) the fecal matter re-establishes the microbiota thereby treating the disease. This long tradition might be the reason why FMT is so well accepted in China [129]. In Europe, the first report of using fecal enema to treat pseudomembranous colitis came in 1958 [130]. Currently, FMT stands in the threshold of becoming a great technology to cure many disorders considered incurable in the past.

4.1 In treating diseases associated with gut microbiota-associated dysbiosis

CDI perhaps was the first condition in which a treatment was attempted using FMT. In 1983, it was shown that by re-establishing the healthy gut microbiota using FMT, mitigation of CDI can be achieved (Schwan 1983 EN). Severe CDI cases can lead to intensive care admission, sepsis, toxic megacolon, and can prove fatal. Colectomy is the standard method of treatment, but the mortality rate is 50%. In a study involving 29 patients who underwent FMT plus vancomycin for severe CDI cases, 62% of the patients were cured in a single FMT, while 38% needed multiple FMTs. Taken together, FMT was highly efficient for CDI infections [131]. The primary and secondary cure rates with FMT using fresh fecal sample to cure CDI is 91 and 98% [132]. FMT from frozen fecal sample also gave similar efficacies in treating CDI [133, 134]. By 2013, FMT was made officially the treatment strategy for CDI [103, 135]. Many pharmaceutical firms are actively working to bring easily consumable CDI-targeted drugs based on FMT. A defined microbial ecosystem therapeutics (MET-1 or RePOOPulate) was developed to cure recurrent CDI [136, 137]. The closest enema-based drug that is awaiting clinical approval is RBX2660, which depends upon the microbial suspension provided from the donor and is formulated for therapeutic delivery. With positive results in phase 2, the drug is currently in phase 3.

Similar to CDI, IBD is also a dysbiotic-associated disease where FMT is a potential therapy. However, in IBD, the use of FMT is a bit complicated and less efficient than in CDI. Early studies using FMT to treat IBD showed very promising result with good microbiota remission reported over long-term follow up [138]. With years, the outcomes started to differ depending upon sample size, treatment approaches, and study designs [139]. Even within IBD patients, remission rates were different. Crohn's disease had a higher remission of 61%, while ulcerative colitis patients had a remission rate of 22%. It is clear that the FMT treatment for IBD is complicated by numerous factors like differences in treatment regimens, stool preparation/formulation, and dosing frequencies. Varied levels of dysbiosis and difference in the microbiota composition between donor and patient also complicate FMT treatments. However, it was reported that FMT with intensive doses and multiple donors induced clinical remission and endoscopic improvement in ulcerative colitis patients, and this treatment had distinctive improvements in the microbiota composition [140]. It was also shown that a second FMT 3 months past the first one greatly improved the efficacy and safety in treating IBD with FMT [141, 142]. Here, the patients received FMT repeatedly in 3 month intervals—in a procedure called step-up FMT. The efficacy of the procedure increased at each step and was best suggested for patients with refractory IBD and immune-related diseases [143, 144]. There are currently 27 ongoing clinical trials using FMT targeting IBD with two additional trials on children with IBD [145].

Cancers like colorectal cancers that are associated with a dysbiotic microbiome opening the possibility for a therapeutic intervention using FMT. It was shown that bacteria like *Enterococcus hirae* and *Barnesiella intestinihominis* strengthen

cyclophosphamide-induced therapeutic immunomodulatory effects in cancer [146]. This has been highlighted very recently with evidence that microbiome influences with body's ability to respond to antibody therapy for cancers [147, 148]. A correlation was observed between commensal microbial composition and clinical response to anti-PD-L1 therapy through abundance of bacterial species like *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium* [149]. When fecal matter from responding patients were transplanted to germ-free mice, the animals were noted with stronger tumor control, augmented T cell responses, and better efficacy [150]. Gut microbiota could also affect anticancer responses with CTLA-4 [151]. The effects of radiation in gut microbiota and the clinical implications of a modified microbial balance postradiotherapy are now being investigated [152]. The microbiota can be modified to improve its efficacy and reduce the toxic burden of these treatments [153]. FMT can be used to reduce the radiation-induced toxicity and the increase the survival rate in irradiated mice. Here, the WBCs, GI tract function, and intestinal epithelial cell integrity were improved [154]. The research advances notwithstanding, therapeutic approaches associated with FMT is still in its nascent stage. However, considerable progress made in this area of research indicate that the application of FMT based therapy to mitigate mortality associated with diseases like cancer is a near possibility.

FMT is also showing great promise in patients undergoing hematopoietic stem cell transplantation surgery. Administration of a series of prophylactic antibiotics during surgery can result in the loss of microbial gut diversity and antibiotic resistant strains like *Streptococcus viridans*, *Enterococcus faecium*, and other Enterobacteriaceae can expand their population in the gut. This loss of microbial diversity during stem cell transplantation is associated with marked increase in mortality [91, 155, 156]. Restoration of a healthy microbiota by eliminating the dominant pathogenic microorganisms therefore becomes very important strategy from a therapeutic point of view. FMT involving a consortium of obligate anaerobic commensal bacteria containing especially *Barnesiella* is shown to eliminate *E. faecium* in mice [157], opening up a new therapeutic approach for stem cell transplant patients.

4.2 Collection, preparation, and delivery of FMT samples

Collection and preservation of the stool samples carry the primary importance in FMT. Freshly collected feces can either be immediately used, lyophilized, or cryopreserved. The efficacy of FMT in treating CDI using fresh or frozen feces varied but not significantly [158]. The cure rate was 100% in patients receiving fresh feces, 83% for the lyophilized group, and 78% for patients receiving frozen feces. But the efficacy was more pronounced in treating IBD, and this was demonstrated to be caused due to loss of bacteria in frozen feces [143]. The laboratory preparation methods of FMT is also critical for the success of FMT. Recent studies have reported that some preparation methods can stress the living microbial cells affecting the efficacy of FMT [159] emphasizing the need for extreme care. For example, *Faecalibacterium prausnitzii* is affected when the fecal sample is exposed to oxygen. Currently, the preparation methods can be classified into "rough filtration" (RF), "filtration plus centrifugation" (FPC), and "microfiltration plus centrifugation" (MPC) [141, 142]. Manual preparation methods takes about 6 hours to complete [160]. With the introduction of automated systems and close cooperation between laboratory scientists and clinicians, the time period of preparation from "defecation to freezing" has been shortened to 1 hour [160]—has effectively increased the efficacy of FMT when tested against IBD patients [143]. Current FMT delivery technologies include delivery of the microbiota to

upper, mid, and the lower gut [161]. Oral intake of capsular microbiota delivers it to the upper gut [134, 162]. A suspension of microbiota infusion can be transferred to the small intestine beyond the second duodenal segment through endoscopy [163], nasojejunal tube [143], mid-gut transendoscopic enteral tubing (TET) [164], and percutaneous endoscopic gastro-jejunostomy (PEG-J) [161]. The TET procedure for microbiota transplant is considered very successful [164]. Delivery of microbiota to the lower gut could be through colonoscopy, enema, distal ileum stoma, colostomy, and colonic TET [161]. Colonic TET is recommended for patients needing frequent FMT.

Several groups are developing stool products that can be packaged, transported, commercialized, and easily administered by physicians or consumed by patients. These products range from basic (frozen or freeze-dried stool) to more advanced products like capsules of synthetic stool grown in culture and assembled. The most basic products, from stool banks like OpenBiome and Advancing Bio, provide hospitals with screened frozen material ready for clinical use. More advanced are products like RBX2660, a cryopreserved filtered microbiota derived from stool of selected donors and administered via an enema system. The most advanced is a lyophilized powder that can be reconstituted by rectal infusions developed by CIPAC Therapeutics.

4.3 Precision microbiome reconstitution

The lack of regulatory protocol and stiff resistance from clinicians treating chronically ill patients has dampened efforts to introduce FMT as a viable therapy. This led to the development of the concept of "precision microbiome reconstitution," where a single bacterium can be used to restore colonization resistance in *C. difficile* patients [105]—providing a more targeted approach where a consortia of specific bacterial strains are identified to treat a particular diseased condition, and this will enable greater specificity and quality control. In germ-free mice, a murine isolate belonging to the family *Lachnospiraceae* partially restored colonization resistance against *C. difficile* [165]. An elaborate study using mouse models, clinical studies, metagenomic analysis, and mathematical modeling identified *C. scindens* as an intestinal bacterium associated with resistance to *C. difficile*. *C. scindens* produces growth inhibitory or spore germination inhibitory secondary bile acids to inhibit *C. difficile*. Furthermore, colonic induction of anti-inflammatory T regulatory cells can be used to develop immunity against dysbiotic conditions. A community of 17 strains including *C. scindens* induced the development of anti-inflammatory T regulatory cells, and this reduced colitis [67]. They also identified that the concentration of short-chain fatty acids increased upon the colonization of these 17 isolates. The fact that short-chain fatty acids modulated a Treg cell response suggested a common pathway by which different microbes modulated an induction of Treg cells. This opportunity was utilized to identify many more strains mostly belonging to bacteroides that are capable to induce an immune response that can restore colonization resistance from a dysbiotic condition [68]. However, even though a single strain may be able to resist a single organism of interest, a community of organisms reflecting the diversity of microbiota might be needed to restore baseline colonization resistance. This specific targeted approach is used by pharmaceutical firms to develop targeted drugs—for example, Seres Therapeutics is developing SER-109—comprising bacterial spores enriched and purified from healthy stool and packaged into capsules. The product can restructure a dysbiotic gut to a healthy microbiome. Vedanta Biosciences are identifying and developing

bacterial strains that can suppress chronic gut inflammation. Similarly, a microbial assemblage derived from stool and grown in culture called RePOOPulate has been developed to treat CDI infections.

4.4 The importance of SAFE FMT

Safety of the patient should be of prime importance when an FMT procedure is considered, especially if the patient is having a poor immune status [166, 167]. Middle gut FMT procedures can cause vomiting and aspiration [168]. The nasojejunal tube could put the patient at high risk of aspiration and should be conducted with anesthesia [143]. There is enough evidence that the long-term safety of the patient should be considered as well. Generally, a tested donor fecal sample is used for FMT. However, this carries the disadvantage that unwanted or potentially pathogenic bacterial phenotypes maybe carried from donors to recipients. A particular case was reported where the patient developed new onset obesity after obtaining a stool sample from a heterologous donor [169]. Using the patient's own stool sample can avoid the problems associated with donor stool samples. Here, a fecal sample of the patient is banked in the hospital before any procedure that requires antibiotic treatment. The banked sample may provide the vital resource to avoid hospital-acquired infections and to replenish the patient's own microbiota. Preservation of the patient's own or donor feces pose a second challenge [170]. There are reports that fecal matter from patients with colon cancer promoted tumorigenesis in germ-free and carcinogenic mice. Potential cardiometabolic, autoimmune, and neurological disease also have been discussed. All these points to the tough screening and regulations are needed before a donor is selected for fecal sample prior FMT.

However, recent reports suggest that FMT is gaining wide acceptance among patients. A survey showed that among patients of Crohn's disease who received FMT, 56% showed satisfactory clinical efficacy, 74% showed willingness for a second FMT, and 89% expressed willingness to recommend FMT to other patients [171]. Also, the cost efficacy of FMT has been demonstrated worldwide [172–176, 177]. FMT is still very far from being implemented into routine therapy. The technique needs to undergo rigorous process of standardization before the therapy becomes applied in daily practice. Nevertheless the importance of gut microbiota in maintenance of a healthy lifestyle is demonstrated without doubt. In future therapeutic approaches including antibiotic therapy should take into consideration the impact it has on the gut microbiota and the clinicians should be mindful of the impact of the devastating secondary effects of these therapeutic approaches on the patient.

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Author details

Shabarinath Srikumar* and Séamus Fanning
UCD-Centre for Food Safety, School of Public Health, Physiotherapy and Sports Science, University College Dublin, Ireland

*Address all correspondence to: srikumar.shabarinath@ucd.ie

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