Gut Microbiota and Health
The intestinal microbiota promotes three levels of protection against enteric infection. (I) Saturation of colonization sites and competition for nutrients by the microbiota limit pathogen association with host tissue. (II) Commensal microbes prime barrier immunity by driving expression of mucin, immunoglobulin A (IgA) and antimicrobial peptides (AMPs) that further prevents pathogen contact with host mucosa. (III) Finally, the microbiota enhances immune responses to invading pathogens. This is achieved by promoting IL-22 expression by T cells and NKP46+ cells, which increases epithelial resistance against infection, as well as priming secretion of IL-1β by intestinal monocytes (M0) and dendritic cells (DCs), which promotes recruitment of inflammatory cells into the site of infection. In conditions in which the microbiota is absent, such as following antibiotic treatment, there is reduced competition, barrier resistance and immune defense against pathogen invasion.
The discovery of antibiotics in the last century is one of the most significant achievements of modern medicine. Pathogens that once devastated entire civilizations, such as *Mycobacterium tuberculosis*, could finally be controlled, suggesting a triumph over infectious disease. However, the rampant rise of antibiotic resistance among pathogens, compounded by a drying pipeline of novel antibiotic development by pharmaceutical companies, has rendered current therapeutic strategies ineffective. As such, it is speculated that we are entering a post-antibiotic era where pathogens once again reign with limited opposition and a minor scrape may pose the risk of a fatal infection [1,2]. To combat the renewed threat of pathogenic microorganisms, clinical approaches toward eradicating infectious disease must evolve.
... **certain enteric pathogens** are able to outcompete commensal microbes by actively triggering host inflammation which favors pathogen invasion and dissemination.

*C. rodentium*, *Campylobacter jejuni*, and *Salmonella enterica* serovar Typhimurium (STm) appear to induce inflammation as part of their infectious process, and increasing intestinal inflammation actually promotes disease.

Further, these reports surprisingly demonstrate that **pathogen-induced inflammation** adversely affects the microbiota, **reducing the numbers of beneficial bacteria**, which protect us from infections.

**Collectively, there is growing evidence for the notion that pathogens and symbiotic bacteria are engaged in an ‘evolutionary combat’, with the host serving as the battlefield.**
RegIIIg is a C-type lectin that possesses antimicrobial activity against Gram-positive microbes.

MAMPs
microbial associated molecular patterns

TLRs
pathogen recognition receptors
Defects in host–microbial symbiosis may predicate susceptibility to infection

... factors that determine an individual's susceptibility to infectious disease remain largely unknown.
The evidence summarized in this review suggests that **disruption of the microbiota through environmental influences** may compromise immune function, leading to increased susceptibility to infectious disease.

In particular, we propose that antibiotic use **may paradoxically promote bacterial and viral infections** by depleting immune-promoting gut bacteria.

For example, antibiotics are routinely administered in the hospital to patients admitted for various non-bacterial illnesses. Not only can this practice select for antibiotic-resistant microbes (an extensively reported phenomenon), but may also lead to nosocomial infections by reducing the ability of the immune system to fight infections.

Furthermore, antibiotic use over several generations may reduce gut bacteria diversity in entire populations, a notion proposed by the ‘disappearing microbiota’ hypothesis. In cases where antimicrobial use is justified, we speculate that the administration of commensal-derived products that promote immunity may represent a viable companion therapy to antibiotics … **efforts that support microbiome-mediated protection may be an effective approach to achieve resistance to infectious disease in the postantibiotic era.**
Bacteriotherapy for the treatment of intestinal dysbiosis caused by \textit{Clostridium difficile} infection

\textbf{Faecal microbiota transplantation (FMT)} has been used for more than five decades to treat a variety of intestinal diseases associated with pathological imbalances within the resident microbiota, termed dysbiosis.

FMT has been particularly effective for treating patients with recurrent \textit{Clostridium difficile} infection who are left with few clinical options other than continued antibiotic therapy.

Our increasing knowledge of the structure and function of the human intestinal microbiota and \textit{C. difficile} pathogenesis has led to the understanding that FMT promotes intestinal ecological restoration \textbf{and highlights the microbiota as a viable therapeutic target.}

However, the use of undefined faecal samples creates a barrier for widespread clinical use because of safety and aesthetic issues.

An emerging concept of bacteriotherapy, the therapeutic use of a \textbf{defined mixture of harmless, health-associated bacteria}, holds promise for the treatment of patients with severe \textit{C. difficile} infection, and possibly represents a paradigm shift for the treatment of diseases linked to intestinal dysbiosis.
Disturbances in the intestinal microbiota caused by infections and antibiotics have profound effect on the microbiota’s composition and function and can predispose the individual to **antibiotic-associated diarrhoea (AAD)**. In the last decade, the incidence of morbidity and mortality from *Clostridium difficile* infection (CDI), the leading cause of AAD, increased largely due to the emergence and **global spread of fluoroquinolone- resistant variant** of *C. difficile*.

First line **treatment** for severe *C. difficile* infections include metronidazole or vancomycin although in 15–35% of these cases, a recurrence (relapse or reinfection) follows the cessation of antibiotic therapy.

**FMT** has been mainly used as an alternative treatment for patients with persistent, recurrent *C. difficile* infection and involves the restoration of the intestinal microbiota through instillation of homogenized faecal suspension from a healthy donor.

**FMT** has also been utilized in the treatment of diseases associated with intestinal dysbiosis such as inflammatory bowel diseases (IBD; manifested as Crohn’s disease and ulcerative colitis), irritable bowel syndrome (IBS) and obesity.
A proposed model for recurrent *C. difficile* infection and the restoration of the intestinal microbiota through FMT or bacteriotherapy. Intestinal homeostasis (a) is characterized by large microbial diversity in the microbiota and health-associated metabolites. Antibiotic exposure disrupts the microbiota (b-c) by destroying the microbial community leading to reduction in the diversity and loss of colonization resistance. The microbiota usually expands in diversity (d) after antibiotics are stopped to restore diversity. Antibiotic disruption makes individuals hyper-susceptible to *C. difficile* colonization potentially leading to chronic infection and persistent dysbiosis (e). After treatment of CDI with antibiotics such as vancomycin, further microbiota disruption (f) occurs potentially leading to recurrent CDI after discontinued use of the antibiotic. FMT or bacteriotherapy disrupts intestinal dysbiosis leading to resolution of CDI (g) and increase in species diversity (d) and restores intestinal homeostasis.

Figure modified from [48].
C. difficile produces highly resistant and transmissible spores that can potentially persist in the gut of infected individuals or contaminate skin or environmental surfaces.

These spores are resistant to antibiotics and can recolonize and germinate after antibiotic therapy leading to recurrent disease. Asymptomatic carriers are also a source of spores that can promote transmission and persistence at both the hospital and global levels.

...patients with recurrent CDI had decreased proportional abundance of Bacteroidetes and increased Proteobacteria and Verrucomicrobia.

... the use of new antibiotic, fidaxomicin was found to have lower rates of recurrence of C. difficile infection associated with the non-epidemic strain compared to vancomycin, an effect that is attributed to its lower activity against commensal and beneficial gut microbes

FMT
In this process, homogenized faeces from a healthy donor is infused through colonoscopy, enema or nasogastrically to an individual with C. difficile disease to restore the intestinal microbiota and thereby eradicate CDI. The donor, usually a healthy individual/relative, is screened for contagious pathogenic agents such as Salmonella spp., Staphylococcus aureus, C. difficile and HIV and other infections or inflammatory conditions.
The intestinal microbiota of *C. difficile* patients treated with FMT is characterized by expansions in species diversity characterized by an increase in *Bacteroides*, *Roseburia* and *Faecalibacterium* and a reduction in *Enterobacteriaceae*.

**Rational design of a defined bacteriotherapy**

The use of undefined faecal samples for FMT creates a barrier for widespread clinical use, mainly because of the **amount of time needed** to prepare and **screen donor samples**, **patient safety issues**, **non-standardization** of the treatment procedure and general doctor and patient **aversion**. Therefore, there is an unmet clinical need to **design a combination of harmless, health associated bacteria** as a viable therapeutic option.
FMT is increasingly being accepted as a treatment for recurrent CDI, but large, randomized double-blinded studies are needed. However, beyond FMT, bacteriotherapy using standardized mixtures of beneficial bacteria could potentially be used in the future for the treatment of recurrent CDI and other diseases associated with dysbiosis in the intestinal microbiota such as IBD, IBS and obesity.
Faecalibacterium prausnitzii and human intestinal health

*Faecalibacterium prausnitzii* is the most abundant bacterium in the human intestinal microbiota of healthy adults, representing more than 5% of the total bacterial population.

Over the past five years, an increasing number of studies have clearly described the importance of this highly metabolically active commensal bacterium as a component of the healthy human microbiota.

Changes in the abundance of *F. prausnitzii* have been linked to dysbiosis in several human disorders.

Administration of *F. prausnitzii* strain A2-165 and its culture supernatant have been shown to protect against 2,4,6- trinitrobenzenesulfonic acid (TNBS)-induced colitis in mice.
Correlation have been found between the level of *F. prausnitzii* and obesity and Coeliac disease.
Proposed anti-inflammatory mechanisms of *F. prausnitzii*. 1. The supernatant of *F. prausnitzii* blocks NF-κB activation induced by a pro-inflammatory stimulus [21**]. 2. Butyrate produced by *F. prausnitzii* inhibits NF-κB activation in mucosal biopsies. 3. *F. prausnitzii* components might interact with CD103+ dendritic cells (DCs) in the lamina propria and stimulate their migration to mesenteric lymph nodes (MLN) and the induction of Tregs. 4. M cell transcytosis of *F. prausnitzii* in organized lymphoid structures may induce Tregs. 5. The capacity of *F. prausnitzii* to induce high amounts of IL-10 in antigen presenting cells may enhance the suppressive activity of Foxp3+ Tregs and block Th17 cells induced by pro-inflammatory stimuli.
*F. prausnitzii*, is a major EOS (extremely oxygen sensitive) component of the intestinal microbiota which has been largely ignored until recently. Its low prevalence in many intestinal disorders, particularly in IBD patients, suggests its potential as an indicator of intestinal health.

*F. prausnitzii* is a butyrate producer and has demonstrated antiinflammatory effects in vitro and in vivo using a mouse colitis model making it a key member of the microbiota that may contribute to intestinal homeostasis. Thus, modulation of *F. prausnitzii* abundance, for example using prebiotics and/or probiotics and/or formulations that permit survival through the upper part of the intestinal tract might have prophylactic or therapeutic applications in human health.

For instance, the fiber inulin has well-characterized impact on microbiota composition inducing specific and significant increase in *Bifidobacterium* and *F. prausnitzii*.

Moreover the rapid detection of *F. prausnitzii* abundance in feces warrants further investigation as a biomarker of intestinal health.
Gut Microbiota from Twins Discordant for Obesity Modulate Metabolism in Mice

We transplanted fecal microbiota from adult female twin pairs discordant for obesity into germ-free mice fed low-fat mouse chow, as well as diets representing different levels of saturated fat and fruit and vegetable consumption typical of the U.S. diet.

Increased total body and fat mass, as well as obesity-associated metabolic phenotypes, were transmissible with uncultured fecal communities and with their corresponding fecal bacterial culture collections.

Cohousing mice harboring an obese twin’s microbiota (Ob) with mice containing the lean co-twin’s microbiota (Ln) prevented the development of increased body mass and obesity-associated metabolic phenotypes in Ob cage mates ... was diet-dependent.

Monozygotic (MZ) or dizygotic (DZ) twins discordant for obesity provide an attractive model for studying the interrelations between obesity, its associated dietary and lifestyle risk factors, and the gut microbiota/microbiome. In the case of same-sex twins discordant for a disease phenotype, the healthy co-twin provides a valuable reference control to contrast with the co-twin’s disease associated gut community.
LF-HPP, low in fat (4% by weight) and high in plant polysaccharides
Diet-Specific Effects

LoSF-HiFV, low–saturated fat, high fruits and vegetables
HiSF-LoFV, saturated fats and the lower of fruits and vegetables
LoSF-HiFV diet

- Ruminococcus torques
- Clostridium symbiosum
- Clostridium sp. NML 04A032
- Firmicutes unclassified
- Akkermansia muciniphila
- Bacteroides caccae
- Parabacteroides merdae
- Paraprevotella clara
  - Bacteroides uniformis*
  - Bacteroides vulgatus*
- Butyricimonas virosa

Absolute Spearman's correlation coefficient $\rho$

- Blue indicates enrichment in mice colonized with an Ob microbiota
- Red indicates enrichment in mice colonized with or invaded by members of a Ln microbiota
Culture collections generated from human microbiota samples can transmit donor phenotypes of interest (body composition and metabotypes).

If these derived culture collections can transmit a phenotype, the stage is set for studies designed to determine which culturable components of a given person’s gut community are responsible.

Sequenced culture collections generated from human gut microbiota donors also provide an opportunity to model and further address basic issues such as the determinants of invasiveness including the mechanisms by which invasion is impacted by diet composition, as well as the mechanisms by which invading components affect microbial and host metabolism.

This issue is important for identifying next-generation probiotics, prebiotics, or a combination of the two (synbiotics).

Moreover, the ability to generate a culture collection, from an individual—whose composition is resolved to the gene level, whose properties can be validated in preclinical models, and whose “manufacture” is reproducible—may provide a safer and more sustainable alternative to fecal transplants for microbiome-directed therapeutics.
Fusobacterium nucleatum Potentiates Intestinal Tumorigenesis and Modulates the Tumor-Immune Microenvironment

Metagenomic analyses indicate that symbiotic *Fusobacterium* spp. are associated with human colorectal carcinoma, but whether this is an indirect or causal link remains unclear. We find that *Fusobacterium* spp. are enriched in human colonic adenomas relative to surrounding tissues and in stool samples from colorectal adenoma and carcinoma patients compared to healthy subjects.
In the ApcMin/+ mouse model of intestinal tumorigenesis, *Fusobacterium nucleatum* increases tumor multiplicity and selectively recruits tumor-infiltrating myeloid cells, which can promote tumor progression.
Although barrier defects expose the intestinal mucosa to the entire luminal microbial milieu, *Fusobacterium* spp. Become the most highly enriched bacterium in colorectal tumors relative to adjacent tissue.

This enrichment may be attributable to the **strong adhesive and invasive** abilities of fusobacteria for epithelial cells.

Alternatively, **metabolic specializations** may endow fusobacteria with a competitive advantage in the evolving tumor milieu. *Fusobacterium nucleatum* is an asaccharolytic bacterium; therefore, unlike the Enterobacteriaceae, it will not compete for glucose, a preferred substrate for tumor metabolism.

Instead, fusobacteria can utilize **amino acids** and **peptides** as nutrient sources in the tumor microenvironment.

In addition, *F. nucleatum* strains, unlike many strict anaerobes of the intestinal lumen, possess a **rudimentary electron transport chain**, endowing them with a limited ability to respire oxygen. Thus, *F. nucleatum* may be able to persist and slowly replicate in the hypoxic tumor microenvironment.

**Adhesive molecules that contribute to invasiveness** in *F. nucleatum* can promote bacterial aggregation and biofilm formation that also enhance oxygen tolerance. Products of fusobacterial metabolism may make the tumor microenvironment more tumor permissive over time by directly promoting tumor cell proliferation, blood vessel growth, or immune cell infiltration.